The effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State

by

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2010015382

Submitted in fulfilment of the requirements in respect of the Master's Degree Physiology in the Department of Basic Medical Sciences in the Faculty of Health Sciences at the University of the Free State

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June 2019
Bloemfontein
South Africa

Abstract

Objective: Texting has become central to social life, especially among young adults. It has been shown that texting has an adverse effect on physiological functioning. This study investigated the effect of receiving mobile text messages on salivary cortisol levels in undergraduate Physiology students.

Methods: This protocol was set as an experimental, crossover, quantitative study. Respondents (men age: M = 20.5, SD = 1.34; women age: M = 20.7, SD = 1.69) participated in the study over two consecutive study days, receiving the intervention (receiving mobile text messages) on one day and acting as their own control on the other day. Self-reported data and saliva samples were collected during the study to assess salivary cortisol levels. Anxiety, depression and stress levels as well as the respondents' subjective experience of the study were determined. Text frequency (number of text messages received) and text emotions (words with a neutral, positive or negative connotation) were varied among respondents.

Results: Salivary cortisol levels did not differ significantly between the intervention and control days. High anxiety levels were associated with increased salivary cortisol levels. No associations with salivary cortisol levels were documented in low to moderate anxiety levels, stress, depression or how respondents subjectively experienced the intervention. There was no significant difference between text frequency, text emotion and the change in cortisol levels on the intervention day.

Conclusion: The results in this study indicate that receiving mobile text messages did not elicit a significant cortisol response in respondents.

Key words: texting, salivary cortisol, anxiety, depression, stress, subjective experience, text frequency, text emotion, lecture, Hospital Anxiety and Depression Scale, Perceived Stress Scale.

Declarations

I. I, Francois Petrus Venter declare that the Master's research dissertation that I herewith submit at the University of the Free State, is my independent work and that I have not previously submitted it for a qualification at another institution of higher education.

II. I, Francois Petrus Venter hereby declare that I am aware that the copyright is vested in the University of the Free State.

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Francois Petrus Venter

Acknowledgements

I would like to thank my supervisors, Dr Tonie Gerber and Mrs Roné Vorster-De Wet. I value you for who you are and the relationship we share. Thank you for this journey and the journey ahead. You live with integrity.

To my parents and brother: Thank you for raising me to embrace challenges and to see them as opportunities. Thank you for showing me the value of hard work. Thank you for teaching me to be disciplined. Thank you for viewing me as perfect, even though I most certainly am not. I love you.

To my wife: Thank you for understanding and loving me; although difficult, you have mastered both. Thank you for supporting me in all that I endeavour. The Lord knew who to bless me with and I am eternally thankful. I love you.

To my Creator: Thank You for undeserved grace, every day of my life.

Table of contents

List of Tables

List of Figures

Abbreviations

AD	Adenylyl cyclase
ATP	Adenosine triphosphate
ACTH	Adrenocorticotropic hormone
AFT	After the experiment
ABRs	Auditory brainstem responses
BBB	Blood brain barrier
BEF	Before the experiment
BP	Blood pressure
CAL	Calibration
CBF	Cerebral blood flow
CONT	Control day
CRH	Corticotropin-releasing hormone
CAR	Cortisol awakening response
cAMP	Cyclic adenosine monophosphate
DNA	Deoxyribonucleic acid
DPOAE	Distortion product optoacoustic emission
ELISA	Enzyme-linked immunosorbent assay
E	Epinephrine
FSH	Follicle-stimulating hormone
GAD	Generalised anxiety disorder
GLUT	Glucose transporter

GRs	Glucocorticoid receptors
HSREC	Health Sciences Research Ethics Committee
HRV	Heart rate variability
HADS-A	Hospital Anxiety and Depression Scale: Anxiety
HADS-D	Hospital Anxiety and Depression Scale: Depression
H^+	Hydrogen ion
HPA	Hypothalamus-pituitary-adrenal cortex axis
Ig	Immunoglobulin
IL	Interleukin
INT	Intervention day
LH	Luteinising hormone
MDD	Major depressive disorder
MCR-2	Melanocortin receptor 2
mRNA	Messenger ribonucleic acid
MRs	Mineralocorticoid receptors
NE	Norepinephrine
OD	Optical density
PSS	Perceived Stress Scale
PEPCK	Phosphoenolpyruvate carboxykinase
K^+	Potassium
POMC	Pro-opiomelanocortin
RANK	Receptor activator of nuclear factor κB
RANKL	Receptor activator of nuclear factor κB ligand

RF-EMF	Radiofrequency electromagnetic waves
rCBF	Regional cerebral blood flow
RSA	Respiratory sinus arrhythmia
ROS	Reactive oxygen species
SGK	Serum and glucocorticoid-induced kinase
SMS	Short message service
Na^+	Sodium
SAR	Specific absorption rate
SCN	Suprachiasmatic nucleus
SD	Standard deviation
TMB	Tetramethylbenzidine
TSH	Thyroid-stimulating hormone
TSST	Trier social stress test
TNF	Tumour necrosis factor
UK	United Kingdom
USA	United States of America
VP	Vasopressin
V2R	Vasopressin 2 receptor

Chapter 1 INTRODUCTION

1.1 Background

One of the most often used mobile devices at present is the mobile phone (1). Although mobile phones have countless features, this study examined one of its oldest and most simple features - texting, which is used by virtually all mobile phone users (2). Texting is a method of communication that allows short messages to be sent and received between mobile phones.

It has been determined that United States of America (USA) citizens aged between 18-34 years may individually send more than 2000 text messages per month (3). Junco (4) found that on average, university students send 97 text messages per day; 71% are sent whilst doing academic work (p.2236), while Burns *et al.* (5) found that more than half (53%) of students report that they text during a lecture (p.808).

Numerous authors reported on the adverse effects that texting has on attention, academic performance, academic work, recall and academic distractibility (3,6–8).

It has also been reported that more university students, in comparison to non-university students, in the USA and South Africa, own mobile phones (4,9). Even though most of these figures are estimates in the American context, it is highly applicable to the South African community. In a report by PEW Research Center, Poushter *et al.* (9) found that in 2014, adults in South Africa who owned mobile phones were as common as in the USA (89%) (p.2), with Ghana (83%) and Kenya (82%) not far behind. South Africa has the most smartphone users in Africa (p.3). Chiumbu (10) also reports that in Sub-Saharan Africa, South Africa has one of the highest incidences of mobile phone usage (p. 193). Furthermore, by 2010 Africa had 500 million mobile phone subscribers, with South Africa, Kenya, Nigeria and Ghana dominating the market (11). More recently, 2018, the report from PEW Research Center indicated that 91% of adults in South Africa own a mobile phone (12). Interestingly and worth noting, the survey also found that the activity most often performed on mobile phones, was sending text messages (p.2). North *et al*. (13) conducted a survey at the University of Cape Town to measure the attitudes, opinions and behaviours of students in regard to mobile phone use (p.9). They found that texting was the main reason students used mobile phones. Of the 362 students that completed the survey, 65% sent more than 21 text messages per day.

Having established that texting forms a major part of the lives of young adults (including students), attention is turned to the impact that texting potentially has as a distractor in the classroom.

Lawson *et al*. (14) stated that distractors, did exist even before mobile phones, in the university classroom. Previously students read newspapers, engaged in conversations or even daydreamed, but the advancement of technology has broadened the reach of possible distractors (p.119). The use of smartphones or laptops has brought the world into the classroom. Students can access the internet, Facebook and their personal emails, which Lawson *et al*. (14) label as powerful distractors (p.119).

Texting in the classroom setting has become a distractor for lecturers as well as fellow students (15). This is supported by an informal poll by The Chronicle of Higher Education, where the results showed that mobile phone use was regarded as the greatest classroom distraction (16). Multi-tasking behaviour in the classroom leads to increased distraction (focusing on texting rather than the lecture), academic decline and an increase in incomplete homework. This may result in lower memory recall during assessments (3,6–8). In previous psychological studies, researchers had set up a simulated lecture (some respondents text during the lecture while others do not) and then measure academic performance (after the lecture) by way of a quiz on the lecture content (3).

In this study, salivary cortisol is the measured variable and not academic performance, since the negative impact on academic performance has already been well established (4,6,17).

The content of text messages may elicit various psychological responses; for example, a text perceived as hurtful may cause distancing behaviour in close relationships (18). Furthermore, it has been found that text messages with negative emotion words, may persuade romantic partners to evaluate their relations as less satisfactory, while positive emotion words in texting lead to increased friendship satisfaction (19). From this information, it is clear that the content of text messages affects psychological aspects such as relationship satisfaction. Since psychological stimuli often manifest in a physiological response that affects cortisol secretion, it is important to determine word choice in text messages when measuring salivary cortisol levels.

Affective norms for English words have been determined by Bradley *et al.* (20). These words were rated in terms of pleasure, arousal and dominance. Cuming (21) measured gender

differences in a South African population aged 19-25 years between males and females in the recall of neutral, positive emotion and negative emotion words (p.36). The general finding of Cuming's study was that there was no significant difference between males and females in the recall of positive and negative words, although females tended to recall more neutral words than males (21).

In the book *"The Organized Mind: Thinking Straight in the Age of Information Overload"* neuroscientist and author Daniel J Levitin (22) makes a few very valid points. He explains that the use of mobile phones is constant because they are always in close proximity to the owner. This is unlike regular telephones, which only periodically affect the owner. He explains that email is thought of as outdated by people under the age of 30 years. Young people prefer texting because it provides all the functions email provides. Furthermore, people feel obliged to immediately respond to text messages, since it has become a social responsibility (22). From this information, it is evident that mobile phones have become a necessity without which students will struggle to cope.

An epidemiological study by Adam *et al.* (23) revealed that stressful stimuli activate the hypothalamus-pituitary-adrenal cortex axis (HPA) and lead to an increase in cortisol secretion. Increased cortisol levels are associated with physiological, social or psychological stress. Cortisol functions to initiate various bodily adaptations to counteract stressors. These adaptations are essential during true stressors, such as physical injury or prolonged periods without food, for example. They are, however, improper when no physical harm or physiological imbalances are present. This often occurs during psychosocial stress that sets in motion the exact same stress responses (p.2). Some adaptations during the stress response include: increased glucose production from non-carbohydrate sources (gluconeogenesis) and the utilisation of free fatty acids for adenosine triphosphate (ATP) production; inflammation inhibition (24) and vascular changes as a result of increased vascular responsiveness to catecholamines (25). Texting also affects human physiology (26–28).

Various factors influence cortisol secretion in one individual at any given time. Some noteworthy factors include: age, diurnal rhythm, gender, the menstrual cycle, pregnancy, oral contraceptives, smoking, drug misuse, exercise, adrenal disorders and stress (29). For its ability to counteract the burdens of stress, Clements (29) explains that cortisol has become the research focus of many scientists, since a physiological or psychological stressor ultimately stimulates its release (p.212).

Cortisol follows a highly regulated diurnal rhythm in normal circumstances: we know that stress, amongst other factors, may alter cortisol secretion. The diurnal rhythm of cortisol is regulated by the suprachiasmatic nuclei (SCN), which regulate the rhythmic release of corticotropin-releasing hormone (CRH) from the hypothalamus (30). The subsequent increase in adrenocorticotropic hormone (ACTH) release from the anterior pituitary under control of CRH brings about increased cortisol release from the adrenal cortex (23).

Salivary cortisol represents free unbound cortisol (5-10% of total cortisol) and has become the gold standard of cortisol assessment during real life (ambulatory) conditions (31). Collection of salivary cortisol is safe, non-invasive and painless (29). Hence, salivary cortisol provides an avenue to a plethora of physiological and psychological research.

This study therefore aimed to analyse the effect of texting; specifically the action of receiving mobile text messages. In this context, the possible interaction between the distractibility of receiving mobile text messages during a lecture and increasing stress was investigated by measuring salivary cortisol levels. In this study, salivary cortisol was the measured variable and not academic performance, since the negative impact on academic performance has already been well established (4,6,17).

1.2 Problem statement

Texting during academic lectures is a major incidence in universities that affects students and lecturers alike. It is known that texting affects attention and academic performance (3,6–8). Although sound evidence exists that texting impacts human physiology, little is known regarding the effect of receiving mobile text messages during an academic lecture on salivary cortisol secretion.

1.3 Research question

The research question was, “What effect does receiving mobile text messages during a lecture have on cortisol secretion in undergraduate Physiology students?”

1.4 Sub-questions

To answer the research question, the following sub-questions were formulated:

a) What are the general and texting characteristics of the sample population?
b) Will stress, anxiety and depression have a moderating effect on cortisol secretion while receiving mobile text messages?

c) Will subjective feedback on the experiment correlate with objectively measured cortisol data?
d) Will text frequency and the use of neutral, positive or negative emotion words in text messages have a moderating effect on cortisol secretion?

1.5 Methodology

This protocol was set as an experimental, crossover, quantitative study. A detailed review on the methodology of this study is provided in Chapter 3, section 3.5. Salivary cortisol levels were measured to determine the effect of the experiment on salivary cortisol levels. Questionnaires were used to collect demographical data and to measure data regarding stress, anxiety, depression and respondents' subjective experience of the study (cf. Appendix F).

1.6 Organisation of dissertation

This dissertation consists of five chapters; the first of which is the introduction. Chapter 2 reviews literature related to areas of this study. Chapter 3 provides detailed methodology and restates the problem statement, research question and sub-questions. This chapter also describes the study design, target population, sampling, screening, instruments used, sample collection, study procedures, data management and analysis, statistical analysis, laboratory procedures, reliability, validity, ethical considerations, the time schedule, budget and the implementation of this study. In Chapter 4 the results pertaining to this study are relayed. In Chapter 5 the findings of this study are discussed in detail and amalgamated to existing literature. The limitations of the study and the conclusion are included in the discussion chapter and ends with a brief summary of this study in English and Afrikaans.

1.7 Significance of the study

The results from this study add to the body of knowledge about the effect of receiving mobile text messages on salivary cortisol levels within an undergraduate student population during a lecture setting. Saliva cortisol sampling provided valid and accurate objective data. Factors, contributing to the body of knowledge, were captured in questionnaires measuring stress, anxiety, depression and respondents' subjective experience of the experiment. Text frequency and emotive connotation of words also added another dimension to this study. These mediating factors were compared to the objective data obtained from salivary cortisol data.

Chapter 2 LITERATURE REVIEW

2.1 Introduction

Chapter 2 presents a thorough review that was conducted around the main topics of the physiological effect of mobile phone exposure, and cortisol. The latter investigation includes the anatomy, physiological functioning, secretion and diurnal rhythm of cortisol. The effect of heightened cortisol levels, the role cortisol plays in depression and anxiety and salivary cortisol as a biomarker are reviewed. Thereafter, the origins of texting are briefly reported and the physiological effect of texting and mobile phone use is reviewed, followed by a review on current knowledge with regard to receiving mobile text messages during a lecture and the effect it has on salivary cortisol levels.

2.2 Physiological effects of mobile phone exposure

In modern times, humans are surrounded by technological developments. Except for the most remote places on earth, technology is part of society. There is, however, concern as to whether people are at risk because of the electrical environment around them (32). In this literature review, the focus is placed on mobile phone radiation and the possible adverse health effects it may have on human physiology. Although it is an extensively researched topic, conclusive evidence that mobile phones negatively impact health has not been registered (33). Contemporary research, however, finds stronger associations between the use of mobile phones and various health-related problems (34,35). Continuous research on mobile phone radiation is imperative because mobile phones are the one tool that people constantly have with them. Mobile phones are usually in close proximity to the body and may therefore have a direct effect on physiological functioning (36). The long-term effects that mobile phones have on normal physiological functioning are only now starting to transpire (37). In this chapter, the effects of mobile phone radiation on the neurological, cardiovascular, auditory and reproductive systems are analysed. Before the specific effects of mobile phone radiation on human physiology are unpacked, a general discussion on the classification of mobile phone radiation will be discussed.

2.2.1 Mobile phone radiation

Mobile phones emit radiofrequency electromagnetic waves (RF-EMF), which form part of the lower end of the electromagnetic spectrum (38). Although mobile phones transmit low

frequencies, the possible harmful effect they have on human physiology is a topic of debate (33).

RF-EMF form part of non-ionizing radiation and it has been reported that the effects of mobile phone radiation may be of a thermal or non-thermal nature (32).

2.2.2 Thermal effects of mobile phone exposure

Since mobile phones emit RF-EMF, it may influence body temperature homeostasis. Body core temperature may be generalised within a physiological range between 35.8°C-37.3°C (25).

Thermal effects of RF-EMF causes local increase in tissue temperature in a process called dielectric heating. According to the Collins Dictionary (39), dielectric heating is a form of heating in which electrically insulating material is heated by being subjected to an alternating electric field. The extent to which exposure occurs is dependent on several physical factors (frequency of exposure, distance to mobile phone) and biological factors (temperature, humidity, tissue surface area in contact with mobile phone) (32). It has been established that radio frequencies ranging from 1 MHz-10 GHz can be absorbed by exposed body tissues and may increase body temperature (38). In this process, various molecules (mostly water) in the body absorb photons which are then transformed to thermal energy (32,40). The body responds to heat gain by altering thermoregulatory measures such as sweating and heat loss through blood flow (radiation and evaporation). Anderson *et al.* (41) found that holding a mobile phone against the cheek for six minutes increased the skin temperature on the side of the face by 2.3°C (p.1). They concluded that the temperature increase was due to heat conduction from the mobile phone. When skin temperature is increased, eccrine sweat glands respond by secreting sweat, since the environmental temperature exceeds skin temperature. Through evaporation of sweat, the skin temperature returns to normal. Another mechanism to lose the gained heat is via radiation from the skin to the surrounding environment. If thermal heating is sufficient to increase core body temperature, blood plasma absorbs heat gained from dielectric heating and distributes it to skin where it is eliminated to the surrounding environment by radiation (24,42). It has been determined that the maximum temperature elevation in the closest brain region to the mobile phone antenna experiences a mere 0.11°C temperature increase, even though the power absorption of mobile phone radiation in the brain may penetrate as deep as 5cm (43,44). The skull provides a thick barrier and thus plays an important role in diminishing EMF penetration (45).

Although thermal radiation from mobile phones is possible, stringent laws to ensure that the specific absorption rate (SAR) is below the threshold to cause damage, regulate mobile phone producers. SAR is the amount of energy absorbed by tissues and organs averaged over the whole body. It is expressed as a coefficient of power per tissue mass (W/kg). An SAR of >4W/kg causes bodily harm (32). According to the Federal Communications Commission, the SAR limit for exposure by mobile phones is 1.6W/kg. As stated above, it should be noted that SAR is averaged over the whole body. Some organs, like the brain and skin, experience more frequent radiation by mobile phones (46); thus the SAR, although regulated, may be higher than the prescribed 1.6W/kg on a specific exposed organ. For instance, the International Commission on Nonionizing Radiation Protection has set the localised SAR value of the head and trunk at 2W/kg (47). So it is true that localised SAR in the head is higher than that of the averaged whole body value, but (as previously stated), only a small increase in temperature is observed in the brain region closest to the mobile phone antenna (43,44). Therefore, it may be concluded that the thermal effects of mobile telephones are minimal. Although it is generally accepted that thermal effects are minimal (48), when a mobile phone is held against the head while talking, symptoms of thermal radiation are commonly a sensation of increased heat around the ear and facial skin (37). Thus, it is a form of conduction heating from the device (mobile phone) to the skin. One author states that it is an indisputable fact that mobile phone radiation cause heat around the area where the phone is placed (32). It remains to be seen if thermal radiation receives more interest once legislation for SAR values becomes more stringent.

2.2.3 Non-thermal effects of mobile phone exposure

In 2001, Repacholi *et al.* (49) reported results that indicated that the non-thermal effects of mobile phone radiation was minimal. In the last 15 years, though, research has increasingly turned its focus to non-thermal radiation (49). Various authors are focusing their research on the non-thermal effect of mobile phone radiation (37,46,50–53).

Non-thermal radiation, as the name implies, does not cause an increase of temperature. Instead, it affects various tissues in different permutations such as oxidative stress caused by reactive oxygen species (ROS); increased capillary permeability; interfering with plasma membrane potentials; altering red blood cell elasticity; and interfering with artificial cardiac pacemakers of the heart.

This section preludes the in-depth discussion on non-thermal effects of RF-EMF. These include, but are not limited to neurological, cardiovascular, auditory and reproductive systems. It has been established that mobile phones are regulated to prevent thermal radiation, but current research turns its focus to specific biological responses to non-thermal radiation (36).

2.2.4 Neurological effects

Under normal physiological conditions the HPA responds to stressful stimuli. When activated, CRH is released by the hypothalamus which activates the release of ACTH by the anterior pituitary gland, ACTH stimulates the release of cortisol from the adrenal cortex (42). Typically, the production of CRH and ACTH is inhibited by negative feedback of increasing levels of cortisol. Chronic stressful stimuli affect the normal physiological response and lead to oversensitivity in the physiological system, causing an increase in circulating cortisol (54).

2.2.4.1 Vacuolisation in brain tissue and blood brain barrier permeability

Shahabi *et al.* (55) recently found that prolonged RF exposure in adult male wistar rats caused increased plasma levels of ACTH and cortisol levels. Furthermore, they found that the zona fasciculata of the adrenal cortex thickened because of increased cell size and perimeter after prolonged RF exposure. They found increased vacuolisation in brain tissue, while the number and size of vacuoles also increased after two months of RF exposure (p. 1269).

Research has also indicated that there is a possible link between mobile phone radiation and albumin leakage across the blood brain barrier (BBB) (37).

Recently, long-standing results of a study conducted by Persson *et al.* (56) were countered by others. In the Persson *et al.* study it was found that mobile phone radiation indisputably effects BBB permeability of albumin in Fischer 344 rats. The three countering studies aimed to reproduce the 1997 study and they found no increased effects on albumin extravasation (57).

In 2009, Nittby *et al.* (58), carried out experiments on mobile phone exposure in rats. The rats were exposed to two hours of mobile phone radiation and tested for albumin leakage across the BBB. They found that immediately after exposure and even seven days after exposure there was a marked increase in albumin extravasation (leakage of a substance) across the BBB (p.103). According to Eberhardt *et al.* (46), these findings were strongly supported by previous research, where researchers found that albumin extravasation was seen as long as 14 days after exposure; they also found that extraverted albumin was taken up by neuronal cells or caused neuronal damage (p.215).

In opposition to these findings, Grafström *et al.* (59), found no increase in albumin extravasation or neuronal damage in rats (p.19). The exact mechanisms that allow the passage of albumin across the BBB are debated. Up until 2008 it was not known how albumin leaks across the BBB; how it is taken up by neurons, or how neuronal damage is caused (46). It is proposed that albumin leakage may occur through paracellular transport (channels between endothelial cells) possibly by way of alteration in tight junctions of endothelial cells. Another proposed mechanism of passage may be through transcellular transport (movement across the plasma membrane) by way of pinocytosis and transcytosis (58).

Other studies assessed BBB permeability using the low molecular mass marker Evans Blue (57). The one found no association between mobile phone radiation and BBB permeability (60), while the other found support thereof (61).

2.2.4.2 Cerebral blood flow

Research has indicated a possible link between mobile phone exposure and cerebral blood flow (CBF) (43).

CBF is remarkably constant because of its autoregulation ability, ensuring that blood flow to the brain remains constant despite wide fluctuations in blood pressure (BP). There are limits to the brain's auto-regulatory functions, though. Below a pressure of 60mmHg, vessel vasodilation cannot further compensate, so CBF decreases and brain damage may occur. At pressures greater than 180mmHg, vasoconstriction of cerebral vessels give way and CBF increases, which if continued may lead to brain oedema. The normal amount of blood that flows to the brain is 50-65 milliliters/ 100g of brain tissue/minute. This normally constitutes 15% of total cardiac output (24,62).

In an experiment by Aalto *et al.* (43) they tested the effect of a mobile phone (held in a natural talking position) on regional cerebral blood flow (rCBF) using positron emission tomography. They found that rCBF decreased only in the fusiform gyrus in the hemisphere of the brain (left) where the mobile phone was positioned. They also found that rCBF increased bilaterally (both hemispheres) in the medial and superior frontal gyri (p.887). It is interesting that in their concluding remarks they state that their results do not provide conclusive evidence that mobile phone usage would be more harmful than normal cognition. Normal cognition also temporarily changes rCBF (43).

In contradiction to Aalto *et al.* (43), Kwon *et al.* (51) found that rCBF was not affected when placing the mobile phone against the left ear, right ear and forehead (p.254). The authors of this study proposed that the time intervals of exposure may provide reason for contradictory findings. The duration of exposure by Kwon *et al.* (51) was significantly less than that of the study by Aalto *et al.* (43).

There are few evaluations of the effects of stress on the vascular system of the brain (54). It has been found that acute psychological stress caused increased CBF in the ventral right prefrontal cortex and left insula/putamen (63). In an experiment by Lee *et al.* (64), it was found that rats with chronic stress had a diminished hemodynamic response in the hindpaw region of the somatosensory cortex (p.5), when the hindpaw was electrically stimulated.

Endothelial dysfunction in cerebral circulation is affected by chronic stress (54). Figure 2.1 summarises the effect that chronic stress has on cerebrovascular dysfunction.

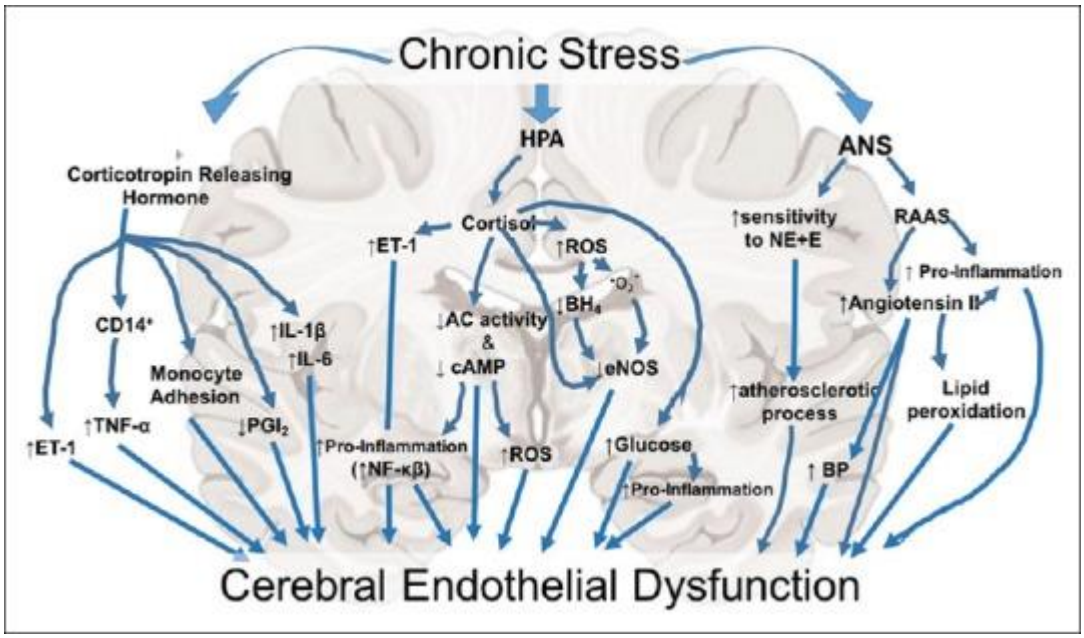


Figure 2.1 Systematic representation of the potential downstream effects of chronic stress exposure on the cerebrovasculature system (54).

2.2.4.3 Brain glucose metabolism

The brain primarily utilises blood-derived glucose as its primary source of fuel. The brain's capacity to store glycogen, in contrast to other organs, is temporary and inadequate. It is proposed that the brain only stores a two-minute back-up supply of glycogen. To fully appreciate the brain's need for glucose, the following holds true: "This organ, constituting only 2% of total body weight, consumes 50% of blood glucose during resting conditions" (24,42).

There is evidence that supports increased brain glucose metabolism during RF-EMF exposure (48). It was found that brain glucose metabolism increased in brain regions closest to the antenna of the mobile phone (48). The methodology of this study was questioned by Kwon *et al.* (50), who reported in their previous research that a decrease in brain glucose metabolism was seen in the temporal and temporoparietal cortex in the hemisphere exposed to mobile phone irradiation (p.2293).

2.2.4.4 Tumours of the nervous system

There is a speculation about the effect that mobile phones might have on tumours of the nervous system (35).

It has been reported that there is an association between gliomas and the usage of a mobile phones in rural areas. Reasons postulated was that base stations are further away in rural areas, where mobile phone have a greater power output for proper transmission (44). Gliomas do not affect tissues outside the brain and spinal cord and they account for 60% of brain tumours (44). These findings seem to conclude that mobile phone radiation may cause gliomas, but not all studies have found similar results (48). One study found that there exists an association between astrocytomas and mobile phone exposure ipsilateral (same side) to the side of exposure (52). The general consensus, until a few years ago, was that mobile phone exposure does not cause brain tumours (44,48). However, in 2015, Morgan *et al.* (35) made a strong statement by titling their review article: "*Mobile phone radiation causes brain tumors and should be classified as a probable human carcinogen (2A) (Review)*". This statement was based on a study by Coureau *et al.* (34) who in their retrospective case control study analysed 253 cases of gliomas, 194 cases of meningiomas and 892 matched controls. They (34) found that there was a positive correlation between long-term mobile phone users and brain tumour development (p.1). This is in stark contrast to findings by Schüz *et al.* (65) who found no association between mobile phone use and an increased risk for the development of brain tumours. It does seem at present that there is strong new evidence that mobile phone radiation is more evident in brain tumour formation than previously thought.

It is known that a risk factor for developing meningioma is large exposures to ionizing radiation, thus researchers thought that mobile phone (non-ionizing) radiation might also be a risk factor. As in the case of gliomas, research findings a few years ago suggested no association between mobile phone radiation and meningiomas (44). Morgan *et al.* (35) disagree

on this front (p.1865), but in a recent study by Carlberg *et al.* (66) no association was once again found (p.3093).

Since the auditory nerve is in close contact to the mobile phone while talking, it receives a large amount of the power emitted by the phone. Hardell *et al.* (52) found that there is a causal relationship between the development of an acoustic neuroma and mobile phone usage (p.85).

2.2.5 Cardiovascular system

Homocysteine, a risk factor for heart disease and increased cardiovascular activity which may lead to endothelial damage is observed when the autonomic nervous system and HPA are overstimulated (54). Increased levels of cortisol lead to decreased levels of nitric oxide, that play an important role in maintaining mean arterial BP (42). According to Burrage *et al.* (54), chronic psychological stress may lead to intimal-medial thickening, the gradual onset of arterial stiffness and atherosclerosis (p.46).

