RELATIONSHIPS BETWEEN GLUCOCORTICOID HORMONES AND PHYSIOLOGICAL PARAMETERS IN FEMALE ASIAN ELEPHANTS (*Elephas maximus*)

TREEPRADAB NORKAEW

DOCTOR OF PHILOSOPHY
IN VETERINARY SCIENCE

GRADUATE SCHOOL
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A THESIS SUBMITTED TO CHIANG MAI UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN VETERINARY SCIENCE

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Examination Committee:

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(Dr.Boripat Siriaroonrat)

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25 December 2018

To my parents who inspires and motivates me.

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Treepradab Norkaew

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25 December 2018

หัวข้อดุษฎีนิพนธ์ ความสัมพันธ์ระหว่างฮอร์โมนกลูโคคอร์ติคอยด์และพารามิเตอร์ทางสรีรวิทยาในช้างเอเชียเพศเมีย (*Elephas maximus*)

ผู้เขียน นางสาวตรีประดับ หน่อแก้ว

ปริญญา ปรัชญาดุษฎีบัณฑิต (วิทยาศาสตร์การสัตวแพทย์)

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บทคัดย่อ

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาความสัมพันธ์ระหว่างความสมบูรณ์ของร่างกายกับหน้าที่ทางสรีรวิทยาและผลกระทบของฤดูกาลท่องเที่ยวต่อสภาวะความสมบูรณ์ของร่างกาย การทำงานของต่อมหมวกไต ระดับไขมัน และการเผาผลาญของช้างเพศเมีย (ทั้งหมดเท่ากับ 33 เชือก) ที่อาศัยอยู่ในภาคเหนือของประเทศไทย โดยทำการประเมินความสมบูรณ์ของร่างกาย (BCS) (ความสมบูรณ์ของร่างกาย; 1 = ผอมมาก, 5 = น้ำหนักเกินมาก) และความเข้มข้นของกลูโคคอร์ติคอยด์เมตาบอไลท์ในอุจจาระ (FGM) 2 ครั้งต่อสัปดาห์ เป็นเวลา 1 ปี เพื่อให้ทราบถึงความแตกต่างด้านฤดูกาลในช้างที่ใช้ในการท่องเที่ยวในประเทศไทยจำนวน 33 เชือก และศึกษาความสัมพันธ์ระหว่างความสมบูรณ์ของร่างกายและการทำงานของต่อมหมวกไตต่อระดับไขมัน [คอเลสเตอรอลรวม (TC) ไลโพโปรตีนความหนาแน่นต่ำ (LDL) ไลโพโปรตีนความหนาแน่นสูง (HDL) และไตรกลีเซอไรด์ (TG)] และพารามิเตอร์ทางเมตาบอลิก [อินซูลิน กลูโคส ฟรุกโตซามีน และอัตราส่วนระหว่างกลูโคสต่ออินซูลิน (G:I)] นอกจากนี้ผู้วิจัยได้ทำการเปรียบเทียบเมตาบอลิคฮอร์โมน ระดับไขมันและความเข้มข้นของกลูโคคอร์ติคอยด์เมตาบอไลท์ในอุจจาระกับคะแนนความสมบูรณ์ของร่างกาย ระหว่างช่วงฤดูท่องเที่ยว (พฤศจิกายน - กุมภาพันธ์) และช่วงนอกฤดูการท่องเที่ยว (มีนาคม - ตุลาคม) ในปางช้าง 5 ปาง จากการศึกษาพบว่าความสมบูรณ์ของร่างกายมากที่สุดอยู่ระหว่าง 3-3.5 (60.6%) รองลงมาได้แก่ ความสมบูรณ์ของร่างกาย = 4 (น้ำหนักเกิน) ที่ 27.3% และ ความสมบูรณ์ของร่างกาย = 4.5-5 (น้ำหนักเกินมาก) ที่ 12.1% และไม่พบช้างที่ความสมบูรณ์ของร่างกาย <2 ความสมบูรณ์

ของร่างกายในช่วงฤดูฝนและฤดูหนาวสูงกว่าในช่วงฤดูร้อน ในทำนองเดียวกัน จากการศึกษาพบว่า ระดับของกลูโคคอร์ติคอยด์เมทาบอไลท์ในอุจจาระ ไตรกลีเซอไรด์ ไลโพโปรตีนความหนาแน่นสูง ไลโพโปรตีนความหนาแน่นต่ำ และอินซูลินในช่วงฤดูฝน และ/หรือ ฤดูหนาวสูงกว่าในช่วงฤดูร้อน ($p<0.05$) ในทางตรงกันข้าม ระดับคอเลสเตอรอลรวมและกลูโคสมีค่าต่ำสุดในฤดูฝน อีกทั้งยังพบว่ากลูโคคอร์ติคอยด์เมทาบอไลท์ในอุจจาระมีความสัมพันธ์เชิงลบกับปัจจัยด้านสิ่งแวดล้อมสองปัจจัย ได้แก่ อุณหภูมิและปริมาณน้ำฝน แต่ไม่พบความสัมพันธ์ระหว่างกลูโคคอร์ติคอยด์เมทาบอไลท์ในอุจจาระกับความชื้น ความสมบูรณ์ของร่างกายมีความสัมพันธ์เชิงบวกกับ คอเลสเตอรอลรวม ไลโพโปรตีนความหนาแน่นต่ำและไลโพโปรตีนความหนาแน่นสูง กลูโคคอร์ติคอยด์เมตาบอไลท์ในอุจจาระมีความสัมพันธ์กับคอเลสเตอรอลรวม ไลโพโปรตีนความหนาแน่นสูง กลูโคสและอินซูลิน ($p<0.05$) อีกทั้งยังพบว่าทั้งความสมบูรณ์ของร่างกายและกลูโคคอร์ติคอยด์เมตาบอไลท์ในอุจจาระมีความสัมพันธ์เชิงลบกับอัตราส่วนระหว่างกลูโคสต่ออินซูลิน ($p<0.05$) อย่างไรก็ตาม ไม่พบความสัมพันธ์ระหว่างความสมบูรณ์ของร่างกายกับกลูโคคอร์ติคอยด์เมตาบอไลท์ในอุจจาระในช้างที่ใช้ในการศึกษา ระดับเมตาบอลิค ระดับไขมัน (ยกเว้นไตรกลีเซอไรด์) ความสมบูรณ์ของร่างกายและกลูโคคอร์ติคอยด์เมตาบอไลท์ในอุจจาระในช่วงฤดูการท่องเที่ยวแตกต่างกันอย่างมีนัยสำคัญเมื่อเทียบกับนอกฤดูการท่องเที่ยว การศึกษานี้แสดงให้เห็นถึงความสัมพันธ์เชิงบวกระหว่างจำนวนนักท่องเที่ยวกับความสมบูรณ์ของร่างกาย ไตรกลีเซอไรด์และอินซูลิน ($p<0.05$) ในทางกลับกันจำนวนนักท่องเที่ยวมีความสัมพันธ์เชิงลบกับระดับกลูโคส ($p<0.05$) ยิ่งไปกว่านั้น จากการวิจัยพบว่าคอเลสเตอรอลรวมและไตรกลีเซอไรด์ มีความสัมพันธ์เชิงบวกกับช่วงเวลาการทำงาน ในขณะที่ระยะทางการเดินมีความสัมพันธ์เชิงบวกกับระดับคอเลสเตอรอลรวม ระยะเวลาการทำงานและระยะทางการเดินมีความสัมพันธ์เชิงลบกับระดับกลูโคส ฟรุกโตซามีน และอินซูลิน ปริมาณอาหารหลัก (หญ้าและต้นข้าวโพด) มีความสัมพันธ์กับกลูโคคอร์ติคอยด์เมตาบอไลท์ในอุจจาระ ความสมบูรณ์ของร่างกาย และระดับอินซูลิน ปริมาณอาหารเสริม (เช่น กล้วย อ้อย และฟักทอง) มีความสัมพันธ์เชิงบวกกับระดับกลูโคคอร์ติคอยด์เมตาบอไลท์ในอุจจาระ กลูโคส ฟรุกโตซามีน และอินซูลิน ในแต่ละปางค่าความสมบูรณ์ของร่างกาย คอเลสเตอรอลรวม ไลโพโปรตีนความหนาแน่นสูง อินซูลิน และกลูโคสมีความแตกต่างกัน ในปางที่ช้างไม่มีกิจกรรมการทำงานพบอัตราส่วนระหว่างกลูโคสต่ออินซูลินต่ำที่สุด อีกทั้งยังพบว่าในช่วงฤดูการท่องเที่ยว มีระดับกลูโคคอร์ติคอยด์เมตาบอไลท์ในอุจจาระสูงกว่าช่วงนอกฤดูการท่องเที่ยว (ยกเว้นเพียง 1 ปาง) จากการใช้ความสมบูรณ์ของร่างกายและกลูโคคอร์ติคอยด์เมตาบอไลท์ในอุจจาระเป็นตัวแปลผลในการวิเคราะห์การถดถอย พบว่า ค่าความสมบูรณ์ของร่างกายและกลูโคคอร์ติคอยด์เมตาบอไลท์ในอุจจาระที่เพิ่มขึ้นมี

ความสัมพันธ์ต่อระดับไขมันและสภาวะทางเมตาบอลิกในช้าง และยิ่งไปกว่านั้น จำนวนนักท่องเที่ยว ชั่วโมงการทำงาน ปริมาณอาหารเสริมอาจส่งผลต่อสภาวะร่างกาย ระดับความเครียด ระดับเมตาบอลิค และระดับไขมันในช้างได้ ดังนั้นการมีน้ำหนักเกิน และการได้รับปัจจัยกระตุ้นต่างๆ อาจสงผลต่อการเพิ่มขึ้นของการทำงานของต่อมหมวกไตซึ่งอาจก่อให้เกิดผลเสียต่อสุขภาพ ดังนั้น ควรมีการเปลี่ยนแปลงการจัดการที่จะส่งผลเสียต่อตัวช้าง ในขณะที่ควรเพิ่มกิจกรรมของช้างที่จะช่วยป้องกันสภาวะน้ำหนักเกิน

Dissertation Title Relationships Between Glucocorticoid Hormones and Physiological Parameters in Female Asian Elephants (*Elephas maximus*)

Author Miss Treepradab Norkaew

Degree Doctor of Philosophy (Veterinary Science)

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ABSTRACT

This study investigated relationships between body condition and physiological function and tourist season effects on body condition, adrenal function, lipid profiles and the metabolic status of female elephants (n=33) in northern Thailand. We evaluated the body condition score (BCS; 1=thinnest, 5=fattest) every other months and fecal glucocorticoid metabolite (FGM) concentrations twice monthly in 33 tourist elephants in Thailand for a 1-year period to assess seasonal variations, and determined how lipid profiles [total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides (TG)] and metabolic parameters [insulin, glucose, fructosamine, glucose to insulin ratio (G:I)] related to measures of body condition and adrenal function. Furthermore, we compared metabolic hormones, lipid profiles, and FGM concentrations to body condition scores between High (November–February) and Low (March–October) tourist seasons at five elephant camps. The most prevalent body condition score was 3-3.5 (60.6%), with 27.3% at body condition score=4 (overweight) and 12.1% at body condition score = 4.5-5 (very overweight); no elephants had a body condition score <2. Body condition score were higher in rainy and winter seasons compared to summer, with FGM, TG, HDL, LDL, and insulin also higher in the rainy and/or winter seasons ($p<0.05$). In contrast, TC and glucose were lowest in the rainy season. FGM measures were negatively associated with two environmental factors: temperature and rainfall, but not

humidity. Positive correlations were found between body condition score and TC, LDL, and HDL, and between FGM and TC, HDL, glucose, and insulin ($p<0.05$), whereas BCS and FGM were both negatively associated with the G:I ($p<0.05$). However, there was no relationship between body condition score and FGM among the camp elephants. Differences between High and Low tourist season months were found for all measures of lipid and metabolic status, body condition score, FGM, with the exception of TG. There were positive associations between tourist number and body condition score, TG, and insulin ($p<0.05$). In contrast, tourist number was negatively correlated to glucose ($p<0.05$). There were positive associations between work time, TC, and TG, whereas walking distance was positively related to TC. Both work time and walking distance were negatively correlated to glucose, fructosamine and insulin. The amount of primary diet (grasses and cornstock) fed was correlated to FGM, body condition score, and to insulin. Diet supplement amount (e.g., bananas, sugar cane, and pumpkin) also was positively correlated with FGM, glucose, fructosamine, and insulin concentrations. There were camp differences of body condition score, TC, HDL, insulin and glucose being among the highest, and G:I being the lowest in the one camp with no work activities for elephants. Moreover, all but one camp had higher FGM during the High compared to the Low tourist season. Using body condition score and FGM measures as outcome variables in separate regression models, this study found high body condition score and elevated FGM concentrations were associated with altered lipid profiles and metabolic status in elephants. Furthermore, body condition, stress levels, metabolic markers, and lipid profiles in captive elephants may be affected by visitor numbers, work time, and the amount of supplementary foods offered by tourists. Thus, being overweight and exposed to factors that increase adrenal activity could adversely affect health status, requiring alterations in management for some individuals, whereas exercise appears to have a protective effect against obesity.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ACUC	Institutional Animal Care and Use Committee
BCS	Body condition score
CETP	Cholesteryl ester transfer protein
CHOD-POD	Cholesterol oxidase-peroxidase
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CMU	Chiang Mai University
CRH	Corticotropin releasing hormone
CV	Coefficient of variation
DNA	Deoxyribonucleotide
e.g.	Exempli gratia, for example
EIA	Enzyme immunoassay
et al.	Et alia, and others
etc.	Et cetera, and other similar things
FA	Fatty acids
FGM	Fecal glucocorticoid metabolite
FRUC	Fructosamine
FVM	Faculty of Veterinary Medicine
g	gram
GCs	Glucocorticoids
GEE	Generalized estimating equations
GLU	Glucose
GLUT4	Glucose transporter type 4
GPO-POD	Glycerokinase peroxidase-peroxidase
GREs	Glucocorticoid responsive elements
G:I	Glucose to insulin ratio
h	Hour

LIST OF ABBREVIATIONS (continued)

HbA1c	Hemoglobin A1c
HDL	High density lipoproteins
HPA axis	Hypothalamic – pituitary –adrenal axis
HRH	Her Royal Highness
i.e.	Id est, that is
IL	Interleukin
INS	Insulin
IUCN	International Union for Conservation of Nature
kg	Kilogram
km	Kilometer
L	Liter
LCAT	lecithin-cholesterol acyltransferase
LDL	Low density lipoproteins
m	Meter
min	Minute
mm	Millimeter
mM	Millimolar
mmol	Millimole
NF-kB	Nuclear factor kappa light chain enhancer of activated B cells
n	Nano
PEPCK	Phosphoenolpyruvate carboxykinase
RH	Relative humidity
RNA	Ribonucleotide
RT	Room temperature
SAA	Acute-phase serum amyloid A
SEM	Standard error of mean

LIST OF ABBREVIATIONS (continued)

TC	Total cholesterol
TG	Triglycerides
THI	Thermal–humidity index
TNF	Tumor necrosis factor
U.S.	The United State
UK	The United Kingdom
VLDL	Very low density lipoprotein
11β-HSD 1	11β hydroxysteroid dehydrogenase type 1
11β- HSD 2	11β hydroxysteroid dehydrogenase type 2
μ	micro

LIST OF SYMBOLS

%	Percentage
&	Ampersand, and
<	Less than
=	Equality
>	Greater than
±	Plus-minus, a probability of being within the stated interval
∼	Approximately equal
°C	Degree Celsius
F	F-value, Kruskal–Wallis rank sum test
P	P-value

ข้อความแห่งการริเริ่ม

1) งานวิจัยนี้เป็นการศึกษาการประเมินความสัมพันธ์ระหว่าง BCS และปัจจัยด้านเมตาบอลิกในช้างเอเชียและเป็นการศึกษาแรกในการประเมินระดับไขมันในช้างสายพันธ์ดังกล่าว

2) งานวิจัยนี้เป็นการศึกษาแรกที่แสดงให้เห็นถึงความสัมพันธ์ระหว่างภาวะโภชนาการกับระดับฮอร์โมนความเครียดในช้าง

3) งานวิจัยนี้เป็นการศึกษาแรกที่แสดงให้เห็นถึงความสัมพันธ์ของปัจจัยด้านเมตาบอลิกและระดับไขมันในช้างเอเชียภายใต้การดูแลของมนุษย์ในประเทศไทยกับระยะทางการเดิน เวลาในการทำงานและปริมาณอาหารเสริมที่ให้แก่ช้างในฤดูการท่องเที่ยวและนอกฤดูการท่องเที่ยว

4) งานวิจัยนี้จะทำให้เข้าใจสภาวะเมตาบอลิกและระดับไขมันในช้างได้อย่างครบถ้วน รวมถึงการจัดการปางช้างแบบท่องเที่ยวให้ดีขึ้นเพื่อช่วยรับมือกับปัญหาที่อาจเกิดขึ้น ปัญหาโรคอ้วนและการส่งเสริมสุขภาพช้างที่ยั่งยืน

STATEMENTS OF ORIGINALITY

1. This study was to evaluate relationships between BCS and metabolic factors in Asian elephants, and the first to assess lipid profiles in relation to body condition in this species.

2. This was the first study to examine relationship between nutritional status and stress hormone levels in elephants

3. This is the first study to investigate metabolic and lipid parameters in Asian elephants under human care in Thailand in relation to walking distance and working time, and provisioning of supplementary diet items by tourists during the High and Low tourist seasons

4. This research will result in a complete understanding of metabolic conditions and lipid profiles in elephants and facilitate better management of tourist camp elephants to combat a potentially serious problem, obesity, and promote a healthy, sustainable population.

CHAPTER 1

Introduction

The Asian elephant (*Elephas maximus*) is an endangered species (IUCN 2008) and has been listed in Appendix 1 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) since 1973 (CITES 2018). The captive elephant population has also declined along with that of the wild elephants. Asian elephants are a long-lived species, and produce only a few calves in their lifetime. Thus, it is important to better understand factors affecting health and reproduction to prevent further population declines.

Poor health and reproduction in captive elephants are both suspected of being linked at least in partially to obesity as a result of either too little exercise or feeding foods too high in calories, or both ;(Clubb *et al.*, 2009; Morfeld *et al.*, 2016; Morfeld *et al.*, 2017). There are numerous documented links between obesity, metabolic and lipid problems in other species, including humans, companion and domestic animals (Jeusette *et al.*, 2005; Jung *et al.*, 2014; Watson *et al.*, 1990). Abnormally elevated blood glucose, TG, and cholesterol have been linked to a number of disease problems, collectively called metabolic syndrome, such as hypertension, hyperlipidemia, insulin resistance, and type 2 diabetes (Lanktree *et al.*, 2013; Lee *et al.*, 2012). In contrast, few studies have examined these health factors in relation to body condition in elephants. Most Thai tourism camps encourage the feeding of elephants by tourists, often with high calorie treats, such as sugar cane and bananas. Many camps have trouble breeding elephants, and birth rates are not keeping up with death rates. Thus, a better understanding of how body condition and metabolic function affect the health of tourist camp elephants is needed.

In addition to obesity, stress can also affect metabolic health and lipid parameters. Glucocorticoids (GCs) are endogenous stress hormones that affect nearly every organ and tissue in the body, modulating various physiological processes including energy homeostasis (metabolism), immunology, behavior, reproductive function, cell proliferation, and survival (Patel *et al.*, 2014). While important for maintaining metabolic equilibrium, excessive GCs exposure for prolonged periods can have devastating effects on health. Working elephants in Thailand interact with the public in a variety of ways, including performing in shows, trekking, bathing, and painting. Often these activities are not closely monitored or regulated, and could be sources of stress to individual animals. Tourist activities have been shown to compromise welfare and negatively affect behavior and physiology in other species, resulting in increased hiding behaviors (Birke, 2002; Stevens *et al.*, 2013), heightened vigilance (Clark *et al.*, 2012; Larsen *et al.*, 2014), stereotypies (Mallapur *et al.*, 2002; Wells, 2005), poor body condition (Maréchal *et al.*, 2016), and elevated cortisol (Davis *et al.*, 2005; Pifarré *et al.*, 2012). Most studies of this type have been conducted in western zoo animals, with few focusing on captive animals in range countries, especially elephants and particularly those in tourism. High numbers of work hours have been associated with increased adrenal corticoid output (Lundberg *et al.*, 2002; Strzelec *et al.*, 2011), and a number of negative health effects, such as poor body condition (Ayalew *et al.*, 2018). However, others report positive health effects of work (Urtasun *et al.*, 2018), so relationships between work, health and well-being are not always clear (van der Hulst, 2003), and likely depend on the type of work being done, how the work is perceived by the individual, and if the work intensity exceeds a biological threshold.

Objective

The aims of this study were 1) to examine relationships between body condition score and fecal glucocorticoid metabolite (FGM) concentrations on metabolic function (insulin, glucose, fructosamine, glucose to insulin ratio) and lipid profiles (total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein) in female Asian elephants in Thailand, 2) to examine the effect of tourist numbers and working hours on adrenal, lipid and metabolic function in working elephants in female Asian elephants. The hypothesis was that high body condition score and high stress elephant exhibit more characteristics of metabolic aberrations and obesity than low body condition elephant and tourist numbers and working hours have an effect on adrenal, lipid and metabolic function. This project will facilitate better management of captive elephants to promote their sustained survival and combat potentially serious problems such as obesity and metabolic abnormalities.

CHAPTER 2

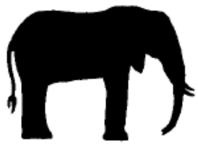
Literature Review

2.1 Biology of Elephant

2.1.1 Classification of elephant

There are three living species of elephants, classified in 2 genera; *Loxodonta* and *Elephas*. Based on the accessible morphological evidence, *Loxodonta*, described by the living African elephant, appears to be more primitive than *Elephas,* described by the living Asian elephants (Fowler *et al.*, 2008) (Table 2.1). There are four subspecies of Asian elephants, the Sri Lankan subspecies (*Elephas maximus maximus*) the mainland subspecies (*Elephas maximus indicus*), the Borneo subspecies (*Elephas maximus borneensis*), and the Sumatran subspecies (*Elephas maximus sumatranus*). Subspecies of Asian elephants are distinguished by geographic distribution and several physical characteristics. *E. m. sumatranus* is the most primitive of the three subspecies, *E. m. maximus* the most derived, and *E. m. indicus* an intermediate form.

Table 2.1 Comparing between the African (left) and the Asian (right) Elephant (Fowler and Mikota, 2008).

		
Weight	4,000-7,000 kilograms (8,820-15,430 pounds)	2,000-5,500 kilograms (4,410-11,020 pounds)
Height at shoulder	3-4 meters (10-13 feet)	2-3.5 meters (6 feet 7 inches-11 feet 6 inches)
Skin	More Wrinkled	Smoother
Number of ribs	Up to 21 pairs	Up to 20 pairs
Highest point	At top of shoulder	At top of head
Size of ears (pinnae)	Larger, do exceed height of neck	Smaller, do not exceed height of neck
In mature individuals dorsal of pinnae	Fold medially	Fold laterally
Shape of back	Concave	Convex or level
Shape of belly	Slopes diagonally downwards from front to back	Either almost horizontal or "sagging " in the middle
Shape of head	No compression; no bulges, no dish 	Compressed antero-posteriorly; has dorsal bulges, dished forehead 
Teeth	Lozenge-shaped loops 	Narrow compressed loops 
Food	Mostly browser	Mostly grazer
Tusks	Both sexes possess tusks; larger in males	Males usually carry tusks; in females tusks are vestigial or absent
Trunk	Has more rings (annulated), less rigid	Appears to have less annulation, more rigid
Tip of trunk	Two "fingers" 	One "finger" 
Number of nail-like structures (toes)	Forefeet 4 or 5 Hind feet 3, 4 or 5	Forefeet 5 Hind feet 4 or 5

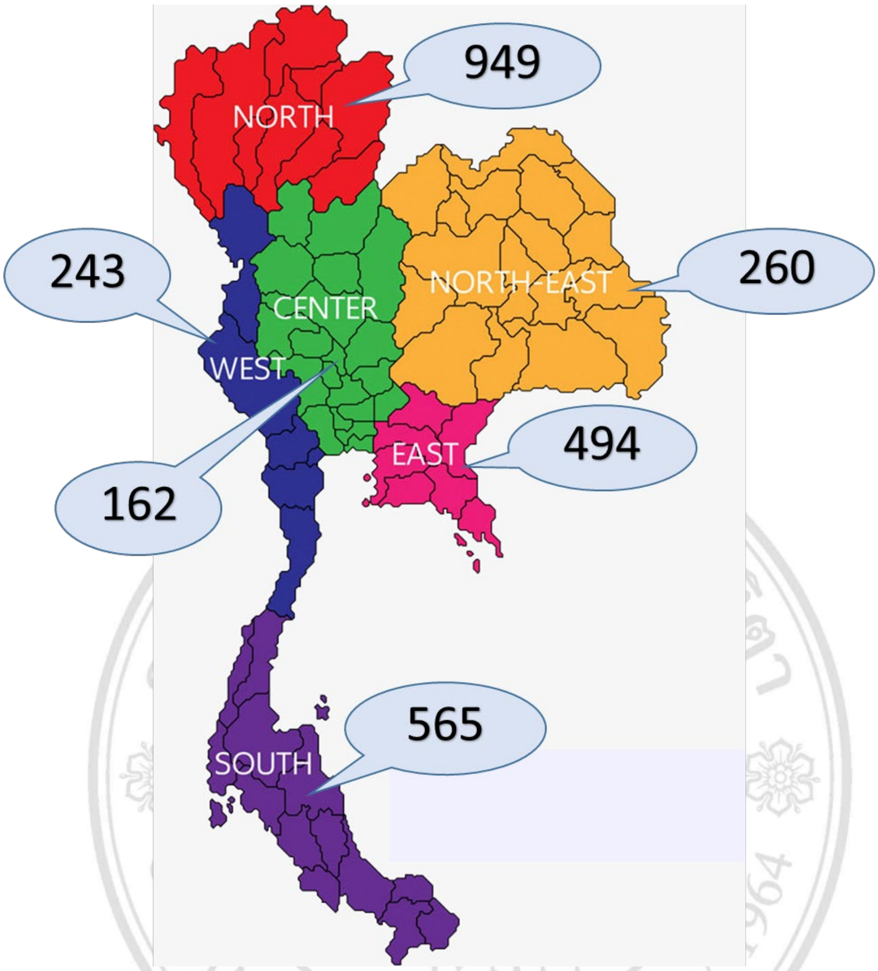


Figure 2.1 Number of semi-captive elephants in each region of Thailand.

2.1.2 Geographic Range

In Thailand, wild elephant population is estimated at 3,100-3,600 individuals distributed in 69 protected areas. There are about 3,783 captive elephants with almost 95% of them privately owned and working in 223 tourism sites throughout the country mainly in the north, especially in Chiang Mai (892 elephants) (Asian Elephant Specialist Group, 2017) (Figure 2.1).

2.2 Animal welfare

Animal welfare has to do with the feelings experienced by animals: the absence of strong negative feelings, usually called suffering and (probably) the presence of positive feelings, usually called pleasure (Duncan, 2005). Clark et al. (1997) reported that, interest in and concern about well-being has been increasing because of changing views towards non-human animals, societal attitudes, legislation and conduct of quality scientific research and testing (Clark *et al.*, 1997). The research data from a variety of fields, such as animal biology and behaviour, stress biology and psychoneuroimmunology, increasingly support a holistic view of well-being. Existing data suggest an interactive system linking internal psychologic, neurologic, physiologic, immunologic, endocrine and biochemical events with the external psychosocial and physical environment (Clark et al., 1997). A key concept when evaluating animal welfare that was initially developed in 1965 was that of the "Five Freedoms" that animals should possess (Brambell, 1965). These were later modified by Webster, (2005), gaining widespread acceptance in regulatory, industry, and advocacy bodies, including incorporation into many codes of practice (Table 2.2) (Webster, 2005). Similar concepts have been developed and amplified into a five-domains model (Table 2.3), which focuses on nutrition, environment, health, and behavior, as well as an overarching mental component (Mellor *et al.*, 2015).

Table 2.2 The five freedoms.

The five freedoms, as defined by Webster 2005
1. Freedom from hunger or thirst by ready access to fresh water and a diet to maintain full health and vigor.
2. Freedom from discomfort by providing an appropriate environment including shelter and a comfortable resting area.
3. Freedom from pain, injury, or disease by prevention or rapid diagnosis and treatment.
4. Freedom to express (most) normal behavior by providing sufficient space, proper facilities, and company of the animal's own kind.
5. Freedom from fear and distress by ensuring conditions and treatment that avoid mental suffering.

Table 2.3 The Five Domains model and promoting positive welfare states.

Physical/Functional Domains

Survival-Related Factors						Situation-Related Factors	
1: Nutrition		2: Environment		3: Health		4: Behaviour	
Restrictions on:	*Opportunities to:*	*Unavoidable/imposed conditions*	*Available conditions:*	*Presence of:*	*Little or no:*	*Exercise of 'agency' impeded by:*	*'Agency' exercised via:*
Water intake Food intake Food quality Food variety	Drink enough water Eat enough food Eat a balanced diet Eat a variety of foods	Thermal extremes Unsuitable substrate Close confinement Atmospheric pollutants: CO_2, ammonia, dust, smoke	Thermally tolerable Suitable substrate Space for freer movement Fresh air	Disease: acute, chronic Injury: acute, chronic; husbandry mutilations Functional impairment: due to limb amputation, or lung, heart, vascular, kidney, neural or other problems	Disease Injury Functional impairment	Invariant, barren environment (ambient, physical, biotic) Inescapable sensory impositions Choices markedly restricted	Varied, novel, engaging environmental challenges Congenial sensory inputs Available engaging choices
Voluntary overeating Force-feeding	Eating correct quantities	Unpleasant/strong odours Light: inappropriate intensity Loud/otherwise unpleasant noise	Pleasant/tolerable odours Light intensity tolerable Noise exposure acceptable	Poisons	Poisoning	Constraints on environment-focused activity Constraints on animal-to-animal interactive activity	Free movement Exploration Foraging/hunting Bonding/reaffirming bonds Rearing young Playing Sexual activity
		Environmental monotony: ambient, physical, lighting	Normal environmental variability	Obesity/leanness	Body condition appropriate		
		Unpredictable events	Predictability	Poor physical fitness: muscle de-conditioning	Good fitness level	Limits on threat avoidance, escape or defensive activity Limitations on sleep/rest	Using refuges, retreat, or defensive attack Sleep/rest sufficient

Affective Experience Domain

5: Mental State							
Negative	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*
Thirst Hunger (general) Hunger (salt) Malnutrition malaise Bloated, over full Gastrointestinal pain	Wetting/quenching pleasures of drinking Pleasures of different tastes/smells Pleasure of salt taste Masticatory pleasures Postprandial satiety Gastrointestinal comfort	*Forms of discomfort:* Thermal: chilling, overheating Physical: joint pain, skin irritation Physical: stiffness, muscle tension Respiratory: e.g. breathlessness Olfactory Auditory: impairment, pain Visual: glare/darkness eye strain Malaise from unnatural constancy	*Forms of comfort:* Thermal Physical Respiratory Olfactory Auditory Visual Variety-related comfort	Breathlessness Pain: many types Debility, weakness Sickness, malaise Nausea Dizziness Physical exhaustion	Comfort of good health and high functional capacity Vitality of fitness	Anger, frustration Boredom, helplessness Loneliness, isolation Depression Sexual frustration Anxiety, fearfulness, panic, anger Neophobia Exhaustion	Calmness Engaged, in control Affectionate sociability Maternally rewarded Excitation/playfulness Sexual gratification Secure/protected/confident Likes novelty Energised/refreshed

Welfare Status

2.3 Welfare assessment

Scientists have searched for standard goal to assess welfare. The various ways to measure stress have been proposed. A single measure of stress might not be a reliable source of indicator and it is usually more informative to combine multiple indicators of stress to assess animal welfare (Kumar *et al.*, 2012). Popular measures of stress such as hormonal profiles can be complemented with behavioural and immunological changes.

Injury, disease and abnormality are generally known as a major sources of suffering and poor welfare (Dawkins, 2003). The physiological measures that have been used include short term responses such as increases in heart rate, body temperature, respiratory rate, increases in adrenalin secretion, corticosteroids (sometimes called "stress" hormones), plasma glucose, lactate and free fatty acids (Broom *et al.*, 1993) and long term responses such as enlargement of the adrenal glands and depression of immune function (Reiche *et al.*, 2004). For example, hunted deer had higher serum levels of creatine kinase, aspartate aminotransferase, lactate dehydrogenase, plasma glucose, lactate, sodium, cortisol and endorphins compared to deer that had been shot with rifles (Bateson *et al.*, 1997). The problem is that many of the physiological indicators of 'welfare' currently in use are in fact autonomic responses that indicate activity rather than being specific to poor welfare. Heart rate, respiratory rate and level of corticosteroids, rise during, excite, exercise, and in anticipation of food. New technology that enables us to look directly at brain activity or to use urine, saliva or fecal measurements of stress hormone levels (Peeters *et al.*, 2011; Thanos *et al.*, 2009; Touma *et al.*, 2005) will, it is to be hoped, overcome some of these problems in the future.

2.3.1 Glucocorticoid measurement

Recently, non-invasive methods to measure fecal hormonal metabolites in elephants and other mammals have increased considerable importance, because of the relative ease of fecal sample collection of wild animals in their natural habitat. Further, the measurement of glucocorticoid in fecal samples is useful in determining the stress of captive Asian elephants (Kumar *et al.*, 2014).

Serum and fecal glucocorticoids are commonly measured for evaluating stress responsiveness in captive or restrained elephants (Kumar *et al.*, 2014; Norkaew *et al.*, 2018; Proctor *et al.*, 2015; Rasmussen *et al.*, 2008; Viljoen *et al.*, 2008). The study of stress hormones is essential to the understanding and evaluation of normal behavior, stress control, well-being, pregnancy status, and reproductive activity in elephants (Brown, 2000; Kajaysri *et al.*, 2014). Normally, before excretion in feces, the main glucocorticoid hormones are rapidly and extensively changed in form to glucocorticoid metabolites. Assays of fecal glucocorticoid or its metabolites in female elephants are validated for indicators of adrenal activity (Wasser *et al*., 2000). Moreover, fecal glucocorticoid assays have been validated and used for evaluating the reproductive status of elephants (Brown, 2000). Measurement of fecal glucocorticoid is a practical, reliable, and noninvasive method for this, because samples can be collected relatively easily in captive or semi-captive elephants without disturbing effects. Enzyme immunoassay (EIA) is the most common test for monitoring reproductive function, because it does not involve disposal of radioactive material or specialized and expensive equipment (Graham *et al.*, 2001).

2.4 Glucocorticoids and stress responses

Stress is defined as a state of threatened or perceived as threatened homeostasis. The body and mind respond to stress by stimulating an intricate repertoire of physiological and behavioral central nervous system and peripheral adaptive responses, which, if insufficient or excessive stress, may effect on physiological functions, such as growth, metabolism, circulation, reproduction, and the immune response (Charmandari *et al.*, 2005).

The central control stations of the stress system are located in the hypothalamus and the brain stem and include the parvocellular corticotropin releasing hormone (CRH) (Tsigos *et al.*, 2002). The hypothalamic - pituitary - adrenal (HPA) axis, together with the efferent sympathetic/adrenomedullary system, represent the effector limbs, via which the brain influences all body organs during exposure to threatening stimuli (Fig 2.2). Corticotropin-releasing hormone, which is released from the hypothalamus in response to stress, activates the HPA axis and stimulates the release of adrenocorticotropic hormone from the pituitary gland into the systemic circulation. The principle target of adrenocorticotropic hormone is the adrenal cortex, where it stimulates the release of cortisol, a glucocorticoid hormone. Cortisol has vital physiological functions, many of which are relevant to diabetes mellitus; indeed, glucocorticoid hormones are so named owing to their influence on glucose homeostasis (Hackett *et al.*, 2017)

Glucocorticoids exert their effects through their ubiquitous cytoplasmic receptors (Kino *et al.*, 2001). On ligand binding, the glucocorticoid receptors translocate into the nucleus, where they bind with specific glucocorticoid responsive elements (GREs) within the DNA to regulates the expression of glucocorticoid-responsive genes positively or negatively, depending on the GRE sequence and promoter context (Nemeroff *et al.*, 2018). The activated receptors also inhibit, through protein – protein interactions, other transcription factors, such as NF-kB, which are positive regulators of the transcription of several genes involved in the activation and growth of immune and other cells. Furthermore, glucocorticoids change the stability of messenger RNAs and hence the translation of several glucocorticoid-responsive proteins, as well as the electrical potential of neuronal cells (Charmandari *et al*, 2005).

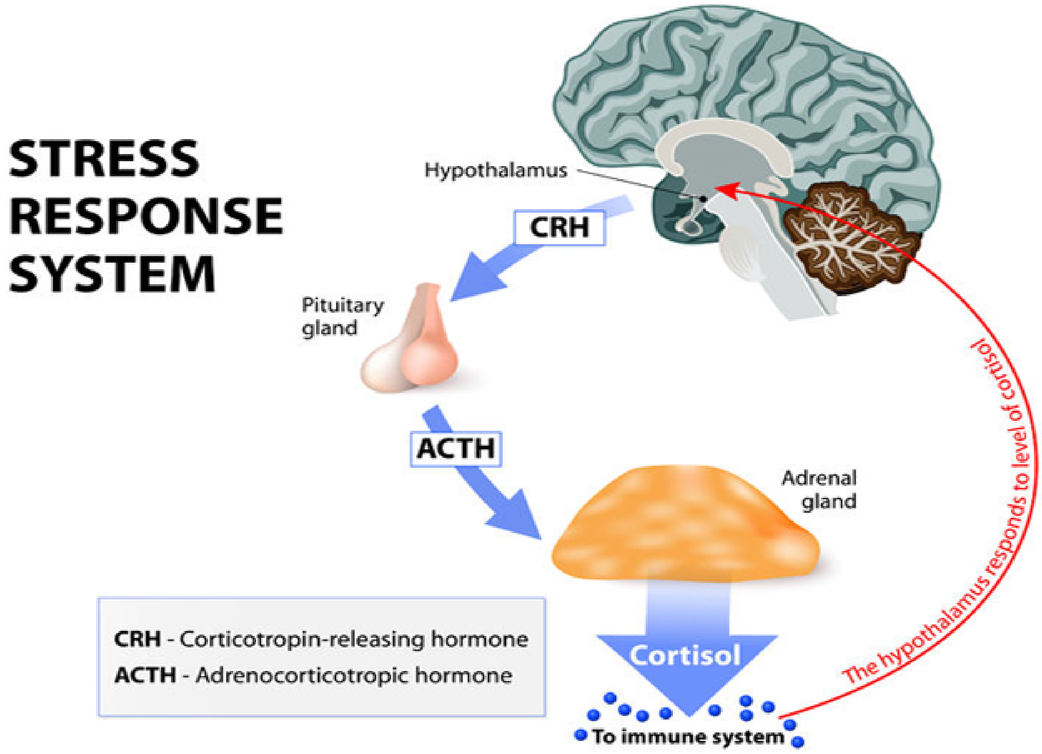


Figure 2.2 Stress response system (http://www.liveinthenow.com).

2.5 Effect of glucocorticoids on metabolic function

Glucocorticoids (GCs) are endogenous stress hormones that affect nearly every organ and tissue in the body, modulating various physiological processes including energy homeostasis (metabolism), immunology, behavior, reproductive function (Khonmee *et al*., 2014a; Khonmee *et al*., 2014b), cell proliferation, and survival (Patel *et al.*, 2014). The physiological effects of GCs are increased glucose production from hepatic cells (Vegiopoulos *et al.*, 2007), reduced glucose uptake into muscle and adipose tissue (Sakoda *et al.*, 2000; Vegiopoulos and Herzig, 2007), increased breakdown of muscle and fat to supply additional substrates for glucose production (Munck, 1971), and constraint of insulin release from cells (Lambillotte *et al.*, 1997). The abundant actions of glucocorticoids were acknowledged upon identification of the phenotypes of adrenocortical deficiency and adrenocortical excess. Adrenocortical insufficiency is characterized by hypoglycemia, low blood pressure, weight loss and anorexia (Addison 1885). In contrast, elevated and sustained cortisol secretion results in central obesity, increased overall body fat, postural hypertension, insulin resistance and a preference for fatty foods (Rabasa *et al.*, 2016).

2.5.1 Glucose and insulin

Glucocorticoids increase blood glucose levels by inhibiting the translocation of glucose transporter type 4 (GLUT4) to cell membranes, and in turn repressing peripheral glucose uptake in muscle and adipose tissue (Sakoda *et al*, 2000) (Figure 2.3). It activates gluconeogenesis while increasing the expression of gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK) (Chakravarty *et al.*, 2005). Glucocorticoids also increase hepatic responsiveness to the gluconeogenetic hormone glucagon and increase the release of substrates for gluconeogenesis from peripheral tissues (particularly muscle) (Jayakumar, 2012). Furthermore, cortisol enhances hepatic glycogen synthesis and storage. However, these effects are insulin-dependent and may result in increased insulin secretion in states of chronic glucocorticoid excess (Jayakumar, 2012).

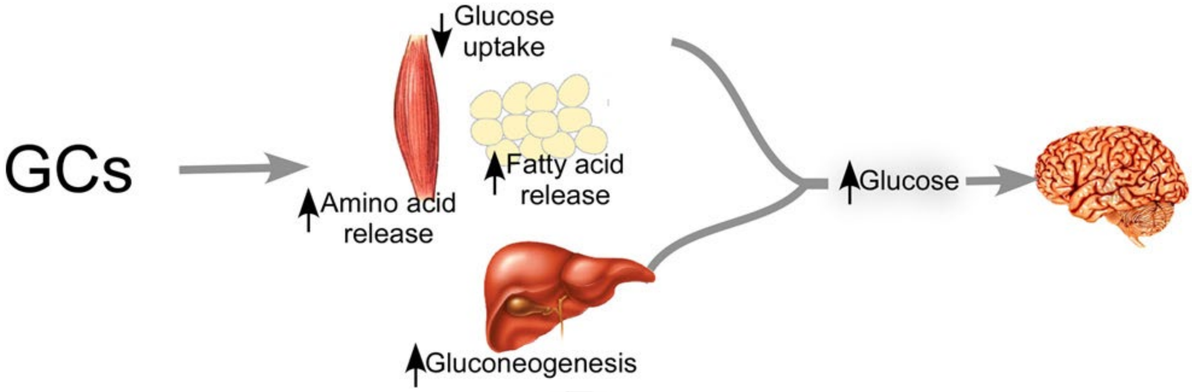


Figure 2.3 Schematic overview of metabolic actions of cortisol (Patel *et al*., 2014).

2.5.2 Fructosamine

Measures of average daily glucose level are not effective estimates of serum glucose levels in the long-term. Rather, the current approach to measuring average glucose is through analysis of fructosamine (Sacks *et al.*, 2011). Serum fructosamine is formed through non-enzymatic irreversible reactions between glucose and serum proteins. Fructosamine concentration directly depends on the blood protein concentrations and their composition (Loste *et al.*, 2001) and on the plasma glucose concentration. The half-life of albumin is 20 days, therefore, fructosamine levels are indirect measures of blood glucose levels occurring 2-3 weeks earlier. The assessment of fructosamine is simple, effective and inexpensive. Previous studies found that fructosamine has a close relationship with glucose and can detect abnormalities blood glucose levels as well (Mittman *et al.*, 2010).

2.5.3 Glucose to insulin ratio

Biological actions of insulin are essential for regulation and maintenance of glucose homeostasis. Insulin resistance (typically defined as decreased sensitivity or responsiveness to the metabolic actions of insulin) plays an important role in the pathophysiology of diabetes (Kahn *et al.*, 2006). Insulin resistance is also associated with obesity (Kahn *et al.*, 2006) as well as hypertension, coronary artery disease, and dyslipidemias (McFarlane *et al.*, 2001). Standard methods of assessing insulin sensitivity include the euglycemic-insulin clamp and the frequently sampled iv glucose tolerance test. These tests are time, labor, and cost intensive and are not practical for large scale studies, screening, or routine assessment. The fasting glucose insulin ratio (G:I) has been proposed as a useful screening test for insulin resistance in adult women with PCO (Legro *et al.*, 1998). Legro *et al.* determined that a fasting glucose to insulin ratio correlated strongly (r = 0.73) with insulin sensitivity using the F frequently sampled iv glucose tolerance test (Legro *et al.*, 1998). The strongest correlations were found between glucose/insulin ratio and insulin sensitivity (Manibo *et al.*, 2001; Vuguin *et al.*, 2001) and fasting insulin (Vuguin *et al.*, 2001). A number of studies have suggested that the fasting glucose to insulin ratio may represent another useful method for assessing insulin resistance (Legro *et al.*, 1998; Manibo *et al*. 2001; Vuguin *et al.*, 2001).

2.6 Effect of glucocorticoids on lipid parameters

Glucocorticoids affect fat metabolism by activating lipolysis, which leads to the release of free fatty acids into the circulation, and by stimulating the differentiation of preadipocytes to adipocytes. Moreover, it has been shown that glucocorticoids play a role in the redistribution of body fat from the periphery to visceral depots (Rebuffe-Scrive, 1988). At the times of starvation or insufficiency of energy intake, glucocorticoids stimulate the mobilization of energy substrates from peripheral fat through lipolysis, while promoting the accumulation of fat in abdominal depots, which can contribute to obesity over time (Dallman *et al.*, 2004).

2.6.1 Triglyceride (TG)

Glucocorticoids are steroid hormones that maintain crucial and complex actions in TG metabolism. During fasting and starvation, increased levels of glucocorticoids in circulation stimulate lipolysis in adipocytes, and TG is hydrolyzed to fatty acids (FA) and glycerol. Free Fatty acid are translocated to skeletal muscles and the liver to be oxidized and used as energy, whereas glycerol becomes the substrate for hepatic gluconeogenesis (Wang *et al.*, 2012).

While glucocorticoids are important for the regulation of lipid homeostasis in different physiological states, excess and/or chronic glucocorticoid exposure can cause lipid disturbances, such as dyslipidemia, obesity, and accumulate fat in the liver (Arnaldi *et al.*, 2010). Moreover, excess chronic glucocorticoids promote the circulating free fatty acid and stimulate ectopic lipid accumulation in skeletal muscles and the liver, and all of these factors are associated with insulin resistance. Whereas, repressing action of glucocorticoid, such as by suppressing 11β hydroxysteroid dehydrogenase type 1 (11β-HSD 1, which converts inactive glucocorticoids to active ones) or activating 11β- HSD 2 (which converts active glucocorticoids to inactive ones), improves lipid profiles and insulin sensitivity (Berthiaume *et al.*, 2007; Taylor *et al.*, 2009).

2.6.2 Cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins (HDL)

Low-density lipoproteins and high-density lipoproteins both transport cholesterol in the blood. But the main functional difference between low-density lipoproteins and high-density lipoproteins is they contribute cholesterol to different parts of the body. Low-density lipoproteins, the primary carriers of cholesterol, bring cholesterol to cells throughout the body and can cause cholesterol to build up within arteries. This build up can finally lead to arterial blockage and promote risk for cardiovascular disease. High-density lipoproteins, contrarily, can prevents excessive accumulation of cholesterol in the body because HDL carry cholesterol away from the organs and transport it back to the liver (Lund-Katz *et al.*, 2010).