2.2.5.1 Blood

Plasma as one of the components of blood plays a vital role in temperature regulation (67). Plasma plays a critical role in heat absorption of mobile phones and would therefore theoretically have an effect on perfusion and cortisol secretion.

In an experiment by Havas (53), it was observed that red blood cells clump together in what is known as Rouleau formation after a ten-minute session of talking on a cordless phone transmitting at 2.4 GHz. Compared to modern mobile phones that transmit at 2.3 GHz (p.79). Rouleau formation decreases the total surface area of red blood cells. Rouleau formation is when red blood cells clump together in a ‘stacked coin’ formation. The rate of waste removal from the cell is also impacted. Symptoms include BP irregularities, headaches, dizziness and extremity (hands and feet) numbness. Plasma proteins such as fibrinogen and globulin increase the rate of Rouleau formation. Imbalances between albumin and globulin fractions of the plasma proteins also increase Rouleau formation (68). Another postulation is that electrical potential across the cell membrane decreases and the repelling force between cells are thus diminished (53). Havas (53) stated that there is a need for research on the mechanisms of Rouleau formation (p.79). There is certainly also a need for the effects that mobile phone radiation has on Rouleau formation.

2.2.5.2 Cardiac pacemaker effects

In the late nineties, researchers suggested that mobile phones may interfere with cardiac pacemakers (69). Several studies have since researched the topic (70–73).

Hekmat *et al.* (70) reported that the possible interference (disturbances that effect electrical circuits) between pacemaker and mobile phones have been recognised since 1994 (p.365). Furthermore, Hekmat *et al.* (70) stated that while various authors demonstrated that such interference exists, their results were widespread. This was possibly due to the fact that those studies used different types of cell phones and some pacemakers had feedthrough filters (to relay interfering signals) while others did not (p.365-366).

New generation pacemakers are equipped with special filters that drastically reduce the interactions among artificial cardiac pacemakers and mobile phones (72,73). It was found that interference between pacemakers and mobile phones are only present in 0.3% of patients wearing new generation pacemakers. Furthermore, this only occurs when the mobile phone rings and is located less than 10cm from the pacemaker (72).

It has also been found that third-generation mobile phones do not interfere with permanent implanted pacemakers because they use a higher frequency band to operate in than previous-generation mobile phones (71).

Thus it may be concluded that technological advances, both in pacemakers and mobile phones, have gone a long way to prevent possible interference between them.

2.2.5.3 Heart rate variability

Beat-to-beat variation of heart rate, under normal resting condition, is called heart rate variability (HRV). Variations are typically seen in the 'R-R' interval (the peak of one QRS complex to the peak of the next QRS complex) and is called respiratory sinus arrhythmia (RSA). RSA is further defined as the increase and decrease in heart rate in relation to respiration (increasing during inspiration and decreasing during expiration). Furthermore, RSA is caused by alterations in vagal tone (25).

Many authors have reported that mobile phone radiation impacts HRV (74–76). The findings are as follows:

Andrzejak *et al.* (74) found that during a call on a mobile phone, HRV may be influenced. Furthermore, they observed that parasympathetic tone increased alongside a decrease in

sympathetic tone; these results were determined from HRV. They stated that these result may be due to RF-EMF, but that physically speaking during the call may also have an influence (p.409).

Havas *et al.* (75) found, also using HRV as a test parameter, that a large percentage of their test subjects were moderately to severely sensitive to radiation from a cordless phone (transmitting at 2.4GHz; a modern mobile phone transmits at 2.3GHz). They also found that heart rate increased, HRV is altered and that parasympathetic and sympathetic tone changed (p.265).

In contrast to the above studies, Parazzini *et al.* (76) found no differences in nonlinear dynamics of HRV between subjects that were exposed to 26 minutes of mobile phone radiation and those that were not exposed (p.173). This study specifically analysed nonlinear HRV unlike the previous mentioned studies. Havas *et al.* (75) state that nonlinear results are difficult to predict. Although nonlinear results are difficult to predict, the results of Parazinni *et al.* (76) were supported by others (77,78).

Thus, there is research that found a positive correlation between HRV and mobile phone radiation (74,75), but using a nonlinear approach to measuring HRV yielded no correlation between HRV and mobile phone radiation (76–78). In future studies it would thus be prudent to use the same measurement of HRV in large population studies, so that conclusive results may be found.

2.2.6 Auditory effects

As the ear is in close proximity to a mobile phone during calls in order to receive information it is a prime target organ for mobile phone radiation. It has been reported that extremely low frequency radiation may have adverse effect on auditory function, especially on the organ of Corti and the outer hair cells in this organ (32).

The organ of Corti is the receptor organ for hearing. It rests upon the basilar membrane in the cochlear duct and is made up of sensory hair cells and supporting cells (25). Hair cells are of two types: inner hair cells and outer hair cells. Inner hair cells are the sensory cells that receive sound waves; they convert mechanical movement of the cochlear fluid into electrical signals (action potential) that propagate through the auditory nerve to eventually reach the cerebral cortex, where it is interpreted. Outer hair cells are not receiving cells *per se*. Instead they function in supporting inner hair cell function by deliberately modifying the basilar membrane to finetune reception by inner hair cells (42).

The functioning of outer hair cells is tested by distortion product optoacoustic emission (DPOAE) provocation. In one study it was found that DPOAE decreased after exposure to mobile phone radiation (45). What that means is that outer hair cell function was impaired after the radiation. Contrastingly, Alsanosi *et al.* (45) stated that previous research did not find that mobile phone radiation causes measurable effects on the auditory system when radiated for 10, 15, 20 or 30 minutes. Furthermore, test subjects in this study reported symptoms like headache, dizziness and a sensation of heat on the ear when the phone was placed on the ear (p.145).

Auditory brainstem responses (ABRs) test the functional status of the auditory nerve pathway. This includes the time taken to respond to a stimulus and the intensity of the response (25). Khullar *et al.* (37) tested ABRs to mobile phone radiation. They found no significant impact of short-term exposure to any of the parameters they tested. They noted that future research should turn its attention to the long-term effects mobile phone radiation has on the auditory system (p. S645).

2.2.7 Reproductive system

The concern is that people (especially men) carry their mobile phones in their trouser pocket close to the reproductive organs (36,44,79). There is no consensus as yet whether it is thermal heating of the gonads by mobile phones or the non-thermal emitted radio waves that have an impact (36,44,79). Adams *et al.* (80) addresses this question by positing that if it was purely a thermal (heating) effect rather than a non-thermal (radiation) effect, sperm concentration would mostly be impacted, instead of factors such as sperm motility and viability (p.109). This is another problematic issue for research. In such cases: “How conclusive can the results be if testing either heating or radiation, since these are both present in mobile phones?”

2.2.7.1 Effect of mobile phone radiation in males

The testes perform two important functions:

1) spermatogenesis, the process of sperm production and
2) it secretes male sex hormones, collectively called androgens (25).

The focus of this section is the effect of mobile phone radiation on sperm.

The effect of mobile phone radiation seems to greatly impact spermatozoa motility and viability (81). Adams *et al.* (80) conducted a systemic review of nine articles, which contained 1353 semen samples. Six of the nine studies found that mobile phone radiation negatively impacts sperm motility. In this review it was further noted, analysing five previous studies (with a total of 816 semen samples), that sperm viability also decreases significantly as a result of mobile

phone exposure (p.108). These results were also supported by Carpenter (36) and Merhi (79). Adams *et al.* (80) could not conclude that sperm concentration is adversely affected during mobile phone radiation (p.108). In contrast Carpenter (36) stated that sperm concentration (count) is negatively affected by mobile phone radiation (p.164). It should be mentioned that the review by Adams *et al.* (80) used substantially more data. A general consensus among authors is that duration of exposure contributes greatly to sperm defects (80,81).

Evidence suggests that mobile phone radiation induces ROS formation in spermatozoa (81). A large body of evidence attributes decreased sperm motility, viability and concentration to the formation of ROS formation in spermatozoa (79,81). ROS is formed during mitochondrial oxidative phosphorylation by partial reduction of oxygen and includes molecules like superoxide anion, hydrogen peroxide and hydroxyl radical (82). Specific antioxidants like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and heme oxygenase exist to counteract the effect of ROS (83). When ROS production exceeds the antioxidant defense system of cells it causes oxidative stress, which may damage nucleic acids, lipids and proteins (82). The oxidative stress caused by excessive ROS formation increases deoxyribonucleic acid (DNA) fragmentation, which is implicated in decreased sperm motility and viability (80).

Reviewing the findings of previous animal studies, Merhi (79) stated that mobile phone radiation decreased the fructose levels in adult male rabbits' semen (p.294). This is an interesting finding, since fructose is the primary energy source of sperm (42). This finding could possibly explain why mobile phone radiation decreases sperm motility. Furthermore this particular article stated that more abnormalities of sperm heads were found in RF-EMF exposed male mice than in controls who received no exposure (79).

2.2.7.2 Effect of mobile phone radiation in females

The effect of mobile phone radiation on the reproductive system of females is a topic that needs considerably more research (32). According to Merhi (79), of those studies that have been conducted, results are often diverse and the methodology used by different studies are inconsistent (p.296).

Even though consensus on the effect of mobile phone radiation on the female reproductive systems is lacking in research, there have been a number of findings regarding the granulosa cells of the primary follicle and also the number of primary follicles (79).

Using female rats, it has been found that mobile phone radiation causes DNA single and double strand breaks in granulosa cells of the primary follicle (79). It has also been found in culled 21-day old female rats (who were exposed to mobile phone radiation during the whole period of gestation), that the follicle cell concentration of the right ovary were less than in controls who were not exposed (79).

Furthermore, a significant increase in embryo growth cessation during the first trimester was observed in women (especially those that had a history of embryo growth termination) who were increasingly exposed to mobile phone radiation (32). An embryo is the product of fertilisation (42). Merhi (79) also reported that there may be an increase in endometrial cell apoptosis and oxidative stress after exposure to RF-EMF (p.296).

2.2.7.3 Foetal heart rate and cardiac output

During gestation foetal heart rate gradually increases and reaches a maximum rate of ± 140 beats/minute just before birth (25). In a review by Merhi (79) they reported that one study found a significant increase in foetal heart rate and a significant decrease in cardiac output after the mother was exposed to mobile phone radiation (p.296). The particular study observed 90 pregnant women (84). It is interesting to note that cardiac output decreased, even though heart rate increased, since an increase in heart rate usually accompanies an increase in cardiac output. Rezk *et al.* (84) concluded that mobile phone radiation caused decreased cardiac muscle contractibility, which ultimately caused decreased cardiac output (p.218). In a similar study conducted by Celik *et al.* (85) they found that foetal heart rate was not affected by 10 minutes' exposure of mobile phone radiation to the mother (p.55).

2.3 Cortisol

Cortisol is a hormone secreted by the adrenal glands under the influence of ACTH that is secreted from the anterior pituitary. ACTH secretion in turn is controlled by CRH that is secreted from the hypothalamus (29). This elaborate pathway is called the HPA. Cortisol is often called the stress hormone, since cortisol secretion is markedly increased during periods of stress (42). Although rightly associated with stress, people often overlook the role cortisol plays in many physiological systems (86), for instance carbohydrate, fat and protein metabolism. All three physiological systems are affected by cortisol to increase blood glucose levels (24). Cortisol also affects skin, bone, electrolyte metabolism and various other physiological systems as will be discussed in this literature review. The anatomical arrangements of the HPA provide insight into the location of various tissues that play a role in

cortisol secretion. The general characteristics of cortisol are discussed below, followed by an in-depth analysis of the physiological effects exerted by cortisol levels. The word glucocorticoid may be regarded as synonymous to cortisol.

2.3.1 Anatomical arrangement of the hypothalamus-pituitary-adrenal cortex axis components

2.3.1.1 Hypothalamus

The brain is divided into seven major anatomical divisions. They are the cerebral hemisphere, the diencephalon, the midbrain, the pons, the cerebellum, the medulla and the spinal cord (87).

Central in this review is the diencephalon, which houses the hypothalamus.

The hypothalamus exerts multiple functions, the most notable being the regulation of water metabolism, temperature regulation, appetite control and hormonal regulation (88) via hypothalamic nuclei. Sensory and hormonal input to the hypothalamus result in motor outputs to various regulatory sites, such as the anterior pituitary gland , the posterior pituitary gland, the cerebral cortex, the premotor and motor neurons in the brain stem and spinal cord, and parasympathetic and sympathetic preganglionic neurons (88).

What is of importance in this review is the parvocellular neurosecretory systems within the hypothalamus which regulates hormone release from the anterior pituitary.

Nuclei of the parvocellular system include the arcuate nucleus, the paraventricular nucleus and the medial preoptic area (87). These nuclei are mostly found in the periventricular zone but a few nuclei are also found within the medial zone (89). The paraventricular nucleus releases CRH and vasopressin (VP), both essentially important in the eventual release of cortisol from the adrenal cortex (86).

2.3.1.2 Anterior pituitary

The pituitary gland is situated on the upper surface of the sphenoid bone in a depression called the sella turcica (30) and is divided into two lobes, the anterior pituitary and the posterior pituitary. The posterior pituitary forms the posterior lobe and is an anatomical outgrowth of the hypothalamus (30,87,90). The posterior pituitary is not of great importance in this review but it should be mentioned that the hormones VP and oxytocin are released here. The focus instead lies on the anterior pituitary, which is important in multiple endocrine control systems, including cortisol secretion.

The anterior pituitary gland stems from entirely different tissue than the posterior pituitary and is not considered as brain tissue (90). Instead, it develops from the dorsal invagination of pharyngeal epithelial cells (Rathke's pouch) during embryonic development (30,88). Within the anterior pituitary, five cells types are found: a) somatotropes that produce growth hormone; b) lactotropes that produce prolactin; c) thyrotopes that produce thyroid-stimulating hormone (TSH); d) gonadotropes that produce follicle-stimulating hormone (FSH) and luteinising hormone (LH); and finally e) corticotropes that produce ACTH (42).

2.3.1.3 The hypothalamus-hypophyseal portal system

The hypothalamus and the posterior pituitary are connected to the anterior pituitary by the specialized hypothalamus-hypophyseal portal venous system (91). A portal system is unique since it is a vascular arrangement where venous blood flows from one capillary bed into another capillary bed via a connecting vessel. The hepatic portal system is another example of such a system (42). This is in contrast to blood flow throughout the rest of the circulatory system, where blood flows from an artery into an arteriole, from the arteriole into a capillary network, and the capillary network re-joins to form a venule which flows into a vein (24,42).

The first capillary bed of the hypothalamus-hypophyseal portal system is found in the median eminence (part of the infundibular stalk). The second capillary bed is found in the anterior pituitary (87). Systemic arterial blood enters at the median eminence and forms a capillary network. The capillary network again joins to form a hypothalamus-hypophyseal portal vein. This vein then again forms a capillary network in the anterior pituitary, which re-joins to form a) another hypothalamus-hypophyseal portal vein returning to the first capillary bed, and b) a venule returning to the systemic circulation which provides a route for anterior pituitary hormones to exert their systemic effects (42,88).

2.3.1.4 Adrenal cortex

The adrenal glands lie anterosuperior to the upper part of each kidney and are somewhat asymmetrical; the right adrenal gland is pyramidal in shape and the left adrenal gland is crescent-shaped (91). Furthermore, an adrenal gland consists of two layers: a) the outer cortex which appears yellow in colour, and b) an inner medulla which is much thinner in comparison to the cortex(91). The cortex is further divided into three zones (from the surface inwards): the zona glomerulosa, the zona fasciculata and the zona reticularis.

Glucocorticoids are produced in the two inner zones of the cortex; the zona fasciculata (major source of glucocorticoid production) and the zona reticularis (meagre sources of

glucocorticoids production). The glucocorticoid hormones are divided into cortisol and corticosterone, of which cortisol is secreted in greater abundance (42,88).

2.3.2 Physiological functioning of the hypothalamus-pituitary-adrenal cortex axis

2.3.2.1 Principle of hypothalamus-pituitary- peripheral gland axis

Endocrine control, involving a hypothalamus-anterior pituitary-peripheral target endocrine gland axis, is involved in the release of multiple hormones from various peripheral glands (42).

The endocrine axis consists of the hypothalamus, which secretes the CRH and VP; the anterior pituitary secretes ACTH and the peripheral gland secretes cortisol. Thus three levels of control exist (88).

2.3.3 The hypothalamus-pituitary-adrenal cortex axis and cortisol secretion

2.3.3.1 Hypothalamus

In the first level of control, the hypothalamus releases CRH and VP from the paraventricular nucleus (86,92) under the influence of various stimuli: a) physiological stressors such as hypoglycaemia, hypotension, fever, surgery and injury; b) the diurnal rhythm; c) neurotransmitters such as acetylcholine, serotonin, norepinephrine (NE) and endorphins; d) feeding (88,92) and e) neuronal input from the amygdala (93).

2.3.3.2 Anterior pituitary

In the second level of control, the synthesis and secretion of ACTH is stimulated by the binding of CRH and VP to corticotrope cells within the anterior pituitary (30). A G-coupled receptor is activated when CRH binds on the corticotrope plasma membrane to corticotropin-releasing hormone receptor 1 and 2; corticotropin-releasing hormone receptor 1 having a higher binding affinity (94). The activated G-coupled receptor's α-subunit activates adenylyl cyclase (AD), which converts ATP to cyclic adenosine monophosphate (cAMP). cAMP activates protein kinase A by phosphorylation (the addition of phosphate to protein kinase A). Active protein kinase A converts an inactive designated protein to an active designated protein as a result of phosphorylation (42). The active designated protein leads to a cellular response, which in this case is the activation and synthesis of the polypeptide hormone ACTH (42,86,95). CRH increases the amount of ACTH released by the corticotrope cells, whereas VP potentiates the effect of CRH by increasing the amount of CRH responsive corticotrope cells (86).

ACTH is a polypeptide hormone consisting of 39 amino acids. It is synthesised within the anterior pituitary but first appears as the amino acid precursor pro-opiomelanocortin (POMC) (88). POMC is cleaved and forms β-lipoproteins and ACTH (88,96).

2.3.3.3 Adrenal cortex

ACTH is secreted into the systemic venous blood and binds to melanocortin receptor 2 (MCR-2) on the plasma membrane of adrenal cortex cells (94). Binding to this G-coupled receptor results in the AD: cAMP pathway (86) as discussed above. The resultant cellular response accumulates in the release of cortisol and corticosterone, the third level of control.

2.3.3.4 Cortisol

Released free cortisol in blood readily dissolves across the plasma membrane of cells (24). Within the cytosol cortisol binds to glucocorticoid receptors (GRs) and forms a hormone-receptor complex (88); in this case the cortisol-GR complex (97). The cortisol-GR complex, alongside essential transcription factors, interacts with the glucocorticoid response element; a DNA sequence that will either lead to gene expression or repression (24,88). Expressed genes increase messenger ribonucleic acid (mRNA) transcription and proteins are synthesized, whilst repressed genes decrease mRNA transcription and protein formation decreases or temporarily halts (98). As is characteristic of all steroid hormones that effect gene expression and/or repression, it is a timely process for protein synthesis to be concluded. So much so that from the moment cortisol reaches a cell it takes 45-60 minutes to synthesize the predetermined proteins, but it may take hours or days to exert systemic effects (24).

2.3.4 Physiological characteristics of cortisol

2.3.4.1 Synthesis

The binding of ACTH on zona fasiculata and zona reticularis cells containing MCR-2 initiates the activation of an esterase via cAMP and free cholesterol is formed and transported into the mitochondrion. Within the mitochondrion, cholesterol is converted into pregnenolone by cytochrome P450 side chain cleavage enzyme (99). Pregnenolone follows two separate pathways that converge two reactions further. In the first pathway, pregnenolone is converted to 17-Hydroxypregnenolone by 17α-Hydroxylase. 17-Hydroxypregnenolone is converted to 17-Hydroxyprogesterone via 3β-OH dehydrogenase:$\triangle^{5,4}$ isomerase. In the second pathway pregnenolone is converted to progesterone by 3β-OH dehydrogenase:$\triangle^{5,4}$ isomerase. Progesterone is then converted to 17-Hydroxyprogesterone by 17α-Hydroxylase. Thus two separate pathways yield 17-Hydroxyprogesterone which is converted to 11-Deoxycortisol by

21-Hydroxylase. 11-Deoxycortisol is finally converted to cortisol by 11 β-Hydroxylase (99–101).

2.3.4.2 Transport

Once secreted into blood, 90-95% of cortisol is bound to plasma proteins, mostly to the α_2-globulin, cortisol binding globulin (or transcortin) and to a lesser extent cortisol is bound to albumin (24,88). The 5-10% of cortisol not bound to plasma proteins is thus free cortisol (i.e. the cortisol that impacts physiological function at any one time). Since cortisol is largely bound to plasma proteins, elimination from blood is slow and cortisol thus has a relatively long half-life of 70-120 minutes, although authors differ (24,25,88). The half-life of cortisol may seem short in duration to the reader, but compared to epinephrine (E) or NE that each has a half-life of 2-4 minutes, the physiological function of cortisol is relatively prolonged (24).

2.3.4.3 Degradation

The major organ involved in cortisol breakdown is the liver, but the kidneys have been shown to contain enzymes involved in biochemical pathways related to cortisol breakdown (88). Ninety-five percent of cortisol metabolites are excreted in urine:

a) 50% as 5β-tetrahydrocortisol, 5α-tetrahydrocortisol and tetrahydrocortisone;
b) 25% as cortols or cortolones;
c) 10% as C19 steroids; and
d) 10% as cortolic acid or cortolonic acid.

The remaining 5% of metabolites are free unconjugated steroids (88). Of the free unconjugated steroids, ±25% is excreted as bile into the digestive tract and eventually eliminated as faeces. The remaining 75% remains unbound in the blood (i.e. it is not bound to plasma proteins) and thus freely filters into the tubular portions of the nephron to be eliminated in urine (24).

2.3.5 Cortisol diurnal rhythm

Hormonal diurnal rhythm regulation in mammals is controlled by the suprachiasmatic nucleus (SCN), situated in the anterior hypothalamus above the optic chiasm (88). Humoral and neural outputs of the SCN have widespread effects on physiology and behaviour. Even though the SCN is accredited with the underlying clock mechanism, there is evidence that peripheral cells have autonomous control which directly affect diurnal rhythms (102,103). One such example is the intrinsic diurnal rhythm within the adrenal cortex cells (104) that function to prepare time periods where it is most reactive to ACTH (105).

The endogenous clock is encoded at molecular level by autoregulatory transcriptional and translational factors that form positive and negative feedback loops. Region specific cell lines (such as the above-mentioned adrenal cortex cells) may have their own rhythm (104,106). Environmental cues regulate the diurnal system; the most important cue is certainly retinal afferent input (42,107). These environmental cues exert their effect by neurotransmitter/neuromodulator and hormonal pathways that subsequently activate the SCN, which can then reset the diurnal rhythm in autoregulatory feedback loops (108,109).
To understand the positive/negative regulation of the diurnal rhythm, elaboration on diurnal genes is necessary. Diurnal genes undergo rhythmic production (positive feedback) and slow protein degradation after translation that ultimately inhibits gene expression once again (negative feedback). In the primary feedback loop, three basic helix-loop-helix/ Per-Arnt-Sim domains (*CLOCK, Bmal 1 and Npas 2)* contain transcriptional factors that act either as transcriptional activators or suppressors. Basic helix-loop-helix proteins may form homo- or heterodimers with other basic helix-loop-helix proteins. When dimers are formed, DNA-binding motifs form that recognize E-box sequences (DNA sequences found upstream from promoter regions, which act to initiate gene transcription). In this way *Period* and *Cryptochrome* genes are transcribed and once they translocate to the cytoplasm, they form heterodimeric complexes that again translocate to the nucleus and inhibit their own transcription (110).

The diurnal rhythm of cortisol is also regulated by the SCN, which stimulates the rhythmic release of CRH from the hypothalamus (30). The subsequent increase in ACTH release from the anterior pituitary under control of CRH brings about increased cortisol release from the adrenal cortex. Typically, in normal circumstances, cortisol levels fluctuate during a 24-hour period. An early morning maximum, ± 30 minutes after awakening, is observed. This is known as the cortisol awakening response (CAR). Cortisol levels then decline throughout the day and the lowest level is achieved around midnight. An abrupt surge during late sleep leads to the early morning maximum once again (24,30). Under normal circumstances, at early morning maximum, cortisol plasma levels reach ± 25µg/dl and its lowest level around midnight is < 2µg/dl (24,30,88). Interestingly, if sleeping habits are changed, the diurnal rhythm of cortisol correspondingly changes and adapts to the new sleeping habit (24), otherwise the diurnal rhythm stays relatively constant from day to day (111). The plasma level of ACTH, since it

regulates cortisol secretion, also follows the same fluctuation pattern of cortisol with the highest level at early morning, declining during the day and at its lowest around midnight (88).

2.3.6 Physiological effects of cortisol

2.3.6.1 Carbohydrate, protein and fat metabolism

During feeding, insulin secreted from pancreatic β cells triggers the uptake and oxidative catabolism of glucose in the liver, muscle and adipose tissue. Insulin also inhibits glycogenolysis (the breakdown of glycogen to glucose) and gluconeogenesis (112). Under the influence of insulin, glucose is then stored in the liver and muscle as glycogen (interconnected molecules of glucose) and when these storage sites become saturated, the excess glucose is converted to fatty acids and glycerol, which combine and form triglycerides in adipose (fat) tissue (42). Between meals, glucagon secreted by pancreatic α cells antagonises the effect insulin had while feeding and increases blood glucose levels by promoting glycogenolysis (112). Even though glucagon's actions are vitally important in maintaining blood glucose levels during meals, cortisol greatly augments the sustained maintenance of glucose levels (42).

The best-known metabolic effect of cortisol is its ability to stimulate hepatic gluconeogenesis (24). Gluconeogenesis is the formation of carbohydrates from non-carbohydrate sources like amino acids and then storing "new" glucose as glycogen within the liver (24,42). Cortisol achieves gluconeogenesis by increasing the required enzymes to convert amino acids to glucose (24). Cortisol increases the activity of the enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase. PEPCK converts oxaloacetate to phosphoenolpyruvate that ultimately forms glucose. Glucose-6-phosphatase converts glucose-6-phosphate to glucose (99).

Cortisol also promotes protein breakdown in various tissues, most notably skeletal muscle (42). That is to say, the synthesis of protein from amino acids is decreased, whilst the catabolism of protein is increased (24). Increased circulating amino acids serve a two-fold effect: 1) amino acids are converted to glucose in the process of gluconeogenesis, and 2) amino acids serve as the building blocks of tissue repair and protein structure repair if injury has occurred (42).

Cortisol further increases blood glucose levels by decreasing the rate of glucose uptake by most cells, except the brain (42,86). It is not currently known how cortisol decreases cellular glucose uptake, but three possible factors are hypothesised (24). Firstly, glucocorticoids decreases the

insertion of glucose transporter (GLUT) 4 channels into the plasma membrane of especially skeletal muscle cells, thus counteracting the effect of insulin, which promotes GLUT 4 insertion into the plasma membrane (24,42). Secondly, glucocorticoids may depress insulin receptor substrate 1 and phosphatidylinositol 3 kinase, both of which are important in mediating the actions of insulin. Finally, glucocorticoids block the oxidation of nicotinamide-adenine dinucleotide, which must be oxidised to nicotinamide adenine dinucleotide to allow glycolysis, thus glycolysis is reduced and glucose is spared (24).

Under the influence of glucocorticoids, increased gluconeogenesis and decreased glucose uptake by cells raise the blood glucose level (86). In response, insulin secretion is also increased. Although this is the case, insulin is not nearly as effective in lowering blood glucose levels because of the antagonistic effect brought on by glucocorticoids as discussed above. In addition, the mobilisation of fatty acids may impair the glucose storage abilities of insulin (42).

Concerning protein metabolism, it has already been mentioned that cortisol increases the breakdown of protein into amino acid constituents (42). This principal function of cortisol takes place in nearly all cells of the body, with the exception of the liver (25). Cortisol inhibits the synthesis of extrahepatic tissue protein by depressing the formation of RNA and decreasing the extrahepatic tissue uptake of amino acids (24). Cortisol, by mechanisms unknown, mobilises amino acids from extrahepatic tissue and thus increases plasma amino acid levels (24). Contrastingly, cortisol enhances amino acid uptake in hepatocytes and there is a resultant increase in protein (including plasma protein) synthesis within the liver (24,25). In addition, as has previously been stated, amino acids are converted to glucose in the process of gluconeogenesis (42).

Regarding fat metabolism, cortisol also plays a major role. Much the same as amino acid mobilisation, cortisol increases free fatty acids and glycerol concentration in the blood by mobilisation from adipose tissue (24,25,42). Glycerol is converted to glycerol-3-phosphate (99) which enters the glycolytic pathway to yield ATP (24). Functionally more important is the utilisation of fatty acids to provide an alternate energy source (ketones), since the main aim of cortisol is to spare glucose (25). The mechanisms by which cortisol increases fatty acid release from adipose cells are unknown. A postulation is that since glucose uptake into adipose cells decreases under the influence of cortisol, the glucose derivative α-glycerophosphate is also decreased. α-Glycerophosphate is essential to triglyceride formation and maintenance and in its absence, free fatty acids are released (24).

2.3.6.2 Skin connective tissue and muscle

Glucocorticoids affects skin by inhibiting epidermal cell division and DNA synthesis. It also reduces collagen synthesis and production (88). Collagen is further impacted by glucocorticoids since collagenase expression is increased (86). Collagenase are enzymes that digest collagen (95). In muscle, glucocorticoids inhibit protein synthesis and increase protein catabolism, causing muscle atrophy that does not extend to necrosis (25,88). In excessive cortisol secretion, muscle may be degraded to such an extent due to extrahepatic protein uptake, that a person cannot rise from the squatting position due to muscle wasting (24,25).

2.3.6.3 Bone and calcium

Glucocorticoids increase bone resorption by increasing osteoclast activity and decrease bone formation by osteoblasts (25). It has been shown that glucocorticoids in excess inhibit osteoblast differentiation and function, while pharmaceutical levels cause osteoblast apoptosis (113). Osteoclast lifetime is extended because of delayed apoptosis and their function is perpetuated by glucocorticoid-activated changes in osteoblast which increase the expression of receptor activator of nuclear factor κB ligand (RANKL) and repression of osteoprotegerin (113). In normal functioning, the binding of RANKL to receptor activator of nuclear factor κB (RANK) receptors increases osteoclast activity. The binding of OPG to RANKL inactivates RANKL and it cannot bind to RANK receptors, thus decreasing osteoclast activity (42).