The cholesterol transport pathway begins with the generation of preβ HDL by the liver and intestines by interaction of apoA-I with the ABCA1 transporter (Ahsan *et al.*, 2014). After efflux of additional cholesterol from peripheral cells discoidal (pre-β and α4) HDL is converted to spherical HDL (α1-3) after esterification by lecithin-cholesterol acyltransferase (LCAT) Cholesterol is then either returned directly to the liver after interaction with hepatic SR-BI receptors or indirectly after transfer to LDL by cholesteryl ester transfer protein (CETP) and uptake by hepatic LDL receptors (Figure 2.4)

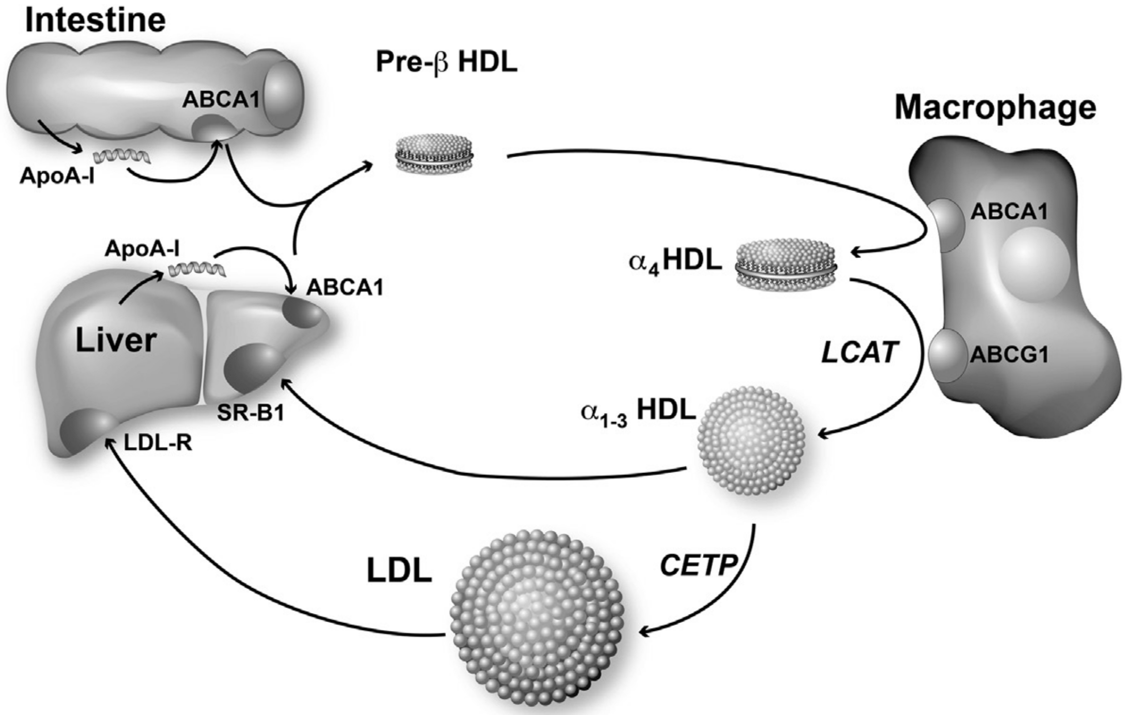


Figure 2.4 Reverse Cholesterol Pathway (Ahsan *et al.*, 2014).

Glucocorticoids are recognized for its role in the regulation of lipolysis that can increase the lipid profiles in the blood circulation. From the study of Dolinsky *et al*., rats treated with dexamethasone (synthetic GCs) showed increased accumulations of TGs in the liver by stimulating TG synthesis and reducing TG lipolysis (Dolinsky *et al.*, 2004). Rats treated with synthetic GCs had increased the amount of plasma TC and TG (Divertie *et al.*, 1991; Guillaume-Gentil *et al.*, 1993). Levels of ApoAI also increased in rats treated with glucocorticoids which increases HDL cholesterol levels as well (Staels *et al.*, 1991). From the third National Health and Nutrition Examination Survey, glucocorticoid administration relates to the rise of HDL and decline of TC/HDL ratio (Choi *et al.*, 2005). In patients with endogenous hypercortisolism (Cushing's disease), TC and LDL cholesterol levels are higher than healthy people. From previous studies, a consensus on the effects of glucocorticoid on TC and TG levels cannot be made, but glucocorticoids have an effect on increasing HDL levels. However, there are no reports of possible links between the intensity of stress responses and blood glucose levels in Asian elephants. Moreover, as yet, a full blood lipid profile (including HDL and LDL) of the Asian elephant is not scientifically documented. Such data is quite useful directly in breeding and general welfare, and indirectly in long-term conservation.

2.7 Body condition score (BCS)

To assess obesity status, various visual BCS indexes have been developed, including for elephants. The first for Asian elephants was based on an 11-point scale (Wemmer *et al.*, 2006). Areas of several body regions were scored : head (temporal depression), scapula (shoulder blade), thoracic region, flank area, lumbar vertebrae and pelvic bone. More recently, simplified BCS indexes have been developed based on 10-point scales of regions, such as head, scapula, pelvic bone and neck (Fernando *et al.*, 2009), or the thoracic region, scapular, pelvic bone and vertebra (Wijeyamohan *et al.*, 2015). Recently, Morfeld et el., (2014) found estimation of visual BCS index and validation of subcutaneous fat by ultrasound measurements in female African elephants (Morfeld *et al.*, 2014). The study used the ribs area, pelvic bone, and the vertebral ridge of the backbone for a BCS index using a 5-point scale. Body condition scoring is important for measuring fat in elephants to predicting obesity-related health and metabolic abnormalities. This is an effective and economical way to assess the factors that affect the body condition of wildlife animals and zoo animals including elephants (Morfeld, Lehnhardt, Alligood, Bolling and Brown, 2014), greater one-horned rhino (*Rhinoceros unicornis*) (Heidegger *et al.*, 2016). Seventy-four percentages of Asian and African elephants in North American zoo have BCS leves of 4 - 5 point scale .Therefore, most zoo elephants are either overweight or obese (Morfeld *et al.*, 2016). This study used the 5-point scale (Table 2.4) adapted from Morfeld et al. 2014 (Morfeld *et al.*, 2014) and Wijeyamohan et al. 2015 (Wijeyamohan *et al.*, 2015) to assess body conditions, with 1 representing the lowest and 5 representing the highest levels of body fat.

2.7.1 Body condition scoring (BCS) and obesity

Body condition scoring is an indirect means of measuring body condition and has become an integral part of assessing body fat in veterinary practices. It is widely used in veterinary medicine as an effective, inexpensive method for quantifying patient condition. Body condition scoring is an individual assessment of subcutaneous body fat stores, based on visual or tactile assessment of muscle tone and key skeletal elements (Donaldson *et al.*, 2004; Kearns *et al.*, 2002). Low scores represent animals with less body fat, and higher scores represent animals with more body fat (obese). Body condition scoring technique are routinely used in the health management of various species including equine, dog and cattle (Henneke *et al.*, 1983; Phythian *et al.*, 2012). This technique has been useful for assessing the degree of obesity in domestic and non-domestic species, including cattle (Schroder *et al.*, 2006), elk (*Cervus elaphus*)(Cook *et al.*, 2001), mule deer (*Odocoileus hemionus*) (Bishop *et al.*, 2009), and pinnepeds (*Eumetopias jubatus*, *Phoca vitulina*) (Mellish *et al.*, 2004).

Table 2.4 Body condition scoring index.

Score	Description
1	Ribs :Individual ribs clearly visible, spaces between ribs are sunken Pelvic Bone :Protrudes, deep depression in front of and behind pelvic bone Backbone :Protrudes from tail head to shoulders, deep depression alongside entire backbone
2	Ribs :Some ribs noticeable, spaces between ribs are not deeply sunken, yet they can still be seen Pelvic Bone :Prominent, prominent depression in front of and behind pelvic bone Backbone :Prominent from tail head to shoulders, deep depression alongside backbone in lumber region
3	Ribs :Not visible, spaces between ribs are not easily visible Pelvic Bone :Clearly visible, depression in front of and behind pelvic bone Backbone :Clearly visible from tail head to shoulders, deep depression alongside backbone in lumber region
4	Ribs :Not visible Pelvic Bone :The edges cannot be demarcated properly, slight sunken in front of and behind pelvic bone Backbone :Visible from tail head to mid-back, slight depression alongside backbone in lumber region
5	Ribs :Not visible Pelvic Bone :Not visible Backbone :Difficult to differentiate, area alongside backbone in lumber region filled in

2.7.2 Effect of obesity on lipid metabolism

Obesity is associated with high level of lipolysis in adipose tissue, and circulating free fatty acid (van Hall *et al.*, 2003). Acute-phase serum amyloid A (SAA), a lipolytic adipokine in humans, stimulates basal lipolysis. The lipolysis has been hypothesized to be an autocrine feedback mechanism by which elevated SAA production from enlarged adipocytes A into the circulation may contribute to insulin resistance. The SAA act through the CLA-1 and the extra-cellular signal regulated kinase signaling pathway to directly stimulate lipolysis (Souza *et al.*, 2003). Alternatively, elevated lipolysis by SAA may be indirect, i.e. through the stimulation of the lipolytic cytokines via IL-6 and TNF-α .Triglyceride (TG) concentration is another metabolic variable, most affected in obesity. It has been proposed that there is tissue resistance to insulin mediated glucose uptake, which in turn accelerates the very low density lipoprotein (VLDL), TG production rate and leads to endogenous hypertriglyceridemia (Barter *et al.*, 1973). Another study evaluating the serum lipid profiles of obese cats revealed that they have higher proportions of VLDL, TG and higher concentrations of LDL than do lean cats (Jordan *et al.*, 2008). In obesity there is decreased lipoprotein lipase-mediated lipolysis of chylomicron-TG and ineffective inhibition of hormone-sensitive lipase-mediated lipolysis in adipose tissue (Lewis *et al.*, 1993). Dyslipidemia and elevated plasma fatty acid concentrations are well-recognized abnormalities in obesity. Excess fatty acid availability early in the postprandial period (when it is normally suppressed by insulin) is estimated to influence glucose uptake by as much as 50% (Yu *et al.*, 2001). SAA has also a direct effect on cholesterol metabolism (Van Lenten *et al.*, 1995). The inter-action of SAA with HDL may diminish the function of HDL as an anti-atherogenic particles (Benditt *et al.*, 1982) and facilitate its degradation (Wu *et al.*, 2004). The increase of adipose tissue derived SAA in obesity may be a link between obesity, low HDL and increased arteriosclerotic. In humans, dyslipidemia are associated with elevated risk of cardiovascular disease (Nelson, 2013).

2.7.3 Effect of obesity on metabolic function

Adipose tissue consists of receptors for a broad number of endocrine hormones, such as insulin, glucagon, growth hormones, thyroid hormones, and glucocorticoids. Consequently, an excess or deficiency of adipose tissue is clearly detrimental from a metabolic homeostasis (Clark *et al.*, 2016). In humans, obesity is associated with a cluster of metabolic abnormalities, termed “the metabolic syndrome” which include insulin resistance, dyslipidemia, hypertension, and diabetes mellitus (Han *et al.*, 2016).

One of the most well-known changes that occurs with excess adiposity is insulin resistance, or a diminished cellular response to a given plasma insulin concentration. The binding of insulin to its cell surface receptors stimulates a signaling cascade that increases glucose uptake and formation of glycogen in muscle, inhibit endogenous gluconeogenesis in the liver, and increases glucose and lipid uptake and inhibits lipolysis in adipose tissue (Saltiel *et al.*, 2001).

In humans, obesity leads to insulin resistance in adipose tissue, liver, and muscle (Conte *et al.*, 2012). The accumulation of fat mass may inhibit the ability of adipose tissue to accomplish its usual metabolic function (adiposopathy) (Bays, 2014). The mechanisms for adipose tissue dysfunction in obesity including;

- Adipocyte hypertrophy lead to oxidative stress, apoptosis, and an inflammatory response that negatively impacts fuel metabolism (Goossens, 2008; Hosogai *et al.*, 2007);

- Decreased adipocyte mitochondrial function (reduction in mitochondrial number, density or function) (Yin *et al.*, 2014);

- Adipokines dysregulation and other bioactive substances, including those involved in insulin sensitivity and inflammation (Maury *et al.*, 2010)

- Abnormal fatty acid trafficking and intracellular accumulation of lipid intermediates that interfere with insulin signaling (McQuaid *et al.*, 2011).

CHAPTER 3

Body condition and adrenal glucocorticoid activity affects metabolic marker and lipid profiles in captive female elephants in Thailand

3.1 Introduction

Healthy populations of elephants are essential to long-term global conservation, especially in the face of declining numbers. Wild elephant numbers throughout Asia have declined at least 50% over the last three generations, and without changes to conservation management, some populations are bound to disappear within the next century (Ingrid *et al.*, 2014). Captive propagation is one strategy to ensure species survival (Conde *et al.*, 2011), especially when conducted in range countries (Thitaram, 2012). However, many Asian elephant (*Elephas maximus*) populations *ex situ* are not self-sustaining due to poor reproduction (Brown, 2014) or health problems (Fowler *et al.*, 2008; Hayward, 2012, Miller *et al.*, 2016).

The elephant is a national symbol of Thailand, and an integral part of Thai and Buddhist culture, and national economics. In the early 1900's, there were ~100,000 captive elephants in Thailand, almost all used for logging (Tipprasert, 2001), a practice that was banned in 1989. Today, there are ~3,500 captive elephants, mostly (95%) privately owned (Asian Elephant Specialist Group, 2017), that are used primarily for tourism. Poor sustainability of captive elephants in Thailand is a concern, and has resulted in increasing efforts to improve breeding and population management (Thitaram, 2012). The majority of tourist elephants today are located in the north and northeast of the country (~60%), primarily in Chiang Mai province where this study was conducted. In species with slow-growth populations (i.e., animals that mature late, produce small numbers of offspring, and have long life spans), sustainability is influenced by factors such as adult survival (Crone, 2001; Heppell *et al.*, 2000; Oli and Dobson, 2003; Young and Keith, 2011) and reproduction (Manlik *et al.*, 2016). Asian elephants are a long-lived species, and produce only a few calves in their lifetime, so it is important to better understand factors affecting health and reproduction to prevent further population declines.

There are a number of ways to evaluate animal health status, apart from routine physical examinations. One is a visual body condition score (BCS), which has become an indirect, effective, and inexpensive tool in veterinary practices for assessing body fat, especially as it relates to problems with obesity (Henneke *et al*., 1983). For Asian elephants, several BCS indexes have been developed using 5- (Morfeld *et al*.,2016), 10- (Wijeyamohan *et al*., 2015) and 11- (Wemmer *et al*., 2006) point scales. In many species, high body condition or obesity can have negative effects on lipid profiles, including elevated serum triglycerides (TG) (Jeusette *et al*., 2005; Watson *et al*., 1990), total cholesterol (TC) (Jeusette *et al*., 2005; Watson *et al*., 1990), low density lipoprotein (LDL) (Jung *et al*., 2014; Sanlier and Yabanci, 2007; Takada *et al*., 1998), and very low density lipoproteins (VLDL) (Jeusette *et al*., 2005; Frank *et al*., 2006). Abnormally elevated blood glucose, TG, and cholesterol have been linked to a number of disease problems, collectively called metabolic syndrome, such as hypertension, hyperlipidemia, insulin resistance, and type 2 diabetes (Lanktree *et al*., 2013; Lee and Sanders, 2012). By contrast, few studies have examined these health factors in relation to body condition in elephants, and those were only on animals in western zoos. For example, negative associations were found between the BCS and glucose to insulin ratio (G:I) in zoo Asian and African elephants (Morfeld and Brown, 2017), and fat-free mass and circulating glucose in African elephants (Chusyd *et al*., 2017), whereas Morfeld and Brown (Morfeld and Brown, 2016) reported a positive correlation between BCS and insulin concentrations in African elephants. Subsequent studies demonstrated the protective effects of increased exercise and feeding diversity on BCS and metabolic function in zoo-housed elephants (Morfeld and Brown, 2017), which could have application to tourist elephants in Thailand that participate in varying types of physical activities. Another favorite activity of tourists is feeding bananas and sugar cane, leading to concerns about whether that is linked to obesity and metabolic problems.

In addition to obesity, stress can also affect metabolic health and lipid parameters. Glucocorticoids (GCs) are key regulators of whole-body homeostasis, and provide an organism with the capacity to resist environmental changes and invasion of foreign substances (Coutinho and Chapman, 2011). Glucocorticoids modulate a large number of physiological actions involved in metabolic, inflammatory, cardiovascular, and behavioral processes (Wang, 2005). Mechanisms by which GCs orchestrate these effects include increasing hepatic glucose production (Vegiopoulos and Herzig, 2007), decreasing peripheral glucose uptake into muscle and adipose tissue (Sakoda *et al*., 2000; Weinstein *et al*., 1998), increasing breakdown of fat and muscle to provide additional substrates for glucose production (Divertie *et al*., 1991; Guillaume-Gentil *et al*., 1993; Hasselgren, 1999), and inhibiting insulin release from pancreatic beta cells (Lambillotte *et al*., 1997). While important for maintaining metabolic equilibrium, excessive GC exposure for prolonged periods can have devastating effects on health. Patients with hyper-GC production exhibit abdominal obesity, increased overall body fat, high blood pressure, insulin resistance, and a preference for fatty foods (Rabasa and Dickson, 2016). Glucocorticoids can affect fat metabolism by stimulating lipolysis, which leads to the release of free fatty acids into circulation (Wang, 2005). Moreover, it has been shown that GCs play a role in the redistribution of body fat from periphery to visceral depots (Rebuffe-Scrive, 1998). Working elephants in Thailand interact with the public in a variety of ways, including performing in shows, trekking, bathing, and painting. Often these activities are not closely monitored or regulated, and could be sources of stress to individual animals. Fecal GC metabolite analysis techniques have been developed for numerous species, which has led to improved *ex situ* management (Touma and Palme, 2005). The ease of fecal collection without animal disturbance, and that data reflect pooled values over time that are less affected by pulsatile or diurnal changes in secretion makes this a particularly attractive approach for wildlife.

In recent years, the welfare of captive elephants has become a topic of intense debate among the lay public, government agencies, and the scientific community. Therefore, the aim of this study was to examine relationships between BCS and fecal GC metabolite (FGM) concentrations on metabolic function (insulin, glucose, fructosamine, G:I) and lipid profiles (TC, TG, HDL, LDL) in tourist camp elephants in Thailand. The hypothesis was that elephants with higher adrenal activity and BCSs will exhibit more metabolic derangements than those with normal BCS and lower FGM concentrations. The goal is to better understand what factors are related to health and welfare of working elephants in Thailand so that evidence-based standards can be developed to create healthy, self-sustaining populations.

3.2 Materials and methods

3.2.1 Environmental data

Weather in Thailand is hot and humid, with three official seasons: summer (16 February–15 May), rainy (16 May–15 October) and winter (16 October–15 February). Information on daily temperature (°C), amount of rainfall (mm/day), and humidity (%), averaged by month, was obtained from The Northern Meteorological Center, Meteorological Department, Ministry of Information and Communication Technology, Chiang Mai, Thailand (Thai Meteorological Department, 2016). A thermal–humidity index (THI) was calculated based on air temperature and relative humidity using the following formula: $\mathrm{THI} = (1.8\times T\mathrm{db}+32) - (0.55-0.0055\times \mathrm{RH}) \times (1.8\times T\mathrm{db}-26)$, where the Tdb is the temperature of air measured by a thermometer freely exposed to the air, but shielded from radiation and moisture, and RH is the relative humidity (%) (Herbut and Angrecka, 2012).

3.2.2 Animals

This study was approved by the Faculty of Veterinary Medicine, Chiang Mai University, Animal Care and Use Committee (FVM-ACUC; permit number S39/2559). Thirty-three adult female Asian elephants (age range, 18–50; mean, 34.2±7.3 years) were housed at five tourist camps within 43–72 km of the Chiang Mai University Veterinary Faculty (latitude 18°47'N, longitude 98°59'E, altitude 330 m) (Table 3.1). At four of the camps, tourists interacted with elephants through riding programs (bareback or with a saddle) and feeding supplementary foods. The fifth camp offered no tourist activities other than observation of elephants in a large field, and being taken for a bath at a local river. Work hours per day was the time elephants were involved in tourist activities, like giving rides or walking to a river for bathing. For saddle and bareback riding, it equated to the number of rounds of riding per day times the minutes per riding round. Elephants were fed primarily corn stalk, *napier grass* (*Pennisetum purpureum*) and bana grass (*Pennisetum purpureum* X, *P. americanum* hybrid) with unlimited access to fresh water. Animals were given an annual physical examination by staff veterinarians, and were in good health during the study.

3.2.3 Body condition scoring

Once every 2 months, rear and side view photographs were taken of each elephant to permit a visual evaluation of the backbone, rib bone and pelvic bone areas, and scored 1-5 (1 = thinnest; 5 = fattest) as described by Morfeld et al. (2016), except that scoring was done in 0.5-point, rather than 1-point, increments. All photos were evaluated by three experienced elephant veterinarians, and the scores averaged. Intra-class correlations determined the inter-assessor reliability was 0.85.

3.2.4 Blood collection

Blood samples (10 ml) were collected from each elephant from an ear vein by elephant camp staff or Chiang Mai University veterinarians twice monthly for 1 year. All elephants were conditioned to the blood sampling procedure. Blood was centrifuged at 1,500 x g for 10 minutes within a few hours of collection, and the serum stored at -20°C until processing and analysis.

3.2.5 Metabolic markers analysis

Serum glucose was measured by a hexokinase method using an automated glucose analyzer (Glucinet T01-149, Bayer, Barcelona, Spain), with quinoneimine measured at 530 nm. Serum fructosamine was measured by a colorimetric method using nitrobluetetrazolium in a Biosystems BA400 clinical chemistry analyzer (©Biosystems S.A., Barcelona, Spain). A solid-phase, two-site bovine insulin enzyme immunoassay (EIA; Cat. No. 10-1113-01; Mercodia, Uppsala, Sweden), validated for elephants, was used to measure serum insulin concentrations (Morfeld and Brown, 2016). Colorimetric responses were determined spectrophotometrically at 450 nm filter with an Opsys MR Microplate Reader (TECAN Sunrise™ microplate reader; Salzburg, Austria). All samples were analyzed in duplicate; intra- and inter-assay CVs were <10% and <15%, respectively.

Table 3.1 Description of each elephant camp in the study (Camps A–E). Information includes number of years the camp has been in operation (camp age), total number of elephants in the camp, number of elephants participating in the study, participating elephant mean age (±SEM) and range, type of work with tourists, hours worked per day, and primary and supplemental food items.

Variable	**Camp A**	**Camp B**	**Camp C**	**Camp D**	**Camp E**
Camp age (years)	9	27	29	14	40
Total elephant number	46	66	52	68	76
Participating elephant number	6	6	6	11	4
Elephant age (years)	28.5±1.8 (22-34)	36.8±2.9 (23-43)	35.3±3.8 (20-45)	35.8±2.3 (25-50)	32.2±3.0 (22-40)
Type of work	Bareback riding	Saddle riding	Saddle riding	No riding	Saddle riding
Work time (hours/day)	1.3	2.3	3.8	0.2	3.3
Diet					
Primary	*Napier grass*, cornstalk	*Napier grass*, cornstalk	*Napier grass*, cornstalk	*Napier grass*, bana grass, cornstalk	*Napier grass*
Supplementary	*Bamboo, sugarcane, banana*	*Banana, sugarcane*	*Banana, sugarcane*	*Hay, banana, watermelon, pumpkin, cucumber*	*Bamboo, sugarcane, banana*

3.2.6 Lipid profile analysis

Serum lipids were quantified using a Mindray BS Series analyzer (Mindray BS-380, Shenzhen Mindray Bio-Medical Electronics Co., Ltd.). Total cholesterol was measured by a cholesterol oxidase-peroxidase (CHOD-POD) method. Triglycerides were measured by a glycerokinase peroxidase-peroxidase (GPO-POD) method, with a sensitivity of 0.1 mmol/L (99.7% confidence). The lowest measurable concentration was 0.1 mmoL/L (99.7% confidence) for TC, and 0.05 mmol/L for both HDL and LDL.

3.2.7 Fecal extraction and GC metabolite analysis

All chemicals were obtained from Sigma Chemical Company (St. Louis, MO), unless otherwise stated. The fecal extraction method was based on Brown et al. [45]. Briefly, wet fecal samples were dried in a conventional oven at 60°C for ~24-48 hours and stored at -20°C until extraction. Frozen dried fecal samples were thawed at room temperature (RT), mixed well and 0.1 g (±0.01) of dry powdered feces placed in a glass tube containing 90% ethanol in distilled water. Samples were extracted twice by boiling in a water bath (96°C) for 20 minutes and adding 100% ethanol as needed to keep from boiling dry. Samples were centrifuged at 1,500 x g for 20 min, and the combined supernatants dried under air in a 50°C water bath. Dried extracts were reconstituted by vortexing for 1 min in 3 ml ethanol, dried again, and then diluted and vortexed in methanol for analysis. Extracts were stored at –20°C until EIA analysis.