Glucocorticoids in excessive amounts favour bone resorption as opposed to bone formation. During adult life, glucocorticoid excess leads to osteopenia and eventually osteoporosis, while in developmental life (the time before adulthood is reached) glucocorticoids impair skeletal growth (86). Another worrying factor regarding corticosteroid therapy (exogenous glucocorticoids) is the onset of osteonecrosis; a condition where bone quality is rapidly deteriorated. The onset of this condition is due to the effect that corticosteroids have on osteocyte activity (88). Osteocytes lie within the lacunar-canalicular network which contains the vasculature that supplies bone with nutrients and acts as a fluid storage network (113). Osteocytes mainly function to maintain bones by recruiting osteoblasts and osteoclasts to sites of bone remodelling, that is to say the site where resorption and formation are set to take place (42,114). The lacunar-canalicular network is a component that lends to bone strength and it has been shown that high levels of glucocorticoids affect the vasculature and the solute exchange between this network and blood (113); thus osteonecrosis may ensue.

Glucocorticoids also induce a negative calcium balance since intestinal calcium absorption is decreased and renal excretion is increased (25,88). To try and compensate for a negative calcium balance, bone resorption increases, perpetuating osteoporosis (42).

2.3.6.4 Electrolyte (sodium, potassium, hydrogen) and water homeostasis

Glucocorticoids enhances the retention of sodium (Na^+) and to a lesser extent increases the excretion of potassium (K^+) (25). In the proximal convoluted tubule, glucocorticoids increase epithelial Na^+ transport (88), probably through the activity of serum and glucocorticoid-induced kinase (SGK), which may stimulate glucocorticoid induced Na^+ retention by increasing Na^+ channel transport (86). Glucocorticoids may also bind to mineralocorticoid receptors (MRs) on aldosterone target cells in the distal convoluted tubule and the collecting duct of the nephron (42,115), enhancing Na^+ absorption and K^+ excretion (115). Under normal physiological condition this seldom happens, since the enzyme 11-Beta hydroxysteroid dehydrogenase 2 in aldosterone target cells rapidly inactivates glucocorticoids. In glucocorticoid excess, 11β-HSD2 may become overwhelmed and thus cannot keep up inactivation, leading to Na^+ retention via the mimic effect (of aldosterone) that glucocorticoids exert on the aldosterone target cell MRs (86,88). Excess glucocorticoid secretion or exogenous glucocorticoid intake may lead to hypertension, hypokalaemia, and muscular weakness as a result of hypokalaemia (25).

Mediated by SGK (which increases Na^+ channel transport), glucocorticoids also increase renal tubule acid hydrogen ion (H^+) secretion, most probably through the Na^+/H^+ antiporter in the proximal tubule (86,116).

Water homeostasis is also affected by glucocorticoids. Through an increase in glucocorticoids, glomerular filtration rate is increased and leads to increased urine output (42,88). VP helps regulate ACTH release from the anterior pituitary and ACTH in turn regulates glucocorticoid release from the adrenal cortex. Glucocorticoids inhibit the release of VP and CRH from the hypothalamus as well as the release of ACTH from the anterior pituitary. In glucocorticoid insufficiency, negative feedback to the hypothalamus is inadequate and VP plasma levels rise (86). Vasopressin 2 receptor (V2R) are found in the basolateral membrane of the distal convoluted tubule and the collecting ducts of the nephron (42). The binding of VP to a V2R receptor activates the cAMP secondary messenger system, which activates protein kinase. Protein kinase stimulates the formation of mRNA that encodes for aquaporin-2 channels (30). The formed aquaporin-2 proteins move within intracellular vesicles towards the luminal

membrane of distal convoluted tubule and collecting duct cells, and are inserted into the luminal membrane distal convoluted tubule (30,42). The result is the permeability to water through the luminal membranes of distal convoluted tubule and collecting duct cells; a feat that can only be achieved under the influence of VP. If VP does not bind to V2R, these tubules stay impermeable to water (24). Thus glucocorticoid insufficiency leads to the retention of water (25).

2.3.6.5 Central nervous system

The brain is an important target organ for glucocorticoids. This is deferred from the behavioural symptoms associated with glucocorticoid deficiency or excess. Some of these symptoms include depression, psychosis and euphoria (88). It has been found that high HPA activity and the resulting increase in plasma cortisol levels is found in many patients with depression (86). Also, glucocorticoids impair cognitive function and memory as a result of hippocampal structural changes (88). Various parts of the brain have MRs and GRs. Specifically MRs are found in the dentate gyrus and pyramidal cells of the hippocampus and GRs are distributed in neurons and neuroglia (86). Recall that glucocorticoids can bind to MRs and exert the same effect that mineralocorticoids would have had on the MRs. This is however blocked by the enzyme 11β-HSD2 that inactivates glucocorticoids (42,114).

Interestingly, no 11β-HSD2 is found in the hippocampus or any other limbic system structures (86), thus glucocorticoids may bind to MRs and exert the same physiological function as mineralocorticoids would. Functionally, evidence suggests that this is appropriate: basal glucocorticoids levels maintain hippocampal neurons excitability via MRs. On the other hand elevated glucocorticoid levels supress hippocampal neuron excitability via GRs (86).

It is known that glucocorticoid deficiency or excess causes hippocampal neuronal damage (86,88). Glucocorticoid excess causes death of cornu ammonis region three neurons in the pyramidal cells of the hippocampus (86). The possible mechanisms by which glucocorticoid excess might cause hippocampal structural changes are dendrite retraction, loss of neuroglia and decreased neurogenesis within the dentate gyrus (117). Glucocorticoids act on the hippocampus in a negative feedback loop (117) and hippocampal neuronal inputs to the hypothalamus depress the HPA (93). Therefore the danger of hippocampal shrinking is twofold: as the hippocampus shrinks, the inhibitory effect it has on the HPA is reduced; inadvertently HPA activity increases and hippocampal volume is further reduced because of increased glucocorticoid release (117).

A decrease in the amount of neurons of the dentate gyrus and pyramidal cells is observed in patients that have undergone a bilateral adrenalectomy (surgical removal of both adrenal glands) (86). Whether this is the result of a decrease in mineralocorticoids or glucocorticoids is unknown.

Glucocorticoids may also cause glaucoma which is characterised by an increase of intraocular pressure as a result of increased aqueous humour production. Glaucoma is compounded by a decrease in aqueous drainage from the trabecular meshwork as a result of matrix deposition around the trabecular meshwork (88).

2.3.6.6 Permissive role in other hormonal activity

Glucocorticoids exert a major permissive role in the activity of various other hormones (25). The effect that cortisol has on gluconeogenesis and glycogen storage within the liver enables E and glucagon to draw from this glycogen store between meals (24,25). At basal levels, glucocorticoids are permissive to E's glycogenolysis effect. Thus, cortisol's effect to increase hepatic glycogen stores is permissively utilised by E and glucagon during fasting or between meals. This provides protection against wide fluctuations in plasma glucose concentration (24,86). Cortisol must be present to allow the catecholamines, E and NE, to activate vasoconstriction (42). Glucocorticoids further bring about vasoconstriction by increasing endothelial sensitivity to Angiotensin II (a potent vasoconstrictor), while decreasing vasodilation by NO. The exact mechanism by which cortisol is permissive to these vasoactive hormones is unknown, but it is believed that cortisol increases arteriolar and venous endothelial receptors for these hormones (86). Glucocorticoids also supress the HPA via an inhibitory effect on TSH secretion and the inhibition of 5' deiodinase which mediates the conversion of thyroxine to triiodothyronine (88). Furthermore glucocorticoids inhibit gonadotropin-releasing hormone pulsatility and subsequently the hypothalamus-anterior pituitary-gonadal axis, decreasing FSH and LH secretion by the anterior pituitary (88).

2.3.6.7 Anti-inflammatory effects and immunosuppression

Inflammation is the result of tissue damage and/or foreign invasion of pathogens into the body. Inflammation of any origin is remarkably similar (42). Inflammation is characterised by: 1) the release of histamine, serotonin, leukotriene, prostaglandin and bradykinin leading to increased capillary permeability; 2) oedema as a result of increased capillary permeability; 3) clotting within interstitial fluid as a result of the leakage of clot-forming fibrinogen, which is converted to fibrin when exposed to thromboplastin in injured tissue; and 4) migration of leukocytes

(white blood cells) into the injured area (24,25). Thus, because of increased blood flow to the injured area and the resultant oedema, inflammation produces heat, redness, swelling and pain in the inflamed area (42).

Glucocorticoids effectively inhibit the onset of inflammation or reverse inflammation if it has already started (25). Cortisol prevents or reverses inflammation by 5 mechanisms: 1) lysosomal membrane stabilisation; 2) decreased capillary permeability; 3) decreased migration and phagocytosis by phagocytes; 4) inhibition of fever; and 5) immune suppression (24).

Cortisol stabilises the membranes of lysosomes. A lysosome may release more than 30 proteolytic enzymes that break down organic molecules in foreign substances such as bacteria by the addition of water (42). Thus, lysosomal activity is greatly increased during inflammation. By stabilising the lysosomal membrane, cortisol inhibits the release of proteolytic enzymes that play a role in the augmentation of inflammation (24).

Cortisol inhibits the cytokine tumour necrosis factor (TNF) that causes the release of histamine from mast cells. Thus the effect histamine has on local vasodilation and increased capillary permeability is supressed (42,88). Cortisol, via its permissive effect on catecholamines, also cause vasoconstriction, thus reducing blood flow to the injured area (25).

Cortisol decreases the formation of substances like prostaglandins and leukotrienes which cause vasodilation, increased capillary membrane permeability and leukocyte migration (24). Concurrently, blood flow to the injured area is further decreased and phagocytosis is decreased because of decreased phagocyte migration. Since fewer phagocytes are activated (by foreign substance ingestion), less cytokines are released. Decreased inflammatory mediation by cytokines further inhibits inflammation formation (24).

Cortisol reduces fever by inhibiting the release of the cytokines interleukin (IL)- 1 & 6 and TNF which are endogenous pyrogens (24,42). If fever is reduced, the systemic vasodilation effect it has also decreases.

The multiple factors that play a role in inflammation make it difficult to give a clear physiological explanation on how glucocorticoids bring about anti-inflammatory effects. A highly likely mechanism is the inhibition of cytokine secretion from immune cells (86). All immune cells have GRs that, when activated, lead to alterations in gene transcription or translation (86). Transcription may be altered directly when glucocorticoids bind to the glucocorticoid response element on DNA or indirectly by first binding to DNA regulator

proteins such as nuclear factor κB, activator protein 1 or signal transducer and activators of transcription (118). It is known that IL-1 production is inhibited at the level of transcription, translation and secretion. TNF and Granulocyte-macrophage colony-stimulating factor production decreases as a result of a reduction in the mRNA's that translate them. IL 2 & 3 are inhibited at the level of translation. Furthermore, other cytokines (prostaglandins and leukotrienes) may be inhibited because the enzyme/s that lead to their production are not synthesized (86). What is clear is the fact that glucocorticoids act at the molecular level of DNA to inhibit cytokine secretion from immune cells (86).

Since inflammation is largely dependent on immune cells, immunosuppression is another mechanism whereby inflammation may be inhibited or reversed. Glucocorticoids decrease the blood counts of lymphocytes (higher decrease of T-lymphocytes than B-lymphocytes), monocytes, eosinophils and basophils (25,86,88). Glucocorticoids act directly on B lymphocytes and inhibit immunoglobulin (Ig) synthesis (88). Antibodies that have entered the blood are known as immunoglobulins and five subclasses exist (42). 1) IgM serve as B-cell receptor, which recognises and binds foreign antigens. 2) IgG binds to antigens that have previously entered the blood. 3) IgE protects against parasitic worms and is involved in allergic reaction. IgE's do not circulate in the blood; instead their tail portions are attached to basophils and mast cells that store a plethora of inflammatory agents in their cytoplasm. When an antigen binds to the arm regions of IgE's, the release of inflammatory agents in basophils and mast cells is induced. One of these substances is histamine, which causes vasodilation and increased capillary permeability. This effect is often experienced during hay fever when fluid leaks through vascular endothelial cells in the nose and causes a blocked nose. 4) IgA is found in secretions of tissue exposed to the external environment like the eyes, the digestive tract, respiratory systems and the urogenital systems. This helps to ward off antigens that reach the body through these means. 5) IgD is present on the surface of B-lymphocytes and most probably functions to recognise foreign antigens (25).

Glucocorticoids inhibit T-lymphocytes by its effect to stimulate apoptosis (cell death). Glucocorticoids also decrease the number of circulating T-lymphocytes by supressing T-lymphocyte production in the thymus and lymph nodes (88).

Glucocorticoids inhibit monocyte differentiation into macrophages (88). Interestingly, glucocorticoids increase the amount of circulating neutrophils (86,88). Natural killer cells, as

the name implies, are naturally occurring immune cells that destroy cancer cells and virus-infected cells (42). Glucocorticoids also inhibit the activity of natural killer cells (86).

The ultimate goal of glucocorticoids in immunosuppression is to prevent pro-inflammatory cytokine release from immune cells (88). If cytokines are released, inflammation occurs. The effects of glucocorticoids are of clinical value in autoimmune diseases and organ transplants because of the anti-inflammatory and immunosuppressive effects they exert (88).

2.3.6.8 Resistance to stress

The culmination of various physiological factors influenced by glucocorticoids is important in resistance to stress. Whether a stressor is of physical or psychological nature, it ultimately leads to stimulation of the HPA, thereby increasing glucocorticoid release (24,42). Cortisol secretion is increased proportionately to the severity of the stressors (42). Stress is inhibited/suppressed by:

a) increased available energy through the processes of gluconeogenesis and fatty acids mobilisation (ketone formation);

b) permissive actions on various hormones; and

c) anti-inflammatory and immunosuppressive actions (25).

2.3.7 Depression, anxiety and cortisol secretion

Over 350 million people are affected by major depressive disorder (MDD) and this disorder is diagnosed in almost two-thirds of people who commit suicide (119). MDD is characterised by a combination of factors which include depressed mood, diminished interest in activities, significant weight loss, insomnia or hypersomnia, psychomotor agitation or retardation fatigue or loss of energy, feelings of worthlessness or excessive or inappropriate guilt, diminished ability to think or concentrate, and recurrent thoughts of death (120). Interestingly though, CRH plays an important role in the pathophysiology of depression (121). Research has implicated chronic stress as one of the primary factors in the development and persistence of depression. Fundamentally and etiologically, depression is a stress-related disorder (122). Negative feedback, which maintains homeostasis in the stress response system, is dysfunctional during chronic stress. This causes increased expression and activation of neural and endocrine CRH, CRH1 and glucocorticoids systems, all which are typical of persons suffering from depression (123). It has been found that depression in middle-aged, young adult and adolescent females is associated with a heightened CAR (cf. Table 2.1) (124). Other studies, however, have found

hypoactivity of the HPA in depressed patients, which is most probably the result of HPA fatigue after various depressive episodes (125).

Anxiety disorders include a broad spectrum of specific phobias, generalised anxiety disorder (GAD) and panic disorder (3). GAD is characterised by a combination of restlessness, being easily fatigued, difficulty in concentration, irritability, muscle tension and sleep disturbance (120). The relationship between CRH and anxiety disorders is not as clear as those between increased CRH and MDD. Initial research found that CRH concentrations in cerebrospinal fluid did not differ between persons with GAD and panic disorder and normal controls, but more recently it has been found that CRH promotes anxiety, since CRH induces fatty acid amide hydrolase, which degrades anandamide, a neurotransmitter which decreases anxiety when stimulating cannaboid type 1 receptors (119).

2.4 Salivary cortisol

2.4.1 A biomarker in psychobiological research

Cortisol is a steroid hormone and can pass freely across the plasma membranes of all cells in the body. This allows cortisol to be measured in body fluids other than blood, for example saliva (126). Salivary cortisol represents unbound cortisol (5-10% of total cortisol) and because of the free hormone concept, cortisol appears in the saliva via passive diffusion. Cortisol concentration in saliva remains unaltered when whole saliva samples (i.e. not gland-specific saliva) are collected (31), regardless of the saliva flow rate or volume (127).

The use of salivary measures in research is not restricted to cortisol, but include a plethora of biochemical markers (128) the reason being that collecting saliva is safe, non-invasive and painless (29). Salivary cortisol has become the gold standard of cortisol assessment during real life (ambulatory) conditions (31).

2.4.2 Measurement of salivary cortisol

Since saliva samples provide a simple avenue to cortisol analysis, researchers often induce a particular stressor to respondents and compare before and after measurements. For example, delivering oral presentations during a lecture significantly increases cortisol secretion from before the oral presentation to after the oral presentation (129). This method of cortisol analysis is termed "cortisol reactivity to momentary stressors" (cf. Table 2.1) (23). To apply this method extremely accurately, the cortisol diurnal rhythm must be determined the day before the stressor. Multiple before and after measurements must be taken. Also, the moment in time

when the stressors are applied should take place at the exact same time across at least two days (29,130). Table 2.1 portrays the various methods of measuring cortisol.

Table 2.1 Diurnal cortisol testing measures: Adapted from Adam *et al.* (23).

Diurnal cortisol testing measure.	Brief description.
CAR:	The slope of cortisol from awakening to 30-45 minutes after awakening. In normal subjects, there is a surge in this time.
Diurnal cortisol slope:	The degree of change in cortisol levels from early morning to late in the evening. This change is usually a decline from morning to evening.
Area under the daytime cortisol curve:	This measurement is an estimation of average cortisol exposure that does not provide any indication of the diurnal rhythm of cortisol. It is established as the area under cortisol data points measured across a single day.
Wakening cortisol:	Cortisol measured at the quickest possible moment after waking.
Specific points during the day:	Cortisol measurements taken at specific times during the day. To be accurate, the waking and bedtime levels also have to be established.
Bedtime cortisol:	Cortisol measured at the time just before sleeping (at night).
Cortisol after a momentary stressor:	Cortisol is measured directly after a stressor. To be effective, the diurnal rhythm of that particular person has to be established.
Cortisol reactivity of daily stressors:	The change in cortisol is measured from day to day. Thus, samples must be taken for multiple days.

2.4.3 Normal values/ranges of salivary cortisol

In normal circumstances, cortisol levels fluctuate during a 24-hour period with an early morning maximum ± 30 minutes after awakening, followed by declining levels throughout the day, and the lowest level is achieved around midnight, followed by an abrupt surge during late sleep leading to the early morning maximum once again. As shown in Table 2.2, the Cortisol Saliva enzyme-linked immunosorbent assay (ELISA) kit manufactured by Immuno-Biologicals Laboratories Inc. America was used in this study and from their research it is clear to see the CAR followed by declining levels through the day (cf. Table 2.2).

Table 2.2 Expected salivary cortisol values (131)

Time after awakening (h)	**Cortisol (Saliva) Range (male/female; > 6 y; n=100; 5%-95% percentile)**					
	Median (µg/dl)	**Range (µg/dl)**		**Median (nmol/L)**	**Range (nmol/L)**	
		5%-	**95%**		**5%-**	**95%**
Awaking	0.343	0.113	0.803	9.47	3.12	22.17
0.5	0.478	0.200	1.076	13.19	5.52	29.70
1	0.384	0.101	0.936	10.60	2.79	25.82
2	0.234	0.083	0.574	6.44	2.29	15.85
5	0.150	0.074	0.355	4.14	2.04	9.79
8	0.116	0.055	0.314	3.20	1.53	8.67
12	0.082	0.032	0.322	2.26	0.87	8.89

2.5 Texting

The short message service (SMS) is a means of short alphanumeric communication that allows messages of no more than 160 characters to be exchanged between mobile devices (132). This form of communication has become known as texting and has become the most widely used service on mobile data networks (133). The idea was born in 1984 when Friedhelm Hillebrand typed random sentences and questions on his typewriter. He counted every character and found that most of the time the message had fewer than 160 characters. Neil Papworth sent the first SMS, followed by a developer at Sema Group Telecoms in 1992 with the words “Merry Christmas” and in 1993 Nokia launched the first mobile phone that could send an SMS. By 2002, over 250 million text messages had been sent worldwide; a number that would rise to 6.1 trillion in 2010 (134).

2.5.1 Physiological effects of texting and mobile phone use

Physiologically, it has been found that texting increases heart rate and respiratory rate. The same study reported that 83% of respondents indicated that they experienced head and neck

pain (26). More recently it has been shown that the degree to which the head is tilted forward affects pressure on the cervical spine (neck); a 60° forward tilt results in 27,2 kg weight pressure on the neck (135). On average, people spend two to four hours of their day tilting their heads down to text, read or perform a mobile phone-related activity. This results in 700-1 400 hours of excess strain on the cervical spine each year (135). As recently as 2017, AlAbdulwahab *et al.* (27) found a strong association between various degrees of neck pain and addiction to mobile phone usage (p.2). Research by Woo *et al.* (136) supported these findings and found a significant association between musculoskeletal disorders in university students and the use of portable handheld devices (p.6). Interestingly enough, head tilting affects posture and it has been found that a contracted posture increases cortisol levels (135,137). Based on a self-administered questionnaire, dental hygiene students in Seoul reported that the most painful areas after mobile phone use were the neck and shoulders. Back pain was also found to correlate with the size of the mobile phone's liquid crystal display (138). In a longitudinal population-based cohort study of young adults aged 20-24 years, Gustafsson *et al.* (139) concluded that texting and reported ongoing symptoms of musculoskeletal disorders of the neck and upper extremities are associated (p.208).

In a study by Seltzer *et al*. (28) it was found that girls who spoke to their mothers' face to face or telephonically and having a comfortable conversation after undergoing a momentary stressor, using the Trier social stress test (TSST), secreted oxytocin and had reduced levels of salivary cortisol. In contrast, girls who only sent an instant message (using computers) to their mothers after the momentary stressor did not secrete oxytocin. They also had similarly high salivary cortisol levels as the control group who did not interact with their mothers at all (p. 4). This points to an interesting finding, "hormonal responses are elicited by human interactions but not by digital interactions" (28). Multi-tasking behaviour creates a dopamine addiction feedback loop and the brain is rewarded because it is constantly stimulated externally (22). Thus it may be concluded that information on texting and the various physiological factors it affects, is widely available (22,28), although literature on texting and the effect it has on salivary cortisol levels is limited (140).

Lin *et al.* (26) suggested that long-term physiological studies in relation to texting need to be conducted (p. 57). To further assert the notion by Lin *et al.* (26), the following holds true; "After many years of research, it has finally been suggested that mobile phone radiation should be classified as a probable human carcinogen" (35). Mobile phones have been in use for 20-odd years and the long-term effects they have on normal physiological functioning are only

now starting to transpire (37). Since salivary cortisol is an excellent biomarker of stress (141), it may provide more insight in regard to the physiological impact of texting.

2.6 Receiving mobile text messages and salivary cortisol

A literature search early in 2019 using EBSCOhost web (MEDLINE with full text, Academic Search Complete, Africa-Wide Information and PsycINFO) failed to yield any results on the effect texting has on cortisol secretion during a lecture. A total of 95 articles were included in the result. The following search terms were used: ("cell phone*" or "mobile phone*" or "mobile telephone*" or cellphone* or "cellular phone*" or texting or "mobile message*" or "text message*" or sms or "short message service*" or whatsapp or wechat) and stress. The title also had to include one of the following terms: ("cell phone*" or "mobile phone*" or "mobile telephone*" or cellphone* or "cellular phone*" or texting or "mobile message*" or "text message*" or sms or "short message service*" or whatsapp or wechat) and stress.

No study measured texting during a lecture and cortisol secretion. Two pilot studies conducted in 2015 measured mobile phone use compared to biological markers. One study found that salivary amylase activity was significantly higher in respondents that used mobile phones more frequently compared to those that used them less frequently (142). The other study measured the HPA response to a phone call in children and adolescents after taking the TSST for children. The pilot included respondents who either owned or did not own a mobile phone. It was found that mobile phone owners had higher salivary cortisol levels at baseline than non-owners. Also, the expected diurnal decrease in salivary cortisol levels from baseline until the end of the experiment did not significantly decrease in mobile phone owners, but there was a significant decrease in non-owners (143). Recently, Hooker *et al.* (140) found that systolic BP was lower for women receiving mobile text messages from a romantic partner during a stressor than in the control group (p.485). In a recent study by Hunter *et al.* (144), women were subjected to a peer social exclusion stressor. They assigned the women to three conditions: no access to a phone, access to a phone but restricted use, and access to a phone with unrestricted use (p.345). It was measured whether if the presence of a smartphone altered the physiological and psychological responses to the stressor and the result was that cortisol levels did not vary by condition (p.348).

2.7 Chapter summary

In this chapter, mobile phone exposure and its physiological effects as well as cortisol and salivary cortisol as a biomarker were discussed. From the literature review, it is clear that

information on the effect of texting on salivary cortisol levels is limited in current literature. More interesting to evaluate is the effect that texting has on salivary cortisol levels during an academic lecture, since this may have various repercussions on students' academic ability, academic performance and importantly, their physiological wellbeing. Furthermore, information on the possible effect that texting has on salivary cortisol levels during an academic lecture, in the South African population, is unknown.

Chapter 3 METHODOLOGY

3.1 Introduction

In this chapter, the aim is to describe the methodology and study design used in this research study. Firstly, the problem statement, research question and the sub-questions are revisited.

3.2 Problem statement

Texting during academic lectures is a major problem in universities, it affects students and lecturers alike. It is known that texting affects attention and academic performance. Although sound evidence exists that texting impacts on human physiology, little is known regarding the effect of receiving mobile text messages during an academic lecture and salivary cortisol secretion.

3.3 Research question

The research question was, "What effect does receiving mobile text messages during a lecture have on cortisol secretion in undergraduate Physiology students, and what possible factors mediate this effect?"

3.4 Sub-questions

To answer the research question, the following sub-questions were formulated.

a) What are the general and texting characteristics of the sample population?
b) Will stress, anxiety and depression have a moderating effect on cortisol secretion while receiving mobile text messages?
c) Will subjective feedback on the experiment correlate with objectively measured cortisol data?
d) Will text frequency and the use of neutral, positive or negative words in text messages have a moderating effect on cortisol secretion?

3.5 Methodology

3.5.1 Study design

This protocol was set as an experimental, crossover, quantitative study. The study collected data over a period of six months. The major advantage of a crossover design is that each respondent acts as his/her own control, and that a smaller number of respondents are required

when compared to parallel-group designs (145). Respondents received mobile text messages, sent by the researcher, during a lecture (intervention) and acted as their own control the previous or next day, when they did not receive text messages.

Respondents participated in the study over two consecutive study days. For each study day, one group received text messages (intervention) while the other group did not receive text messages and vice versa on the next day. Respondents thus acted as their own controls. Saliva was collected before and after the intervention on both days.

A set of predetermined words by Cuming (21) with a neutral, positive or negative connotation were used in the text messages, with each text message accompanied by only one of these words. A list of neutral, positive and negative words used in a South African population aged 19-25 is presented in Appendix E. Nineteen respondents received words with a positive connotation, 16 respondents words with a negative connotation, and 13 respondents words with a neutral connotation. Whether a respondent received words with a neutral, positive or negative connotation were randomly selected.

Respondents were told on which day they will receive the intervention, but the content of the text messages and the exact time during the lecture when they will receive the text messages was not disclosed to respondents beforehand. Respondents were randomly selected.

3.5.2 Target population

The target population for this study was undergraduate students registered for Physiology modules in the Faculty of Natural and Agricultural Sciences, at the Bloemfontein Campus of the University of the Free State. Physiology students were selected because they had lectures on two consecutive days in the same time slot (English lectures on Thursday & Friday 09:10 am-10:00 am and Afrikaans lectures on Monday & Tuesday 08:10 am-09:00 am). They were also selected because they attend their lectures in the Faculty of Health Sciences in the vicinity where the laboratory for the ELISA analysis is situated, which made the collection, storing and execution of the samples more convenient.

3.5.3 Sample population

From the students who completed the consent forms, 50 respondents were randomly selected to participate further in the study. Due to incomplete data, two respondents were excluded from the study.

Inclusion criteria:

a) Aged 18-25 years.
b) Registered Physiology students in the Faculty of Natural and Agricultural Sciences, University of the Free State.

Exclusion criteria:

a) Respondents who had diseases of the adrenal gland.
b) Respondents who used corticosteroid medication in the form of cream/s, oil/s, pill/s or any other substance containing corticosteroids.

3.5.4 Screening

Prospective respondents were approached in September 2016. An information session, during a lecture, was held to distribute (and read) the information leaflet and consent forms to prospective respondents. To prevent the information session from interfering with students' academic programmes and/or responsibilities, appropriate times and venues were arranged with the relevant lecturers.

Respondents participated voluntarily. They were also informed that they could withdraw from the study at any time without stating a reason for their withdrawal.

Respondents who were subject to exclusion criteria were verbally asked not to fill in and sign the consent form. The information leaflet and consent form also explained exclusion criteria to prospective respondents (cf. Appendix A & B). Respondents who were subject to exclusion criteria did not participate in the study.

3.6 Questionnaires

Two questionnaires (cf. Appendix F) were completed on the control and intervention day (INT), namely:

a) Questionnaire 1 collected information on demographical data, lifestyle, stress, anxiety and depression.
b) Questionnaire 2 collected information on respondents' subjective experience of the study.

To compile the questionnaires used in this study, information from validated questionnaires [Hospital Anxiety and Depression Scale (HADS) (146) and Perceived Stress Scale (PSS) (147)] and previous literature were used.

Respondents completed and handed back questionnaire 1 before the study and questionnaire 2 after the study. Respondents completed questionnaire 1 on both days, while questionnaire 2 was only completed on the day that they received the intervention.

The cover page of the questionnaires had a respondent number (e.g. 01) which correlated with the respondent number on the test tubes (cf. Section 3.9).

3.7 Data collection for salivary cortisol analysis

To decrease sample size and still obtain valid and reliable results, studies measuring salivary cortisol often occur on two consecutive days at the same time each day (29,127). Before and after measurements were then taken at exactly the same time on the consecutive days (129). On one day the stressor was applied and on the other (control) day no stressor was applied (129,148).

Respondents provided saliva samples 10 minutes into and at the end of the lecture (e.g. 09:20 am & 10:00 am). Each respondent provided four saliva samples in total; two on the control day (CONT) and two on the day that they received the intervention.

The test tubes were labelled beforehand as follows: the respondent number followed by the letter A or B. A indicated that the sample was collected before the experiment (BEF) and B indicated that the sample was collected AFT. For example; 01A and 01B. The first and second day samples were stored separately at -20°C as indicated by the ELISA kit user's manual (131).

3.8 Procedures before the study days

Respondents were instructed (cf. Appendix C & Table 3.1) to refrain from strenuous exercise, alcohol and non-prescription medication (e.g. Panado, Grandpa or any over the counter medication) for 24 hours before each study day (130). For example:

a) Wednesday (the day before study day 1),
b) Thursday (study day 1), and
c) Until the end of the Friday (study day 2) lecture.

All respondents were asked to refrain from ingesting caffeine six hours before the study because cortisol secretion is decreased by caffeine intake (149) and the half-life of caffeine is 5-6 hours (150). Respondents were asked not to smoke 30 minutes before the study (129), since smokers have higher cortisol levels (151) which may be attributed to nicotine exposure (152) and may have impacted on salivary cortisol results. It was also requested that respondents not

brush their teeth 30 minutes before the study (127), since it might have caused micro injuries and the resulting blood contamination vastly increases salivary cortisol levels (153). Furthermore, respondents were asked not to eat or drink anything (other than water) an hour before and during the study (127), to avoid salivary cortisol contamination (154).

Unfortunately, not all of these prerequisite factors could be controlled. Compliance to investigator instructions was measured in questionnaire 1 (cf. Appendix F, Questionnaire 1, questions 10-16). Respondents who did not comply to study instructions were included in this study. In Table 3.1 instructions for study participation are relayed.

Table 3.1 Instructions for respondents if lectures take place, for example, on a Thursday and Friday.

Wednesday	**Thursday**	**Until the end of your Friday lecture**
• Refrain from strenuous exercise. • Refrain from non-prescription medication (e.g. Panado, Grandpa or any over the counter medication). • Refrain from alcohol.	• Refrain from strenuous exercise. • Refrain from non-prescription medication (e.g. Panado, Grandpa or any over the counter medication). • Refrain from alcohol. Please do not: • Consume substances that contain caffeine for 6h before the study. • Smoke or brush your teeth 30 minutes before the study. • Eat or drink anything except water for 1h before or during the study.	• Refrain from strenuous exercise. • Refrain from non-prescription medication (e.g. Panado, Grandpa or any over the counter medication). • Refrain from alcohol. Please do not: • Consume substances that contain caffeine for 6h before the study. • Smoke or brush your teeth 30 minutes before the study. • Eat or drink anything except water for 1h before or during the study.

3.9 Procedure on the study days

Selected respondents reported to the lecture venue before their lecture. Respondents were given a demonstration on how to provide a saliva sample. Respondents completed questionnaire 1 and handed it back to the researcher. Respondents were then given:

a) Two labelled test tubes and
b) A respondent number.

Respondents who received text messages were instructed to sit at the back of the lecture hall in order not to disturb the other students that were not participating in the study. Respondents who did not receive mobile text messages were instructed to sit in the front. Respondents were also instructed to read the text messages they receive.

Ten minutes into the lecture (e.g. 09:20 am) a green light was switched on and respondents passively drooled into the test tube labelled with the letter A. At this time the intervention started and continued for 20 minutes (until 09:40 am). During this 20 minute period, respondents received either 10, 15 or 20 text messages with a neutral, positive or negative word accompanying each text message (all text messages sent to each individual respondent however, contained words from the same category — either neutral, positive, or negative). The text messages were sent to all respondents simultaneously using BulkSMS.

The last green light switched on at 10:00 am and respondents again passively drooled into the test tube labelled with the letter B. Under normal circumstances, cortisol increases 5-20 minutes after a physiological/psychological stressor, in this case the receiving of mobile text messages. The last saliva sample was taken 20 minutes after the intervention was completed (155–157).

After the lecture students who received the intervention were asked to hand the saliva samples to the researcher and fill in questionnaire 2.

3.10 Methodological and measurement errors

Lectures took place in sizable venues, thus respondents were not seated in close proximity to one another. The group receiving the intervention did not affect the group not receiving the intervention. Respondents who received text messages were seated at the back of the venue. Respondents that did not receive text messages were seated in the front of the venue. Seating respondents in this manner and the small size of the respondent group caused the least disturbance during the lectures.

Respondents received an instructional guide a week before the study, in order to know exactly how the study would be conducted. A thorough understanding of how the study was conducted and when to provide saliva samples ensured that receiving mobile text messages was the measured stressor.

The learning effect (from one day to the next) applied in some crossover designs was not applicable to this study since respondents did not know when they would receive text messages on the INT (158). Since they also did not receive any text messages on the CONT there was nothing to be "learned" for the INT. Respondents were also asked not to discuss the words sent to them with other respondents or anybody else, to prevent the learning effect.

A possible effect of crossover designs is "carry-over" between one day and the next (159). This effect did not apply to this study since cortisol was measured. The half-life of cortisol is 70-120 minutes (24,25,88); thus any cortisol secretion changes possibly brought on by receiving mobile text messages (intervention) will not be relevant on the following day.

Respondents were not allowed to use their own mobile phones and/or any other mobile devices as this may have acted as a confounder. Each respondent in this study was provided with a mobile phone and the mobile phone was only set to vibrate. The mobile phone was placed close to the respondents to be able to hear the mobile phone when vibrating.

The intervention took place during a Physiology lecture and the before and after measurements took place within a period of 40 minutes. Joubert *et al.* (158) state that before and after measurements are strengthened when the duration between them is short, for example before and after a lecture (p.26).

BulkSMS was used, and respondents received the text messages simultaneously. BulkSMS provided features to configure the time that messages were sent and what the content of those messages were. This study made use of this feature to ensure that the timing and content of messages were precise.

Factors as discussed in section 3.5.3 were used to exclude respondents. This study, however, did not exclude respondents who did not adhere to study instructions. Adherence was measured in Questionnaire 1.

3.11 Data management and analysis

3.11.1 Questionnaire

Completed questionnaires were stored in a locked cabinet to which only the researcher had access.

Results were analysed from the information captured. Statistics were determined with the help of a biostatistician from the Department of Biostatistics, University of the Free State.

Questionnaire 1 included (cf. Appendix F):

a) demographical data (questions 1-9);
b) lifestyle data (questions 10-16);
c) HADS (questions 17-30). The HADS consists of two subsections: one measures anxiety (HADS-A) and the other measures depression (HADS-D);
d) PSS (questions 31-40).

3.11.2 Scoring

3.11.2.1 Demographical data

Questions 1-9 of Questionnaire 1 provided descriptive statistics with regard to age, gender, race and lifestyle.

3.11.2.2 Lifestyle

Questions 10-16 of Questionnaire 1 were closed-ended questions measuring study adherence. If any of the questions were marked '*yes*', respondents did not conform to instructions - 'procedures before the study days' (cf. section 3.8). Data obtained from respondents that did not adhere to study instruction were included in the results.

3.11.2.3 The Hospital Anxiety and Depression Scale

The total for the anxiety and depression subsections were calculated by the sum of each item, as seen in Table 3.2.

Normal=0-7, Borderline=8-10 and Abnormal=11-21.

Table 3.2 HADS-A and HADS-D scoring

Question (italics) and responses HADS-A	Scoring	Question (italics) and responses HADS-D	Scoring
17. I feel tense or wound up.		*18. I still enjoy the things I used to enjoy.*	
Most of the time	3	Definitely as much	0
A lot of the time	2	Not quite so much	1
From time to time	1	Only a little	2
Not at all	0	Hardly at all	3
19. I get sort of frightened feeling something awful is about to happen.		*20. I can laugh and see the funny side of things.*	
Very definitely and quite badly	3	As much as I ever could	0
Yes, but not badly	2	Not quite as much now	1
A little, but it doesn't worry me	1	Definitely not so much now	2
Not at all	0	Not at all	3
21. Worrying thoughts go through my mind.		*22. I feel cheerful.*	
A great deal of the time	3	Not at all	3
A lot of the time	2	Not often	2
From time to time but not too often	1	Sometimes	1
Only occasionally	0	Most of the time	0
23. I can sit at ease and feel relaxed.		*24. I feel as if I am slowed down.*	
Definitely	0	Nearly all the time	3
Usually	1	Very often	2
Not often	2	Sometimes	1
Not at all	3	Not at all	0
25. I get a sort of frightened feeling like "butterflies" in the stomach.		*26. I have lost interest in my appearance.*	
Not at all	0	Definitely	3
Occasionally	1	I don't take as much care as I should	2
Quite often	2	I may not take quite as much care	1
Very often	3	I take just as much care as ever	0
27. I feel restless as if I have to be on the move.		*28. I look forward with enjoyment to things.*	
Very much indeed	3	As much as I ever did	0
Quite a lot	2	Rather less than I used to	1
Not very much	1	Definitely less than I used to	2
Not at all	0	Hardly at all	3
29. I get sudden feelings of panic.		*30. I can enjoy a good book or TV program.*	
Very often indeed	3	Often	0
Quite often	2	Sometimes	1
Not very often	1	Not often	2
Not at all	0	Very seldom	3

3.11.2.4 Perceived Stress Scale

The total was calculated by the sum of the 10 items. Questions 34, 35, 37, 38 were reverse scored, as seen in Table 3.3. The total score ranges from 0-40. Higher scores indicate greater levels of stress.

Table 3.3 PSS scoring

	Never	Almost never	Sometimes	Fairly often	Very often
Questions 31, 32, 33, 36, 39 & 40	0	1	2	3	4
Questions 34, 35, 37 & 38	4	3	2	1	0

3.11.2.5 Subjective experience questionnaire

The second questionnaire included data on respondents' subjective experience of the study. It consisted of five Likert items that were scored as indicated in Table 3.4.

The five questions were:

a) Did the text messages draw your attention away from the lecture?
b) Did you anxiously wait for the text messages?
c) Did you wish the text messages would stop?
d) Did you find this experiment stressful?
e) Would you say this experience changed the way you feel about texting?

Table 3.4 Likert item scoring

Scoring	Strongly disagree	Disagree	Undecided	Agree	Strongly agree
Questions 1-5	0	1	2	3	4

3.12 Statistical analysis

After consultation with the biostatistician at the Department of Biostatistics, University of the Free State, it was decided that t-tests and one-way ANOVAS be used to analyse the data.

3.13 Laboratory procedures

3.13.1 Saliva samples

Saliva cortisol analysis was conducted in the Department of Haematology and Cell Biology, Faculty of Health Sciences, University of the Free State. Analysis was done using a cortisol Saliva ELISA kit provided by IBL International (cf. section 3.14 for assay procedure). One millilitre - two millilitres of saliva was required from each respondent for each sample provided.

Salivary cortisol remains stable at room temperature for 10 days (31), but were stored at -20°C as advised by the ELISA kit user's manual (131) within 30 minutes of when the last samples were provided.

On the day of the analysis, saliva samples were thawed at room temperature for 30 minutes. A 300µl volume of saliva was then aliquoted into a new marked test tube. The sample was then centrifuged at 3000 r/m for 10 minutes (131), leaving debris at the bottom of the test tube and a clear workable suspension on top for pipetting.

3.14 Assay procedure

GILSON PIPETMAN® electronic pipets were used for all steps.

The assay procedure was as follows (131):

a) Wash buffer solution was prepared using 400ml of bi-distilled water and 40ml of the buffer concentrate provided in the kit.
b) 50µl of each calibrator (A-F), control (1 & 2) and sample was dispensed in duplicate into appropriate wells using a new disposable tip each time.
c) 100µl of enzyme conjugate was dispensed into each well using a multichannel pipet. The plate was then covered with adhesive foil and shaken carefully.
d) The plate was incubated for 60 minutes at 24°C on an orbital shaker (575rpm).
e) The adhesive foil was removed and the incubation solution discarded. Thereafter the plate was washed four times with the wash buffer. Excess solution was removed by tapping the inverted plate briskly on a paper towel.
f) 100µl of tetramethylbenzidine (TMB) substrate solution was dispensed into each well using a multichannel pipet. The plate was then covered with adhesive foil and shaken carefully.

g) The plate was incubated for 30 minutes at 24°C on an orbital shaker (575rpm).
h) The enzymatic reaction was stopped by dispensing 100µl TMB stop solution into each well using a multichannel pipet. The colour changed from blue to yellow.
i) Optical density (OD) was measured within 5 minutes using a photometer at 450nm (Reference-wavelength: 600-650nm).

All assay steps were performed in range of the ELISA kit user's manual assay procedure (131).

3.15 Reliability

To increase the reliability of the questionnaires, the following criteria were used:

a) The questionnaires used in the study asked simple questions to obtain data from respondents.
b) The questionnaires read easily and there were no questions that were unclear.
c) The questionnaires started with more neutral questions and later moved on to sensitive and more important questions.

This self-composed questionnaire was compiled from the following freely available sources:

Validated questionnaires

a) The PSS is a measure used to determine to what extent situations in a person's life are appraised as stressful. Cronbach's α ranged from 0.84-0.86. Test-retest reliability 0.85. Correlation between other similar measures ranged from 0.52-0.76 (147).
b) The HADS is a measure used to detect states of anxiety and depression (146). Although originally designed for the hospital setting, it has been found to be a good instrument to use in the general population (160). Bjelland *et al.* (161) report that the correlation between the two subscales ranged from 0.40-0.74, Cronbach's α for HADS-A ranged from 0.68-0.93, Cronbach's α for HADS-D ranged from 0.67-0.90 and the correlation between other similar measures ranged from 0.49-0.83.

Development of the questionnaires used in this study were supported by:

a) Kirschbaum *et al.* (162) and Gaab *et al.* (163) used visual analog scales to rate how stressful respondents found the TSST. This study used Likert items (Questionnaire 2) to determine how respondents subjectively felt about receiving mobile text messages.
b) Lifestyle questions (Questionnaire 1, question10-16) were compiled from Entringer *et al.* (130), Preuss *et al.* (164) and Merz *et al.* (129).

3.16 Validity

The use of validated questionnaires and a standardised method to measure salivary cortisol levels ensured the validity of this study.

3.17 Ethical considerations

3.17.1 Approval

An Evaluation Committee on 20/06/2016 and the Health Sciences Research Ethics Committee (HSREC) at the University of the Free State approved the research study protocol on 26/07/2016 (HSREC number: 116/2016). The dean of the Faculty of Natural and Agricultural Sciences and the dean of the Faculty of Health Sciences provided approval for this study to be conducted. This dual approval was necessary due to respondents being students of the various faculties. It was also approved by the head of the Department of Basic Medical Sciences, Physiology lecturers, the Vice-Rector: Academic and the Dean of Student Affairs (cf. Appendix D).

3.17.2 Information and consent

Students were given an information leaflet (cf. Appendix A) and consent form (cf. Appendix B). The information leaflet and consent form were read to students and questions regarding the study was addressed. Students that were willing to participate were asked to sign the consent form. It was explained to all students that participation was voluntary, that the study posed no threat and that they may discontinue participation at any time during the study. Respondents were not remunerated for their participation.

3.17.3 Anonymity

The questionnaires did not require respondents' names, student numbers or ID numbers. Data gathered were stored on a password protected computer, with an antiviral program. Data and all paper documentation will be destroyed five years after the completion of this dissertation.

3.17.4 Confidentiality

All efforts were made to keep personal information confidential. Only the researcher had access to respondents' personal information. Personal information may be disclosed if required by law. Organisations that may inspect and/or copy research records for quality assurance and data analysis include groups such as the Ethics Committee for Medical Research and the Medicine

Control Council. If results are published, this may lead to cohort identification. No individual results are to be made public, thus individual confidentiality will be maintained.

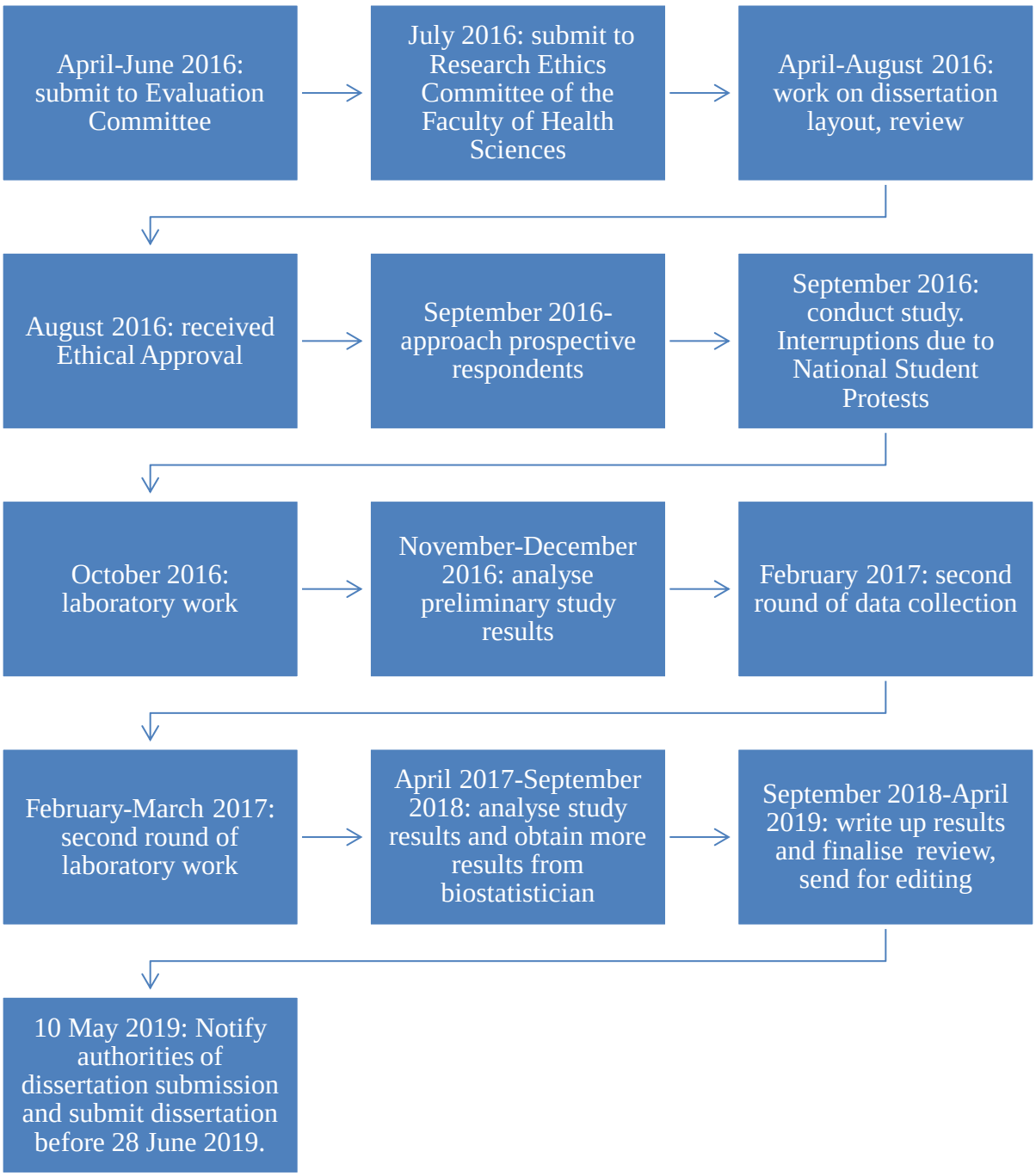
3.17.5 Students and lecturers

To indemnify the researcher and the University of the Free State from academic time loss, a voice recording of each Physiology lecture (in which the study took place) was made. No-one requested a copy of the recording.

To prevent the information session from interfering with students' academic programmes and/or responsibilities, appropriate times and venues were arranged with Physiology lecturers.

Permission to perform the study was also requested from Physiology lecturers (cf. Appendix D).

3.18 Time schedule



3.19 Implementation

Results will be submitted for publication. The results obtained from this study may provide relevant clinical information on psychophysiological stress.

3.20 Chapter summary

This methodology chapter set out a clear framework which enabled the researcher to collect valid and reliable data from the study population of Physiology students at the University of the Free State.

Chapter 4 RESULTS

4.1 Introduction

In this chapter, results of the quantitative statistical analyses of the two questionnaires and salivary cortisol are presented.

4.2 Clarification of abbreviations used

In the methodology chapter sub-questions were formulated to ultimately find the answer to the research question. In this chapter each sub-question is revisited and the results found in this particular study are relayed.

Abbreviations were used in the statistical analysis of the data obtained (cf. Abbreviations). Below follows a list of the abbreviations and an example to aid the reader while reading this chapter.

AFT- After the experiment

BEF- Before the experiment

CONT- Control day

HADS-A- Hospital Anxiety and Depression Scale: Anxiety

HADS-D- Hospital Anxiety and Depression Scale: Depression

INT- Intervention day

PSS- Perceived Stress Scale

A graph having a heading of: "HADS_A_Score_Cont – HADS_A_Score_Int", would imply that data were analysed for the HADS, the anxiety section and data were compared between the control and INT.

4.3 Demographical information

4.3.1 Age, gender, mobile phone data and adherence to study instructions

The first sub-question asked, "*What are the general and texting characteristics of the sample population?"* The results to this question gave a broad overview of the respondents in this study and their texting habits.

Respondents were 22 men and 26 women aged 19-25 years (men: $M = 20.5$, $SD = 1.34$; women: $M = 20.7$, $SD = 1.69$).

Nearly all respondents owned a mobile phone (97.9%) and all respondents could operate a mobile phone (100%).

Respondents reported that 45.8% seldom text, 41.7% never text and 12.5% often text during a lecture.

Respondents reported that 27.7% often receive texts, 63.8% seldom receive texts and 8.50% never receive texts during a lecture.

On the CONT, 64.6% of respondents adhered to study procedures, 35.4% did not adhere to study procedures.

On the INT, 66.7% of respondents adhered to study procedures, 33.3% did not adhere to study procedures.

4.4 Descriptive statistics

In Table 4.1 descriptive statistics for HADS-A scores, HADS-D scores, PSS scores and cortisol are tabulated.

Table 4.1 Descriptive Statistics

The MEANS procedure												
Variable	**N**	**Minimum**	**Lower Quartile**	**Median**	**Upper Quartile**	**Maximum**	**Mean**	**Std Dev**	**Lower 95% CL for Mean**	**Upper 95% CL for Mean**	**Skewness**	**Kurtosis**
HADS_A_score_Cont	48	0.000	4.000	6.000	8.000	16.000	6.229	3.075	5.336	7.122	0.727	1.070
HADS_D_score_Cont	48	0.000	0.500	2.000	6.000	9.000	3.042	2.910	2.197	3.887	0.579	-1.109
PSS_score_Cont	48	3.000	14.000	16.500	20.500	35.000	17.271	6.314	15.438	19.104	0.521	1.604
HADS_A_score_Int	48	0.000	3.500	6.000	7.500	14.000	5.875	3.105	4.973	6.777	0.469	0.132
HADS_D_score_Int	48	0.000	1.000	2.000	6.000	10.000	3.104	2.875	2.269	3.939	0.575	-0.964
PSS_score_Int	48	5.000	13.000	16.000	21.500	33.000	16.854	6.365	15.006	18.702	0.390	0.016
Cortisol_Cont_Bef	48	0.083	0.265	0.401	0.538	1.092	0.419	0.218	0.356	0.482	0.947	1.046
Cortisol_Cont_Aft	48	0.109	0.386	0.525	0.675	1.241	0.564	0.262	0.488	0.641	0.707	0.402
Cortisol_Int_Bef	48	0.095	0.251	0.420	0.553	1.006	0.433	0.222	0.369	0.498	0.607	-0.163
Cortisol_Int_Aft	48	0.072	0.385	0.531	0.869	1.170	0.611	0.314	0.520	0.703	0.346	-1.083
Cortisol_Change_Cont	48	-0.794	0.051	0.125	0.263	0.507	0.145	0.212	0.084	0.207	-1.568	7.084
Cortisol_Change_Int	48	-0.165	0.058	0.139	0.307	0.636	0.178	0.183	0.125	0.231	0.612	-0.047

4.5 Cortisol results

The research question asked, "*How will receiving mobile text messages effect cortisol secretion?"* Various statistical analyses were conducted to determine the effect that texting had on cortisol secretion. Firstly, however, laboratory results are reported in section 4.5.1.

4.5.1 Elisa results

Mean measured OD values for calibration (CAL) A-F (plates 1-5) were in range of the kit validation results (cf. Appendix I, Tables 5.1-5.5). Control 1 & 2 concentrations were in range of the lower and upper limits of the kit's validations for plates 1, 2, 3 and 5. The concentration of control 1 in plate 4 (0.0454 µg/dL) was below the lower limit of the kit validation (0.053 µg/dL).

All samples were tested in duplicate and standard deviation (SD) was determined for CAL A-F (cf. Figure 4.1). No outliers were observed and all SD values were <0.1.

R-squared values for plates 1-5 were all >0.99.

In Figure 4.1 standard curves for each ELISA plate are depicted.

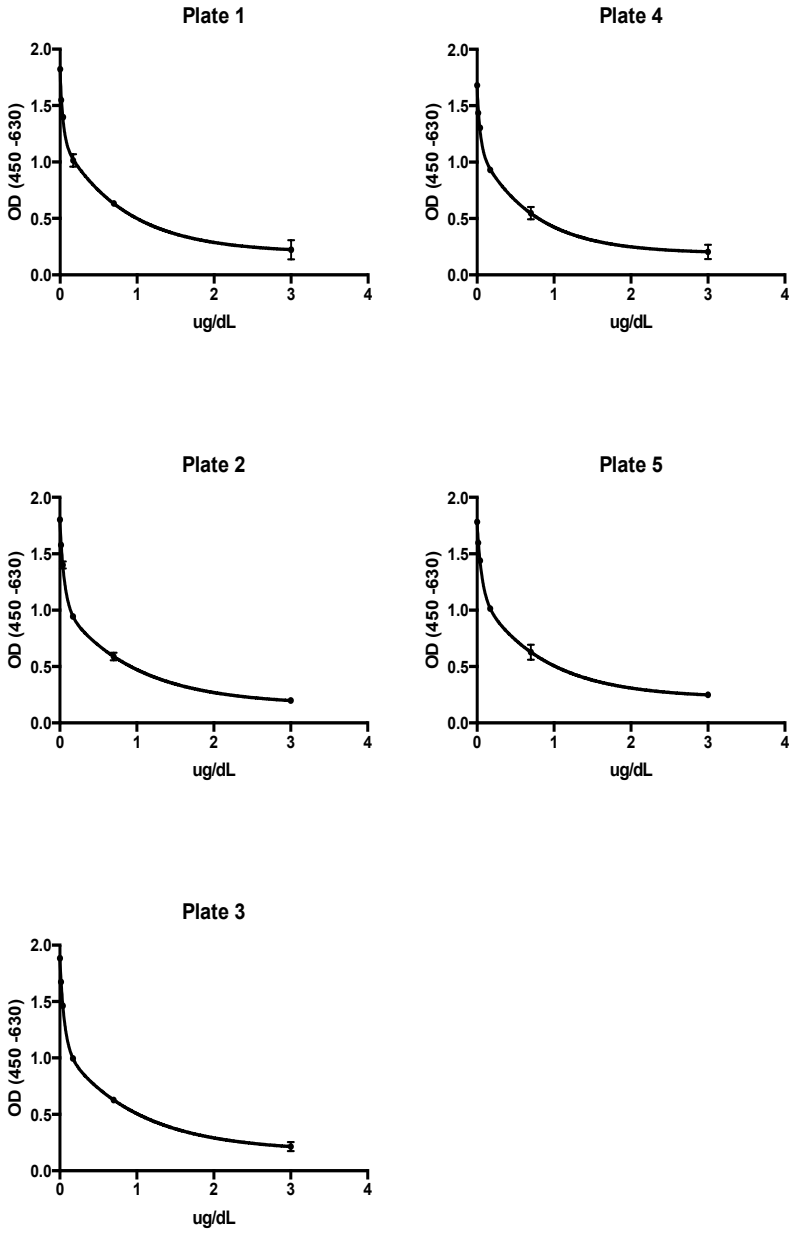


Figure 4.1 Standard curves of plates 1-5

4.5.2 Cortisol statistical analysis

A paired-samples t-test was conducted to compare the cortisol samples taken BEF on the control and INT. There was no significant difference between the CONT (M = 0.42, SD = 0.22) and the INT (M = 0.43, SD = 0.22), $t(47)$ = -0.48, p = .64 (cf. Figure 4.2). The mean cortisol levels BEF on the INT were slightly higher (but not statistically significant) than the mean cortisol levels BEF on the CONT (cf. Figure 4.3).

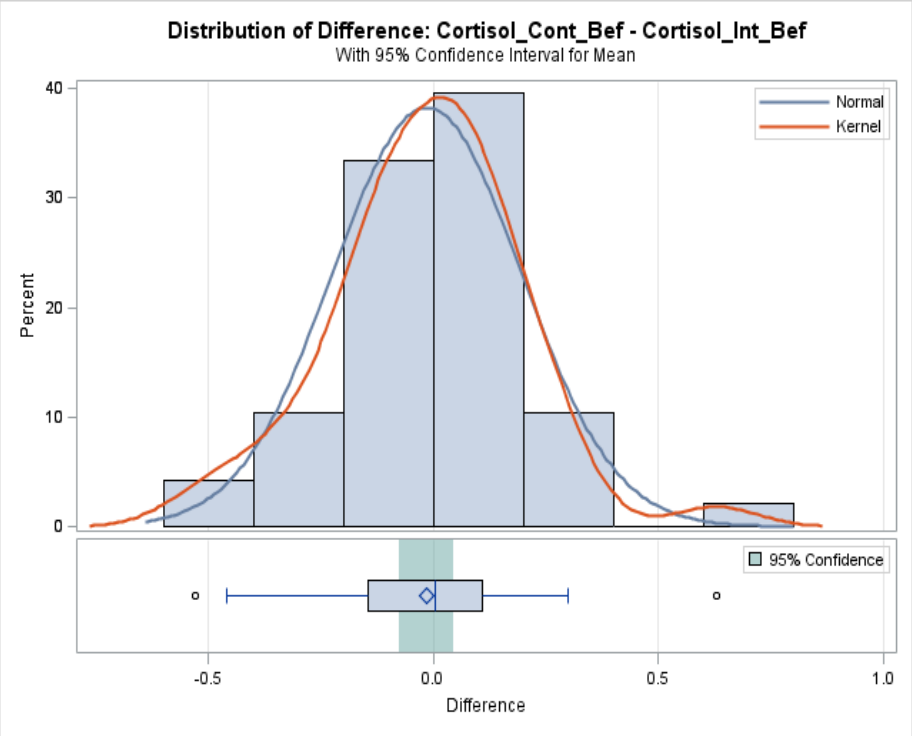


Figure 4.2 Distribution of difference between cortisol levels BEF on the control and INT

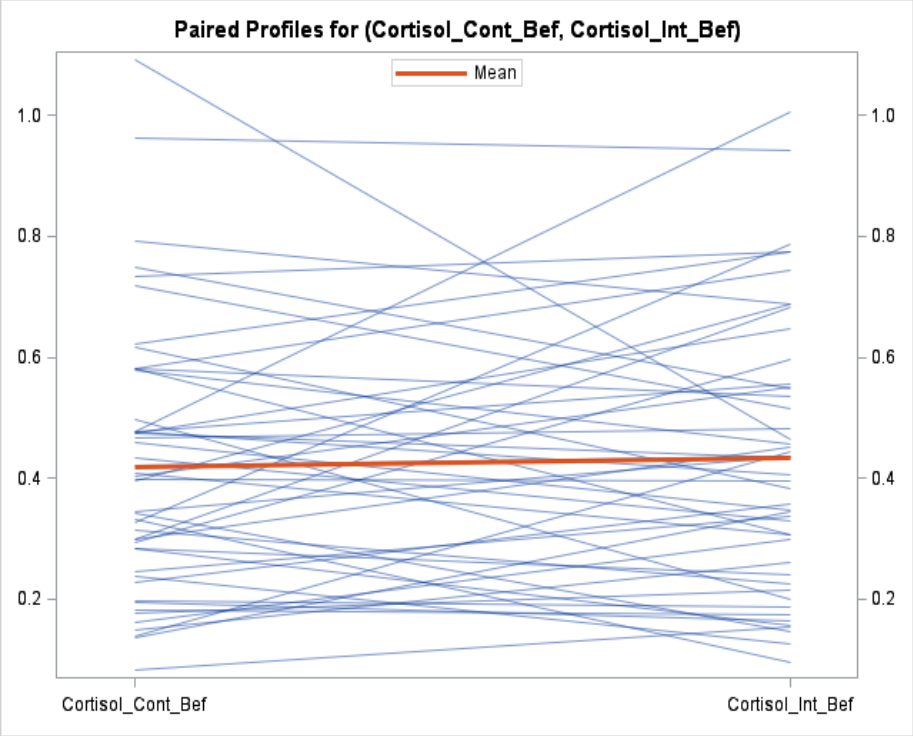


Figure 4.3 Paired profiles plot for cortisol levels BEF on the control and INT

A paired-samples t-test was conducted to compare the cortisol samples taken after the experiment (AFT) on the control and INT. There was no significant difference between the CONT (M = 0.56, SD = 0.26) and the INT (M = 0.61, SD = 0.31), $t(47)$ = -1.11, p = .27 (cf. Figure 4.4). The mean cortisol levels AFT on the INT were slightly higher than the mean cortisol levels AFT on the CONT (cf. Figure 4.5), but not statistically significant.