Concentrations of FGM were determined using a double-antibody enzyme immunoassay (EIA) with a polyclonal rabbit anti-corticosterone antibody (CJM006) that has been validated for Asian elephants (Watson *et al*., 2013). Second antibody-coated plates were prepared by adding 150 µl of anti-rabbit IgG (0.01 mg/ml) to each well of a 96-well microtiter plate, and incubating at room temperature (RT) for 15-24 h. The wells were then emptied and blotted dry, followed by adding 250 µl blocking solution and incubating for 15-24 h at RT. After incubation, all wells were emptied, blotted and dried at RT (Sanpla Dry Keeper, Sanplatec Corp., Auto A-3, Japan) with loose desiccant in the bottom. After drying (humidity <20%), plates were heat-sealed in a foil bag with a 1g desiccant packet, and stored at 4°C until use.

Samples (50 µl), diluted 1:3 in assay buffer, or corticosterone standards (50 µl) were added to appropriate wells. Corticosterone-horseradish peroxidase (25 µl) was immediately added to each well except for non-specific binding wells, followed by 25 µl anti-corticosterone antibody, and incubated at RT for 1 h. Plates were then washed four times with wash buffer (1:20 dilution, 20X Wash Buffer Part No. X007; Arbor Assays, MI) and 100 µl of TMB substrate solution was added, followed by incubation for 45-60 min at RT without shaking. The absorbance was measured at 405 nm by a microplate reader (TECAN). Assay sensitivity (based on 90% binding) was 0.14 ng/ml. Samples were analyzed in duplicate; intra- and inter-assay CVs were <10% and <15%, respectively.

3.2.8 Statistical analysis

Descriptive data were reported as mean ± standard error of the mean (SEM) and camp management variables were presented as a range or a frequency, depending on the type of data. Statistical analyses were performed using R version 3.4.0 (R Development Core Team, 2017). Repeated measures data were analyzed using Generalized Estimating Equations (GEE) to determine: 1) the effects of BCS and FGM on metabolic and lipid panel results; 2) seasonal and climate factor effects on metabolic and lipid function; and 3) relationships among metabolic and lipid panel measures. Differences in mean metabolic and lipid profiles between BCS groups and seasons were further analyzed by Tukey's post-hoc tests after GEE analyses. Correlations between individual FGM and metabolic hormones or lipid measures in each elephant (n=33) were analyzed using Pearson's tests for aggregated data. Mean monthly FGM were compared using GEE followed by a Tukey's test. The significance level was set at α=0.05.

3.3 Results

Descriptive FGM, BCS, metabolic marker, and lipid profile measures are presented in Table 3.2, highlighting the variability in mean and range values across individuals. BCSs ranged from 2 to 5, with none scoring BCS=1 or 1.5. Based on the yearly average of monthly mode values, numbers of elephants in each BCS category were: BCS=2 (N=1), BCS=2.5 (N=0), BCS=3 (N=14), BCS=3.5 (N=6), BCS=4 (N=7), BCS=4.5 (N=3), BCS=5 (N=2). Most elephants (42.4%) were a BCS=3, with 33.3% scoring BSC =4 and 5. Relationships between BCS and FGM on metabolic markers and lipid profiles are presented in Table 3.3. There were significant positive associations between BCS and TC, HDL and LDL. Fecal GC metabolite concentrations also were positively related to TC and HDL, as well as glucose and insulin. Both BCS and FGM were negatively correlated to G:I ratios. In separate Pearson's correlation analyses of individual means (n=33), FGM levels were similarly correlated to TC, LDL, glucose, insulin, and fructosamine ($p<0.05$) (Fig 1). Work time (hours per day) was negatively correlated with FGM concentrations ($r=-0.69$, $p<0.01$), HDL ($r=-0.51$, $p<0.01$), glucose ($r=-0.78$, $p<0.01$), fructosamine ($r=-0.59$, $p<0.01$) and insulin ($r=-0.59$, $p<0.01$), whereas the G:I was positively associated with work time ($r=0.32$, $p<0.05$).

Differences in FGM, metabolic marker and lipid profile measures related to BCS are shown in Table 3.4. Because of limited numbers, elephants were grouped into three BCS classes: 2.0–3.0 (N=15); 3.5–4.0 (N=13); and 4.5–5.0 (N=5) for further analysis. Higher levels of TC and LDL were found in elephants with a BCS of ≥3.5 (overweight/very overweight) compared to those <3.5 ($p<0.05$). Elephants with BCSs of 4.5–5.0 had higher HDL, glucose and insulin levels, and the lowest G:I ratio ($p<0.05$). Triglyceride concentrations in the intermediate BCS group (BCS=3.5-4) were higher than those in BCS=2-3, but similar to BCS=4.5-5. By contrast, FGM concentrations were similar across the BCS groups.

Seasonal effects on measured parameters are summarized in Table 3.5. All but the G:I was significantly affected by season. BCSs were 9-13% higher in elephants during the rainy and winter seasons compared to the summer, with TG, LDL, and insulin being higher in the winter months only. Mean FGM concentrations were ~28% higher in winter compared to the summer and rainy seasons. LDL was also higher in the rainy and winter seasons compared to the summer. By contrast, HDL was highest in the summer, intermediate in the winter and lowest in the rainy season, whereas glucose was high in the winter and summer seasons. The effect of season on longitudinal FGM concentrations is presented in Figure 2, with lower concentrations observed between March and September, representing mid-summer to rainy seasons. Relationships between environmental factors and BCS and FGM concentrations are presented in Table 3.6, with significant correlations noted between BCS and humidity, and between FGM and all environmental factors but humidity. There were significant negative effects of monthly temperature, rainfall, and THI on FGM measures.

Several correlations were noted amongst the metabolic and lipid factors as shown in Table 3.7. Strong positive associations ($p<0.001$) included TC with TG, HDL with LDL, HDL with LDL and insulin, and glucose with fructose and insulin. By contrast, negative relationships ($p<0.01$) were found between the G:I ratio and HDL, glucose, and insulin.

Table 3.2 Descriptive statistics. Mean (±SEM) and range values for body condition scores (BCS), fecal glucocorticoid metabolite (FGM) concentrations, lipid panel measures and metabolic factors in female Asian elephants (n=33) in Thailand.

Parameters	**Mean**	**Min - Max**	**Mean range**
BCS	3.50±0.02	2.00-5.00	2.37-5.00
FGM (ng/g)	50.80±0.89	11.42-194.17	34.51-72.26
TC (mg/dL)	37.40±0.32	10.00-109.00	24.38-48.52
TG (mg/dL)	28.60±0.64	4.00-157.00	18.09-42.96
HDL (mg/dL)	11.60±0.10	2.00-26.00	9.17-18.96
LDL (mg/dL)	27.80±0.29	8.00-107.00	14.30-41.14
Glucose (mg/dL)	88.90±0.75	50.00-180.00	68.91-112.50
Fructosamine (mM)	0.59±0.01	0.38-0.92	0.54-0.67
Insulin (µg/L)	0.75±0.03	0.02-5.91	0.12-2.10
G:I	196.00±6.72	22.67-721.56	83.87-532.32

TC=total cholesterol; TG=triglycerides; HDL=high density lipoproteins; LDL=low density lipoproteins; G:I=glucose to insulin ratio.

Table 3.3 General Estimation Equation analyses. Relationships between health factors and body condition and adrenal steroid activity in female Asian elephants (n=33) in Thailand.

Parameters	BCS			FGM		
	Intercept	**Beta**	**P value**	**Intercept**	**beta**	**P value**
TC (mg/dL)	2.742	0.021	0.001	38.370	0.330	0.009
TG (mg/dL)	3.428	0.004	0.210	50.742	0.005	0.920
HDL (mg/dL)	2.751	0.067	0.001	34.160	1.445	<0.001
LDL (mg/dL)	2.960	0.021	0.002	44.933	0.223	0.076
Glucose (mg/dL)	3.361	0.002	0.430	21.314	0.336	<0.001
Fructosamine (mM)	3.179	0.610	0.570	39.270	19.81	0.230
Insulin (ng/ml)	3.654	0.062	0.570	46.610	6.160	0.004
G:I	3.913	-0.001	0.009	54.248	-0.014	0.038

BCS=body condition score; FGM=fecal glucocorticoid metabolites; TC=total cholesterol; TG=triglycerides; HDL=high density lipoproteins; LDL=low density lipoproteins; G:I= glucose to insulin ratio.

Table 3.4 Body condition effects on adrenal and health makers in elephants. Effect of body condition on mean (±SEM) and range values fecal glucocorticoid metabolite (FGM) concentrations, lipid panel measures and metabolic factors in Asian elephants in Thailand (n=33).

BCS	FGM (ng/g)	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	GLU (mg/dL)	FRUC (mM)	INS (ng/ml)	GI
2.0 - 3.0	58.01±4.02	35.20±0.88^{a}	24.70±1.97^{a}	11.50±0.27^{a}	25.00±0.97^{a}	96.50±3.09^{a}	0.59±0.01^{ab}	0.91±0.12^{a}	165.00±34.60^{ab}
	(20.00–194.00)	(22.00–52.00)	(7.00–85.00)	(7.00–17.00)	(9.00–43.00)	(56.00–180.00)	(0.45–0.80)	(0.04–5.91)	(22.70–483.00)
3.5 - 4.0	58.00±4.28	38.60±1.10^{b}	34.50±2.99^{b}	11.60±0.32^{ab}	29.40±0.98^{b}	92.10±3.50^{a}	0.58±0.01^{a}	0.91±0.14^{a}	183.00±24.10^{b}
	(15.70–159.00)	(22.00–60.00)	(8.00–110.00)	(9.00–21.00)	(13.00–52.00)	(52.00–152.00)	(0.48–0.70)	(0.07–2.92)	(38.20–543.00)
4.5 – 5.0	60.93±8.72	40.60±2.03^{b}	29.90±2.84^{ab}	13.20±0.67^{b}	29.80±1.23^{b}	104.80±5.65^{b}	0.61±0.01^{b}	1.23±0.22^{b}	85.00±10.20^{a}
	(32.00–126.00)	(28.00–55.00)	(10.00-59.00)	(9.00–21.00)	(21.00–38.00)	(58.00–144.00)	(0.57–0.67)	(0.07–2.58)	(40.70–142.00)

^{a,b,c}Values for each variable differ among BCS categories within columns are significantly different ($p<0.05$).

BCS=body condition score; TC=total cholesterol; TG=triglycerides; HDL=high density lipoproteins; LDL=low density lipoproteins; GLU=glucose; FRUC=fructosamine; INS=insulin; G:I=glucose to insulin ratio.

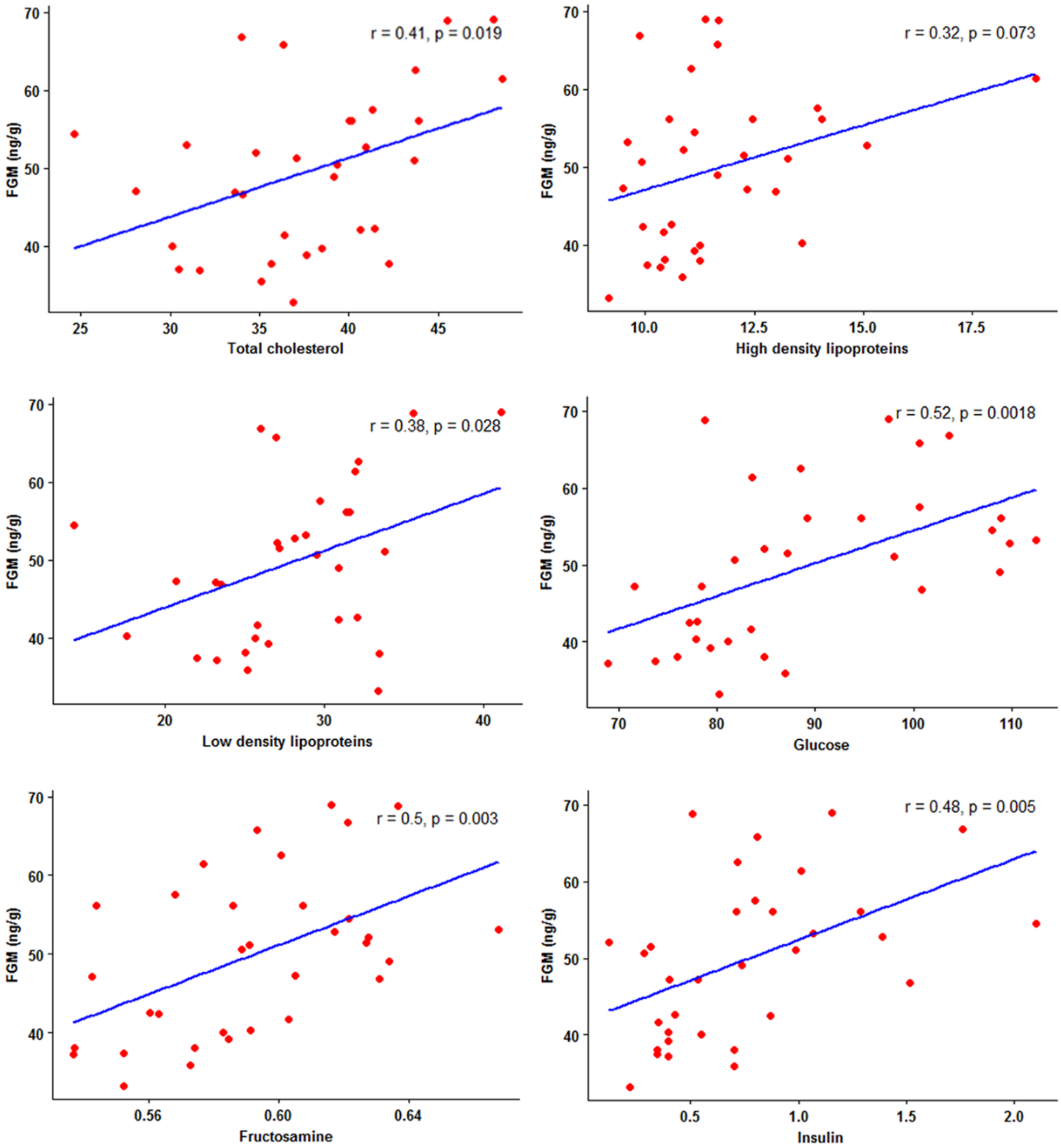


Figure 3.1 Relationships between fecal glucocorticoid metabolite (FGM) concentrations and metabolic and lipid measures**.** Pearson's correlation analyses illustrating relationships between FGM concentrations and (a) total cholesterol, (b) high density lipoproteins, (c) low density lipoproteins, (d) glucose, (e) fructosamine, and (f) insulin in female Asian elephants (n=33) in Thailand.

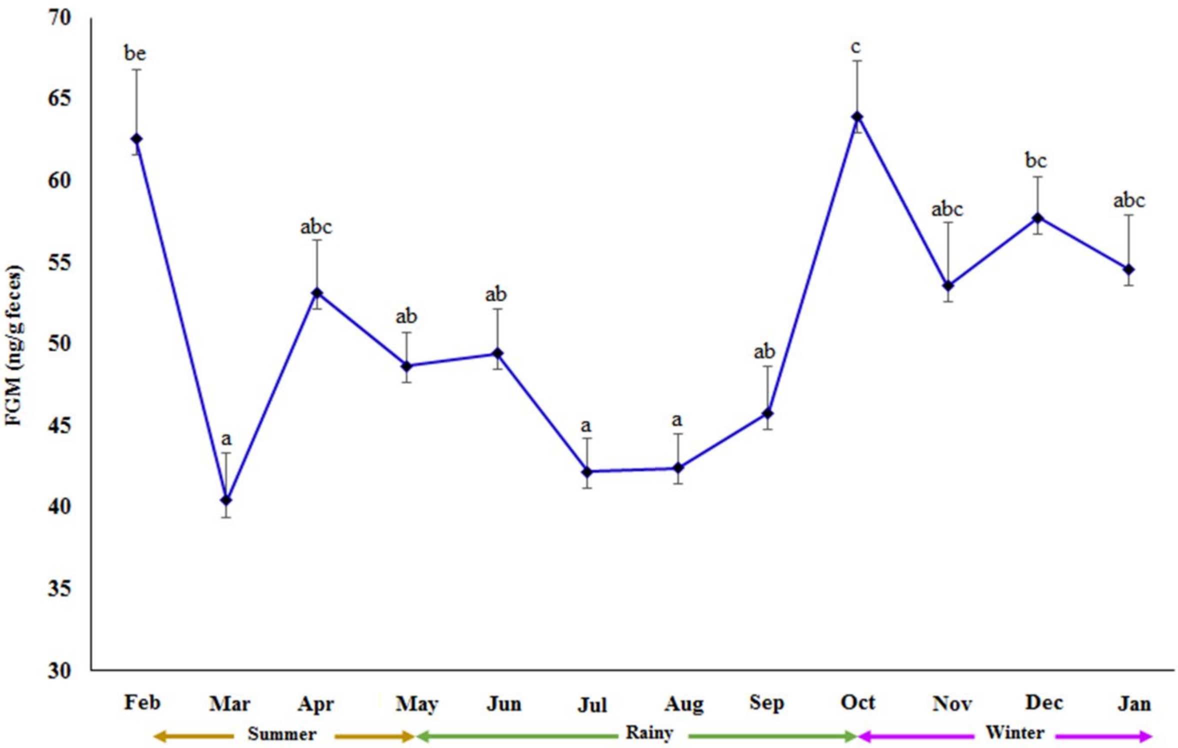


Figure 3.2 Seasonal pattern of fecal glucocorticoid metabolite (FGM) concentrations. Mean (±SEM) monthly fecal glucocorticoid metabolite (FGM) concentrations in female Asian elephants (n=33) across the summer, rainy and winter seasons in Thailand. Superscripts designate significant differences in monthly mean FGM concentrations ($p<0.05$).

Table 3.5 Seasonal effects on body condition, adrenal steroid hormones and health factors in elephants. Mean (±SEM) and range values in body condition scores (BCS), fecal glucocorticoid metabolite (FGM) concentrations, lipid panel measures and metabolic factors across the summer, rainy and winter seasons of female Asian elephants (n=33) in Thailand.

Parameters	**Summer**	**Rainy**	**Winter**
BCS	3.20±0.13[a]	3.48±0.08[ab]	3.62±0.08[b]
	(3.00-4.00)	(2.00-5.00)	(2.00-5.00)
FGM (ng/g)	47.40±1.79[a]	47.3±1.15[a]	60.00±1.95[b]
	(11.42-147.00)	(11.90-132.38)	(15.45-194.17)
TC (mg/dL)	38.70±0.88[b]	36.00±0.45[a]	38.20±0.46[b]
	(20.00-109.00)	(10.00-77.00)	(22.00-60.00)
TG (mg/dL)	25.10±1.70[a]	29.20±0.86[ab]	30.40±1.10[b]
	(4.00-157.00)	(6.00-88.00)	(7.00-110.00)
HDL (mg/dL)	12.50±0.25[c]	11.10±0.13[a]	11.70±0.16[b]
	(2.00-26.00)	(5.00-26.00)	(7.00-21.00)
LDL (mg/dL)	25.80±0.57[a]	27.70±0.56[b]	29.40±0.46[b]
	(12.00-89.00)	(8.00-107.00)	(9.00-67.00)
Glucose (mg/dL)	92.90±1.13[b]	83.60±1.13[a]	92.90±1.56[b]
	(65.00-160.00)	(50.00-180.00)	(52.00-172.00)
Fructosamine (mM)	0.60±0.004[b]	0.59±0.004[ab]	0.58±0.003[a]
	(0.48-0.77)	(0.38-0.92)	(0.45-0.77)
Insulin (ng/ml)	0.76±0.08[ab]	0.59±0.05[a]	0.94±0.09[b]
	(0.02-3.37)	(0.03-2.67)	(0.03-5.91)
G:I	207.00±19.60	188.00±11.90	163.00±12.40
	(34.41-721.56)	(38.17-713.17)	(22.67-612.53)

[a,b,c]Seasonal mean values are significantly different across each row ($p<0.05$). TC=total cholesterol; TG=triglycerides; HDL=high density lipoproteins; LDL=low density lipoproteins; G:I=glucose to insulin ratio

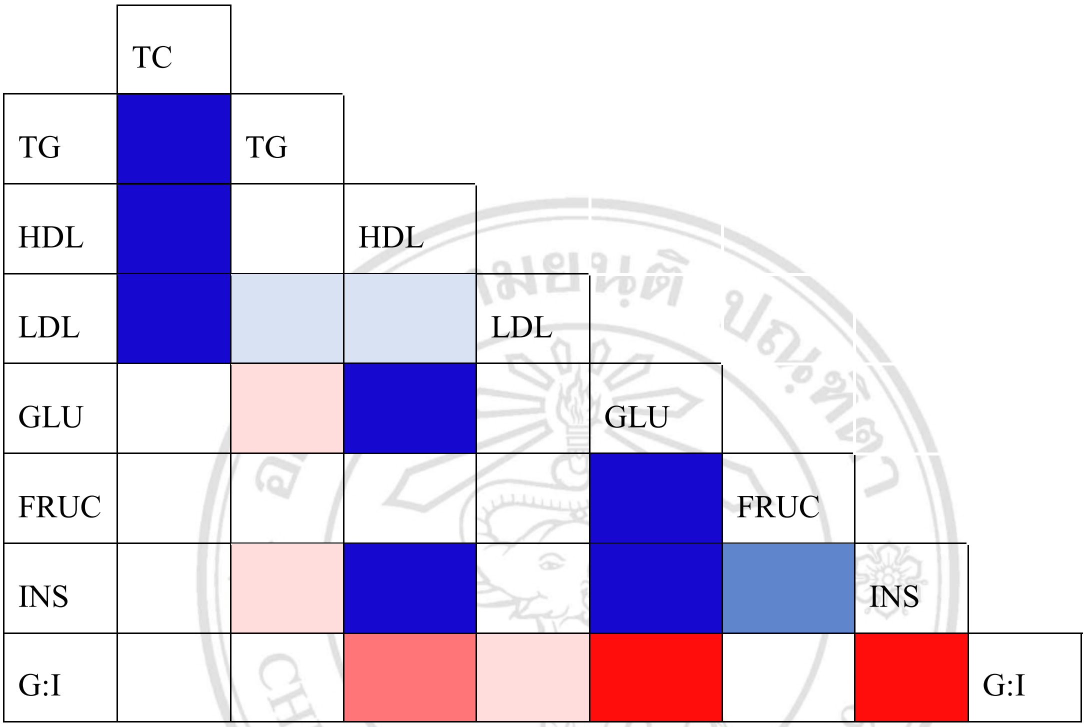
Summer: 16 February–15 May, rainy: 16 May–15 October, winter: 16 October–15 February

Table 3.6 General Estimation Equation analysis of seasonal relationships. Relationships between body condition scores (BCS) and fecal glucocorticoid metabolite (FGM) concentrations and environmental factors in female Asian elephants (n=33) in Thailand.

Parameters	**BCS**			**FGM**		
	Intercept	**Beta**	**P value**	**Intercept**	**Beta**	**P value**
Temperature (°C)	4.238	-0.026	0.23	84.43	-1.211	0.001
Rainfall (mm)	3.310	0.041	0.057	53.65	-0.77	0.028
Humidity (%)	2.307	0.005	0.001	54.21	-0.043	0.600
THI	3.781	-0.003	0.835	103.93	-0.682	0.022

THI=temperature-humidity index

Table 3.7 Relationships among metabolic and lipid factors. Correlation matrix presenting relationships between lipid panel measures and metabolic factors in female Asian elephants (n=33) in Thailand.



***p<0.001 (dark color), **p<0.01 (medium color), *p<0.05 (light color)

Blue=positive correlation

Red=negative correlation

TC=total cholesterol; TG=triglycerides; HDL=high density lipoproteins; LDL = low density lipoproteins; GLU = glucose; FRUC = fructosamine; INS = insulin; G:I=glucose to insulin ratio.