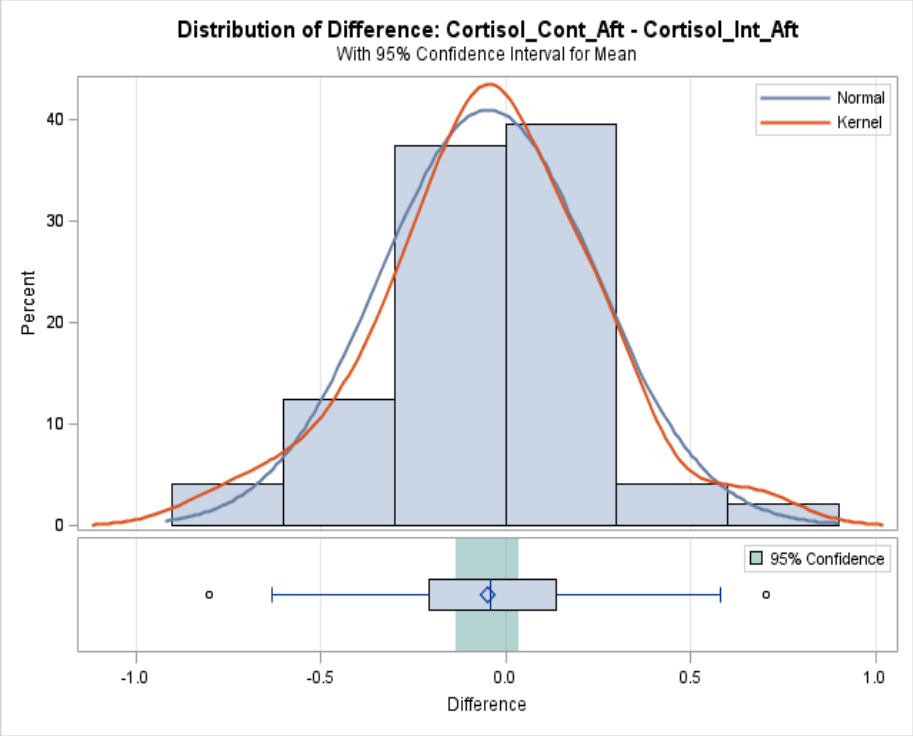


Figure 4.4 Distribution of difference between cortisol levels AFT on the control and INT

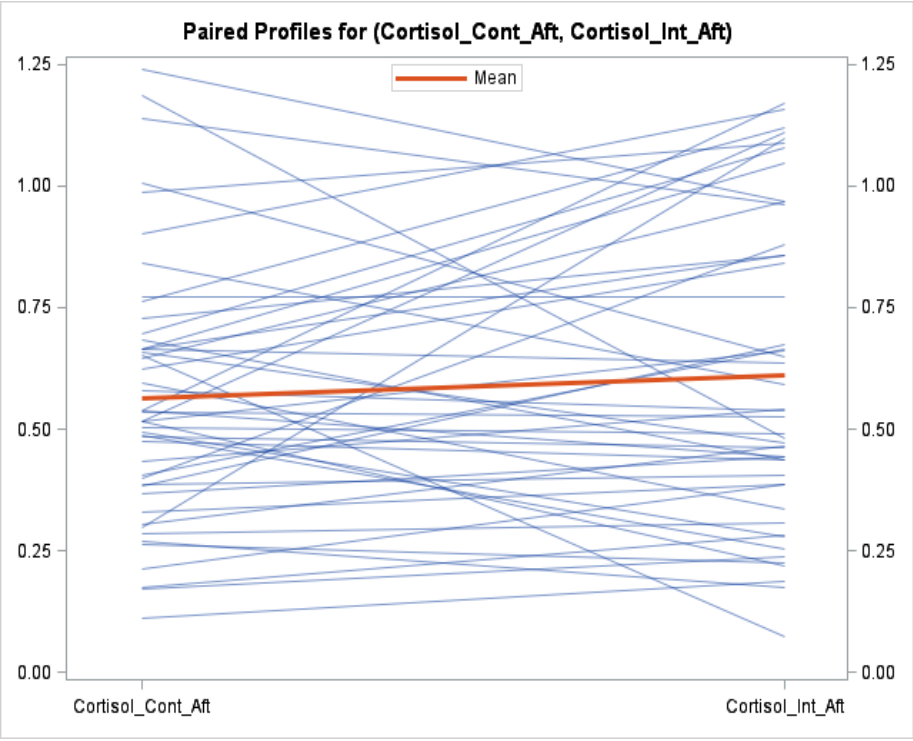


Figure 4.5 Paired profiles plot for cortisol levels AFT on the control and INT

A paired-samples t-test was conducted to compare the cortisol samples taken before and AFT on the CONT. There was a significant difference between the before samples (M = 0.42, SD = 0.22) and the after samples (M = 0.56, SD = 0.26), $t(47) = -4.74$, $p < .001$ (cf. Figure 4.6). The mean cortisol levels AFT on the CONT were higher than the mean cortisol levels BEF on the CONT and are statistically significant (cf. Figure 4.7).

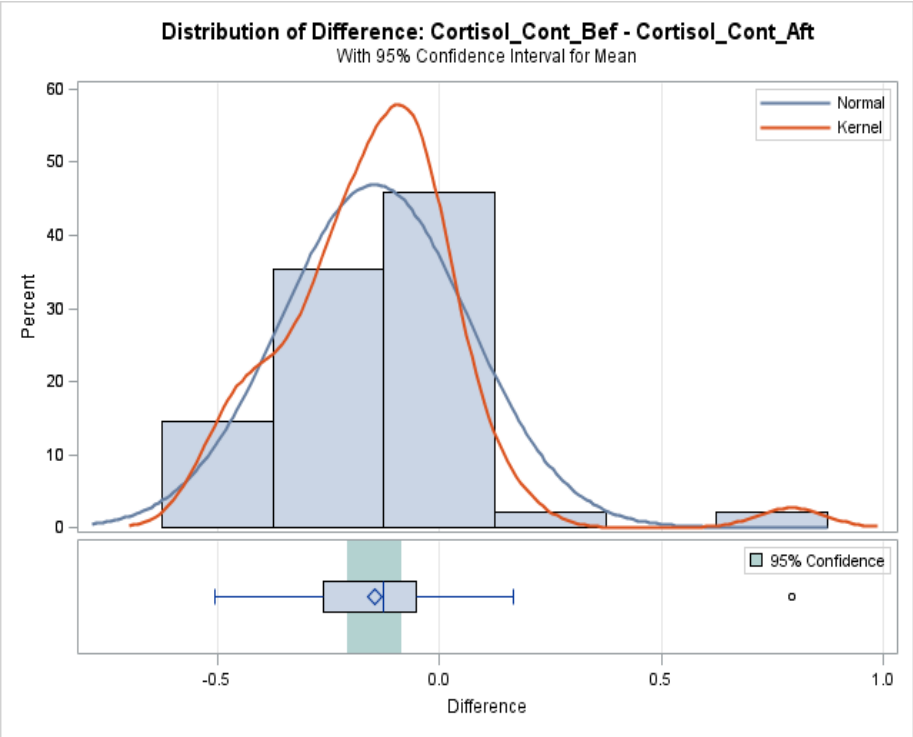


Figure 4.6 Distribution of difference between cortisol levels before and AFT on the CONT

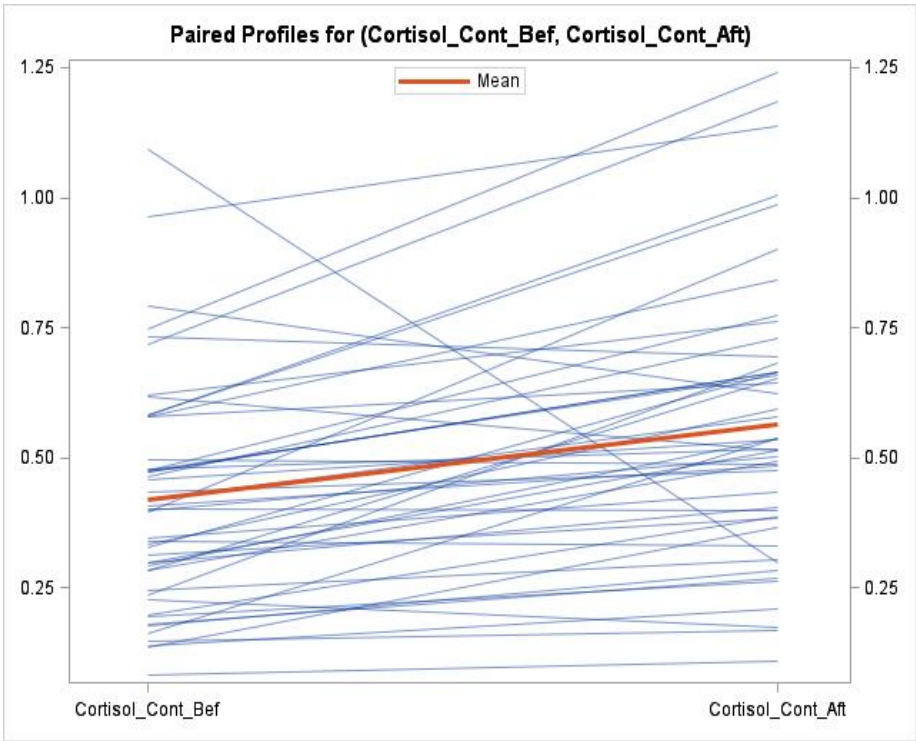


Figure 4.7 Paired profiles plot for cortisol levels before and AFT on the CONT

A paired-samples t-test was conducted to compare the cortisol samples taken before and AFT on the INT. There was a significant difference between the before samples (M = 0.43, SD = 0.22) and the after samples (M = 0.61, SD = 0.31), $t(47) = -6.74$, $p < .001$ (cf. Figure 4.8). The mean for cortisol levels AFT on the INT were higher than the mean cortisol levels BEF on the INT (cf. Figure 4.9), and were statistically significant.

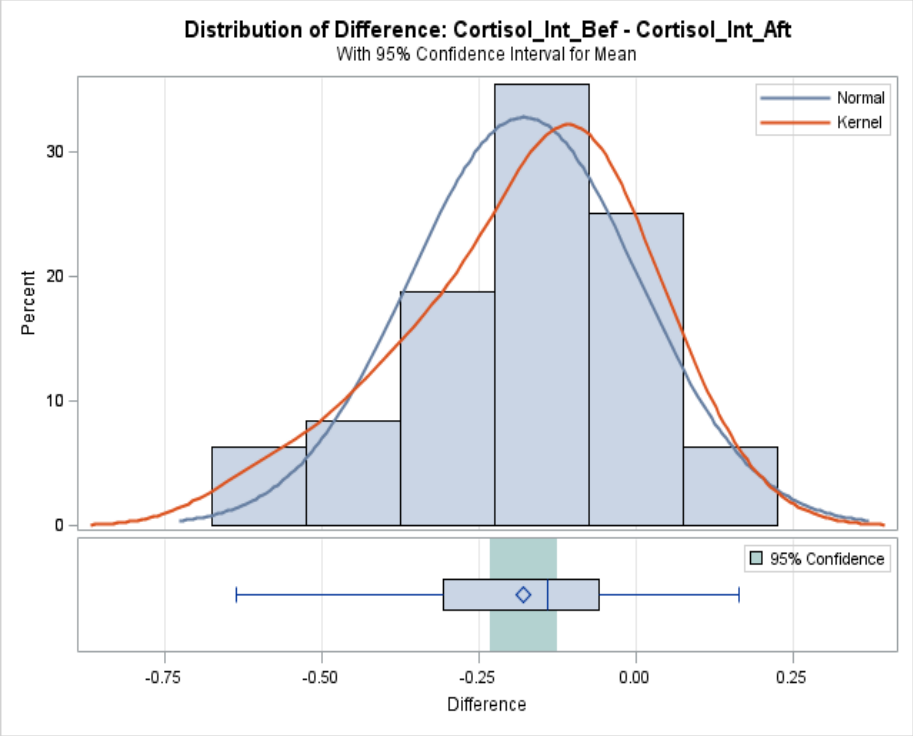


Figure 4.8 Distribution of difference between cortisol levels before and AFT on the INT

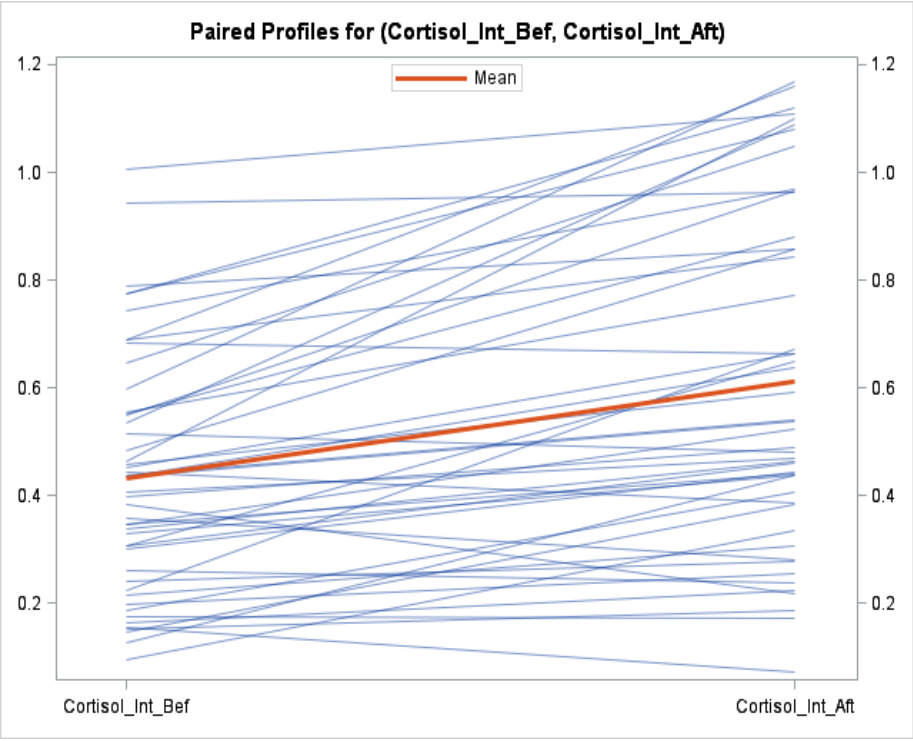


Figure 4.9 Paired profiles plot for cortisol levels before and AFT on the INT

A paired-samples t-test was conducted to compare the change in cortisol levels on the CONT with the change in cortisol levels on the INT. There was no significant difference between the change in cortisol levels on the CONT (M = 0.15, SD = 0.21) and the change in cortisol levels on the INT (M = 0.18, SD = 0.18), $t(47)$ = -0.8, p = .42 (cf. Figure 4.10). The mean for change in cortisol levels on the INT were slightly higher than the mean for change in cortisol levels on the CONT (cf. Figure 4.11).

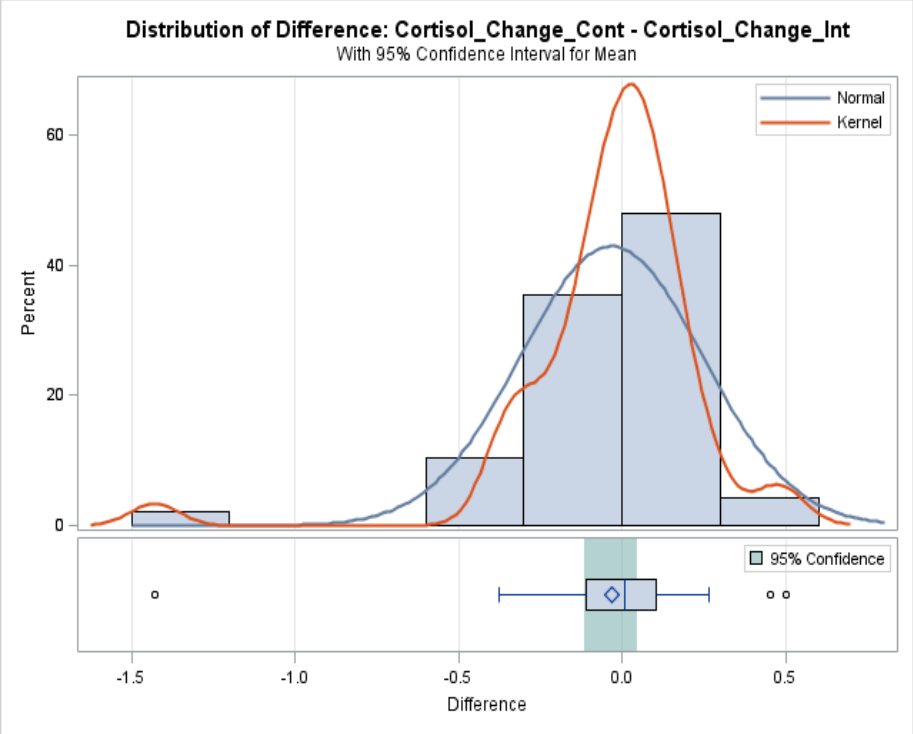


Figure 4.10 Distribution of difference between the change in cortisol levels on the control and INT

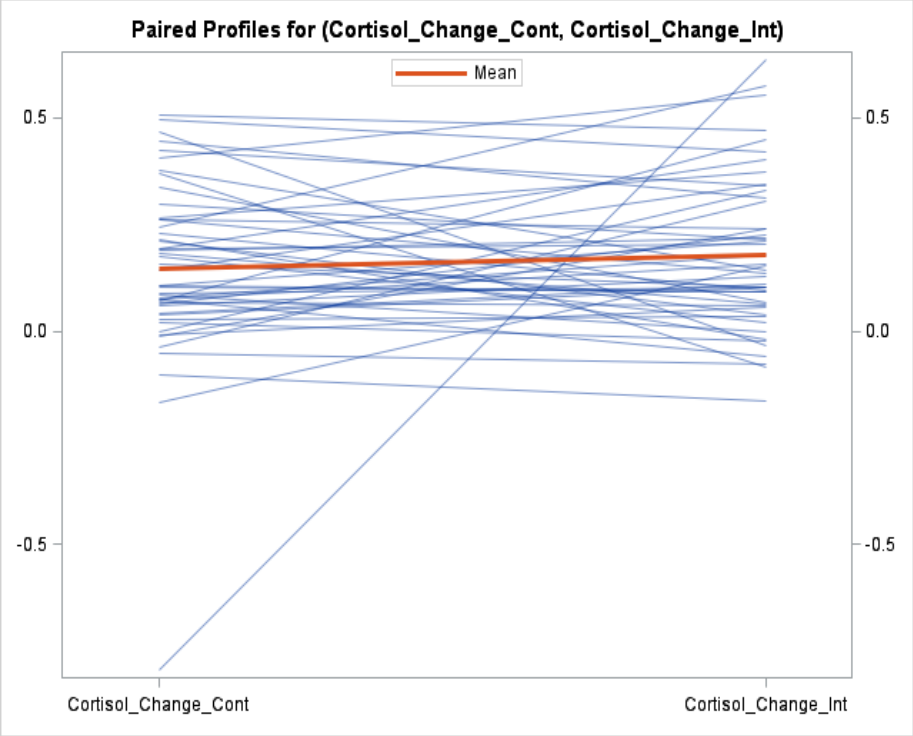


Figure 4.11 Paired profiles plot for the change in cortisol levels on the control and intervention days

4.6 Questionnaires

Questionnaires used measured stress, anxiety and depression in respondents (cf. Appendix F). The information gathered in the questionnaires was used to answer sub-question two: "*Will stress, anxiety and depression have a moderating effect on cortisol secretion while receiving mobile text messages?"* Questionnaire 2 was used to question respondents on their experience of the experiment (receiving mobile text messages during a lecture). The subjective feedback received from respondents was used to determine if it would correlate to the objective data gathered from the cortisol analysis. Various statistical analyses were conducted to determine if any of these factors had a moderating effect on cortisol secretion.

4.6.1 The Hospital Anxiety and Depression Scale-Anxiety

The frequency procedure was used to determine HADS-A scores (normal, borderline and abnormal) within the sample population on the control and INT (cf. Tables 4.2 & 4.3).

Table 4.2 HADS-A Score INT

Table of HADS-A Score INT				
HADS A Score	**Normal**	**Borderline**	**Abnormal**	**Total**
0	1	0	0	1
1	1	0	0	1
2	6	0	0	6
3	4	0	0	4
4	5	0	0	5
5	4	0	0	4
6	7	0	0	7
7	8	0	0	8
8	0	3	0	3
9	0	4	0	4
10	0	2	0	2
12	0	0	1	1
13	0	0	1	1
14	0	0	1	1
Total	36	9	3	48

Table 4.3 HADS-A Score CONT

Table of HADS-A Score CONT				
HADS A Score	**Normal**	**Borderline**	**Abnormal**	**Total**
0	1	0	0	1
1	1	0	0	1
3	8	0	0	8
4	4	0	0	4
5	7	0	0	7
6	7	0	0	7
7	7	0	0	7
8	0	3	0	3
9	0	4	0	4
11	0	0	4	4
12	0	0	1	1
16	0	0	1	1
Total	35	7	6	48

A paired-samples t-test was conducted to compare HADS-A scores between the control and INT. There was no significant difference between the CONT (M = 6.23, SD = 3.01) and the INT (M = 5.88, SD = 3.11), $t(47) = 1.40$, $p = .17$ (cf. Figure 4.12). The mean score on the CONT was slightly higher than the mean score on the INT (cf. Figure 4.13).

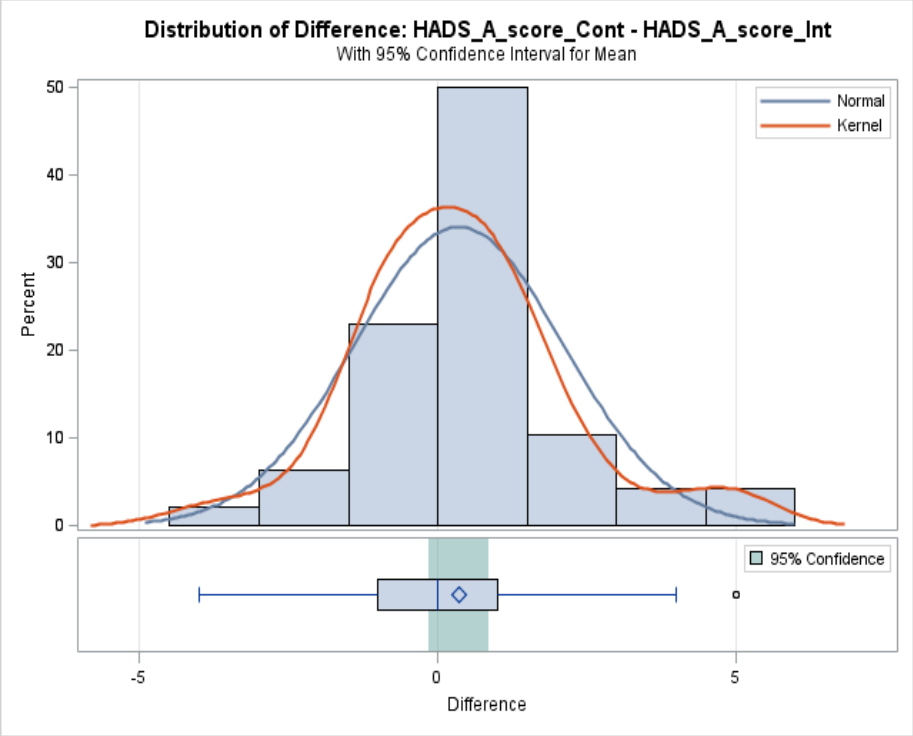


Figure 4.12 Distribution of difference between HADS-A scores on the control and intervention days

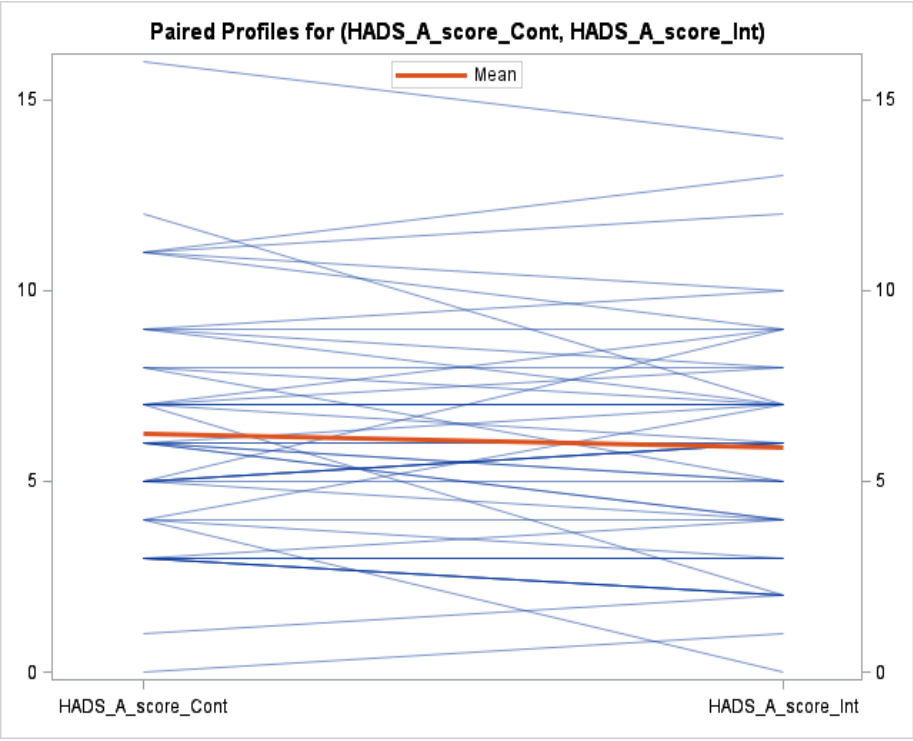


Figure 4.13 Paired profiles plot for reported HADS-A scores on the control and intervention days

A one-way between subjects ANOVA was conducted to compare the effect of HADS-A scores on cortisol levels taken BEF on the control and INT for abnormal, borderline and normal anxiety conditions. There was no significant difference between anxiety and cortisol levels taken BEF for the three conditions on the CONT [F(2, 45) = 0.77, p = .47] (cf. Figure 4.14). There was a significant difference between anxiety and cortisol levels on the INT [F(2, 45) = 4.00, p = .025] (cf. Figure 4.15). Post hoc analyses using Scheffe's post hoc criterion for significance indicated that subjects with abnormal HADS-A scores had higher cortisol levels than subjects who reported normal HADS-A scores. The normal and borderline groups did not significantly differ.