3.4 Discussion

This was the first study to evaluate relationships between BCS and metabolic factors in Asian elephants in any range country, and the first to assess lipid profiles in relation to body condition in this species. We also present new evidence for a relationship between nutritional status and stress hormone levels in elephants, as indicated by associations between FGM concentrations and several measures of metabolic and lipid function. The most prevalent (mode) BCS in Thai elephants was 3–3.5 (60.6%), which is considered ideal (Morfeld and Brown, 2016). Deviations from that were mostly in the higher BSC categories, with 27.3% at BCS=4 (overweight) and 12.1% at BCS=4.5-5 (very overweight); no elephants had a BCS <2. Overall, the working elephants in our study had better body condition than those in western zoos. For example, in the U.S. only 16.5% had a BCS=3; 27% were BCS=4 and 48% were BCS=5 (Morfeld and Brown, 2016), whereas 75% were scored as overweight or very overweight in the U.K. (Harris *et al*., 2008). This could be due to higher amounts of exercise, with tourist elephants engaged in many activities, including trekking, bathing, shows or walking with tourists (Malikhao and Servaes, 2017), so inactivity is less of a concern. In the U.S., elephants that walked more than 14 hours/week had a decreased risk of BCS=4 or 5 (Morfeld and Brown, 2016, Morfeld and Brown, 2017). Feeding diversity (i.e., presenting food in multiple ways) also was related to lower BCS in U.S. studies (Morfeld and Brown, 2016, Morfeld and Brown, 2017). Comparatively, there is little diversity in how tourist camp elephants are fed, which are given fodder throughout the day, even during trekking. Thus, increased exercise during tourist activities likely helps those elephants maintain better body condition, and in this study, elephants that worked more hours per day in the form of saddle or bareback riding had lower BCSs.

Despite the better overall body condition of tourist compared to western zoo elephants, over a third had scores that suggested they were overweight, and these exhibited alterations in lipid and sugar metabolism. A popular activity for tourists is feeding elephants, particularly bananas and sugar cane, which possess high concentrations of sucrose and other soluble sugars that could contribute to weight problems. Blood glucose values in this study agreed with non-fasted levels found in a previous study of Thai elephants (Weerakhun *et al*., 2010). Another finding was that very overweight elephants (BCS=4.5-5) had higher levels of insulin. Insulin plays a central role in the regulation of blood glucose and energy homeostasis; however, high levels are associated with hypertension, obesity, dyslipidemia, and glucose intolerance in humans (Boden, 2015; Modan *et al*., 1987). It was not possible to fast elephants before blood sample collection in this study, and they had access to forage overnight, so we also evaluated the G:I ratio, which has been used for detection of insulin sensitivity in women (Legro *et al*., 1998), with lower values reflecting metabolic abnormalities. In the present study, BCS was predictive of G:I, and elephants with higher scores (BCS=4-5) exhibited significantly lower G:I, a finding similar to U.S. studies (Morfeld and Brown, 2016; Morfeld and Brown, 2017). Comparatively, the overall G:I average value for the tourist elephants (G:I=165) was slightly better than that in the U.S. (G:I=110), although the ranges were similar (23-483 versus 14-430, respectively). In addition to glucose and insulin, serum fructosamine also was measured, which reflects glucose levels over the previous 2-3 weeks, and has been used to monitor and control blood sugar levels in diabetic patients (Sacks *et al*., 2011), dogs and cats (Nelson, 2012). It has been reported to have positive correlations with body weight (Gilor *et al*., 2010), body mass index (Woo *et al*., 1992) and waist circumference (Manjrekar *et al*., 2012) in other species. This is the first report of fructosamine levels in Asian elephants, which were positively correlated with BCS and glucose, and so could be an additional marker of sugar homeostasis. Glycated hemoglobin (HbA1c) is another measure that provides average blood glucose levels over longer, 1-4 month periods, and is more common for monitoring diabetes in humans, although fructosamine tests are better for people with sickle cell or other blood disorders that can affect HbA1c levels (Kosecki *et al*., 2005). We attempted to measure HbA1c in elephants by two ways: high performance liquid chromatography and a HbA1c-DIRECT EIA (BioSystems S.A., Costa Brava, Barcelona, Spain); however, levels were undetectable.

There was a seasonality in BCSs, with the average being lowest in the summer. Other studies have shown a shift towards thinner body condition during dry as compared to wet seasons in free-ranging Asian elephants (Mumby *et al*., 2015). The only climate factor related to BCS was a positive relationship with humidity, although the effect of rainfall approached significance. A variety of climatic drivers of body size declines have been proposed, and in ungulates, an indirect link between climate, resources and ungulate body mass has been established, and may be influenced by climate change (Albon *et al*., 2017; Rughetti *et al*., 2012). Warm springs reduce both relative winter mass loss and summer mass gain in adult bighorn sheep (*Ovis canadensis*), likely due in part to growth rate of plants and duration of access to high-quality forage (Douhard *et al*., 2018). A seasonal shift in the diet of southeast Asian elephants from high-quality grasses in the wet season to poorer quality grass during the dry season (Campos-Arceiz *et al*., 2008, Sukumar and Gadgil, 1988, Western and Lindsay, 1984) could explain the lower BCS during the summer season. Although our study animals were not free-ranging, most fodder was sourced locally, and so would have been subject to seasonal influences. Quantifying the amount and frequency of food provided was beyond the scope of this study, but will be a focus of future studies to identify relationships between nutrient intake, body condition and health.

Serum TG concentrations were within the range reported for Asian elephants (Morfeld and Brown, 2016; Ratnasooriya *et al*., 2006), and lower for females with BCS =2-3, like that of Morfeld et al. (Morfeld and Brown, 2016). Significant relationships between BCS and other lipids were observed, with thinner elephants (BCS=2-3) exhibiting lower TC, HDL, and LDL concentrations than those with higher scores. In humans, a number of metabolic changes are associated with obesity, high BMI or poor eating habits, including elevated TG, TC and LDL levels. Similarly, in dogs, increased plasma TC and TG concentrations are observed in association with obesity (Jeusette *et al*., 2005), and in horses, TG concentration is correlated with body condition scores (Bertin and de Laat, 2017; de Laat *et al*., 2016). Dyslipidemia resulting from poor diets and inadequate exercise constitutes a major risk for development of heart disease and other health problems in women, and is characterized by increased levels of TC, LDL cholesterol and TG, and decreased levels of HDL (Jacobson, 2013; Mearns, 2011, Orozco-Beltran *et al*., 2017). Hyperlipidemia could pose problems for elephants, although incidence of cardiovascular disease appears to be relatively low (Fowler and Mikota, 2008). There was variability in the types and duration of work experienced by elephants across the five camps, with regression analyses indicating significant correlations between work hours and lipid panel results, being negative for HDL, glucose, fructosamine and insulin, and positive for G:I. Moderate amounts of exercise have a positive impact on lipid levels in other species (LeMura *et al*., 2000; Wang and Xu, 2017); thus, exercise may provide a protective effect on lipid and sugar homeostasis in elephants, and should be encouraged.

There was a positive association between FGM and glucose, fructose, insulin, and a negative correlation with G:I ratio, indicating a possible relationship between adrenal function and metabolism, as has been demonstrated in other species (Horner *et al*., 1987; Mudron *et al*., 2005; Wang *et al*., 1995). Elevated and sustained cortisol secretion during chronic stress can lead to central obesity, hypertension, glucose intolerance, and dyslipidemia in humans (Wang, 2005). Administration of exogenous GCs also can increase plasma insulin and TG levels in women, whereas adrenalectomy reduces plasma insulin, glucose and TG levels (Tamashiro *et al*., 2011). Studies to understand how management factors affect stress responses in captive animals are key to improving welfare (Freedman *et al*., 1986), and are beginning to be applied to elephants in westerns zoos (Harris *et al*., 2008; Proctor and Brown, 2015), which will be used as models for subsequent studies of working tourist elephants in Asia.

Last, there was an effect of season on FGM, with higher concentrations during the winter when temperatures and rainfall were lower. Saliva cortisol in zoo Asian elephants in Spain were highest between October and December (Menargues *et al*., 2012), in agreement with our results. The need for more energy to maintain optimum body temperature and ensure survival in cooler temperatures could be related to this finding. Although the climate in Thailand is relatively warm, elephants have been known to shiver on cool winter days (personal observations). In other ungulates, higher GC levels during winter have been found in white-tailed deer (*Odocoileus virginianus*) (Bubenik and Brown, 1989) and mule deer (*Odocoileus hemionus*) (Saltz and White, 1989). Elevated circulating GC levels as a response to cold stress also were documented in reindeer (*Rangifer tarandus*) (Yousef and Luick, 1971) and in farm animals (Dantzer and Mormede, 1983). In our study, FGM concentrations were influenced by several environmental factors, and negatively associated with temperature, rainfall and THI. Interestingly, this pattern differed significantly from that of logging elephants in Myanmar, where FGM levels were positively correlated with rainfall, but not with temperature (Mumby *et al*., 2015). In that study, FGM concentrations were highest in June, July and August, which corresponded to the end of the hot season and start of the monsoon and work season (Mumby *et al*., 2015). In that population, the shift from rest to intense work periods appears to have resulted in a marked increase in adrenal activity. Increased workloads have been associated with increased adrenal corticoid activity in horses and humans (Strzelec et al., 2011; Lundberg and Hellström, 2002). However, the negative relationship between FGM and work hours in this study suggests it may not only be work hours that are affecting adrenal activity in Thai elephants, but perhaps other factors like a greater number of tourists taking advantage of the nicer climate in the winter, all of which should be explored more fully.

3.5 Conclusions

Using BCS and FGM as outcome variables in regression models, high BCS and FGM were predictors of higher and potentially unhealthy metabolic and lipid levels in female Asian elephants. This study provides the first evidence that altered metabolic marker and lipid levels are associated with high BCS and adrenal steroid hormone measures in tourist camp elephants in Thailand, and that problems may be exacerbated during the high tourist season (winter and rainy seasons). Future studies will focus on what factors specifically affect elephant health and well-being, and the potential benefits of limiting the amount of high calorie treats (bananas, sugar cane) given to elephants by tourists, ensuring animals receive appropriate levels of exercise to reduce fat and increase muscle mass, and reducing stress by limiting workloads and numbers of tourists interacting with individual elephants, especially during the high season.

CHAPTER 4

Effect of tourist season and camp management on lipid profiles, body condition and metabolic function in female Asian elephants in Thailand

4.1 Introduction

Elephants have been used in the logging industry in Thailand for centuries; however, a logging ban initiated by the government in 1989 left thousands of elephants and their mahouts unemployed, and brought on the elephant tourism era. Elephants today play an important role in the economy of Thailand, with about 3,500, mostly (95%) privately owned (Asian Elephant Specialist Group, 2017) being used primarily for tourism. Over the past two decades, the number of elephant camps has increased significantly, especially in the northern region, which consists of 40% of camps in the country, mainly in Chiang Mai (37 % of the country) (National Institute of Elephant Research and Health Service, Lampang, Thailand). Elephants participate in a variety of activities, including shows, riding, feeding, and bathing by tourists. In recent years, the welfare of captive elephants has become a topic of intense debate among government agencies, animal rights groups, scientists, and the general public. Management differs across elephant camps with respect to tourist numbers, intensity of activities, nutrition, breeding, restraint, and control, all of which can have impacts on health, behavior and welfare. Tourist activities have been shown to compromise welfare and negatively affect behavior and physiology in other species, resulting in increased hiding behaviors (Birke, 2002; Stevens *et al.*, 2013), heightened vigilance (Clark *et al.*, 2012; Larsen *et al.*, 2014), stereotypies (Mallapur *et al.*, 2002; Wells, 2005), poor body condition (Maréchal *et al.*, 2016), and elevated cortisol (Davis *et al.*, 2005; Pifarré *et al.*, 2012). Most studies of this type have been conducted in western zoo animals, with few focusing on captive animals in range countries, including elephants, and particularly those in tourism.

One of the most popular tourist activities is feeding elephants treats like bananas and sugar cane, although there is a minimal understanding of the potential health impacts. Feeding of unnatural or unhealthy high energy food items can compromise health and fitness (Heuberger *et al.*, 2011), resulting in obesity, metabolic syndromes, and energy imbalances (Epstein *et al.*, 2008; Stelmach-Mardas *et al.*, 2016). Elephant riding, with saddles or bareback, is another popular activity enjoyed by tourists, although it has come under intense criticism in recent years, with TripAdvisor no longer selling tickets to venues where elephant rides are offered. However, in a recent study, the number of working hours related primarily to giving rides was related to lower stress levels, based on fecal glucocorticoid metabolite (FGM) measures, and better body condition in elephants using camp-based information (Norkaew *et al.*, 2018). In other species, however, high numbers of work hours have been associated with increased adrenal corticoid output (Lundberg *et al.*, 2002; Strzelec *et al.*, 2011), and a number of negative health effects, such as poor body condition (Ayalew *et al.*, 2018). However, others report positive health effects of work (Urtasun *et al.*, 2018), so relationships between work, health and well-being are not always clear (van der Hulst, 2003), and likely depend on the type of work being done, how the work is perceived by the individual, and if the work intensity exceeds a biological threshold.

Stress and increased adrenal activity can affect metabolic health and lipid parameters. Glucocorticoids modulate a large number of physiological actions involved in metabolic, inflammatory, cardiovascular, and behavioral processes. Mechanisms by which GCs coordinate these effects include increasing glucose production from hepatic cells (Vegiopoulos *et al.*, 2007), decreasing glucose uptake into muscle and adipose tissue (Sakoda *et al.*, 2000; Weinstein *et al.*, 1998), increasing lipolysis (Divertie *et al.*, 1991), and decreasing insulin release from pancreatic cells (Lambillotte *et al.*, 1997). Working elephants in Thailand interact with the public in a variety of ways. Often these activities are not closely monitored or regulated, and could be sources of stress to individual animals. Recently, Norkaew *et al.* (2018) found relationships between adrenal and metabolic hormones and lipid profiles in Asian elephants, with positive correlations between FGM and total cholesterol (TC), high density lipoprotein (HDL), glucose, and insulin, and negative correlations with the glucose-to-insulin (G:I) ratio. Because it is generally not possible to fast elephants before blood sample collection in tourist camps, and elephants have access to forage overnight, a G:I ratio is calculated, which detects insulin sensitivity in women (Legro *et al.*, 1998), with lower values reflecting metabolic abnormalities. Perhaps related to the feeding of high calorie treats, positive correlations were found between body condition score (BCS) and TC, low density lipoproteins (LDL), and HDL, with a negative correlation between BCS and G:I. That study evaluated relationships on a population level and did not take into account the variation in management across individual camps throughout the year. This study built on that research to examine the effect of tourist numbers and working hours on adrenal, lipid and metabolic function in captive elephants in northern Thailand, and how results across camps compared between the High (November–February) and Low (March–October) tourist season in the region.

4.2 Materials and methods

4.2.1 Animals

This study was approved by the Faculty of Veterinary Medicine, Chiang Mai University, Animal Care and Use Committee (FVM-ACUC; permit number S39/2559). Thirty-three adult female Asian elephants (age range, 18 – 50; mean, 34.2 ± 7.3 years) were housed at five tourist camps within 43–72 km of the Chiang Mai University Veterinary Faculty (latitude 18°47'N, longitude 98°59'E, altitude 330 m) (Table 4.1). At four of the camps, tourists interacted with elephants through riding programs (bareback or with a saddle) and feeding of supplementary foods. The fifth camp offered no tourist activities other than observation of elephants in a large field, and being taken for a bath at a local river. Work time per day was the time elephants were actively involved in tourist activities. For saddle and bareback riding, it equated to the number of rounds of riding per day times the minutes per riding round. Walking distance was the distance elephants were walked per day. Elephants were fed primarily corn stalk*, napier grass* (*Pennisetum purpureum*) and bana grass (*Pennisetum purpureum* X, *P. americanum* hybrid) with limited access to fresh water. Supplementary foods consisted of bamboo, sugarcane, bananas, pumpkins, watermelon and cucumber that were primarily offered by tourists. Animals were given an annual physical examination by staff veterinarians, and were in good health during the study.

4.2.2 Blood collection

Blood samples (10 ml) were collected from an ear vein by elephant camp staff or Chiang Mai University veterinarians twice monthly for 1 year. All elephants were conditioned to the blood sampling procedure. Blood was centrifuged at 1,500 x g for 10 minutes within a few hours of collection, and the serum stored at -20°C until processing and analysis.

4.2.3 Body condition scoring

Once every 2 months, rear and side view photographs were taken of each elephant to permit a visual evaluation of the backbone, rib and pelvic bone areas, and body condition was scored 1-5 (1 = thinnest; 5 = fattest) as described by Morfeld *et al*. (2016), except that scoring was done in 0.5-point, rather than 1-point, increments. All photos were evaluated by three experienced elephant veterinarians, and the scores averaged. Intra-class correlations determined the inter-assessor reliability was 0.85.

4.2.4 Metabolic marker analyses

Serum glucose was measured by a hexokinase method using an automated glucose analyzer (Glucinet T01-149, Bayer, Barcelona, Spain), with quinoneimine measured at 530 nm. Serum fructosamine was measured by a colorimetric method using nitrobluetetrazolium in a Biosystems BA400 clinical chemistry analyzer (©Biosystems S.A., Barcelona, Spain). A solid-phase, two-site bovine insulin enzyme immunoassay (EIA; Cat. No. 10-1113-01; Mercodia, Uppsala, Sweden), validated for elephants, was used to measure serum insulin concentrations (Morfeld *et al.*, 2016). Colorimetric responses were determined spectrophotometrically at 450 nm filter with an Opsys MR Microplate Reader (TECAN Sunrise^{TM} microplate reader; Salzburg, Austria). All samples were analyzed in duplicate; intra- and inter-assay CVs were <10% and <15%, respectively.

4.2.5 Lipid profile analysis

Serum lipids were quantified using a Mindray BS Series analyzer (Mindray BS-380, Shenzhen Mindray Bio-Medical Electronics Co., Ltd.). Total cholesterol was measured by a cholesterol oxidase-peroxidase (CHOD-POD) method. Triglycerides were measured by a glycerokinase peroxidase-peroxidase (GPO-POD) method, with a sensitivity of 0.1 mmol/l (99.7% confidence). The lowest measurable concentration was 0.1 mmol/l (99.7% confidence) for TC, and 0.05 mmol/l for both HDL and LDL.

4.2.6 Fecal extraction and GC metabolite analysis

The fecal extraction technique is described in Norkaew *et al.* (2018). Briefly, wet samples were dried in a conventional oven at 60°C for ~24-48 hours and stored at -20°C until extraction. Frozen dried fecal samples were thawed at room temperature (RT), mixed well and 0.1 g (±0.01) of dry powdered feces extracted twice in 90% ethanol in distilled water by boiling in a water bath (96°C) for 20 minutes and adding 100% ethanol as needed to keep from boiling dry. Samples were centrifuged at 1,500 x g for 20 min, and the combined supernatants dried under air in a 50°C water bath. Dried extracts were reconstituted in methanol and diluted 1:3 in assay buffer (Cat. No. X065, Arbor Assays, Arbor, MI, USA) and stored at –20°C until enzyme immunoassay (EIA) analysis.

Concentrations of FGM were determined using a double-antibody enzyme EIA with a polyclonal rabbit anti-corticosterone antibody (CJM006) validated for Asian elephants (Watson *et al.*, 2013) and described by Norkaew *et al.* (2018). Samples (50 µl) or corticosterone standards (50 µl) were added to appropriate wells. Corticosterone-horseradish peroxidase (25 µl) was immediately added to each well except for non-specific binding wells, followed by 25 µl anti-corticosterone antibody, and incubated at RT for 1 h. Plates were washed four times (1:20 dilution, 20X Wash Buffer Cat. No. X007; Arbor Assays) and 100 µl of TMB substrate solution was added, followed by incubation for 45-60 min at RT without shaking. The absorbance was measured at 405 nm by a microplate reader (TECAN). Assay sensitivity (based on 90% binding) was 0.14 ng/ml. Samples were analyzed in duplicate; intra- and inter-assay CVs were <10% and <15%, respectively.

4.2.7 Statistical analysis

Descriptive data were reported as mean±standard error of the mean (SEM) and camp management variables were presented as a range or a frequency, depending on the type of data. Statistical analyses were performed using R version 3.4.0 (R Development Core Team, 2017). Repeated measures data were analyzed using Generalized Estimating Equations (GEE) to determine how BCS, FGM, metabolic and lipid panel results were affected by tourist numbers, work time, and walking distance. High (November–

February) and Low (March–October) tourist seasons were defined by the Tourism Authority of Thailand. Differences in mean FGM, metabolic, lipid profiles and work type between High and Low tourist seasons were analyzed by Tukey's post-hoc tests. Differences in mean BCS, FGM, metabolic hormones and lipid measures among camp were analyzed using multiple comparison with Tukey's post-hoc tests. Correlations between diet and BCS, FGM and metabolic hormones or lipid measures were analyzed using linear regression tests for aggregated data. Differences in mean BCS, FGM, metabolic hormones and lipid measures between or within camp in High and Low tourist seasons were analyzed using Tukey's post-hoc tests. The significance level was set at $\alpha = 0.05$.

4.3 Results

There was notable variation across the camps in work activities for elephants, with bareback riding for camp A, saddle riding for camp B, C and E, and no riding for camp D. Camp C had the highest walking per day and camp A had the lowest. Camp D elephants did not work at all; tourists watched them at a close distance in a field (Table 4.1). There were significant differences across camps in adrenal activity, metabolic maker and lipid profiles (Table 4.2), with BCS, TC, HDL, insulin and glucose being among the highest, and G:I being among the lowest in camp D, the facility with no work activities for elephants. Glucose and insulin concentrations in elephants at camp A also were high, and during the High season, the G:I in that camp was the lowest. In addition to metabolic factors, FGM concentrations in elephants at camp D also were high, similar to camp A, while those in camps C and E were the lowest. Camp E had the highest TC and TG values, while Camps C and E had the lowest HDL and highest LDL levels.

Table 4.1 Description of each elephant camp in the study (Camps A–E). Information includes number of years the camp has been in operation (camp age), total number of elephants in the camp, number of female elephants participating in the study, participating elephant mean age (±SEM) and range, type of work with tourists, work time and walking distance per day, and primary and supplemental food items.

Variable	Camp A	Camp B	Camp C	Camp D	Camp E
Camp age (years)	9	27	29	14	40
Total elephant number (participating)	46 (6)	66 (6)	52 (6)	68 (11)	76 (4)
Elephant age (years)	28.50±1.80 (22-34)	36.80±2.90 (23-43)	35.30±3.80 (20-45)	35.80±2.30 (25-50)	32.20±3.00 (22-40)
Type of work	Bareback riding	Saddle riding	Saddle riding	No riding	Saddle riding
Total work time (min/day)	66.70±1.58	180.00±7.11	204.00±7.94	0	236.00±8.42
- number of round	1.67±0.04	8.99±0.36	5.11±0.20	0	9.33±0.35
- round time (min/round)	40	20	40	0	20
Total walking distance (km/day)	1.63±0.04	4.48±0.18	5.05±0.21	0	4.77±0.17

Table 4.1 (continued)

Variable	Camp A	Camp B	Camp C	Camp D	Camp E
Diet					
Primary	Napier grass, cornstalk	Napier grass, cornstalk	Napier grass, cornstalk	Napier grass, bana grass, cornstalk	Napier grass
- Amount per day (kg/day)	150±0.00	168±1.70	100±0.00	165±0.00	168±4.70
Supplementary	Bamboo, sugarcane, banana	Banana, sugarcane	Banana, sugarcane	Hay, banana, watermelon, pumpkin, cucumber	Bamboo, sugarcane, banana
- Amount per day (kg/day)	30	10	10	20	5

Ta

Overall, during the High tourist season, elephants exhibited higher FGM, TC, glucose and insulin concentrations than during the Low season (Table 4.3). In particular, insulin levels were 44% higher during the High compared to the Low season. Although glucose also was higher, the G:I did not differ between seasons. Conversely, fructosamine was higher in the Low season. Elephant work time and walking distance in the High season were nearly double those in the Low season, although camp operating hours were unchanged.

Figure 4.1 shows differences among the five camps in FGM and BCS, and between High and Low tourist seasons within each camp. The results showed that all camps except camp E had higher FGM concentrations (18-30%) during the High than the Low tourist season. Camps A and D had the highest, while camps C and E had the lowest FGM levels in both seasons. During the High tourist season, camps A and D had higher FGM than camps C and E. Similarly, during the Low tourist season, camp A had higher FGM than camps B, C and E, while camp D had higher FGM than camps C and E. For BCS, the only camp with a seasonal difference was camp A, with lower condition in the Low season. Camp D had the highest BCS in both High and Low tourist seasons.