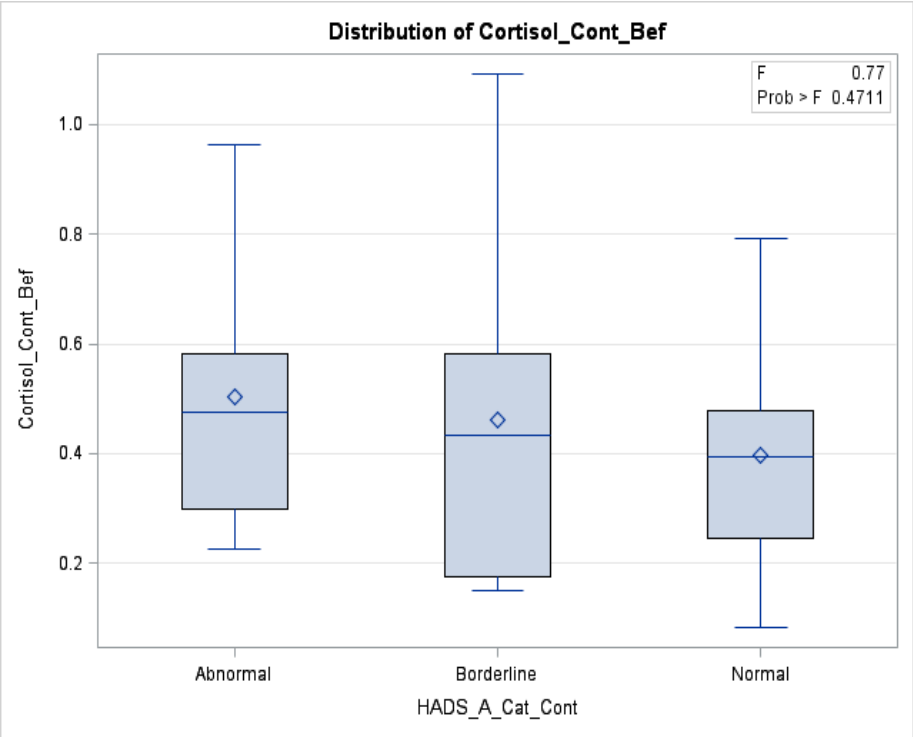


Figure 4.14 Box plot of the distribution between HADS-A scores and cortisol levels BEF on the CONT

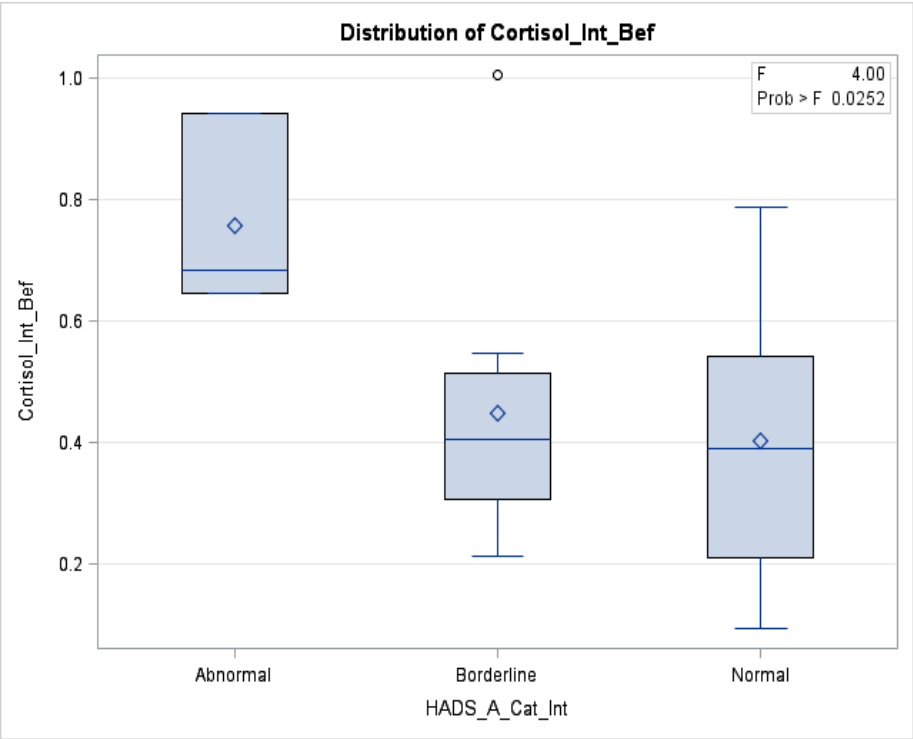


Figure 4.15 Box plot of the distribution between HADS-A scores and cortisol levels BEF on the INT

4.6.2 The Hospital Anxiety and Depression Scale-Depression

The frequency procedure was used to determine HADS-D scores (normal, borderline and abnormal) within the sample population on the control and INT (cf. Tables 4.4 & 4.5).

Table 4.4 HADS-D Score INT

Table of HADS-D Score INT			
HADS D Score	**Normal**	**Borderline**	**Total**
0	11	0	11
1	9	0	9
2	7	0	7
3	1	0	1
4	4	0	4
5	3	0	3
6	4	0	4
7	6	0	6
8	0	2	2
10	0	1	1
Total	45	3	48

Table 4.5 HADS-D Score CONT

Table of HADS-D Score CONT			
HADS D Score	**Normal**	**Borderline**	**Total**
0	12	0	12
1	9	0	9
2	6	0	6
3	3	0	3
4	2	0	2
5	2	0	2
6	5	0	5
7	6	0	6
8	0	1	1
9	0	2	2
Total	45	3	48

A paired-samples t-test was conducted to compare HADS-D scores between the control and INT. There was no significant difference between the CONT (M = 3.04, SD = 2.91) and the INT (M = 3.10, SD = 2.88) , $t(47)$ = -0.35, p = .73 (cf. Figure 4.16). The mean score on the INT was slightly higher than the mean score on the CONT (cf. Figure 4.17).

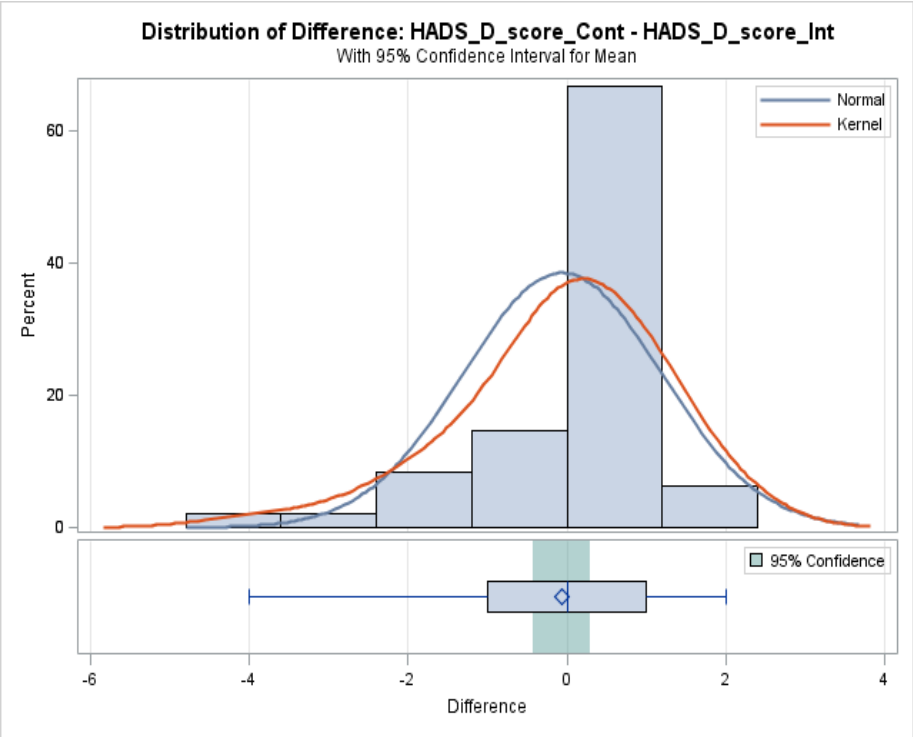


Figure 4.16 Distribution of difference between HADS-D scores on the control and intervention days

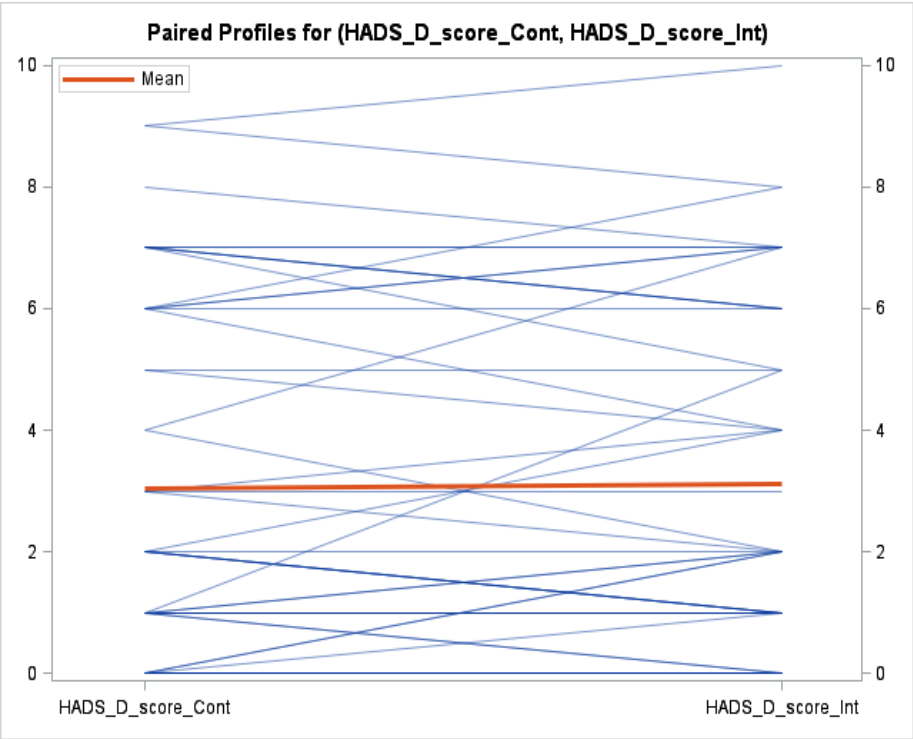


Figure 4.17 Paired profiles plot for reported HADS-D scores on the control and INT

A one-way between subjects ANOVA was conducted to compare the effect of HADS-D scores on cortisol levels taken BEF on the control and INT for borderline and low depression conditions. There was no significant difference between depression and cortisol levels taken BEF for the two conditions on the CONT [F(1, 46) = 3.39, p = .072] or the INT [F(1, 46) = 2.92, p = .094] (cf. Figures 4.18 & 4.19).

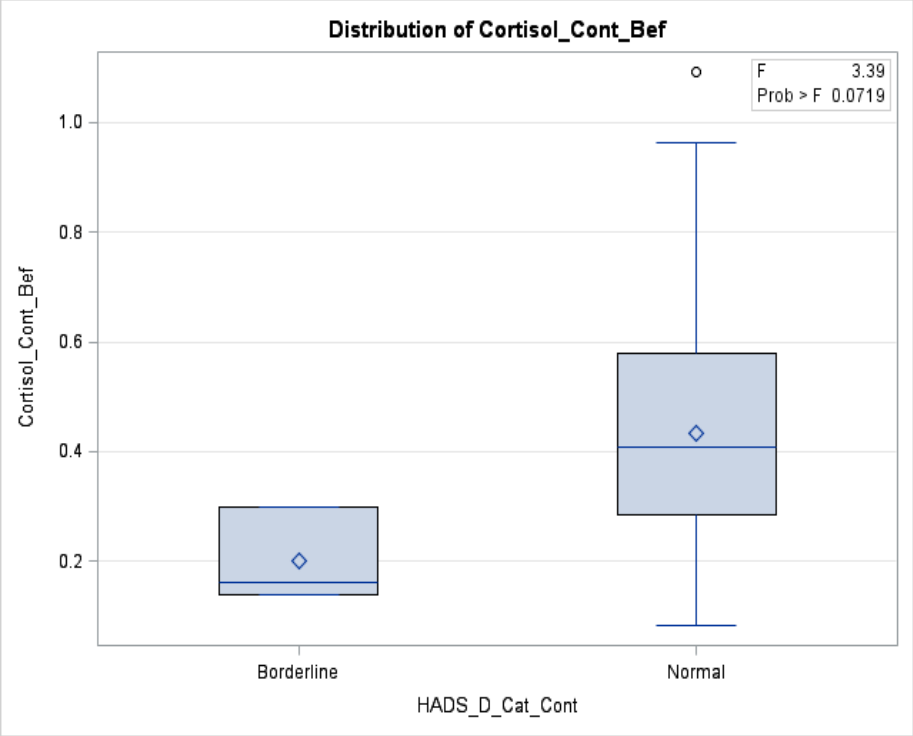


Figure 4.18 Box plot of the distribution between HADS-D scores and cortisol levels BEF on the CONT

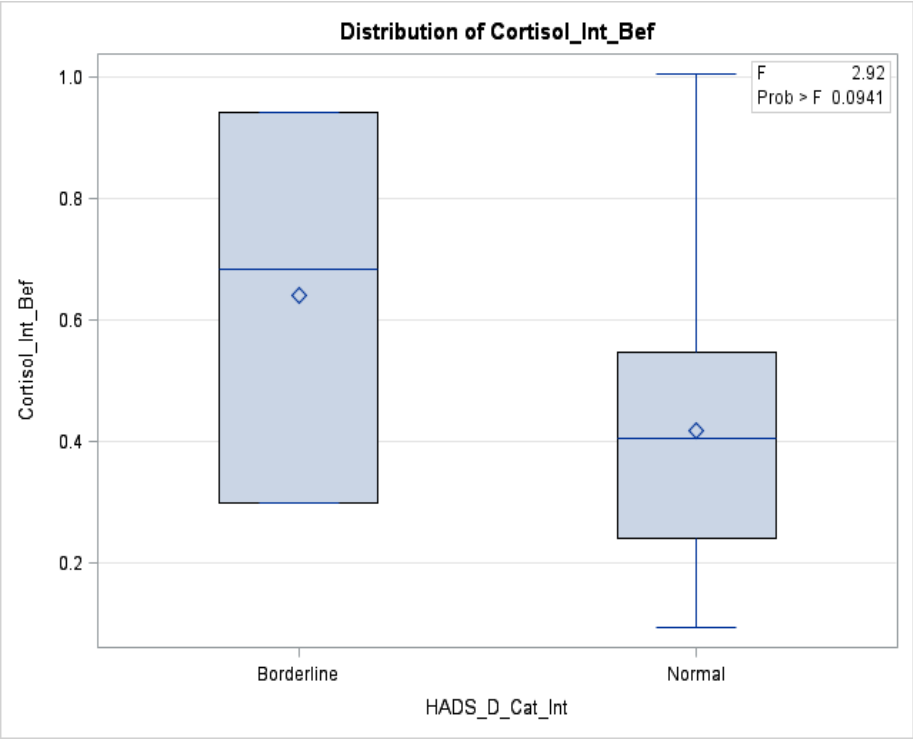


Figure 4.19 Box plot of the distribution between HADS-D scores and cortisol levels BEF on the INT

4.6.3 Perceived Stress Scale

The frequency procedure was used to determine PSS scores (low stress, moderate stress and high stress) within the sample population on the control and INT (cf. Tables 4.6 & 4.7).

Table 4.6 PSS Score INT

Table of PSS Score INT				
PSS Score	**Low Stress**	**Moderate Stress**	**High Stress**	**Total**
5	1	0	0	1
6	2	0	0	2
7	1	0	0	1
9	1	0	0	1
10	1	0	0	1
11	2	0	0	2
12	3	0	0	3
13	4	0	0	4
14	0	7	0	7
15	0	1	0	1
16	0	2	0	2
17	0	2	0	2
18	0	4	0	4
19	0	1	0	1
20	0	1	0	1
21	0	3	0	3
22	0	2	0	2
23	0	3	0	3
24	0	1	0	1
25	0	3	0	3
26	0	1	0	1
32	0	0	1	1
33	0	0	1	1
Total	15	31	2	48

Table 4.7 PSS Score CONT

Table of PSS Score CONT				
PSS Score	**Low Stress**	**Moderate Stress**	**High Stress**	**Total**
3	1	0	0	1
4	1	0	0	1
7	1	0	0	1
9	1	0	0	1
10	1	0	0	1
11	2	0	0	2
13	3	0	0	3
14	0	4	0	4
15	0	4	0	4
16	0	6	0	6
17	0	4	0	4
18	0	3	0	3
19	0	3	0	3
20	0	2	0	2
21	0	1	0	1
22	0	2	0	2
23	0	4	0	4
24	0	1	0	1
26	0	1	0	1
27	0	0	1	1
35	0	0	2	2
Total	10	35	3	48

A paired-samples t-test was conducted to compare PSS scores between the control and INT. There was no statistical significant difference between the CONT (M = 17.3, SD = 6.31) and the INT (M = 16.9, SD = 6.4), $t(47) = 0.89$, $p = .38$ (cf. Figure 4.20). The mean score on the CONT was slightly higher than the mean score on the INT (cf. Figure 4.21).

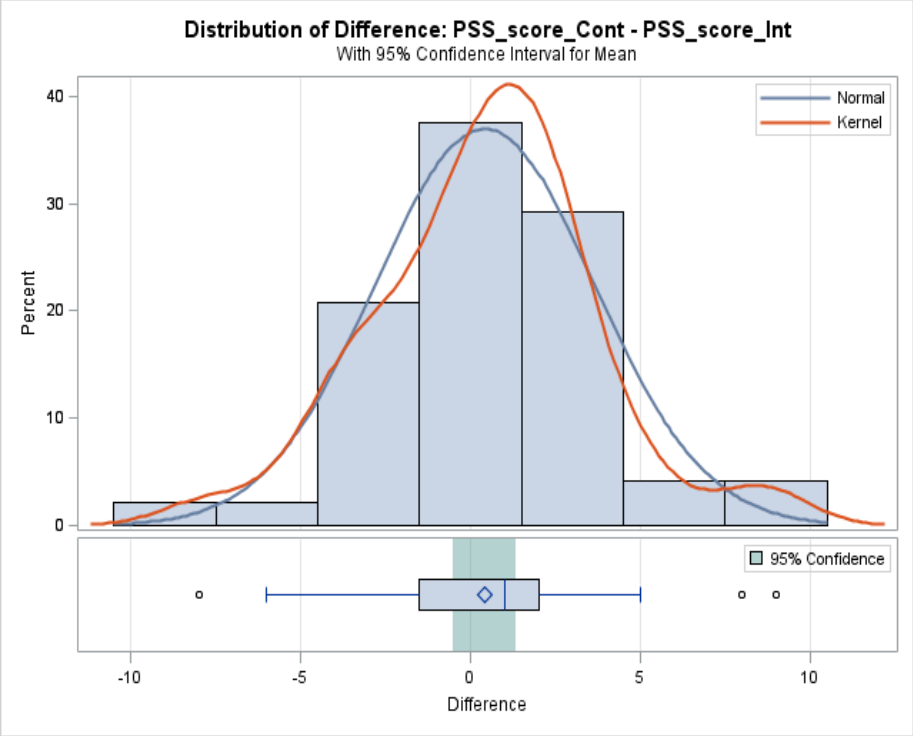


Figure 4.20 Distribution of difference between PSS scores on the control and intervention days

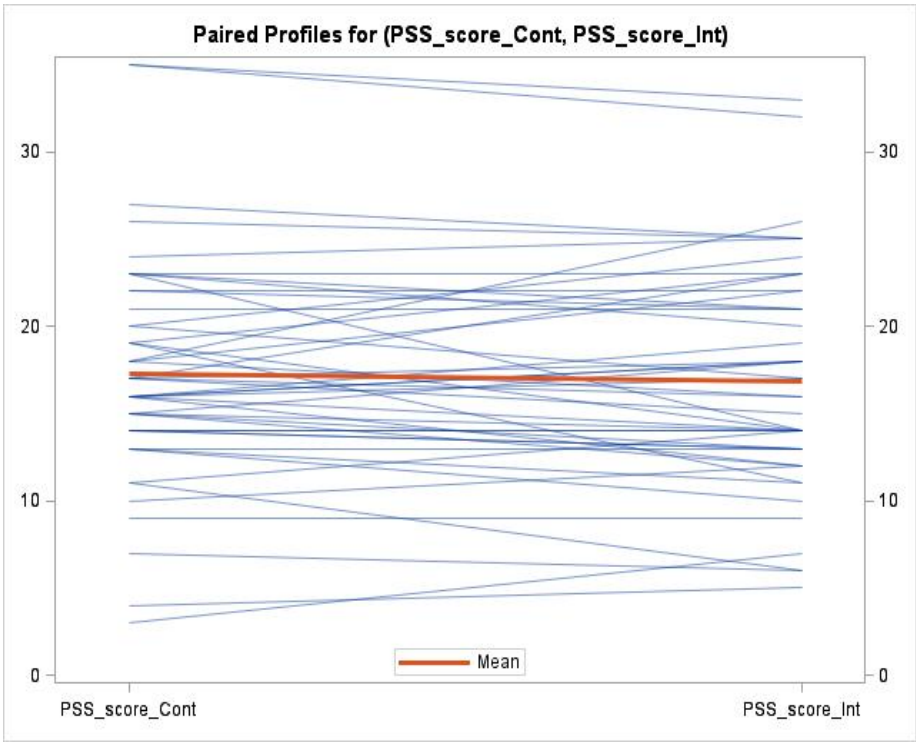


Figure 4.21 Paired profiles plot for reported PSS scores on the control and INT

A one-way between subjects ANOVA was conducted to compare the effect of PSS scores on cortisol levels taken BEF on the control and INT for high stress, moderate stress and low stress conditions. There was no significant difference between stress and cortisol levels taken BEF

for the three conditions on the CONT [F(2, 45) = 0.01, p = .99] or the INT [F(2, 45) = 0.11, p = .90] (cf. Figures 4.22 & 4.23).

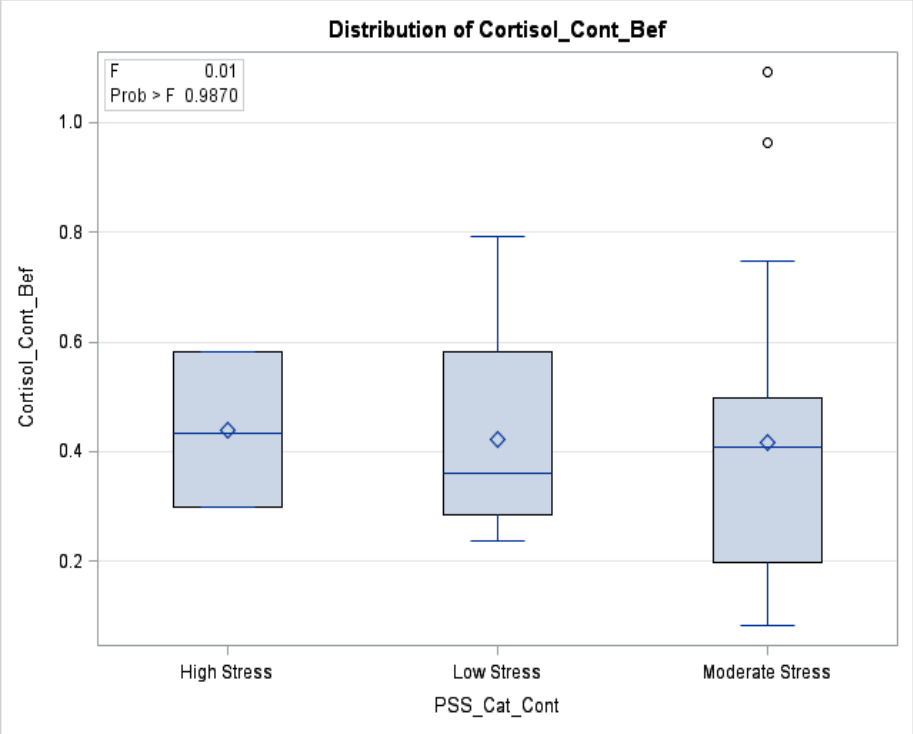


Figure 4.22 Box plot of the distribution between PSS scores and cortisol levels BEF on the CONT

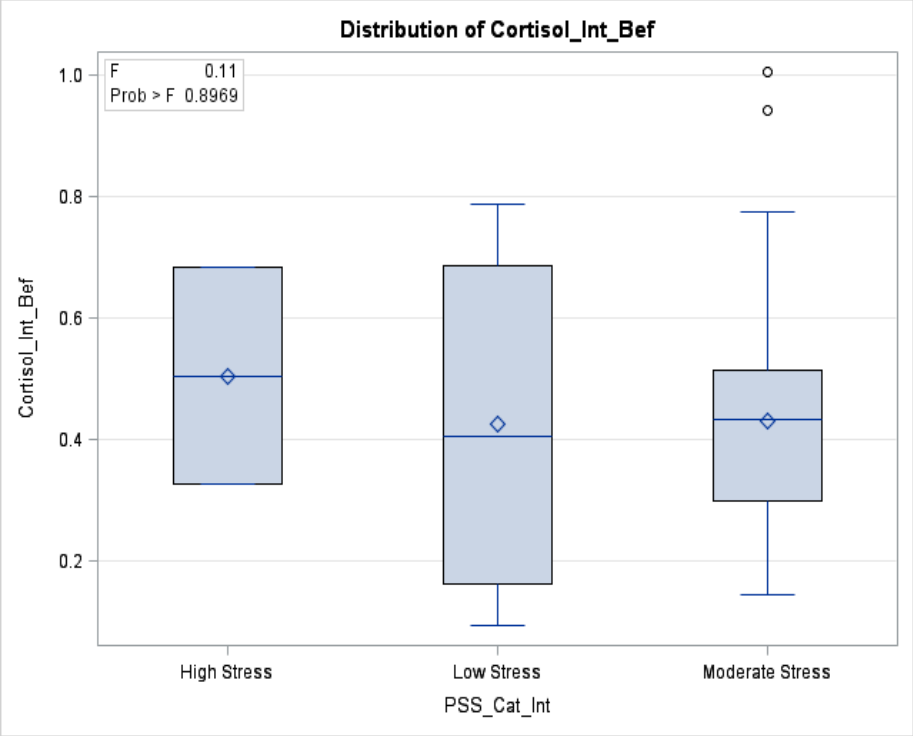


Figure 4.23 Box plot of the distribution between PSS scores and cortisol levels BEF on the INT

4.6.4 Subjective experience of receiving mobile text messages

Respondents completed the subjective experience questionnaire on the INT and rated each question. For statistical analysis, two groups were used; agreement and disagreement. Agreement included *strongly agree* and *agree* responses, disagreement included *strongly disagree*, *disagree* and *undecided* responses (cf. Table 4.8).

Table 4.8 Subjective experience questionnaire responses

Table of Subjective Experience Score					
	Text Messages Caused Distraction From the Lecture	**Anxiously Waited for Text Messages**	**Wished Text Messages Would Stop**	**Found Experience Stressful**	**Feel Different About Texting**
Agreement	38	25	34	5	23
Disagreement	10	23	14	43	25
Total	48	48	48	48	48

An independent-samples t-test was conducted to compare the change in cortisol levels on the INT with respondents' agreement (strongly agreed and agreed) or disagreement (strongly disagree, disagree and undecided) to the question "*Did the text messages draw your attention away from the lecture?"* There was no significant difference in the scores for agreement (M = 0.26, SD = 0.18) and disagreement (M = 0.16, SD = 0.18), t (46) = 1.54, p = .13 (cf. Figure 4.24).

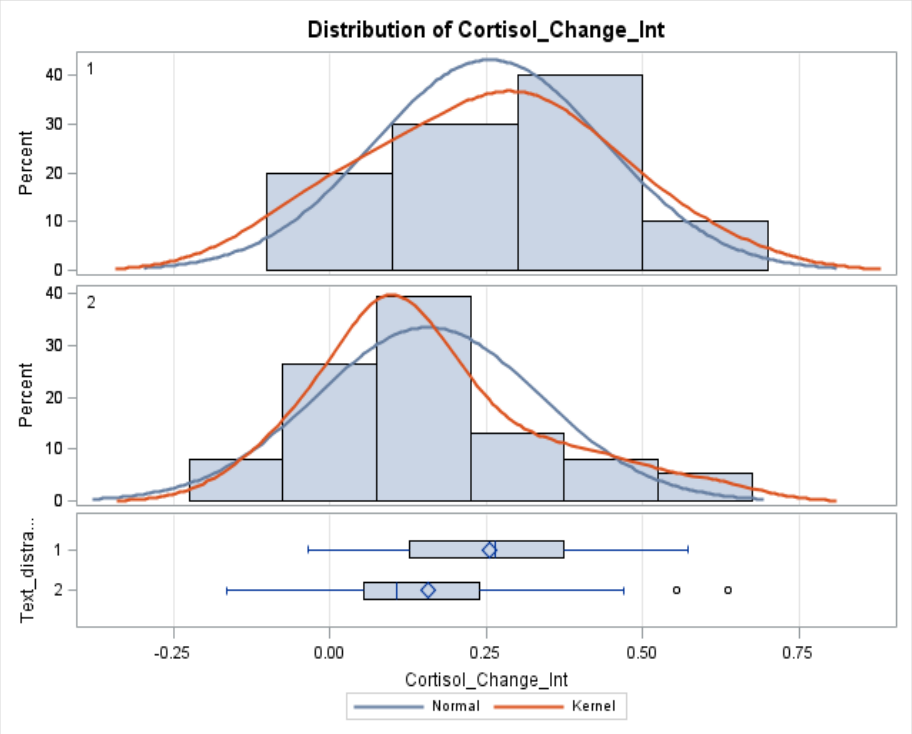


Figure 4.24 Distribution of the change in cortisol levels on the INT and agreement (1) or disagreement (2) of the question "*Did the text messages draw your attention away from the lecture?"*

An independent-samples t-test was conducted to compare the change in cortisol levels on the INT with respondents' agreement (*strongly agreed* and *agreed*) or disagreement (*strongly disagree*, *disagree* and *undecided*) to the question "*Did you anxiously wait for the text messages?"* resulted in no significant difference in the scores for agreement (M = 0.16, SD = 0.18) and disagreement (M = 0.20, SD = 0.18), t (46) = 0.79, p = .43 (cf. Figure 4.25).

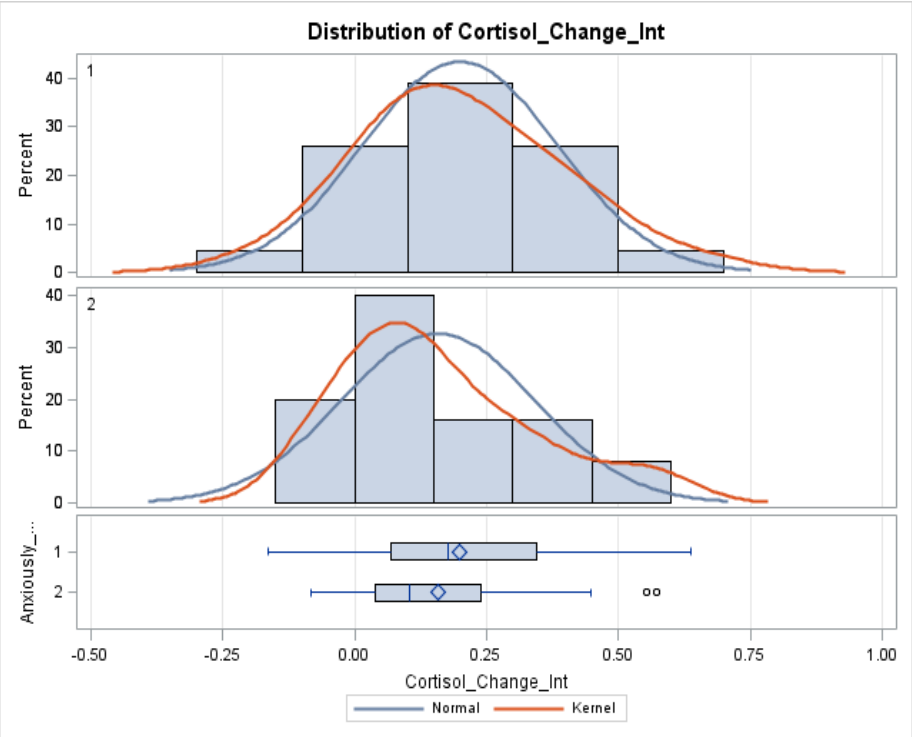


Figure 4.25 Distribution of the change in cortisol levels on the INT and disagreement (1) or agreement (2) of the question "*Did you anxiously wait for the text messages?"*

An independent-samples t-test was conducted to compare the change in cortisol levels on the INT with respondents' agreement (*strongly agreed* and *agreed*) or disagreement (*strongly disagree*, *disagree* and *undecided*) to the question "*Did you wish the text messages would stop*?" resulted in no significant difference in the scores for agreement (M = 0.17, SD = 0.17) and disagreement (M = 0.20, SD = 0.22), t (46) = 0.56, p = .58 (cf. Figure 4.26).