Figure 4.2 illustrates camp and tourist season differences in lipid parameters. The only tourist season differences were TC in camp C and HDL in camp A, where concentrations were higher in the High season. Across camps, camp D had higher HDL than camps B and C during the High season, and the highest of all camps during Low season. For LDL, camps D and E had higher LDL than camps B during the High season, and camps D and E had higher LDL than other camps during the Low season.

Camp and tourist season difference in metabolic parameters are shown in Figure 4.3. Glucose and insulin levels in camps A and D were 14% and 11% higher during the High than the Low tourist season, respectively. During the High season, glucose levels in camp A were ~30% higher than in camps B, C and E, and 16% higher than in camps B, C and E. Glucose levels in camp D were ~32-40% higher in camps B, C and E during the High season and 24% higher than camps B, C and E during the Low season. Moreover, insulin levels in camps A and D during the High season were greater than all other camps. During the High season, insulin levels in camp A were three, five and two times higher than camps B, C and D, respectively. However, during the Low season, camp D had higher insulin levels than camps B and C. Because of the high insulin concentrations, camp A had a low G:I during the High tourist season less, 2.5 times than during the Low season. During the High tourist season, the G:Is in camps A and D were significant lower than in camps B and C. During the Low tourist season, only camp D had higher insulin concentrations than camps B and C. There were significant tourist season differences between fructosamine concentrations in camps B and C.

Relationships between tourist numbers and metabolic marker and lipid profiles were determined using GEE (Table 4.4). There were significant positive associations between tourist number and BCS, TG, and insulin. By contrast, tourist number were negatively correlated to glucose. Relationships between work time and walking distance on metabolic marker and lipid profiles are presented in Table 4.5, and show significant positive associations between work time and TC and TG. Walking distance also was positively related to TC. Both work time and walking distance were negatively correlated to glucose, fructosamine and insulin. Only walking distance was negatively related to FGM.

In separate linear regression analyses of individual means (n=33), the amount of primary diet was correlated to FGM, BCS, and insulin (Table 4.6), whereas the amount of supplementary diet was positively correlated with FGM concentrations, glucose, fructosamine, and insulin.

Table 4.2 Mean (± SEM) and range values in lipid profiles and metabolic factors between high and low tourist seasons in Thailand.

Factor	**High season**	**Low season**
FGM (ng/g)	58.12±2.24[b]	48.33± 1.03[a]
	(19.59-194.17)	(11.42-147.07)
TC (mg/dL)	38.44±0.58[b]	36.83±0.39[a]
	(22.00-109.00)	(10.00-85.00)
TG (mg/dL)	28.00±1.11	29.00±0.82
	(7.00-110.00)	(4.00-157.00)
HDL (mg/dL)	11.90±0.18	11.50±0.12
	(3.00-26.00)	(2.00-26.00)
LDL (mg/dL)	28.70±0.50	27.30±0.40
	(9.00-89.00)	(8.00-107.00)
Glucose (mg/dL)	92.40±1.53[b]	87.20±0.88[a]
	(52.00-172.00)	(50.00-180.00)
Fructosamine (mM)	0.58±0.01[a]	0.60±0.01[b]
	(0.45-0.77)	(0.38-0.92)
Insulin (µg/L)	0.94±0.09[b]	0.65±0.04[a]
	(0.03-5.91)	(0.02-3.37)
G:I	163.00±12.40	195.00±10.50
	(22.70-613.00)	(34.40-722.00)

Table 4.2 (continued)

Factor	High season	Low season
Operation time (hour/day)	6.38±0.05	6.38±0.03
	(5.00-7.00)	(5.00-7.00)
Work time (hr/day)	3.83 ± 0.12^{b}	2.22 ± 0.05^{a}
	(0.00-400.00)	(0.00-400.00)
Walking distance (km/day)	5.52 ± 0.20^{b}	3.11 ± 0.87^{a}
	(0.00-10.00)	(0.00-10.00)

^{a,b}Row values are significantly different (P<0.05)

FGM=fecal glucocorticoid metabolites; TC=total cholesterol; TG=triglycerides; HDL= high density lipoproteins; LDL=low density lipoproteins; G:I=glucose to insulin ratio.

High: November–February, Low: March–October (According to the Tourism Authority of Thailand)

Table 4.3 Mean (±SEM) and range values (min–max) for lipid profiles and metabolic factors in each elephant tourist camp.

Factor	Camp A	Camp B	Camp C	Camp D	Camp E
FGM (ng/g)	60.40 ± 2.43^{c}	49.60 ± 2.40^{b}	39.10 ± 1.41^{a}	58.20 ± 1.75^{c}	39.60 ± 2.06^{a}
	(20.00-142.00)	(11.90-194.00)	(15.40-153.00)	(16.60-173.00)	(12.40-110.00)
BCS	3.25 ± 0.06^{a}	3.44 ± 0.08^{a}	3.21 ± 0.09^{a}	4.17 ± 0.10^{b}	3.43 ± 0.10^{a}
	(3.00–4.00)	(3.00–4.50)	(2.00–4.00)	(3.00–5.00)	(3.00–4.00)
TC (mg/dL)	35.50 ± 0.88^{a}	35.50 ± 0.72^{a}	34.60 ± 0.82^{a}	39.5 ± 0.50^{b}	39.70 ± 0.74^{b}
	(10.00-76.00)	(22.00-76.00)	(20.00-109.00)	(21.00-77.00)	(28.00-72.00)
TG (mg/dL)	25.50 ± 1.36^{a}	29.40 ± 1.62^{a}	25.50 ± 1.33^{a}	28.80 ± 1.27^{a}	36.20 ± 1.74^{b}
	(5.00-88.00)	(8.00-113.00)	(4.00-94.00)	(6.00-157.00)	(11.00-110.00)
HDL (mg/dL)	11.40 ± 0.21^{b}	11.40 ± 0.17^{b}	10.30 ± 0.14^{a}	13.00 ± 0.20^{c}	10.70 ± 0.31^{ab}
	(3.00-18.00)	(7.00-19.00)	(2.00-15.00)	(7.00-26.00)	(3.00-26.00)
LDL (mg/dL)	26.70 ± 0.94^{ab}	25.00 ± 0.66^{a}	26.00 ± 0.91^{a}	29.50 ± 0.43^{bc}	30.40 ± 0.60^{c}
	(11.00-53.00)	(9.00-51.00)	(8.00-107.00)	(15.00-67.00)	(19.00-47.00)

Table 4.3 (continued)

Factor	Camp A	Camp B	Camp C	Camp D	Camp E
Glucose (mg/dL)	96.40±2.27^{b}	78.20±1.23^{a}	78.50±1.43^{a}	100.90±1.29^{b}	79.60±1.47^{a}
	(55.00-172.00)	(52.00-125.00)	(50.00-160.00)	(52.00-180.00)	(55.00-118.00)
Fructosamine (mM)	0.60±0.004^{bc}	0.59±0.006^{b}	0.57±0.005^{a}	0.61±0.003^{c}	0.56±0.006^{a}
	(0.50-0.77)	(0.45-0.92)	(0.47-0.86)	(0.39-0.78)	(0.38-0.70)
Insulin (µg/L)	0.99±0.16^{cd}	0.45±0.05^{ab}	0.34±0.04^{a}	1.08±0.08^{d}	0.66±0.09^{bc}
	(0.03-5.91)	(0.03-1.77)	(0.03-1.98)	(0.02-3.93)	(0.08-2.83)
G:I	171.00±29.20^{ab}	240.00±21.92^{b}	238.00±22.17^{b}	141.00±8.77^{a}	181.00±16.23^{ab}
	(22.70-722.00)	(58.30-712.00)	(47.90-660.00)	(23.70-569.00)	(37.50-446.00)

FGM=fecal glucocorticoid metabolites; BCS=body condition score; TC=total cholesterol; TG=triglycerides; HDL=high density lipoproteins; LD =low density lipoproteins; G:I=glucose to insulin ratio.

Table 4.4 General Estimation Equation analyses. Relationships between tourist number and health factors and adrenal steroid activity in female Asian elephants (n=33) in Thailand.

Parameters	Tourist number		
	Intercept	**Beta ($x10^{-5}$)**	**P value**
FGMs (ng/g)	52.05	-27.90	0.210
BSC	3.19	0.91	0.017
TC (mg/dL)	35.40	6.87	0.140
TG (mg/dL)	25.50	21.50	0.002
HDL (mg/dL)	10.70	1.28	0.280
LDL (mg/dL)	27.10	-0.75	0.870
Glucose (mg/dL)	88.70	-41.80	<0.001
Fructosamine (mM)	0.59	-0.04	0.320
Insulin (ng/ml)	0.72	0.93	0.034
G:I	200.00	75.30	0.470

FGM=fecal glucocorticoid metabolites; BCS=body condition score; TC=total cholesterol; TG=triglycerides; HDL=high density lipoproteins; LDL=low density lipoproteins; G:I=glucose to insulin ratio.

Table 4.5 General Estimation Equation analyses. Relationships between work time and health factors and adrenal steroid activity in female Asian elephants (n = 33) in Thailand.

Parameters	Work time (hr/day)			Walking distance (km/day)		
	Intercept	Beta ($x10^{-2}$)	P value	Intercept	Beta ($x10^{-4}$)	P value
FGMs (ng/g)	51.48	-2.000	0.140	53.73	-13.460	0.015
BCS	3.31	0.018	0.660	3.30	0.106	0.540
TC (mg/dL)	33.82	1.570	0.003	34.40	4.820	0.032
TG (mg/dL)	26.08	1.564	0.032	27.20	3.350	0.250
HDL (mg/dL)	10.74	0.125	0.320	10.80	0.241	0.590
LDL (mg/dL)	25.97	0.574	0.250	26.50	0.925	0.650
Glucose (mg/dL)	87.23	-2.826	0.003	88.60	-14.480	<0.001
Fructosamine (mM)	0.61	-0.016	<0.001	0.61	-0.063	<0.001
Insulin (ng/ml)	0.73	-0.089	0.039	0.81	-0.532	0.002
G:I	204.73	4.230	0.720	188.00	58.200	0.300

FGM=fecal glucocorticoid metabolites; BCS=body condition score; TC=total cholesterol; TG =triglycerides; HDL= high density lipoproteins; LDL=low density lipoproteins; G:I=glucose to insulin ratio.

Table 4.6 Linear regression analyses. Relationships between primary and supplementary diet and health factors and adrenal steroid activity in female Asian elephants (n = 33) in Thailand.

Parameters	**Primary diet**			**Supplementary diet**		
	Intercept	**Beta**	**P value**	**Intercept**	**Beta**	**P value**
FGMs (ng/g)	32.64	0.126	0.037	35.62	0.986	<0.001
BCS	2.67	0.006	0.050	3.43	0.011	0.377
TC (mg/dL)	32.60	0.033	0.313	36.30	0.077	0.525
TG (mg/dL)	21.01	0.051	0.157	32.11	-0.214	0.104
HDL (mg/dL)	9.19	0.016	0.149	10.59	0.002	0.130
LDL (mg/dL)	24.64	0.031	0.463	26.70	0.084	0.464
Glucose (mg/dL)	72.11	0.111	0.119	73.12	0.967	<0.001
Fructosamine (mM)	0.542	0.001	0.081	0.563	0.002	0.008
Insulin (ng/ml)	-0.031	0.005	0.047	0.33	0.026	0.006
G:I	281.38	-0.582	0.258	215.92	-1.382	0.472

FGM=fecal glucocorticoid metabolites; BCS=body condition score; TC=total cholesterol; TG =triglycerides; HDL=high density lipoproteins; LDL=low density lipoproteins; G:I=glucose to insulin ratio.

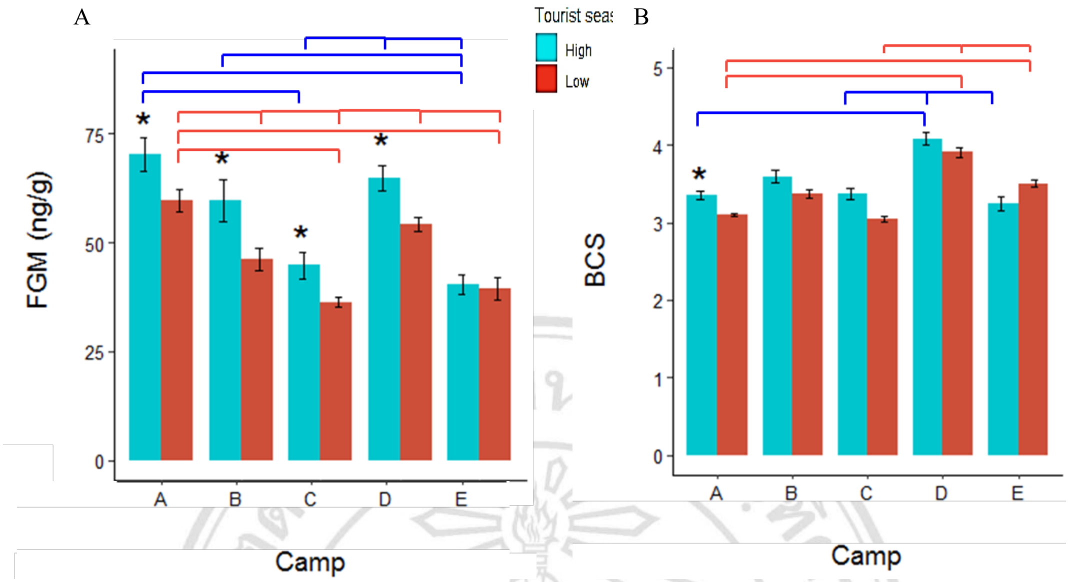


Figure 4.1 Mean±SEM fecal glucocorticoid metabolite (FGM) concentrations (A) and body condition score (BCS) (B) in female Asian elephants (n=33) across the five tourist camps. Red lines indicate significant differences between camps in the High season, and blue lines indicate significant differences between camps in the Low season. Asterisks (*) indicate significant differences between High and Low seasons within the same camp.

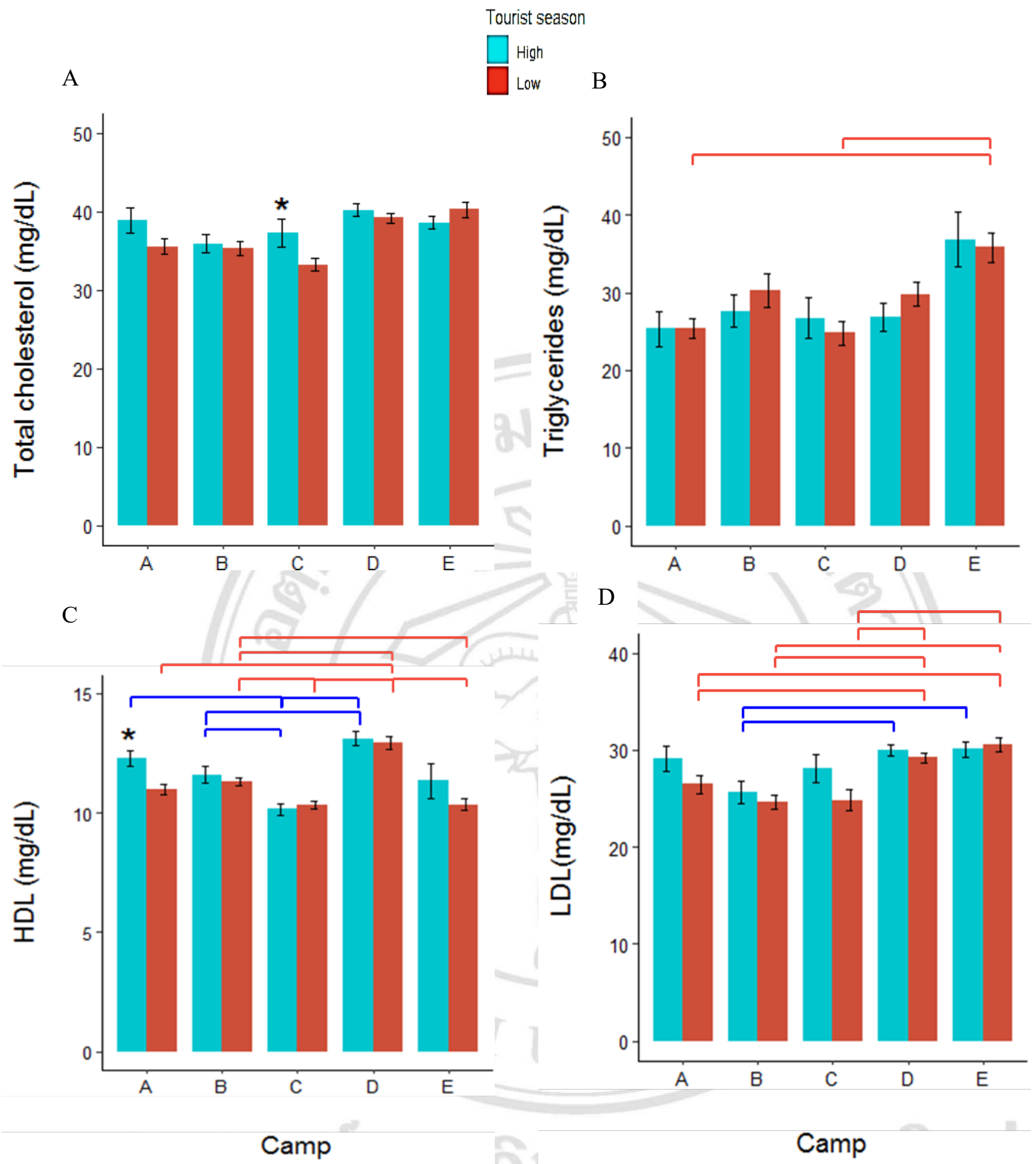


Figure 4. 2 Mean±SEM total cholesterol (TC) (A) and triglyceride (TG) (B) HDL (C) and LDL (D) in female Asian elephants (n=33) across the five tourist camps. Red lines indicate significant differences between camps in the High season, and blue lines indicate significant differences between camps in the Low season. Asterisks (*) indicate significant differences between High and Low seasons within the same camp.

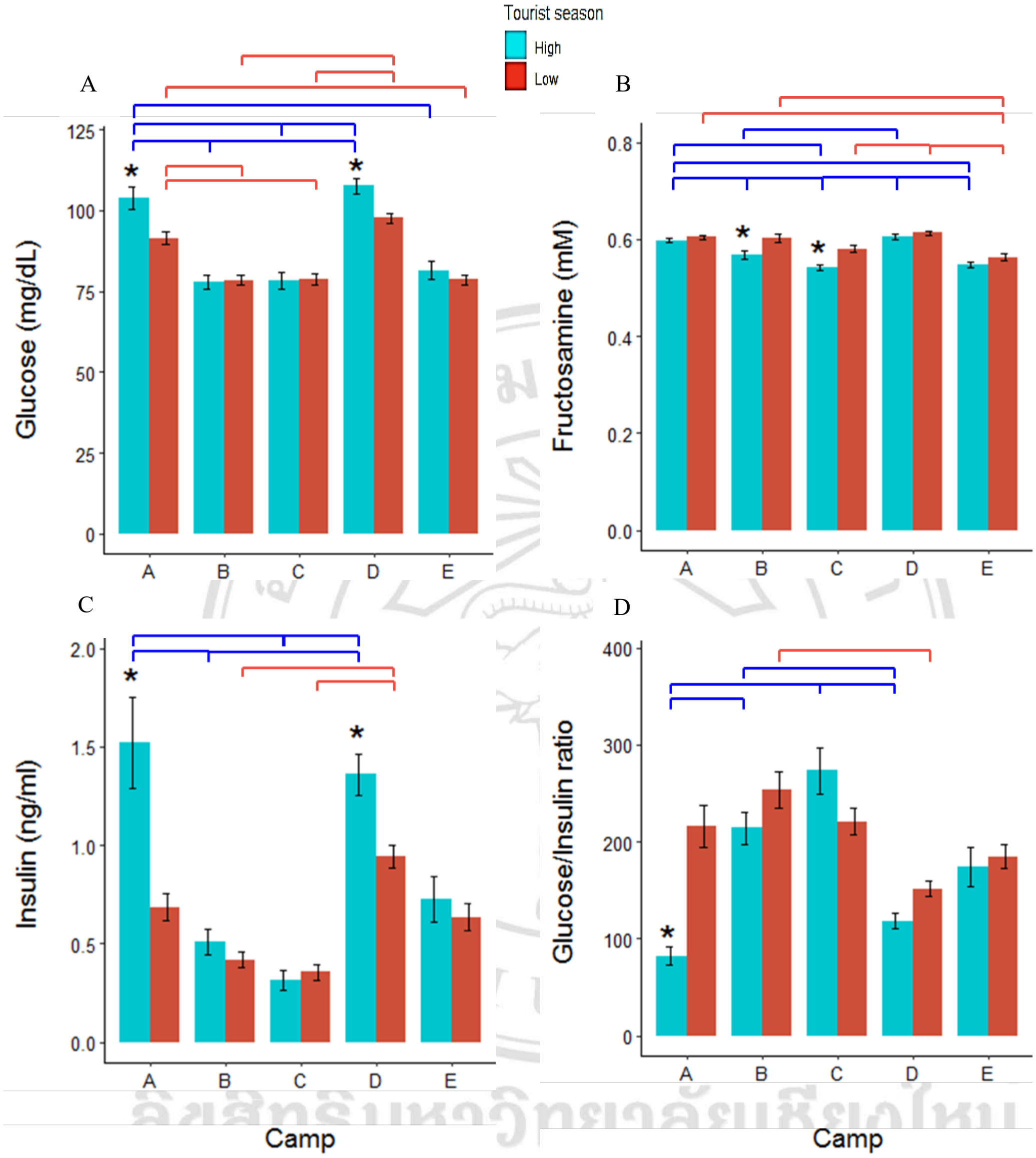


Figure 4.3 Mean±SEM glucose (A) and fructosamine (B) insulin (C) and glucose to insulin ratio (D) in female Asian elephants (n=33) across the five tourist camps. Red lines indicate significant differences between camps in the High season, and blue lines indicate significant differences between camps in the Low season. Asterisks (*) indicate significant differences between High and Low seasons within the same camp.

4.4 Discussion

This is the first study to examine metabolic and lipid parameters in Asian elephants under human care in Thailand in relation to walking distance and working time, and provisioning of supplementary diet items, like bananas and sugar cane, by tourists during the High and Low tourist seasons. It also examined differences across camps in health and welfare measures. There was a significant tourist season effect on health status, with levels of several metabolic markers being higher during the High season. Significant differences across camps in stress and metabolic status further highlight the effect of tourist activities, and how supplemental feeding and lack of exercise may have negative consequences for health. Higher numbers of tourists likely are associated with increases in amounts of food treats offered to elephants, given that feeding is one of the most popular tourist activities.

The type and amount of supplementary food given to elephants varied with each camp, but generally consisted of items with a high sugar content and glycemic index, including bananas (glycemic index=47), sugarcane (50), watermelon (72), and pumpkin (51; only camp D) (Sydney University's Glycemic Research Service). The glycemic index quantifies the widely variable increases in blood glucose after ingestion of different carbohydrates, with larger values associated with the development of metabolic disease (Pfeiffer *et al.*, 2018). High glycemic index foods induce an exaggerated insulin response, which can increase body fat and weight, and lead to insulin resistance, and eventual exhaustion of endocrine pancreatic function and insulin release (Ludwig, 2002; Pawlak *et al.*, 2004). There is growing recognition and concern that obesity and metabolic conditions are negatively impacting the health of many species, including humans, companion and domestic animals. A similar health concern exists for zoo-held species, including elephants, that often are fed diets high in calories and given inadequate exercise (Ange *et al.*, 2001; Clubb *et al.*, 2009; Hatt and Clauss, 2006; Lewis *et al.*, 2010; Morfeld and Brown, 2014; Morfeld *et al.*, 2017). Elephants at two camps in particular, A and D, exhibited glucose and insulin levels that were higher overall compared to the other camps, and 1-2 times greater during the High tourist season. BCS at camp A also was higher during the High season, while the G:I was the lowest during that time, indicative of metabolic derangements (Morfeld and Brown, 2014; Morfeld *et al.*, 2017). These effects appear to be related, in part, to the feeding of greater amounts of supplementary foods at those two camps.