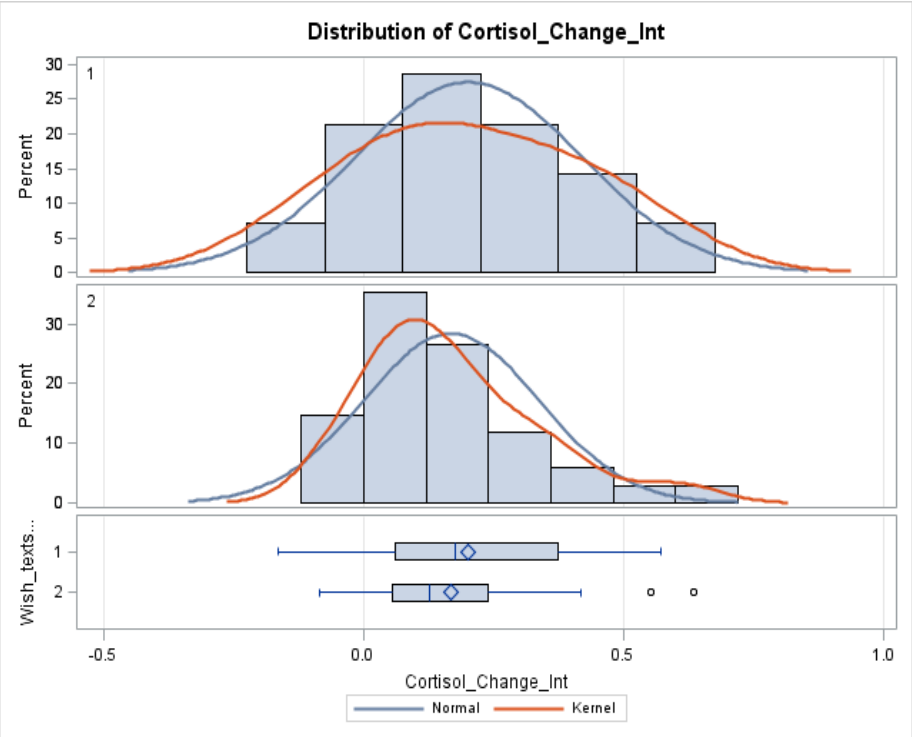


Figure 4.26 Distribution of the change in cortisol levels on the INT and disagreement (1) or agreement (2) of the question "*Did you wish the text messages would stop?"*

An independent-samples t-test was conducted to compare the change in cortisol levels on the INT with respondents' agreement (*strongly agreed* and *agreed*) or disagreement (*strongly disagree, disagree* and *undecided*) to the question "*Did you find this experiment stressful?*" resulted in no significant difference in the scores for agreement (M = 0.11, SD = 0.042) and disagreement (M = 0.19, SD = 0.19), t (46) = 0.90, p = .37 (cf. Figure 4.27).

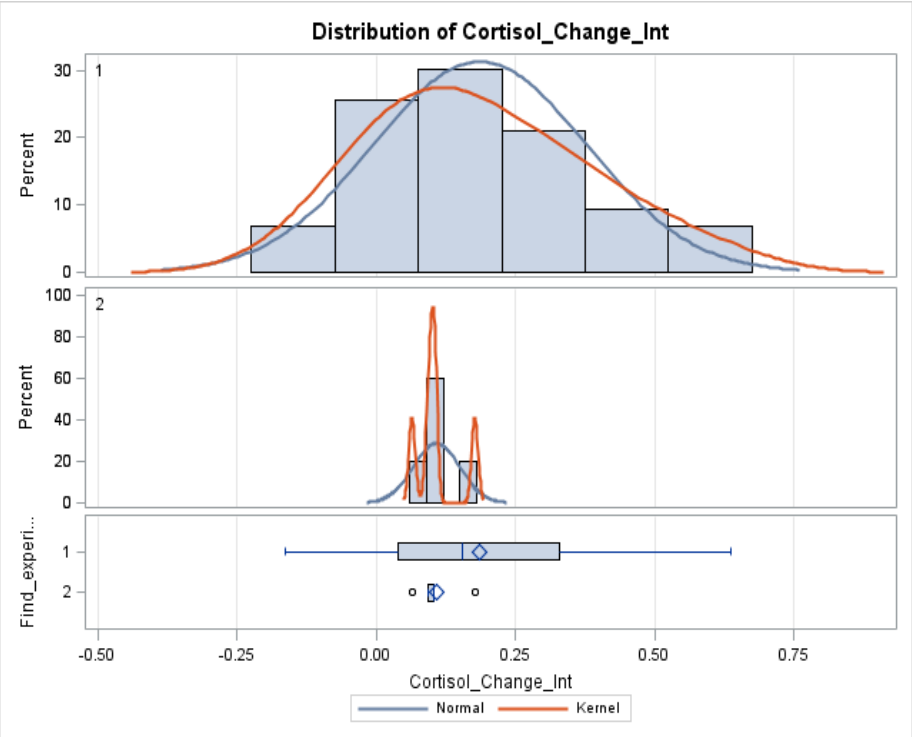


Figure 4.27 Distribution of the change in cortisol levels on the INT and disagreement (1) or agreement (2) of the question "*Did you find this experiment stressful?"*

An independent-samples t-test was conducted to compare the change in cortisol levels on the INT with respondents' agreement (*strongly agreed* and *agreed*) or disagreement (*strongly disagree*, *disagree* and *undecided*) to the question "*Would you say this experience changed the way you feel about texting?*" resulted in no significant difference in the scores for agreement ($M = 0.15$, $SD = 0.18$) and disagreement ($M = 0.21$, $SD = 0.18$), $t(46) = 1.09$, $p = .28$ (cf. Figure 4.28).

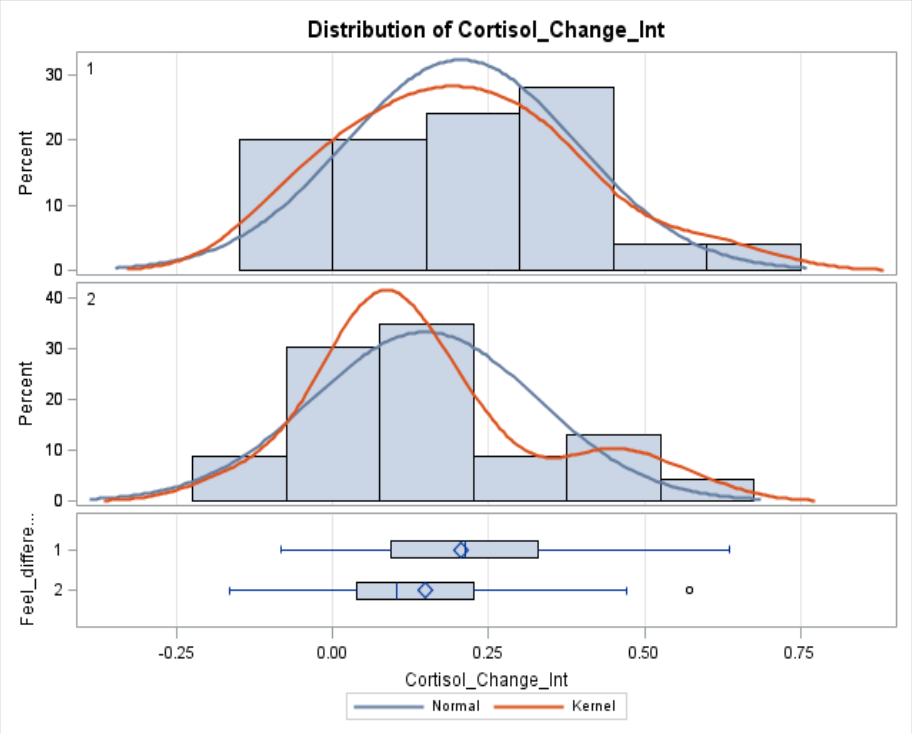


Figure 4.28 Distribution of the change in cortisol levels on the INT and disagreement (1) or agreement (2) of the question "*Would you say this experience changed the way you feel about texting*?"

4.7 Text frequency and emotion

The final sub-question considered if text frequency and the use of neutral, positive or negative words in text messages will have a moderating effect on cortisol secretion.

A one-way between subjects ANOVA was conducted to compare the effect of text frequency on the change in cortisol levels on the INT for conditions of 10 text messages, 15 text messages or 20 text messages. There was no significant difference between text frequency and the change in cortisol levels on the INT for the three conditions [F(2, 45) = 2.50, p = .093] (cf. Figure 4.29).

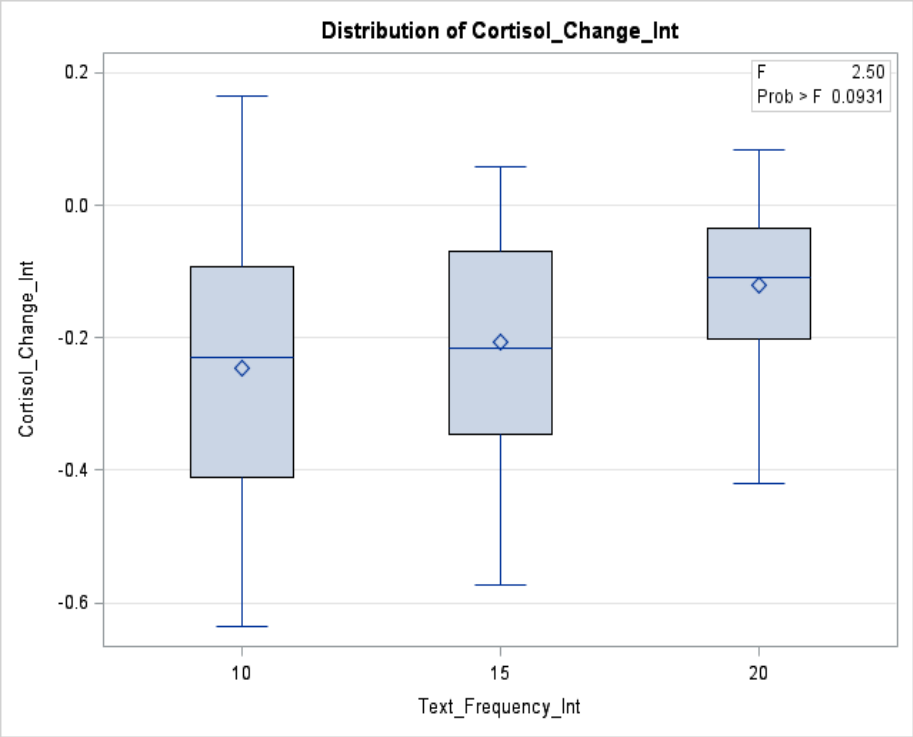


Figure 4.29 Box plot of the distribution between text frequency and the change in cortisol levels on the INT

A one-way between subjects ANOVA was conducted to compare the effect of text emotion on the change in cortisol levels on the INT for negative words, neutral words or positive word conditions. There was no significant difference between text emotion and the change in cortisol levels on the INT for the three conditions, [F(2, 45) = 0.35, p = .71] (cf. Figure 4.30).

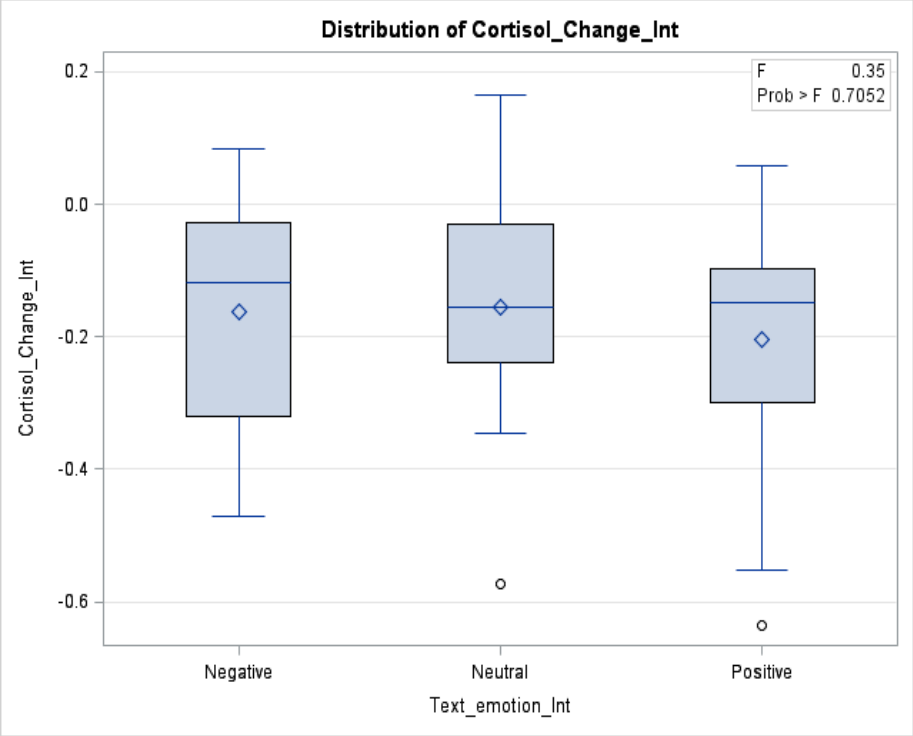


Figure 4.30 Box plot of the distribution between text emotion and the change in cortisol levels on the INT

4.8 Chapter summary

In this chapter the results of demographical information, cortisol levels, questionnaires, the subjective experience of receiving text messages, text frequency and emotion were relayed.

Chapter 5 DISCUSSION

5.1 Introduction

In this chapter, the results presented in Chapter 4 are discussed, followed by the limitations of this study, the final conclusion and summary.

5.2 Demographical information

5.2.1 Age

General and texting characteristics were collected using a questionnaire. Respondents (men age: $M = 20.5$, $SD = 1.34$; women age: $M = 20.7$, $SD = 1.69$) participated in the study This was to be expected, since mostly second-year students participated in the study, and at South African institutions, the average age for second-year students is in the order of twenty years.

5.2.2 Mobile phone data

Interestingly, 97.9% of the respondents owned a mobile phone. This is vastly more than the 89% of South African adults who owned a mobile phone in 2014 as reported by PEW Research Center (9) and the 91% of adults who own a mobile phone as reported in the 2018 PEW Research Center report (9,12). The 2018 PEW Research Center reported that people with a higher educational status are more likely to own a mobile phone. In Kenya it was found that 95% of higher educated people owned a mobile phone (12), which correlates with the finding in this study. In a review by Womack *et al.* (165) it is noted that various studies have found that over 95% of undergraduate students own mobile phones (p.4). Thus, it can be assumed that more students own mobile phones when compared to the general adult population of South Africa. Even though not all respondents owned a mobile phone, all of them knew how to operate a mobile phone. South Africans have certainly embraced mobile phone usage, and mobile phone usage among students is comparable to first world countries.

Most (87.5%) of the respondents in this study reported that they seldom or never send/receive text messages during lectures. This is well supported by North *et al.* (13), who also found, using questionnaires, that the majority of respondents in their study, conducted at the University of Cape Town, did not receive calls during a lecture (p.130) and found it annoying if other students made or received calls during lectures.

5.2.3 Study adherence

In this research and other studies, cortisol research depends on protocol adherence (141). Non-adherence is a factor that can affect sampling (166). Adherence to pre-specified study procedures was disappointing for this study, with 35.4% of respondents not adhering on the CONT and 33.3% not adhering on the INT. It was exceedingly difficult to determine how low adherence could have affected the results obtained in this study.

5.3 Cortisol results

A paired-samples t-test showed that there was no significant difference between cortisol levels taken BEF on the control and intervention days. Similarly, there was no significant difference between salivary cortisol levels taken AFT on the control and intervention days. These results were to be expected, since cortisol follows a strong diurnal rhythm (167). A recent study by Short *et al.* (111), testing the correspondence between hair, saliva and urine cortisol levels, reported that cortisol levels in hair samples showed a strong month-to-month stability, saliva cortisol a modest long-term stability and urine cortisol (measured on a week–to-week basis) had strong associations from one week to another (p.7).

Due to the diurnal rhythm of cortisol, there was a significant difference between salivary cortisol levels taken before and AFT on the control and intervention days. On both the control and intervention days, salivary cortisol levels increased from the BEF results to AFT results. This is in contrast to the diurnal rhythm of cortisol which typically dictates that cortisol levels are high on awakening, increases to a peak 30-45 minutes after awakening and then gradually declines throughout the day, reaching a nadir around midnight (24,30,88,167). Studies, similar to this study, investigated the effect of momentary stressors on cortisol levels and found dissimilar results (129,148). Merz *et al.* (129) investigated whether holding oral presentations during a university course would increase cortisol levels from before the presentation to after the presentation (p.1). They indeed found that cortisol levels increased on the oral presentation day, but declined on the CONT, where no oral presentation took place. In this particular study, salivary cortisol levels increased from before to AFT on both the control and intervention days. Also investigating the effect of oral examinations, Schoofs *et al.* (148) found that on average, cortisol levels were significantly increased on the examination day than on the CONT (p.55). In this study it was also observed that the cortisol concentrations were higher on the intervention INT than the CONT, although not as significant as in the study conducted by Merz *et al* (129). Atypical cortisol diurnal rhythm was observed in this particular study, instead of

declining salivary cortisol levels, cortisol increased from BEF to AFT. The most probable explanation for this might be that merely participating in this study was somewhat stressful to respondents. As previously mentioned, Hunter *et al.* (144) found that cortisol levels did not vary by condition (p.348) when subjecting women to a peer social-exclusion stressor (p.345). As in this study, the stressor used by Hunter *et al.* (144) was not stressful enough to elicit a significant cortisol response.

5.4 Questionnaires

5.4.1 Hospital Anxiety and Depression Scale-Anxiety and Depression

Anxiety and depression indicators were measured on the CONT and the INT using the HADS questionnaire. No significant difference between scores were observed on the days. This attests that respondents reported scores accurately across the intervention and control days. The mean score between the two days for the anxiety scale was 6.05 and the mean score for the depression scale was 3.07, indicating that the respondent group on average reported normal levels for anxiety and depression. Interestingly, when compared to normative data collected in the United Kingdom (UK) (168) and Germany (169) for the HADS, the respondents in this particular study scored higher on the anxiety scale compared to the German population and lower than the UK population. Depression scores were lower than those reported in the UK and Germany. It should be noted that the studies in the UK and Germany consisted of a considerably larger pool of respondents, since the sole purpose of these studies were to determine normative values for the HADS. The respondents in these studies were from various sociodemographic backgrounds in comparison to the respondents in the current study, who were all students aged 19-25 years (168,169). This could possibly indicate why the depression scores was lower in the current study, since the students were still young and most have yet to experience stressors associated with post university life.

As was reported in the results chapter, one-way ANOVA between subject were conducted to compare the scores reported on the HADS-A and HADS-D with the respondent's salivary cortisol levels BEF started. Depending on the scores reported on the HADS, a respondent can either have normal, borderline or abnormal scores for anxiety and depression. A significant difference was found between anxiety scores and salivary cortisol levels on the INT. A post hoc analysis using Scheffe's post hoc criterion indicated that subjects who reported abnormal anxiety scores had higher salivary cortisol levels than subjects who reported normal scores. Vreeburg *et al.* (170) also found that anxiety disorder was associated with a higher CAR

(p.340). Although there are inconsistencies in research findings, anxiety disorder has been linked to increased CRH release which in turn stimulates HPA activity (170). While there is extensive literature linking MDD to the HPA, the association between the HPA and anxiety disorders is not well established (125,170,171). In depression, hyperactivity of the HPA is mostly observed, however in some patients hypoactivity is observed, most probably as a result of HPA fatigue resulting from various depressive episodes (125). Anxiety often precedes MDD (125,172) and these disorders share clinical features, it is proposed that there may thus be a common physiological basis (125). Unlike Faresjö *et al.* (173) who found a significant association between HADS-D scores and cortisol levels in young Greek adults living in a stressful social environment (p.5), the current study did not find an association between elevated cortisol levels and abnormal depression scores on the HADS-D, since no respondents reported abnormal HADS-D scores.

5.4.2 Perceived Stress Scale

Stress was measured on the control and intervention days using the PSS and there was no significant difference between scores reported on the days, indicating accurate reporting between the days. The mean score between the two days was 17.06, indicating that the respondent group on average reported moderate stress levels. Although the experimental group was rather small, it is concerning that the current experimental group reported moderate stress levels. Denovan *et al.* (174) measured stress using the 10-item PSS in 524 social science students in the UK and found that the mean reported score for the sample population was 19.79 (p.5). Using the 20-item perceived stress questionnaire, Heinen *et al.* (175) measured stress in 321 first-year medical students from the University of Hamburg, Germany and found that the mean reported score was 0.40; significantly higher than that of the level of age related Germans in general (mean 0.30) (p.6). Both studies were conducted in 2017. Thus, the respondents in the current study were less stressed than their contemporary peers attending university in the UK and Germany. The sample population in this study was much smaller than those in the UK and Germany, which could possibly explain the discrepancy. Another important factor to consider is the ethnic diversity of the South African population and how stress affects different ethnic groups in South Africa.

A one-way between subject ANOVA yielded no significant difference between stress and salivary cortisol levels taken BEF on the CONT. In this study, it was found that on average, respondents reported moderate stress via the PSS; this could possibly explain why the

association between stress and salivary cortisol levels was insignificant, since stress leads to elevated cortisol levels. In a study among nursing students, Akbari *et al.* (176) measured the relationship between spiritual wellbeing and depression, stress and anxiety with cortisol levels. Likewise, they did not find any meaningful correlation between stress scores and cortisol levels (p.1), unlike Faresjö *et al.* (177) who found that women who had high perceived stress, had higher cortisol levels (p.1).

5.4.3 Subjective experience of receiving mobile text messages

The respondents in this study completed a questionnaire on their subjective experience of the experiment. The majority of respondents reported that the text messages caused a distraction from the lecture while they anxiously waited for the text messages and wished that they would stop, indicating that, subjectively, receiving text messages during a lecture is a hinderance to students. In an earlier study, students who had never owned a smartphone were given one for a whole year. At the start of the study, 63% believed that the smartphone would play a large part in their academic achievement. By the end of the year, students had a negative perspective of mobile phone use in the academic setting, citing that they had become addicted to the mobile phone and that it distracted them from their education (165). In the current study, 79% of respondents reported that the text messages distracted them from the lecture. As Kuznekoff *et al.* (15) explains, learning is a process and when there is a resource that competes with the process of learning, it has a negative effect on learning (p.236). They further explain that texting causes a divide in attention which distracts attention from the on-task behaviour, i.e. learning (p.236). It has been found that students feel relatively neutral about using a mobile phone in the classroom. This is in spite of recognising that texting causes a distraction in an academic environment (165).

In another study it was found that 77% of business students believe that learning in the classroom is seldom or never affected by mobile phones, while 76% believe it seldom assists in learning (178). This is a contradicting finding: on the one hand it is believed that mobile phone seldom or never affects learning, and on the other hand it is thought that using a mobile phone does not assist in learning. In this study, 52% of respondents reported that they anxiously waited for the text messages, while 71% wished the text messages would stop. On the other hand, the majority currently reported that they did not find the experience stressful and that the experiment did not change the way they feel about texting. Since the majority of students subjectively reported that they did not find the experience stressful, it supports the salivary

cortisol analysis, which reported no significant difference between salivary cortisol levels between the control and intervention days.

Independent t-tests were conducted to compare the change in salivary cortisol levels on the INT with respondents' agreement or disagreement scores to the questions on the subjective experience questionnaire. There was no significant difference between agreement or disagreement and the change in salivary cortisol levels on the INT. Agreement was defined by "*strongly agree*" and "*agree*" responses to the questions posed in the subjective experience questionnaire, while disagreement was defined by "*strongly disagree*", "*disagree*" and "*undecided*" responses.

5.5 Text emotion and frequency

This study explored how words with a neutral, positive or negative emotional connotation would affect salivary cortisol levels. Literature regarding the content of text messages and cortisol levels is limited. Presently it was found that text message content did not significantly impact the change in salivary cortisol levels on the INT. The impact of the content of text messages has been investigated in behavioural studies (179). It has been shown in adolescents that text messages with affective attitudes regarding physical activity increased energy expenditure during a two-week period, compared to the control group who received neutral text messages (179). It has also been found that texting is an accepted form of support in adolescent obesity interventions, with respondents reporting that positive and encouraging text messages are supportive, whereas text messages that mention unhealthy foods or behaviours acted as a trigger to consume the unhealthy food and engage in the unhealthy behaviours (180). It is notable that the content of text messages affect behaviour but to what extent still needs to be researched further. This was emphasised by Hooker *et al.* (140) in 2018 who did pioneering work on receiving mobile text messages from a romantic partners during a stressor and the effect it had on BP and heart rate (p.490). In their review they only found one other study, by Otway *et al.* (181), that measured the effect of receiving mobile text messages against physiological parameters (p.1). In the review conducted at present, extra research studies measuring the effect of receiving mobile text message on physiological parameter were found (26,140,144). Interestingly, it is worth noting that two of these studies were published in 2018 by Hooker *et al.* (140) and Hunter *et al.* (144), of which Hooker additionally was a co-author. In contrast, the psychological effect of receiving mobile text messages in relation to changes

in health behaviour, for example diabetes self-management and weight loss, have been thoroughly studied (140,182).

Lastly, this study explored how text frequency would affect salivary cortisol levels. It was found that text frequency did not significantly affect the change in salivary cortisol levels on the INT. It has been found that the frequency, content and wording of text messages are very important in mobile health (mHealth) intervention studies (183). In the context of this study, it was exceedingly difficult to determine if the frequency of text messages was optimised. The respondents received either 10, 15 or 20 text messages during the intervention. As far as could be ascertained, this was the first study to vary text frequency, in a lecture situation, and compare it to salivary cortisol levels.

5.6 Limitations

This study was conducted at a single university using only respondents who enrolled for Physiology modules.

Selection bias should be considered when interpreting the results of this study, as all respondents were Physiology students.

Since literature on the effect of receiving mobile text message on salivary cortisol levels is limited, it was recognised that there may be improvement in future study designs and methodology.

5.7 Conclusion

Texting has become central to the social life of mankind, especially young adults, who send or receive on average 109.5 messages per day (184). It has been shown that texting has an adverse effect on physiological functioning. This includes, but is not limited to, musculoskeletal disorders of the neck and upper extremities (139), increases in heart and respiratory rate (26) and first carpometacarpal joint arthritis (185). Text message reminders are also used to good effect in mHealth programmes to support and improve health care and public health (178). Research regarding the physiological effect that receiving mobile text messages has on stress (140) and particularly cortisol as an indicator of stress is limited. This particular study further narrowed down the physiological effect of receiving mobile text messages to a student population in a lecture environment. This study explored the multi-faceted variables and came to the following conclusions.

This study's primary goal was to determine how receiving mobile text messages affected salivary cortisol levels. What made this study unique was the addition of questionnaires that measured stress, anxiety, depression and the subjective experience of respondents toward the experiment. This study also incorporated text frequency and text emotion into the study design. These factors were compared to salivary cortisol levels to obtain substantial information around the subject.

Regarding the general and texting characteristics of the sample population, currently it was found that respondents were men and women aged between 18-25 years. Nearly all respondents owned a mobile phone and all respondents knew how to operate a mobile phone. The majority of respondents reported that they seldom or never send or receive text messages during a lecture. Overall the adherence to study procedures in this study was low.

It was found that receiving mobile text messages during a lecture had no significant effect on cortisol secretion. Salivary cortisol levels did increase from BEF to AFT, on the control and intervention days, with the increase being slightly higher on the INT, indicating that the lecture may have caused stress among students. Albeit, no statistical significance was reported.

Stress and depression did not have a moderating effect on cortisol secretion while receiving mobile text messages. Although this study indicated that anxiety levels had a moderating effect on cortisol secretion, those respondents who reported abnormally high anxiety scores also had higher salivary cortisol levels.

Respondents provided subjective feedback on their experience of the experiment by answering relevant questions like if text messages caused distraction from the lecture. None of the reported answers correlated with the cortisol data.

Text frequency and the use of neutral, positive or negative words accompanying the text messages had no moderating effect on cortisol secretion.

Thus, it can be concluded that receiving mobile text messages during a lecture did not significantly affect cortisol secretion and the mediating factors that were analysed in this study did not have a significant mediating effect on cortisol secretion. Although the results found in this study were mostly non-significant, this was the first study to measure the effect that receiving mobile text messages has on salivary cortisol levels during a lecture. The uniqueness of this study is also the fact that validated questionnaires were used to measure stress, anxiety, depression and the subjective experience of respondents toward the experiment.

Texting forms part of the daily lives of millions of people, therefore it is of the utmost importance to continue research on the physiological effects of texting. From the work done currently, it is clear that there is a lack of research on receiving mobile text messages and the effect it may have on stress.

5.8 Recommendations for future research

This study incorporated text frequency and text emotion into the study design. Future research can endeavour to optimise text message frequency and emotion when comparing it to physiological parameters.

Grouping of responses into an agreement and disagreement category was used in this study. Future studies may consider conducting a one-way ANOVA analysis using the responses (e.g. *strongly agree, agree, undecided, disagree, strongly disagree*) instead of independent t-tests using categories.

Future research should consider using SMS reminders, pre-programmed wristwatches and other technological devices to assist respondents with protocol adherence.

Replication studies with altered study designs and different sample populations are strongly suggested. It is also suggested that future research use a setting other than a lecture.

5.9 Summary

Research has shown that nearly all students own a mobile phone. Mobile phones have countless features. This study examined one of its oldest and most simple features – texting, which is used by virtually all mobile phone owners. Texting is a method of communication that allows short messages to be transmitted between mobile phones. South Africa is the country with the most smartphone users in Africa and the activity most performed on mobile phones is sending text messages. Many authors report on the adverse effects that texting has on attention, academic performance, academic work recall and academic distractibility, to name a few. It has been determined that texting can negatively impact on various physiological systems, yet texting as a tool can be used to great effect in health intervention studies and other research fields.

The purpose of this research was to study the effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State. In total, 48 individuals participated in this study by completing questionnaires and providing cortisol samples in the form of saliva during a physiology lecture. Respondents acted as their own controls in a crossover design. On one day they received the intervention (receiving mobile text messages), while on the other day they did not receive the intervention.