Fecal glucocorticoid metabolite concentrations also differed among camps, with A and D again being significantly different in having the highest concentrations compared to the other camps. One possible explanation is that elephants in camps A and D received less exercise than those in the other camps. Exercise has been shown to reduce stress, anxiety, and depression in a number of species (Coulter *et al.*, 2009), and can counter many of the physiological decrements of aging, and reduce risks for diseases linked to chronic elevations in cortisol (Traustadottir *et al.*, 2005). Results of this study suggest that greater walking distances per day in tourist camp elephants may have a positive effect not only on BCS, but on adrenal status as well, which agrees with an earlier study (Norkaew *et al.*, 2018). Thus, camps A and D appear to operate in a way that results in overall higher glucocorticoid levels and poorer metabolic health. These camps were notably different in the activities elephants were exposed to. In camp A, the main activity was bareback riding, but interestingly, in camp D, tourists merely watched elephants in close proximity. What these two camps have in common are fewer hours of exercise (0 – 67 versus ≥180 hours/d) and higher amounts of supplementary items being fed (20 – 30 versus 5 - 10 kg/day). Thus, these results suggest that if elephants are not to be used for riding or other forms of physical exercise, then care must be taken not to overfeed them, and to limit the amounts of high calorie treats in particular.

Across camps, there was no relationship between tourist numbers and FGM concentrations, which agrees with studies suggesting that not all animals perceive human presence as a stressor (Fowler, 1999; Knapp *et al.*, 2013), although other studies have linked tourism-related activities to higher glucocorticoids in visited areas (Ellenberg *et al.*, 2007; Thiel *et al.*, 2008; Wells, 2005). One explanation for the High tourist season on effect on FGM is that elephants engage in more activity, and so are exposed to more stimuli that may increase adrenal glucocorticoid output. So higher FGM during the High season may not be related directly to increased numbers of tourists per se, but perhaps to factors like seasonal climate effects (Mumby *et al.*, 2015; Norkaew *et al.,* 2018), other management factors (Dwyer *et al.*, 2004; Moberg *et al.*, 2000), or the health status of individual animals (Moberg, 2000).

4.5 Conclusion

Using a generalized estimating equation (GEE) method, we found differences in concentrations of metabolic factors, lipid profiles and FGM concentrations between High and Low tourist seasons. Nutrition, management and tourist numbers may affect metabolic, lipid panel and FGM concentrations. We conclude that elephant well-being can be promoted by limiting the amount of high calorie treats given by tourists, ensuring animals receive appropriate levels of exercise to reduce fat and increase muscle mass, and reducing stress by moderating the numbers of tourists interacting with individual elephants, especially during the High season.

CHAPTER 5

Summary

Healthy populations of elephants are essential to long-term global conservation, especially in the face of declining numbers. It is important to better understand factors affecting health and welfare of working elephants in Thailand to prevent further population declines.

This study provides the first evidence that altered metabolic marker levels are associated with high BCS in tourist camp elephants in any range country, and the first to assess lipid profiles in relation to body condition in this species. We also present new evidence for a relationship between nutritional status and stress hormone levels in elephants, as indicated by associations between FGM concentrations and several measures of metabolic and lipid function. Moreover, nutrition, management and tourist numbers can affect metabolic, lipid panels and FGM concentrations.

Moreover, this is the first study to examine metabolic and lipid parameters in Asian elephants under human care in Thailand in relation to walking distance and working time, and provisioning of supplementary diet items, like bananas and sugar cane, by tourists during the High and Low tourist seasons. It also examined differences across camps in health and welfare measures. There was a significant tourist season effect on health status, with levels of several metabolic markers being higher during the High season. Significant differences across camps in stress and metabolic status further highlight the effect of tourist activities, and how supplemental feeding and lack of exercise may have negative consequences for health. Higher numbers of tourists likely are associated with increases in amounts of food treats offered to elephants, given that feeding is one of the most popular tourist activities. From our results we conclude that elephant well-being can be promoted by limiting the amount of high calorie treats given by tourists, ensuring animals receive appropriate levels of exercise to reduce fat and increase muscle mass, and reducing stress by limiting workloads and numbers of tourists interacting with individual elephants, especially during the high season. However, there appeared to be a protective effect of exercise, with hours of daily exercise being associated with better body condition and lipid and metabolic profiles, so elephant trekking per se may not be as bad for welfare as some animal activist organizations claim.

Future studies should be focused on how to improve elephant's health condition or nutrition, especially in camps with the incidence of abnormality in metabolic and lipid levels; will BCS improve by increasing of elephant's activities, and whether limitation and/or changing of supplement type will decrease the level of blood glucose or insulin in camp with high prevalence. Furthermore it would be a great benefit to study about the exact daily energy intake and nutrition composition which are considered effect elephant's health. From our study in female, evidence suggested that being overweight may lead to potentially negative health consequences, leading to questions about whether bull elephants are prone to similar obesity-related risks. Moreover, variable of elephant's activities were not investigated in this study even though high level of blood glucose and insulin were revealed in camps with less activity or working hours. Thus, future research should be determine how specific activities, like shows and riding, effect elephants on an individual levels. Likewise, this study was conducted in only five camps, increasing of sample sizes are essential to complete the better understanding of elephant's health affecting factors and welfare to promote a healthy, sustainable population of Thailand iconic species.

Recommendations for elephant camps in the future

Elephant camps that use elephants for tourism should be aware of elephant's welfare. The researcher proposed the management of elephant camps in some aspects including

1. The mahout should observe overall health of elephant every day.

2. Elephant camps should have a large health check at least once a year by collecting blood, urine and fecal samples to screen health and diseases. Assessment of a elephant's body condition score (BCS) gives a visual estimate of body fat reserves to estimate the change of body condition at least every two months.

3. Determine type of the work, duration and work time and allow elephants to rest during working day.

4. Providing an appropriate diet (primary and supplementary diet) based on age, body condition, activity and medical problems.

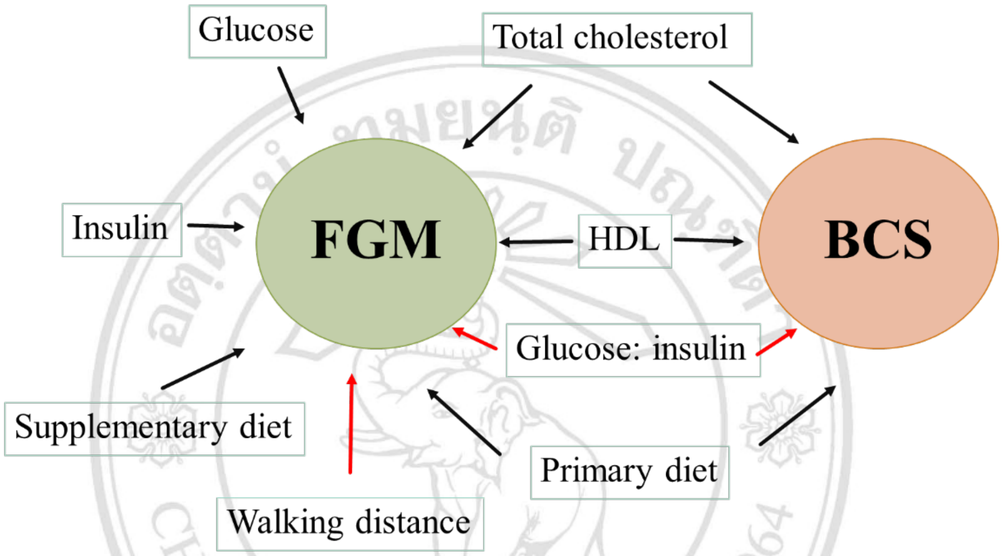


Figure 5.1 Summary of body condition and adrenal glucocorticoid activity associated with physiological outcomes. The red arrow and black arrow indicate positive and negative correlation respectively. BCS = body condition score; FGM = fecal glucocorticoid metabolites; HDL = high density lipoproteins.

REFERENCES

Ahsan L, Ossoli AF, Freeman L, Vaisman B, Amar MJ, Shamburek RD, Remaley AT (2014) Chapter 7 - role of lecithin: Cholesterol acyltransferase in HDL metabolism and atherosclerosis. In Komoda T ed, The HDL handbook (second edition), Boston: Academic Press, pp 159-194

Albon SD, Irvine RJ, Halvorsen O, Langvatn R, Loe LE, Ropstad E, et al. (2017) Contrasting effects of summer and winter warming on body mass explain population dynamics in a food-limited Arctic herbivore. *Glob Chang Biol* 23(4):1374-89

Arnaldi G, Scandali VM, Trementino L, Cardinaletti M, Appolloni G, Boscaro M (2010) Pathophysiology of dyslipidemia in cushing's syndrome. *Neuroendocrinology* 92 Suppl 1: 86-90

Asian Elephant Specialist Group (2017). Asian elephant range states meeting, final report, Asian Elephant Range States Meeting, Asian Elephant Specialist Group, Jakarta, Indonesia, April 18-20, 2017

Ayalew H, Hailemelekot M, Taye M (2018) Monitoring of body weight, body condition and observation of wound on working equines in Huletejuenese district, east Gojjam, amhara national regional state, Ethiopia. *Ethiop Vet J* 22: 40-58

Barter PJ, Nestel PJ (1973) Precursors of plasma triglyceride fatty acids in obesity. Metabolism - Clinical and Experimental 22: 779-785

Bateson P, Bradshaw EL (1997) Physiological effects of hunting red deer (*Cervus elaphus*). *Proc Biol Sci* 264: 1707-1714

Bays H (2014) Central obesity as a clinical marker of adiposopathy; increased visceral adiposity as a surrogate marker for global fat dysfunction. *Curr Opin Endocrinol Diabetes Obes* 21: 345-351

Benditt EP, Hoffman JS, Eriksen N, Parmelee DC, Walsh KA (1982) Saa, an apoprotein of HDL: Its structure and function. *Ann N Y Acad Sci* 389: 183-189

Berthiaume M, Laplante M, Festuccia WT, Cianflone K, Turcotte LP, Joanisse DR, Olivecrona G, Thieringer R, Deshaies Y (2007) 11beta-HSD1 inhibition improves triglyceridemia through reduced liver VLDL secretion and partitions lipids toward oxidative tissues. *Am J Physiol Endocrinol Metab* 293: E1045-1052

Bertin FR, de Laat MA (2017) The diagnosis of equine insulin dysregulation. *Equine Vet J* 49(5):570-6.

Birke L (2002) Effects of browse, human visitors and noise on the behaviour of captive orang utans. *Anim Welfare* 11: 189-202

Brambell FWR (1996) Report of the technical committee to enquire into the welfare of animals kept under intensive livestock husbandry systems. H.M. Stationery Office pp 1-87

Broom DM, Johnson KG (1993) Stress and animal welfare. Springer Netherlands, pp 87-110

Brown JL. Comparative reproductive biology of elephants (2014) *Adv Exp Med Biol* 53:135-69.

Brown JL (2000) Reproductive endocrine monitoring of elephants: An essential tool for assisting captive management. *Zoo Biol* 19: 347-367

Brown JL, Wasser SK, Wildt DE, Graham LH (1994) Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces1. *Biol Reprod* 51: 776-786

Boden G (2015) Chapter 8 - Insulin resistance and inflammation: Links between obesity and cardiovascular disease. In: Dokken BB, editor. Glucose Intake and utilization in pre-diabetes and diabetes. Boston: Academic Press, pp 95-101

Bubenik G, Brown R (1989) Seasonal levels of cortisol, triiodothyronine and thyroxine in male axis deer. *Comp Biochem Physiol A Physiol* 92(4):499-503.

Campos-Arceiz A, Lin TZ, Htun W, Takatsuki S, Leimgruber P (2008) Working with mahouts to explore the diet of work elephants in Myanmar (Burma). *Ecol Res* 23(6):1057-64

Chakravarty K, Cassuto H, Reshef L, Hanson RW (2005) Factors that control the tissue-specific transcription of the gene for phosphoenolpyruvate carboxykinase-c. *Crit Rev Biochem Mol Biol* 40: 129-154

Charmandari E, Tsigos C, Chrousos G (2005) Endocrinology of the stress response. *Annu Rev Physiol* 67: 259-284

Choi HK, Seeger JD (2005) Glucocorticoid use and serum lipid levels in us adults: The third national health and nutrition examination survey. *Arthritis Rheum* 53: 528-535

Chusyd DE, Brown JL, Hambly C, Johnson MS, Morfeld K, Patki A, et al (2017) Adiposity and reproductive cycling status in zoo African elephants. *Obesity (Silver Spring)* 26(1):103-10

Clark M, Hoenig M (2016) Metabolic effects of obesity and its interaction with endocrine diseases. *Vet Clin North Am Small Anim Pract* 46: 797-815

Clark FE, Fitzpatrick M, Hartley A, King AJ, Lee T, Routh A, Walker SL, George K (2012) Relationship between behavior, adrenal activity, and environment in zoo-housed western lowland gorillas (*Gorilla gorilla gorilla*). *Zoo Biol* 31: 306-321

Clark JD, Rager DR, Calpin JP (1997) Animal well-being. I. General considerations. *Lab Anim Sci* 47: 564-570

Clubb R, Rowcliffe M, Lee P, Mar K, Moss C, Mason G (2009) Fecundity and population viability in female zoo elephants: Problems and possible solutions.

Conde DA, Flesness N, Colchero F, Jones OR, Scheuerlein A (2011) An emerging role of zoos to conserve biodiversity. *Science* 331(6023):1390-1

Conte C, Fabbrini E, Kars M, Mittendorfer B, Patterson BW, Klein S (2012) Multiorgan insulin sensitivity in lean and obese subjects. *Diabetes Care* 35: 1316-1321

Coulter PA, Dickman K, Maradiegue A (2009) The effects of exercise on stress in working women. *J Nurse Pract* 5: 408-413

Coutinho AE, Chapman KE (2011) The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol Cell Endocrinol* 335(1):2-13

Crone EE (2001) Is survivorship a better fitness surrogate than fecundity? *Evolution* 55(12):2611-4

Dallman MF, la Fleur SE, Pecoraro NC, Gomez F, Houshyar H, Akana SF (2004) Minireview: Glucocorticoids--food intake, abdominal obesity, and wealthy nations in 2004. *Endocrinology* 145: 2633-2638

Davis N, Schaffner CM, Smith TE (2005) Evidence that zoo visitors influence hpa activity in spider monkeys (*Ateles geoffroyii rufiventris*). *Appl Anim Behav Sci* 90: 131-141

Dawkins MS (2003) Behaviour as a tool in the assessment of animal welfare. *Zoology (Jena)* 106: 383-387

de Laat MA, Hampson BA, Sillence MN, Pollitt CC (2016) Sustained, low-intensity exercise achieved by a dynamic feeding system decreases body fat in ponies. *J Vet Intern Med* 30(5):1732-8

Divertie GD, Jensen MD, Miles JM (1991) Stimulation of lipolysis in humans by physiological hypercortisolemia. *Diabetes* 40: 1228-1232

Dolinsky VW, Douglas DN, Lehner R, Vance DE (2004) Regulation of the enzymes of hepatic microsomal triacylglycerol lipolysis and re-esterification by the glucocorticoid dexamethasone. *Biochem* J 378: 967-974

Donaldson MT, McFarlane D, Jorgensen AJ, Beech J (2004) Correlation between plasma alpha-melanocyte-stimulating hormone concentration and body mass index in healthy horses. *Am J Vet Res* 65: 1469-1473

Douhard M, Guillemette S, Festa-Bianchet M, Pelletier F (2018) Drivers and demographic consequences of seasonal mass changes in an alpine ungulate. *Ecology* 99(3):724-34

Duncan IJ (2005) Science-based assessment of animal welfare: Farm animals. *Rev Sci Tech* 24: 483-492

Ellenberg U, Setiawan AN, Cree A, Houston DM, Seddon PJ (2007) Elevated hormonal stress response and reduced reproductive output in yellow-eyed penguins exposed to unregulated tourism. *Gen Comp Endocrinol* 152: 54-63

Epstein LH, Paluch RA, Beecher MD, Roemmich JN (2008) Increasing healthy eating vs. reducing high energy-dense foods to treat pediatric obesity. *Obesity (Silver Spring)* 16: 318-326

Fernando P, Janaka H, Ekanayaka SK, Nishantha H, Pastorini J (2009) A simple method for assessing elephant body condition. *Gajah* 31: 29-31

Fowler GS (1999) Behavioral and hormonal responses of magellanic penguins (*Spheniscus magellanicus*) to tourism and nest site visitation. *Biol Conserv* 90: 143-149

Fowler M, Mikota SK (2008) Biology, medicine, and surgery of elephants. Wiley.

Frank N, Elliott SB, Brandt LE, Keisler DH (2006) Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance. *J Am Vet Med Assoc* 228(9):1383-90.

Freedman MR, Horwitz BA, Stern JS (1986) Effect of adrenalectomy and glucocorticoid replacement on development of obesity. *Am J Physiol* 250(4 Pt 2):R595-607

Gilor C, Graves TK, Lascelles BD, Thomson AE, Simpson W, Halpern DS (2010) The effects of body weight, body condition score, sex, and age on serum fructosamine concentrations in clinically healthy cats. *Vet Clin Pathol* 39(3):322-8

Goossens GH (2008) The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. *Physiol Behav* 94: 206-218

Graham L, Schwarzenberger F, Möstl E, Galama W, Savage A (2001) A versatile enzyme immunoassay for the determination of progestogens in feces and serum. *Zoo Biology* 20: 227-236

Guillaume-Gentil C, Assimacopoulos-Jeannet F, Jeanrenaud B (1993) Involvement of non-esterified fatty acid oxidation in glucocorticoid-induced peripheral insulin resistance in vivo in rats. *Diabetologia* 36: 899-906

Hackett RA, Steptoe A (2017) Type 2 diabetes mellitus and psychological stress — a modifiable risk factor. *Nat Rev Endocrinol* 13: 547

Han TS, Lean ME (2016) A clinical perspective of obesity, metabolic syndrome and cardiovascular disease. *JRSM Cardiovascular Disease* 5: 2048004016633371-2048004016633371

Harris DM, Sherwin DC, Harris PS (2008) The welfare, housing and husbandry of elephants in UK zoos. Report to DEFRA

Hasselgren PO (1999) Glucocorticoids and muscle catabolism. *Curr Opin Clin Nutr Metab Care* 2(3):201-5

Hayward GS (2012) Conservation: clarifying the risk from herpesvirus to captive Asian elephants. *Vet Rec* 170(8):202-3

Heidegger EM, von Houwald F, Steck B, Clauss M (2016) Body condition scoring system for greater one-horned rhino (*Rhinoceros unicornis*): Development and application. *Zoo Biol* 35: 432-443

Henneke DR, Potter GD, Kreider JL, Yeates BF (1983) Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J* 15: 371-372

Heppell SS, Caswell H, Crowder LB (2000) Life histories and elasticity patterns: perturbation analysis for species with minimal demographic data. *Ecology* 81(3):654-65

Herbut P, Angrecka S (2012) Forming of temperature-humidity index (THI) and milk production of cows in the free-stall barn during the period of summer heat. *Anim Sci Pap Rep* 30(4):363-72

Heuberger R, Wakshlag J (2011) The relationship of feeding patterns and obesity in dogs. *J Anim Physiol Anim Nutr* 95: 98-105

Horner HC, Munck A, Lienhard GE (1987) Dexamethasone causes translocation of glucose transporters from the plasma membrane to an intracellular site in human fibroblasts. *J Biol Chem* 262(36):17696-702

Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, Furukawa S, Tochino Y, Komuro R, Matsuda M et al. (2007) Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes* 56: 901-911

Ingrid CS, Gilles PM, Greg B (2014) Population viability of captive Asian elephants in the Lao PDR. *Endanger Species Res* 24:1-7

IUCN. Elephas maximus (2008) The IUCN Red List of Threatened Species. doi: www.iucnredlist.org.

Jacobson TA (2013) Lipoprotein(a), cardiovascular disease, and contemporary management. *Mayo Clin Proc* 88(11):1294-311

Jayakumar A (2012) Greenspan's basic and clinical endocrinology. *Yale J Biol Med* 85: 559-559

Jeusette IC, Lhoest ET, Istasse LP, Diez MO (2005) Influence of obesity on plasma lipid and lipoprotein concentrations in dogs. *Am J Vet Res* 66: 81-86

Jordan E, Kley S, Le NA, Waldron M, Hoenig M (2008) Dyslipidemia in obese cats. *Domest Anim Endocrinol* 35: 290-299

Jung HS, Chang Y, Eun Yun K, Kim CW, Choi ES, Kwon MJ, Cho J, Zhang Y, Rampal S, Zhao D et al. (2014) Impact of body mass index, metabolic health and weight change on incident diabetes in a Korean population. *Obesity (Silver Spring)* 22: 1880-1887

Kajaysri J, Nokkaew W (2014) Assessment of pregnancy status of Asian elephants (*Elephas maximus*) by measurement of progestagen and glucocorticoid and their metabolite concentrations in serum and feces, using enzyme immunoassay (eia). *J Vet Med Sci* 76: 363-368

Kearns CF, McKeever KH, John-Alder H, Abe T, Brechue WF (2002) Relationship between body composition, blood volume and maximal oxygen uptake. *Equine Vet J Suppl* 34: 485-490

Khonmee J, Brown JL, Rojanasthien S, Thumasanukul D, Kongphoemphun A, Siriaroonrat B, Tipkantha W, Punyapornwithaya V, Thitaram C (2014a) Seasonality of fecal androgen and glucocorticoid metabolite excretion in male goral (*Naemorhedus griseus*) in thailand. *Anim Reprod Sci* 146: 70-78

Khonmee J, Brown JL, Taya K, Rojanasthien S, Punyapornwithaya V, Thumasanukul D, Kongphoemphun A, Siriaroonrat B, Tipkantha W, Pongpiachan P et al. (2014b) Assessment of ovarian activity in captive goral (*Naemorhedus griseus*) using noninvasive fecal steroid monitoring. *Theriogenology* 82: 997-1006

Kino T, Chrousos G (2001) Glucocorticoid and mineralocorticoid resistance/hypersensitivity syndromes. *J Endocrinol* 169: 437-445

Knapp CR, Hines KN, Zachariah TT, Perez-Heydrich C, Iverson JB, Buckner SD, Halach SC, Lattin CR, Romero LM (2013) Physiological effects of tourism and associated food provisioning in an endangered iguana. *Conserv Physiol* 1: cot032

Kosecki SM, Rodgers PT, Adams MB (2005) Glycemic monitoring in diabetics with sickle cell plus beta-thalassemia hemoglobinopathy. *Ann Pharmacother* 39(9):1557-60

Kumar V, Palugulla Reddy V, Kokkiligadda A, Shivaji S, Umapathy G (2014) Non-invasive assessment of reproductive status and stress in captive Asian elephants in three south Indian zoos. *Gen Comp Endocrinol* 201: 37-44

Kumar B, Manuja A, Aich P (2012) Stress and its impact on farm animals. *Front Biosci (Elite Ed)* 4: 1759-1767

Lambillotte C, Gilon P, Henquin JC (1997) Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. *J Clin Invest* 99: 414-423

Lanktree MB, Joy TR, Hegele RA (2013) Chapter 83 - the metabolic syndrome. In Ginsburg GS, Willard HF eds, Genomic and personalized medicine (second edition): Academic Press, pp 1006-1016

Larsen MJ, Sherwen SL, Rault J-L (2014) Number of nearby visitors and noise level affect vigilance in captive koalas. *Appl Anim Behav Sci* 154: 76-82

Lee L, Sanders RA (2012) Metabolic syndrome. Pediatrics in Review 33: 459-468

Legro RS, Finegood D, Dunaif A. (1998) A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 83(8):2694-8

LeMura LM, von Duvillard SP, Andreacci J, Klebez JM, Chelland SA, Russo J (2000) Lipid and lipoprotein profiles, cardiovascular fitness, body composition, and diet during and after resistance, aerobic and combination training in young women. *Eur J Appl Physiol* 82(5-6):451-8

Lewis GF, Uffelman KD, Szeto LW, Steiner G (1993) Effects of acute hyperinsulinemia on VLDL triglyceride and VLDL Apob production in normal weight and obese individuals. *Diabetes* 42: 833-842

Loste A, Marca MC (2001) Fructosamine and glycated hemoglobin in the assessment of glycaemic control in dogs. *Vet Res* 32: 55-62

Ludwig DS (2002) The glycemic index: Physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA* 287: 2414-2423

Lundberg U, Hellström B (2002) Workload and morning salivary cortisol in women. *Work Stress* 16: 356-363

Lund-Katz S, Phillips MC (2010) High density lipoprotein structure-function and role in reverse cholesterol transport. *Subcell Biochem* 51: 183-227

Malikhao P, Servaes L (2017) Elephants in tourism. Sustainable and practical approaches to captive elephant welfare and conservation in Thailand. In: Malikhao P, editor. Culture and communication in Thailand. Singapore: Springer Singapore p. 127-38

Mallapur A, Chellam R (2002) Environmental influences on stereotypy and the activity budget of Indian leopards (*Panthera pardus*) in four zoos in southern India. *Zoo Biol* 21: 585-595

Manlik O, McDonald JA, Mann J, Raudino HC, Bejder L, Krutzen M, et al. (2016) The relative importance of reproduction and survival for the conservation of two dolphin populations. *Ecol Evol* 6(11):3496-512.

Manjrekar P, Hegde A, Shrilaxmi, D'Souza F, Kaveeshwar V, Jose A, et al (2012) Fructosamine in non-diabetic first degree relatives of type 2 diabetes patients: Risk assessor. *J Clin Diagn Res* 6(5):770-3.

Maréchal L, Semple S, Majolo B, MacLarnon A (2016) Assessing the effects of tourist provisioning on the health of wild barbary macaques in morocco. *PLoS ONE* 11: e0155920

Maury E, Brichard SM (2010) Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol* 314: 1-16

McQuaid SE, Hodson L, Neville MJ, Dennis AL, Cheeseman J, Humphreys SM, Ruge T, Gilbert M, Fielding BA, Frayn KN et al. (2011) Downregulation of adipose tissue fatty acid trafficking in obesity. A Driver for Ectopic Fat Deposition? *Diabetes* 60: 47-55

Mearns BM (2011) Targeting levels and functions of blood lipids in the prevention of CVD. *Nat Rev Cardiol* 8:179-80

Mellor DJ, Beausoleil NJ (2015) Extending the 'five domains' model for animal welfare assessment to incorporate positive welfare states. *Anim Welf* 24: 241-253

Menargues MA, Urios V, Liminana R (2012) Seasonal rhythms of salivary cortisol secretion in captive Asian elephants (*Elephas maximus*). *Gen Comp Endocrinol* 176(2):259-64

Miller MA, Hogan JN, Meehan CL (2016) Housing and demographic risk factors impacting foot and musculoskeletal health in African elephants (*Loxodonta africana*) and Asian elephants (*Elephas maximus*) in north American zoos. *PLoS ONE* 11(7):e0155223

Mittman N, Desiraju B, Fazil I, Kapupara H, Chattopadhyay J, Jani CM, Avram MM (2010) Serum fructosamine versus glycosylated hemoglobin as an index of glycemic control, hospitalization, and infection in diabetic hemodialysis patients. *Kidney Int Suppl* 117:S41-5

Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, et al (1985) Hyperinsulinemia. A link between hypertension obesity and glucose intolerance. *J Clin Invest* 75(3):809-17

Morfeld KA, Brown JL (2017) Metabolic health assessment of zoo elephants: Management factors predicting leptin levels and the glucose-to-insulin ratio and their associations with health parameters. *PLoS ONE* 12(11):e0188701

Morfeld KA, Brown JL (2016) Ovarian acyclicity in zoo African elephants (*Loxodonta africana*) is associated with high body condition scores and elevated serum insulin and leptin. *Reprod Fertil Dev* 28(5):640-7

Morfeld KA, Meehan CL, Hogan JN, Brown JL (2016) Assessment of body condition in African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants in north American zoos and management practices associated with high body condition scores. *PLoS ONE* 11: e0155146

Morfeld KA, Lehnhardt J, Alligood C, Bolling J, Brown JL (2014) Development of a body condition scoring index for female African elephants validated by ultrasound measurements of subcutaneous fat. *PLoS ONE* 9: e93802

Moberg GP (2000) Biological response to stress: Implications for animal welfare. The biology of animal stress: basic principles and implications for animal welfare. CABI publishing, New York, USA, pp 1-21

Moberg GP, Mench JA (2000) The biology of animal stress: Basic principles and implications for animal welfare. CABI publishing, New York, USA, pp 199-226

Morfeld KA, Brown JL (2016) Ovarian acyclicity in zoo African elephants (*Loxodonta africana*) is associated with high body condition scores and elevated serum insulin and leptin. *Reprod Fertil Dev* 28: 640-647

Mumby HS, Mar KU, Thitaram C, Courtiol A, Towiboon P, Min-Oo Z, Htut-Aung Y, Brown JL, Lummaa V (2015) Stress and body condition are associated with climate and demography in Asian elephants. *Conserv Physiol* 3: cov030

Munck A (1971) Glucocorticoid inhibition of glucose uptake by peripheral tissues: Old and new evidence, molecular mechanisms, and physiological significance. *Perspect Biol Med* 14: 265-269

Mudron P, Rehage J, Sallmann HP, Höltershinken M, Scholz H (2005) Stress response in dairy cows related to blood glucose. *Acta Vet Brno* 74:37-42.

Nelson RH (2013) Hyperlipidemia as a risk factor for cardiovascular disease. *Primary care* 40: 195-211

Nelson RW. 8 - Endocrine, metabolic, and lipid disorders A2 - Willard, Michael D. In: Tvedten H, editor. Small Animal Clinical Diagnosis by Laboratory Methods (Fifth Edition). Saint Louis: W.B. Saunders; 2012. p. 156-90.

Nemeroff CB, Marmar C (2018) Post-traumatic stress disorder. Oxford University Press.

Norkaew T, Brown JL, Bansiddhi P, Somgird C, Thitaram C, Punyapornwithaya V, Punturee K, Vongchan P, Somboon N, Khonmee J (2018) Body condition and adrenal glucocorticoid activity affects metabolic marker and lipid profiles in captive female elephants in Thailand. *PLoS ONE* 13: e0204965

Oli MK, Dobson FS (2003) The relative importance of life-history variables to population growth rate in mammals: Cole's prediction revisited. *Am Nat* 161(3):422-40

Orozco-Beltran D, Gil-Guillen VF, Redon J, Martin-Moreno JM, Pallares-Carratala V, Navarro-Perez J, et al. (2017) Lipid profile, cardiovascular disease and mortality in a Mediterranean high-risk population: The ESCARVAL-RISK study. *PLoS ONE* 12(10):e0186196

Patel R, Williams-Dautovich J, Cummins CL (2014) Minireview: New molecular mediators of glucocorticoid receptor activity in metabolic tissues. *Mol Endocrinol* 28: 999-1011

Pawlak DB, Kushner JA, Ludwig DS (2004) Effects of dietary glycaemic index on adiposity, glucose homoeostasis, and plasma lipids in animals. *The Lancet* 364: 778-785

Peeters M, Sulon J, Beckers JF, Ledoux D, Vandenheede M (2011) Comparison between blood serum and salivary cortisol concentrations in horses using an adrenocorticotropic hormone challenge. *Equine Vet J* 43: 487-493

Pfeiffer AFH, Keyhani-Nejad F (2018) High glycemic index metabolic damage – a pivotal role of gip and glp-1. *Trends Endocrinol Metab* 29: 289-299

Phillipou G, Seaborn CJ, Phillips PJ (1988) Re-evaluation of the fructosamine reaction. *Clin Chem* 34(8):1561-64

Phythian CJ, Cripps PJ, Michalopoulou E, Jones PH, Grove-White D, Clarkson MJ, Winter AC, Stubbings LA, Duncan JS (2012) Reliability of indicators of sheep welfare assessed by a group observation method. *Vet J* 193: 257-263

Pifarré M, Valdez R, González-Rebeles C, Vázquez C, Romano M, Galindo F (2012) The effect of zoo visitors on the behaviour and faecal cortisol of the mexican wolf (*Canis lupus baileyi*). *Appl Anim Behav Sci* 136: 57-62

Proctor C, Brown J (2015) Influence of handling method on adrenal activity in zoo African and Asian elephants. *J Zoo Aquar Res* 3: 1-1

Rabasa C, Dickson SL (2016) Impact of stress on metabolism and energy balance. *Curr Opin Behav Sci* 9: 71-77

Rasmussen HB, Ganswindt A, Douglas-Hamilton I, Vollrath F (2008) Endocrine and behavioral changes in male African elephants: Linking hormone changes to sexual state and reproductive tactics. *Horm Behav* 54: 539-548

Ratnasooriya WD, Alwis GKH, Vijesekara RD, Amarasinghe RM, Perera D (2006) Lipid profile of captive Sri Lanka elephants. *Gajah* 24:45-9.

Rebuffe-Scrive M (1988) Steroid hormones and distribution of adipose tissue. *Acta Med Scand Suppl* 723: 143-146

Reiche EM, Nunes SO, Morimoto HK (2004) Stress, depression, the immune system, and cancer. *Lancet Oncol* 5: 617-625

Rughetti M, Festa-Bianchet M (2012) Effects of spring-summer temperature on body mass of chamois. *J Mammal* 93(5):1301-7

Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, Lernmark A, Metzger BE, Nathan DM (2011) Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Diabetes Care* 34: e61-e99

Sakoda H, Ogihara T, Anai M, Funaki M, Inukai K, Katagiri H, Fukushima Y, Onishi Y, Ono H, Fujishiro M et al. (2000) Dexamethasone-induced insulin resistance in 3t3-l1 adipocytes is due to inhibition of glucose transport rather than insulin signal transduction. *Diabetes* 49: 1700-1708

Saltiel AR, Kahn CR (2001) Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414: 799

Saltz D, White GC (1991) Urinary cortisol and urea nitrogen responses to winter stress in mule deer. *J Wildl Manage* 55(1):1-16.

Sanlier N, Yabanci N (2007) Relationship between body mass index, lipids and homocysteine levels in university students. *J Pakistan Med Assoc* 57(10):491-5

Souza SC, Palmer HJ, Kang YH, Yamamoto MT, Muliro KV, Paulson KE, Greenberg AS (2003) TNF-alpha induction of lipolysis is mediated through activation of the extracellular signal related kinase pathway in 3t3-l1 adipocytes. *J Cell Biochem* 89: 1077-1086

Staels B, van Tol A, Chan L, Verhoeven G, Auwerx J (1991) Variable effects of different corticosteroids on plasma lipids, apolipoproteins, and hepatic apolipoprotein mrna levels in rats. *Arterioscler Thromb* 11: 760-769

Stelmach-Mardas M, Rodacki T, Dobrowolska-Iwanek J, Brzozowska A, Walkowiak J, Wojtanowska-Krosniak A, Zagrodzki P, Bechthold A, Mardas M, Boeing H (2016) Link between food energy density and body weight changes in obese adults. *Nutrients* 8: 229

Stevens J, Thyssen A, Laevens H, Vervaecke H (2013) The influence of zoo visitor numbers on the behaviour of harbour seals (*Phoca vitulina*). *J Zoo Aquar Res* 1:31-34

Strzelec K, Kankofer M, Pietrzak S (2011) Cortisol concentration in the saliva of horses subjected to different kinds of exercise. *Acta Veterinaria Brno* 80: 101-105

Sukumar R, Gadgil M (1988) Male-female differences in foraging on crops by Asian elephants. *Anim Behav* 36(4):1233-5

Takada H, Harrell J, Deng S, Bandgiwala S, Washino K, Iwata H (1998) Eating habits, activity, lipids and body mass index in Japanese children: the Shiratori Children Study. *Int J Obes* 22(5):470-76.

Tamashiro KL, Sakai RR, Shively CA, Karatsoreos IN, Reagan LP (2011) Chronic stress, metabolism, and metabolic syndrome. *Stress* 14(5):468-74

Taylor AI, Frizzell N, McKillop AM, Flatt PR, Gault VA (2009) Effect of ru486 on hepatic and adipocyte gene expression improves diabetes control in obesity-type 2 diabetes. *Horm Metab Res* 41: 899-904

Tsigos C, Chrousos GP (2002) Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *J Psychosom Res* 53: 865-871

Thanos PK, Cavigelli SA, Michaelides M, Olvet DM, Patel U, Diep MN, Volkow ND (2009) A non-invasive method for detecting the metabolic stress response in rodents: Characterization and disruption of the circadian corticosterone rhythm. *Physiol Res* 58: 219-228

Thiel D, Jenni-Eiermann S, Braunisch V, Palme R, Jenni L (2008) Ski tourism affects habitat use and evokes a physiological stress response in capercaillie Tetrao urogallus: A new methodological approach. *J Appl Ecol* 45: 845-853

Thitaram C (2012) Breeding management of captive Asian elephant (*Elephas maximus*) in range countries and zoos. *Jpn J Zoo Wildl Med* 7(3):91-6.

Tipprasert P editor (2001) Elephants and ecotourism in Thailand. The international workshop on the domesticated Asian elephant food and agriculture organization of the United Nations, Regional Office for Asia and the Pacific

Touma C, Palme R (2005) Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. *Ann N Y Acad Sci* 1046:54–74.

Traustadottir T, Bosch PR, Matt KS (2005) The HPA axis response to stress in women: Effects of aging and fitness. *Psychoneuroendocrinology* 30: 392-402

Urtasun A, Nuñez I (2018) Healthy working days: The (positive) effect of work effort on occupational health from a human capital approach. *Socl Sci Med* 202: 79-88

van der Hulst M (2003) Long workhours and health. *Scand J Work Environ Health* 29: 171-188

van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, Hiscock N, Moller K, Saltin B, Febbraio MA et al. (2003) Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J Clin Endocrinol Metab* 88: 3005-3010

van Lenten BJ, Hama SY, de Beer FC, Stafforini DM, McIntyre TM, Prescott SM, La Du BN, Fogelman AM, Navab M (1995) Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of hdl against LDL oxidation in aortic wall cell cocultures. *J Clin Invest* 96: 2758-2767

Vegiopoulos A, Herzig S (2007) Glucocorticoids, metabolism and metabolic diseases. *Mol Cell Endocrinol* 275: 43-61

Viljoen J, Ganswindt A, Palme R, Reynecke H, du Toit J, Langbauer Jr W (2008) Measurement of faecal glucocorticoid concentrations in free-ranging African elephants: Baseline values from the Kruger national park. *Koedoe* 50: 18-21

Wang Y, Xu D (2017) Effects of aerobic exercise on lipids and lipoproteins. *Lipids Health Dis* 16:132

Wang JC, Gray NE, Kuo T, Harris CA (2012) Regulation of triglyceride metabolism by glucocorticoid receptor. *Cell Biosci* 2: 19

Wang M (2005) The role of glucocorticoid action in the pathophysiology of the metabolic syndrome. *Nutr Metab (Lond)* 2(1):3

Wang CN, McLeod RS, Yao Z, Brindley DN (1995) Effects of dexamethasone on the synthesis, degradation, and secretion of apolipoprotein B in cultured rat hepatocytes. Arterioscler *Thromb Vasc Biol* 15(9):1481-91

Wasser SK, Hunt KE, Brown JL, Cooper K, Crockett CM, Bechert U, Millspaugh, JJ, Larson, S, Monford SL (2000) A generalized faecal glucocorticoid Assay for use in a diverse array of non-domestic mammalian and avian species. *Gen Comp Endocrinol* 120: 260–75.

Watson R, Munro C, Edwards KL, Norton V, Brown JL, Walker SL (2013) Development of a versatile enzyme immunoassay for non-invasive assessment of glucocorticoid metabolites in a diversity of taxonomic species. *Gen Comp Endocrinol* 186: 16-24

Watson TD, Packard CJ, Shepherd J, Fowler JN (1990) An investigation of the relationships between body condition and plasma lipid and lipoprotein concentrations in 24 donkeys. *Vet Rec* 127(20):498-500

Weerakhun S, Wichianrat Y, Laophakdee T, Juntako P, Torsri T (2010) Blood glucose levels in Asian elephants of Thailand. *KKU Vet J* 20(2):208-17.

Weinstein SP, Wilson CM, Pritsker A, Cushman SW (1998) Dexamethasone inhibits insulin-stimulated recruitment of glut4 to the cell surface in rat skeletal muscle. *Metabolism* 47: 3-6

Webster J (2005) Animal welfare: Limping towards, ed In: A practical approach to redressing the problem of our dominion over the animals. Blackwell.

Wells DL (2005) A note on the influence of visitors on the behaviour and welfare of zoo-housed gorillas. *Appl Anim Behav Sci* 93: 13-17

Wemmer C, Krishnamurthy V, Shrestha S, Hayek LA, Thant M, Nanjappa KA (2006) Assessment of body condition in Asian elephants (*Elephas maximus*). *Zoo Biology* 25: 187-200

Western D, Lindsay WK (1984) Seasonal herd dynamics of a savanna elephant population. *Afr J Ecol* 22(4):229-44

Wijeyamohan S, Treiber K, Schmitt D, Santiapillai C (2015) A visual system for scoring body condition of asian elephants (*Elephas maximus*). *Zoo Biology* 34: 53-59

Woo J, Cockram C, Lau E, Chan A, Swaminathan R (1992) Influence of obesity on plasma fructosamine concentration. *Clin Chem* 38(11):2190-2

Wu A, Hinds CJ, Thiemermann C (2004) High-density lipoproteins in sepsis and septic shock: Metabolism, actions, and therapeutic applications. Shock 21: 210-221

Yin X, Lanza IR, Swain JM, Sarr MG, Nair KS, Jensen MD (2014) Adipocyte mitochondrial function is reduced in human obesity independent of fat cell size. *J Clin Endocrinol Metab* 99: E209-E216

Young KE, Keith EO (2011) A comparative analysis of cetacean vital rates using matrix population modeling analysis of cetacean vital rates. *In J of Appl Sci Techol* 1(6):266-77.

Yousef MK, Luick JR (1971) Estimation of thyroxine secretion rate in reindeer, Rangifer tarandus: Effects of sex, age and season. *Comp Biochem Physiol A Physiol* 40(3):789-95

Yu KC, Cooper AD (2001) Postprandial lipoproteins and atherosclerosis. *Front Biosci* 6: D332-354

LIST OF PUBLICATIONS

1) **Norkaew T**, Brown JL, Bansiddhi P, Somgird C, Thitaram C, Punyapornwithaya V, Punturee K, Vongchan P, Somboon N, Khonmee J (2018) Body condition and adrenal glucocorticoid activity affects metabolic marker and lipid profiles in captive female elephants in Thailand. *PLoS One* 13: e0204965

2) **Norkaew T**, Brown JL, Bansiddhi P, Somgird C, Thitaram C, Punyapornwithaya V, Punturee K, Vongchan P, Somboon N, Khonmee J (2019) Influence of season, tourist activities and camp management on body condition, testicular and adrenal steroids, lipid profiles, and metabolic status in captive Asian elephant bulls in Thailand. *PLoS One* In press

3) **Norkaew T**, Ohno H, Sriburee P, Tanabe K, Tharavichitkul P, Takarn P, Puengchan T, Bumrungsri S, Miyazaki Y (2013) Detection of environmental sources of *Histoplasma capsulatum* in Chiang Mai, Thailand, by nested-PCR. *Mycopathologia* 176: 395-402

4) Khayhan K, Hagen F, **Norkaew T**, Puengchan T, Boekhout T, Sriburee P (2017) Isolation of *Cryptococcus gattii* from a *Castanopsis argyrophylla* tree hollow (mai-kaw), Chiang Mai, Thailand. *Mycopathologia* 182: 365-370

APPENDIX A

The information of each elephant camp in the study

order	Camp	Code	Sex	Age
1	A	A6	female	22
		A7	female	25
		A10	female	30
		A11	female	30
		A13	female	30
		A14	female	34
2	B	B7	female	37
		B8	female	43
		B9	female	37
		B10	female	41
		B11	female	40
		B12	female	23
3	C	C7	female	45
		C8	female	40
		C9	female	35
		C10	female	42
		C11	female	30
		C12	female	20
4	D	D1	female	46
		D2	female	33
		D4	female	41
		D5	female	39
		D7	female	31

order	Camp	Code	Sex	Age
4	D	D8	female	34
		D9	female	50
		D11	female	30
		D13	female	25
		D14	female	35
		D15	female	30
5	E	E5	female	35
		E7	female	40
		E8	female	30
		E12	female	26

APPENDIX B

Protocol for corticosterone (CJM006) EIA

Day 1:

1. Plate Coating

   • Use NUNC Maxisorb plates

   • Add 33.33 µl antibody stock (1:100, -20°C) to 5 ml coating buffer (working dilution 1:15,000).

   • Add 50 µl per well of antibody solution to plate.

   • Do not coat column 1 – start at A2 and go down each column (see plate map).

   • Pipet all solutions in this order.

   • Tap plates gently to ensure that coating solution covers the bottom of each well.

   • Label and cover with acetate plate sealer and leave overnight (no less than 12 hrs) at 4°C.

Day 2

2. Standards

   • Standard values are used: 1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.8, and 3.9 pg/well.

   • Dilute standard working stock (20 ng/ml or 1000 pg/well) serially (2-fold) by using 250 µl stock plus 250 µl EIA buffer.

3. Samples/Controls

   • Dilute urine or fecal samples in dilution buffer to appropriate dilution.

   • Prepare high and low controls.

4. HRP

   • Corticosterone-HRP working dilution is 1:15,000.

   • Add 33.33 µl of HRP working stock to 5 ml EIA buffer to make the working dilution (keep this solution cool).

5. Plate washing

   • Wash the plate 5 times with wash solution.

• Blot the plate on paper towel to remove excess wash solution.

6. Plate loading

• Pipet 50 µl of standard, control and sample per well as quickly and accurately as possible, according to the plate map.

• Add 50 µl of diluted Corticosterone HRP (step 4) to all wells that contain standard, control or sample. Avoid splashing.

• No more than 10 minutes should pass during this process.

• Cover plates with acetate plate sealer and incubate at room temperature for exactly 2 hours.

7. Plate washing

• Wash the plate 5 times with wash solution.

• Blot the plate on paper towel to remove excess wash solution.

• Plates are fairly stable at this point and can be left upside on bench top until all plates are washed (no more than 20 minutes).

8. Substrate

• Prepare ABTS substrate immediately before use.

• Combine 40 µl0.5 M H_2O_2, 125 µl 40 mM ABTS and 12.5 ml substrate buffer, and mix well.

• Add 100 µl ABTS substrate to all wells that contain standard, control, or sample.

• Cover with acetate plate sealer and incubate at room temperature with shaking.

• Plate color development will vary based on age of HRP and/or Ab, but should be no greater than one hour.

9. Plate reading

• Optical density (OD) of 0 wells should read 1 or less.

• Optimal readings for 0 wells: >0.7 to < 1 OD.

• Read at 405 nm (reference 540 nm).

Corticosterone stock preparation – per plate

1. Antibody

• Dilute Corticosterone CJM006 at a dilution of 1:100 by adding 20 µl of stock to 2 ml coating buffer.

• Aliquot 500 µl into O-ring vials and store at -20°C.

• Store antibody stock at -80°C.

2. HRP Conjugate

• Dilute corticosterone horseradish peroxidase (HRP) 1:100 by adding 25 µl of stock to 2.475 ml EIA buffer for a working stock and store at 4°C.

• Store HRP stock at -80°C.

3. Standards

• Weigh out 50 mg corticosterone (Steraloids) and add to 50 ml EtOH for a 1 mg/ml primary stock.

• Dilute 1 mg/ml primary stock 1:100 by adding 100 µl to 10 ml EtOH for a 10 µg/ml secondary stock.

• Dilute 10 µg/ml secondary stock 1:500 by adding 100µl to 49.9 ml EIA buffer for a 20 ng/ml (1000 pg/well*) working stock.

• Aliquot working stock and store all stocks at -20°C.

*a well is equal to 50µl, the amount used in the assay.

4. Controls

• Use urine or extracted fecal samples with a high progestin level to make controls.

• Make a pool of high corticosterone level urine or extracted feces (~20ml).

• Serially dilute pool and run on assay.

• Find the dilutions that bind at ~70% and ~30%.

• Use the pool to make up 2 separate stocks for low and high controls using the dilutions that bound at 70% and 30% respectively.

• Make up enough controls to run on at least 500 assays (for this you may need more than 20 ml used for the pool. Any species will do as long as it has high corticosterone levels).

CURRICULUM VITAE

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Publications

Norkaew T, Brown JL, Bansiddhi P, Somgird C, Thitaram C, Punyapornwithaya V, Punturee K, Vongchan P, Somboon N, Khonmee J (2018) Body condition and adrenal glucocorticoid activity affects metabolic marker and lipid profiles in captive female elephants in Thailand. *PLoS One* 13: e0204965

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