Data collection by questionnaires obtained demographical data, measured anxiety, depression and stress levels and how the respondent subjectively experienced the intervention. The data were compared to objectively measured salivary cortisol levels for each respondent using a technique called ELISA. Cortisol is a hormone that gives an indication of stress levels.

Analysis of salivary cortisol revealed that cortisol is a hormone that fluctuates according to a diurnal rhythm. Levels peak after waking up and then slowly decline during the day, reaching a minimum at around midnight. This diurnal rhythm remains relatively constant from day to day. In this study the salivary cortisol levels in most of the respondents increased from when they provided the first sample to when they provided the second sample, which was later in the day. The respondents' salivary cortisol levels did not differ significantly between the control and intervention days.

Results of the questionnaires compared to the salivary cortisol levels revealed that respondents with higher levels of anxiety had higher salivary cortisol levels. Low to moderate anxiety

levels, stress, depression and how respondents subjectively experienced the intervention revealed no associations with salivary cortisol levels.

Receiving mobile text messages and the impact it has on physiology is not well documented in the literature. This study was one of a few studies to examine the effect of receiving mobile text messages on salivary cortisol levels and, as far as could be ascertained, the first to measure the effect of receiving mobile text messages on salivary cortisol levels in a lecture setting.

Key words: texting, salivary cortisol, anxiety, depression, stress, subjective experience, text frequency, text emotion, lecture, HADS, PSS.

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5.10 Opsomming

Navorsing toon dat bykans alle studente ’n selfoon besit. Selfone het talle funksies; hierdie studie het een van die oudste en eenvoudigste funksies ondersoek naamlik teksboodskappe, wat deur die meeste selfooneienaars gebruik word. Teksboodskappe is ’n kommunikasiemetode wat toelaat dat kort boodskappe gestuur en ontvang kan word tussen selfone. Suid-Afrika is die land met die meeste slimfoongebruikers in Afrika en die aktiwiteit waaraan die meeste tyd bestee word op ’n selfoon, is om boodskappe te stuur. Baie skrywers rapporteer oor die uitwerking wat teksboodskappe op aandag, akademiese prestasie, onthou van akademiese werk en akademiese afleibaarheid het, om maar enkeles te noem. Dit is reeds bepaal dat teksboodskappe verskeie fisiologiese sisteme negatief kan aanraak, en tog kan teksboodskappe as hulpbron met groot sukses in studies oor gesondheidsintervensies en in ander navorsingsvelde gebruik word.

Die doel van hierdie navorsingstudie was om vas te stel watter effek die ontvang van boodskappe op speekselkortisolvlakke in Fisiologiestudente van die Universiteit van die Vrystaat het. In totaal het 48 individue deelgeneem aan die studie deur vraelyste in te vul en speekselmonsters gedurende ’n lesing te voorsien. Deelnemers het as hulle eie kontroles gedien in ’n oorkruis-navorsingsontwerp. Op een dag het hulle die ingryping ondergaan (om boodskappe te ontvang), en op die ander dag het hulle nie die ingryping ontvang nie.

Data-invordering het vraelyste gebruik om demografiese inligting in te samel. Angs, depressie, stres, en deelnemers se subjektiewe ervaring van die ingryping is ook gemeet. Hierdie data is vergelyk met objektief-gemete speekselkortisolvlakke vir elke deelnemer deur gebruik te maak van ’n tegniek genaamd ELISA. Kortisol is ’n hormoon wat ’n aanduiding van stres gee.

Analise van speekselkortisolvlakke het getoon dat kortisol ‘n hormoon is wat fluktureer volgens ’n daaglikse ritme. Vlakke piek nadat ’n mens wakker word en daal dan geleidelik deur die dag tot by ’n laagtepunt rondom middernag. Die daaglikse ritme bly relatief konstant van dag tot dag. In hierdie studie het speekselkortisolvlakke by meeste van die deelnemers gestyg van wanneer hulle die eerste monster gelewer het tot by die tweede monster later die dag. Die deelnemers se speekselkortisolvlakke het nie merkwaardig verskil tussen die kontrole- en ingrypingdae nie.

Resultate van die vraelyste in vergelyking met speekselkortisolvlakke het getoon dat deelnemers met hoër vlakke van angs ook hoër speekselkortisolvlakke gehad het. Lae tot

matige angsvlakke, stres, depressie en hoe deelnemers die ingryping subjektief ervaar het, het geen ooreenkomskorrelasie met speekselkortisolvlakke gehad nie.

Om teksboodskappe te ontvang en die effek wat dit op menslike fisiologie het, is nie voldoende in die literatuur bestudeer nie. Hierdie studie is een van slegs ’n handjievol studies om die effek wat die ontvang van boodskappe op speekselkortisolvlakke het, te bepaal. Sover vasgestel kon word is dit die eerste studie om die effek wat die ontvang van boodskappe op speekselkortisolvlakke het in ’n lesingmilieu te bepaal.

Sleutelwoorde: teksboodskappe, speekselkortisol, angs, depressie, stres, subjektiewe ondervinding, boodskapfrekwensie, boodskapemosie, lesing, HADS, PSS.

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Appendix A: Information document

Study title:

The effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State

Introduction:

I, Francois Petrus Venter, am doing research on texting (receiving mobile text messages) and how it influences salivary cortisol levels. Research is the process to learn the answer to a question. In this study we want to learn whether receiving mobile text messages leads to increased salivary cortisol levels.

Invitation to participate:

Hereby, I ask that you participate in this research study.

What does the study entail?

This study is based on a crossover design. Your salivary cortisol levels will be measured before the intervention (receiving mobile text messages) and after the intervention on two consecutive lecture days (e.g. Thursday and Friday). The methods of testing cortisol will be non-invasive (i.e. you will not be exposed to any harm). All that is required are four samples of your saliva. If you were to participate in this study you will be required to adhere to a few measures.

If you participate in this study it will be expected of you: not to use alcohol, not to perform strenuous exercise and not to use non-prescription medication (e.g. Panado, Grandpa or any over-the-counter medication) the day before and on study days.

On both study days you will be asked : not to smoke (if you smoke) for 30 minutes before the study, not to consume coffee or other caffeine-containing products six hours before the study, not brush your teeth 30 minutes before the study and not eat or drink (except water) anything one hour before the study and during the study.

If you participate in this study you will be asked not to discuss the content of the text messages with other respondents or anybody else.

If you participate in this study it will be expected of you to report to room B204 (Francois Retief Building, Faculty of Health Sciences) 30 minutes before your lecture on the day of the study. The study will last for one hour and 20 minutes, thus you will be able to leave after your lecture. For you to be eligible to participate in the study you will have to answer two questionnaires.

There is no risk involved if you participate in this study and you will receive no benefits or incentives if you participate in this study.

You as participant will be given information on the study while involved in the study.

Your participation is voluntary, and refusal to participate will have no consequences. You may discontinue participation at any time without penalty.

If you have been diagnosed with a disease of the adrenal gland (Cushing's disease, Addison's disease etc.) or you use corticosteroid medication in the form of cream/s, oil/s, pill/s etc. you may unfortunately not participate in this study. If this applies to you please do not fill in the consent form.

Confidentiality:

All efforts will be made to keep your personal information confidential. Absolute confidentiality, though, cannot be guaranteed. Your personal information may be disclosed if required by law. Organisations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Ethics Committee for Medical Research and the Medicines Control Council.

If results are published, this may lead to cohort identification.

All efforts will be made to keep your personal information confidential.

Contact details of researcher:

For further information or if you would like to report study-related adverse events please feel free to contact me at venter.fp@gmail.com or 0837807299.

Titel van die studie:

The effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State.

Inleiding:

Ek, Francois Petrus Venter, doen navorsing oor die effek van ‘texting’ (om boodskappe te ontvang op ’n selfoon) op kortisolvlakke. Navorsing is die ontwikkeling van ’n antwoord op ’n vraag. In die studie wil ons vasstel of die ontvang van selfoonboodskappe kortisolvlakke verhoog.

Uitnodiging om deel te neem:

Hiermee nooi ek jou uit om deel te neem aan die studie.

Waaroor gaan die studie?

Die studie volg ’n oorkruis-toetsingsontwerp. Jou kortisolvlak sal voor die toetsing (om boodskappe te ontvang) en na die toetsing gemeet word op twee opeenvolgende lesingsdae (bv. Donderdag en Vrydag). Die metode wat gebruik gaan word is nie-indringend (d.w.s. jy sal nie blootgestel word aan enige gevaar nie). Al wat van jou gevra word is vier speekselmonsters.

Ajy aan hierdie studie sou deelneem, sal daar van jou verwag word om nie die dag voor en op die dae van die studie alkohol te gebruik nie, nie strawwe oefening te doen nie en geen ‘nie-voorskrif’ medikasie (bv. Panado, Grandpa of enige oor-die-toonbank medikasie) te gebruik nie.

Op albei studiedae sal jy ook gevra word : om indien jy rook vir 30 minute voor die studie nie te rook nie, nie enige middels wat kaffeïen bevat ses ure voor die studie in te neem nie, nie jou tande 30 minute voor die studie te borsel nie, en een uur voor en gedurende die studie nie enigiets te eet of drink (behalwe water) nie.

As jy aan hierdie studie sou deelneem, saljy gevra word om nie die inhoud van die boodskappe met ander deelnemers of enigiemand anders te bespreek nie.

Dit sal van jou verwag word om op die dag van die studie 30 minute voor die lesing by kamer B204 (Francois Retiefgebou, Fakulteit Gesondheidswetenskappe) aan te meld. Die studie sal een uur en 20 minute duur, dus kan jy na die lesing verdaag. Vir jou om deel te neem aan hierdie studie word daar verwag dat jy twee vraelyste invul.

Daar is geen gevaar betrokke indien jy sou besluit om deel te neem aan hierdie studie nie. Jy sal ook geen vergoeding ontvang indien jy sou besluit om deel te neem nie.

As deelnemer sal jy inligting oor die studie ontvang.

Jou deelname is vrywillig en nie-deelname sal geen nadelige gevolge dra nie. Jy mag enige tyd onttrek van die studie sonder dat jy benadeel sal word.

Indien jy met ’n siekte van die bynier (Cushing se siekte, Addison se siekte ens.) gediagnoseer is, of kortikosteroïede in die vorm van ’n room, olie, pil ens. gebruik, mag jy ongelukkig nie aan hierdie studie deelneem nie. As dit op jou van toepassing is, moet asseblief nie die ingeligte toestemming invul nie.

Vertroulikheid:

Alle pogings sal aangewend word om jou persoonlike inligting vertroulik te hou. Volstrekte vertroulikheid kan egter nie gewaarborg word nie. Jou persoonlike inligting mag openbaar word as die wet dit sou vereis. Organisasies wat jou rekords mag ondersoek en dupliseer vir kwaliteitversekering en data-analise, sluit in groepe soos die Etiekkomitee vir Mediese Navorsing en die Medisynebeheerraad.

As uitslae van die studie gepubliseer word, mag dit lei tot kohort identifikasie. Alle pogings sal aangewend word om jou persoonlike inligting vertroulik te hou.

Kontakbesonderhede van die navorser:

Vir verdere inligting of as jy ’n studieverwante nadelige gebeurtenis wil rapporteer, kontak my gerus by venter.fp@gmail.com of 0837807299.

Appendix B: Informed consent

STUDY TITLE:

The effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State.

RESEARCHER: Francois Petrus Venter

You have been asked to participate in a research study. You have been informed about the study by the researcher.

To participate in this study please read the following stipulations:

a) I declare that the research protocol has been described to me in the information document and I have received a copy for my own records.
b) I declare that I have taken note of the information document verbally read to me.
c) I declare that if I participate in this study, I will receive a copy of the consent form, which I signed, for my own records.
d) I declare that I have been informed that no financial gain can be claimed if I participate in this study.
e) I declare that I have been informed that the study poses no known threat of injury.
f) I declare that if for some reason injury may occur I cannot hold the researcher, the University of the Free State or any person/s accountable to the research project liable.
g) I declare that the personal information I provide is correct and that it will be handled with confidentiality.
h) I declare that my participation in this research project is voluntary.
i) I declare that if I decide to participate I may discontinue participation at any time without consequence.

You may contact the researcher at venter.fp@gmail.com or 0837807299 any time if you have questions about the research.

You may contact the Secretariat of the Faculty of Health Sciences Research Ethics Committee at telephone number (051) 4052812 if you have questions about your rights as a research subject.

If you have been diagnosed with a disease of the adrenal gland (Cushing's disease, Addison's disease etc.) or you use corticosteroid medication in the form of cream/s, oil/s, pill/s etc. you may unfortunately not participate in this study. <u>If this applies to you please do not fill in the consent form.</u>

I …… (*enter your full names and surname here*) hereby give my full consent to participate in this study.

_____ _____

Signature of Participant Date

TITEL VAN DIE STUDIE:

The effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State.

NAVORSER: Francois Petrus Venter

Jyis gevra om aan ’n navorsingstudie deel te neem. Jy is deur die navorser ingelig oor die studie.

Om deel te neem aan hierdie studie lees asseblief die volgende bepalings:

a) Ek verklaar dat die navorsingstudie aan my verduidelik is deur middel van die inligtingsdokument en dat ek ’n afskrif vir my eie rekords ontvang het.
b) Ek verklaar dat ek kennis neem van die inligtingsdokument wat mondeling vir my voorgelees is.
c) Ek verklaar dat indien ek aan die studie deelneem, ek ’n kopie van die ingeligte toestemmingsvorm, wat ek geteken het, vir my eie rekords sal ontvang.
d) Ek verklaar dat ek ingelig is dat geen finansiële vergoeding geëis kan word indien ek aan die studie deelneem nie.
e) Ek verklaar dat ek ingelig is dat die studie geen bekende beseringsgevaar inhou nie.
f) Ek verklaar dat indien enige besering wel plaasvind, ek nie die navorser, die Universiteit van die Vrystaat of enige persoon/persone betrokke by die navorsing aanspreeklik sal hou nie.
g) Ek verklaar dat die persoonlike inligting wat ek verskaf korrek is en dat dit vertroulik hanteer sal word.
h) Ek verklaar dat my deelname aan hierdie studie uit eie wil geskied.
i) Ek verklaar dat indien ek aan hierdie studie deelneem, ek enige tyd mag onttrek sonder enige gevolge.

Die navorser kan gekontak word by venter.fp@gmail.com of 0837807299 indien daar vrae is oor die navorsing.

Kontak die Sekretariaat van die Gesondheidswetenskappe Etiekkomitee, UV indien jy vrae het oor jou regte as navorsingsdeelnemer by (051) 4052812.

Indien jy met 'nbyniersiekte (Cushing se siekte, Addison se siekte ens.) gediagnoseer is of indien jy kortikosteroïede in die vorm van 'n room/rome, olie/s, pil/le ens. gebruik mag jy ongelukkig nie aan hierdie studie deelneem nie. <u>As dit van toepassing is op jou, moet asseblief nie die ingeligte toestemmingvorm invul nie.</u>

Ek …… (*skryf jou volle name en van hier*) gee hiermee my volle toestemming om deel te neem aan hierdie studie.

_____ _____

Handtekening van deelnemer Datum

Appendix C: Instructional guide for respondents

You have been selected to participate in a research study that analyses the possible effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State.

For you to successfully complete participation, there are some requirements that you will have to adhere to. These requirements have been explained to you in the "Information document". This Instructional Guide will explain the requirements again.

Please provide 1-2ml of saliva for each sample. Hint: press the tip of your tongue against your teeth; it helps to stimulate saliva secretion.

EXAMPLE: if you participate in the study on a Thursday and a Friday, follow these instructions.

The day before the study (Wednesday):

- Please refrain from using alcohol.
- Please refrain from taking non-prescription medication (e.g. Panado, Grandpa or any over–the-counter medication).
- Please refrain from strenuous exercise.

On the day of the study (Thursday and until after the Friday lecture):

- Please refrain from using alcohol.
- Please refrain from taking non-prescription medication (e.g. Panado, Grandpa or any over-the-counter medication).
- Please refrain from strenuous exercise.
- Please refrain from consuming any substance that contains caffeine six hours before the study.
- If you smoke, please do not smoke for 30 minutes before the study.
- Please refrain from brushing your teeth for 30 minutes before the study.
- Please do not eat or drink (except water) anything an hour before or during the study.
- Report to room B 204 half an hour before your lecture. B 204 is situated on the second floor of Block B in the Francois Retief Building (Faculty of Health Sciences), next to Visimed (computer lab). If you are unsure of how to reach this venue, please feel free

to contact the researcher who will schedule a suitable time to show you the venue beforehand.

- You will be told if you receive text messages on that day or not.
- Fill in questionnaire 1.
- Once you have answered all the questions, please hand it to the researcher.
- The researcher will give you the following: two test tubes labelled A and B and a wristband with your participant number on. Please do not remove the wristband until the last study day is complete. Please remember the number.
- Proceed to your lecture.
- Take a seat at a chair where a red poster is present if you will be receiving mobile text messages. Take a seat at a chair where a blue poster is present if you will not be receiving mobile text messages.
- On the desk you will find a mobile phone on the right hand side of the desk as well as further instructions.

Instructions during the study:

  - 1) For the duration of this lecture, please do not use or pick up the mobile phone on the right hand side of the desk, except when a text message comes through. If a text message comes through, please pick up the mobile phone and read the message, but please do not reply or use the mobile phone for anything other than reading the text message. The mobile phone will only vibrate; please do not set it to loud.
  - 2) For the duration of the lecture, please do not eat or drink anything.
  - 3) The green light situated in the front centre of the room indicates when you have to spit into a test tube. When the light switches on, please spit 1-2 ml of saliva into a test tube (the test tube will indicate millilitres). The green light will switch on two times during the lecture: ten minutes into the lecture (e.g. 09:20 am) and at the end of the lecture (e.g. 10:00 am). Please spit into the test tube labelled A at 09:20 am and then into the test tube labelled B at 10:00 am.
  - Please fill in Questionnaire 2 after the lecture (it only takes a minute or two).
  - Give your saliva samples and Questionnaire 2 to the researcher.

- Please do not use your own mobile phone or any other mobile device (Ipad, laptop etc.) during the experiment.

- Sit calmly in your seat for the duration of the lecture and listen to the lecture.

Instruksies aan deelnemers

Jy is gekies om deel te neem aan ’n navorsingstudie wat die effek van “texting” op kortisolvlakke in tweedejaar-Fisiologiestudente aan die Universiteit van die Vrystaat toets.

Daar is ’n paar vereistes waaraan jy moet voldoen om jou in staat te stel om aan hierdie navorsingstudie deel te neem. Die vereistes is aan jou voorgelê in die inligtingsdokument. In hierdie gids word die vereistes weereens aan u voorgelê.

Voorsien asseblief 1-2ml speeksel vir elke monster. Wenk: druk die punt van u tong teen jou tande, dit help om speeksel te vorm.

Byvoorbeeld: as jy Donderdag en Vrydag aan die studie deelneem, volg die instruksies hieronder:

Op die dag voor die studie (Woensdag):

- Moet geen alkohol gebruik nie.
- Moet geen nie-voorskrif medikasie (Bv. Panado, Grandpa of enige oor-die-toonbank medikasie) gebruik nie.
- Moet nie enige strawwe oefening doen nie.

Op die dag van die studie (Donderdag en tot ná die Vrydaglesing):

- Moet geen alkohol gebruik nie.
- Moet geen nie-voorskrif medikasie (Bv. Panado, Grandpa of enige oor die toonbank medikasie) gebruik nie.
- Moet nie enige strawwe oefening doen nie.
- Weerhou jouself asseblief daarvan om enige middel wat kaffeïen bevat ses ure voor die studie in te neem.
- Indien jy rook, moet asseblief nie vir 30 minute voor die studie rook nie.
- Weerhou jouself asseblief daarvan om jou tande ’n halfuur voor die studie te borsel.
- Moet asseblief niks eet of drink (behalwe water) ’n uur voor en gedurende die studie nie.
- Meld asseblief ’n halfuur voor jou lesing by kamer B204 aan. Kamer B204 is geleë op die tweede vloer van Blok B in die Francois Retief-gebou (Fakulteit Gesondheidswetenskappe), langs Visimed (rekenaar lab). As jy nie seker is hoe om by

die lokaal uit te kom nie, skakel gerus die navorser wat dan vooraf vir jou die lokaal sal gaan wys.

- Jysal gesê word of jy vandag gedurende die lesing boodskappe sal ontvang of nie.
- Vul asseblief Vraelys 1 in.
- Sodra jy klaar al die vrae beantwoord het, gee dit asseblief vir die navorser.
- Die navorser sal vir jou die volgende gee: twee proefbuise genommer A en B en ’n armband met jou deelnemersnommer op. Moet asseblief nie die armband verwyder tot die laaste studiedag voltooi is nie. Onthou asseblief die nommer.
- Gaan na jou lesing toe.
- Sit by ’n stoel met ’n rooi plakkaat op indien jy boodskappe gaan ontvang. Sit by ’n stoel met ’n blou plakkaat op indien jy nie vandag boodskappe gaan ontvang nie.
- Op die tafel sal jy ’n selfoon aan die regterkant vind, sowel as verdere instruksies.
- Instruksies gedurende die lesing:
    - 1) Gedurende die hele lesing, moet asseblief nie die selfoon aan die regterkant van die tafel optel nie, behalwe as daar ’n boodskap deurkom. As daar ’n boodskap deurkom, tel asseblief die selfoon op en lees die boodskap, maar moet asseblief nie iets terugstuur nie. Moet asseblief ook nie die selfoon vir enigiets anders gebruik nie. Die selfoon sal net vibreer, moet dit asseblief nie stel om te lui nie.
    - 2) Moet asseblief nie gedurende die lesing iets eet of drink nie.
    - 3) Die groen lig, voor in die middel van die lesinglokaal, dui aan wanneer jy in ’n proefbuis moet spoeg. Wanneer die lig aangeskakel word, spoeg asseblief 1-2ml in ’n proefbuis (die proefbuis sal milliliters aandui). Die groen lig sal twee keer aanskakel gedurende die lesing: tien minute in die lesing (bv. 09:20 vm) en na die lesing (bv. 10:00 vm). Spoeg asseblief in proefbuis A om 09:20 vm en in proefbuis B om 10:00 vm.
    - Voltooi asseblief Vraelys 2 ná die lesing (dit vat net ’n minuut of twee).
    - Gee asseblief jou speekselmonsters en Vraelys 2 vir die navorser.
- Moet asseblief nie jou eie selfoon of enige ander toestel tydens die eksperiment gebruik nie (iPad, skootrekenaar ens.).
- Sit tydens die lesing kalm in jou sitplek en luister na die lesing.

Appendix D: Letters of permission

Dean of the Faculty of Natural and Agricultural Sciences, Vice-Rector: Research, Head of Department of Basic Medical Sciences, Lecturer in the Department of Basic Medical Sciences

University of the Free State

Dear Prof. Vermeulen, Prof. Witthuhn, Dr. Van Zyl, Ms. Vorster, Mr. Muller

I am in the process of planning a research project for my Master's degree in Physiology. I would like to determine whether receiving mobile text messages influence salivary cortisol levels in Physiology students.

Study title:
The effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State.

Aim of the study:
To determine whether receiving neutral, positive or negative mobile text messages during a lecture might have an effect on salivary cortisol levels in Physiology students.

The research objectives are:

a) To assess whether receiving mobile text messages during a lecture has an effect on salivary cortisol levels.
b) To assess whether the use of neutral, positive or negative words in text messages during a lecture has a moderating effect on salivary cortisol levels.
c) To assess, using a questionnaire, whether demographical data, stress, anxiety, depression and lifestyle factors influence the possible effect that receiving mobile text messages have on salivary cortisol levels.
d) To assess, using a questionnaire, whether participants found the receiving of mobile text messages during a lecture stressful.

An information leaflet will be handed out and read to students. They will be assured that their participation is voluntary and poses no harm. Respondents will not be compensated.

Respondents' personal information will be kept confidential. 35 respondents will participate in this study and each will be asked to provide four saliva samples. Data will be gathered over two weeks and the laboratory analyses will take two weeks.

Results will be published.

Please find the attached protocol for your perusal.

Yours sincerely

F.P. Venter
Department of Basic Medical Sciences
Faculty of Health Sciences
Contact number: 0837807299
Email:venter.fp@gmail.com

Appendix E: List of neutral, positive and negative words

Neutral words	Positive words	Negative words
Ankle	Angel	Anger
Bake	Baby	Burn
Column	Comedy	Cruel
Detail	Dazzle	Devil
Frog	Fun	Fire
Glass	Gift	Guilty
Habit	Happy	Hatred
Industry	Intimate	Intruder
Market	Miracle	Murderer
Paper	Party	Pain

Appendix F: Questionnaires

The effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State

Research Study
Questionnaire 1

Please note that by completing the questionnaire you are voluntarily participating in this research study. Your personal information will be kept anonymous and confidential. You may withdraw from this study at any given moment, should you wish to. The results of this study may be published.

THANK YOU FOR YOUR PARTICIPATION.

Participant nr._____

INSTRUCTIONS FOR COMPLETING THIS QUESTIONNAIRE

Most questions are followed by a list of answers. Please **choose the answer that mostly relates to you** and indicate your choice in one of the circles to the left.

FOR EXAMPLE:

I enjoy attending lectures early in the morning.

◯ Never
⊗ Almost never
◯ Sometimes
◯ Fairly often
◯ Very often

Question 1-9: General information

1 How old are you? _____

2 Indicate your gender.

- ◯ Male
- ◯ Female

3 Prefered language for future communication.

- ◯ English
- ◯ Afrikaans

4 Indicate your race.

- ◯ Black
- ◯ Coloured
- ◯ Indian
- ◯ White
- ◯ Other

5 Do you know how to operate a mobile phone (cellphone)?

- ◯ Yes
- ◯ No

6 Do you own a mobile phone?

- ◯ Yes
- ◯ No

7 How often do you send text messages during a physiology lecture?

- ◯ Never
- ◯ Seldom
- ◯ Often
- ◯ Always

8 How often do you receive text messages during a physiology lecture?

- ◯ Never
- ◯ Seldom
- ◯ Often
- ◯ Always

9 Do you consume alcohol?

- ◯ Never
- ◯ Once a week
- ◯ Twice a week
- ◯ Three times a week
- ◯ More than three times a week

Question 10-16: Information on **RECENT** activities.

10 Have you brushed your teeth in the last 30 minutes?

- ◯ Yes
- ◯ No

11 If you smoke, have you smoked during the last 30 minutes?

- ◯ Yes
- ◯ No

12 Have you eaten anything in the last hour?

- ◯ Yes
- ◯ No

13 In the last 6 hours, have you consumed anything that contains caffeine (e.g. coffee)?

- ◯ Yes
- ◯ No

14 In the past 24 hours, have you performed strenuous execise?

- ◯ Yes
- ◯ No

15 In the past 24 hours, have you taken any medication not prescribed to you? If yes please specify below.

- ◯ Yes
- ◯ No

16 In the past 24 hours, have you consumed any substance that contains alcohol (e.g. beer, wine, cough medicine etc.)?

- ◯ Yes
- ◯ No

Question 17-30: Regarding your mood the **PAST WEEK**, how do you feel about the following statements?

17 I feel tense or wound up.

- ◯ Most of the time
- ◯ A lot of the time
- ◯ From time to time
- ◯ Not at all

18 I still enjoy the things I used to enjoy.

- ◯ Definitely as much
- ◯ Not quite so much
- ◯ Only a little
- ◯ Hardly at all

19 I get sort of frightened feeling something awful is about to happen.

- ◯ Very definitely and quite badly
- ◯ Yes, but not badly
- ◯ A little, but it doesn't worry me
- ◯ Not at all

20 I can laugh and see the funny side of things.

- ◯ As much as I ever could
- ◯ Not quite as much now
- ◯ Definitely not so much now
- ◯ Not at all

21 Worrying thoughts go through my mind.

- ◯ A great deal of the time
- ◯ A lot of the time
- ◯ From time to time but not too often
- ◯ Only occasionally

22 I feel cheerful.

- ○ Not at all
- ○ Not often
- ○ Sometimes
- ○ Most of the time

23 I can sit at ease and feel relaxed.

- ○ Definitely
- ○ Usually
- ○ Not often
- ○ Not at all

24 I feel as if I am slowed down.

- ○ Nearly all the time
- ○ Very often
- ○ Sometimes
- ○ Not at all

25 I get a sort of frightened feeling like "butterflies" in the stomach.

- ○ Not at all
- ○ Occasionally
- ○ Quite often
- ○ Very often

26 I have lost interest in my appearance.

- ○ Definitely
- ○ I don't take as much care as I should
- ○ I may not take quite as much care
- ○ I take just as much care as ever

27 I feel restless as if I have to be on the move.

- ○ Very much indeed
- ○ Quite a lot
- ○ Not very much
- ○ Not at all

28 I look forward with enjoyment to things.

- ○ As much as I ever did
- ○ Rather less than I used to
- ○ Definitely less than I used to
- ○ Hardly at all

29 I get sudden feelings of panic.

- ○ Very often indeed
- ○ Quite often
- ○ Not very often
- ○ Not at all

30 I can enjoy a good book or TV program.

- ○ Often
- ○ Sometimes
- ○ Not often
- ○ Very seldom

Question 31-40: ask you about your thoughts and feeling the **LAST MONTH**.

31 In the last month, how often have you been upset because of something that happened unexpectedly?

- ○ Never
- ○ Almost never
- ○ Sometimes
- ○ Fairly often
- ○ Very often

32 In the last month, how often have you felt that you were unable to control the important things in your life?

- ○ Never
- ○ Almost never
- ○ Sometimes
- ○ Fairly often
- ○ Very often

33 In the last month, how often have you felt nervous and "stressed"

- ◯ Never
- ◯ Almost never
- ◯ Sometimes
- ◯ Fairly often
- ◯ Very often

34 In the last month, how often have you felt confident about your ability to handle personal problems?

- ◯ Never
- ◯ Almost never
- ◯ Sometimes
- ◯ Fairly often
- ◯ Very often

35 In the last month, how often have you felt that things were going your way?

- ◯ Never
- ◯ Almost never
- ◯ Sometimes
- ◯ Fairly often
- ◯ Very often

36 In the last month, how often have you found that you could not cope with all the things that you had to do?

- ◯ Never
- ◯ Almost never
- ◯ Sometimes
- ◯ Fairly often
- ◯ Very often

37 In the last month, how often have you been able to control irritations in your life?

- ◯ Never
- ◯ Almost never
- ◯ Sometimes
- ◯ Fairly often
- ◯ Very often

38 In the last month, how often have you felt that you were on top of things?

- ◯ Never
- ◯ Almost never
- ◯ Sometimes
- ◯ Fairly often
- ◯ Very often

39 In the last month, how often have you been angered because of things that were outside of your control?

- ◯ Never
- ◯ Almost never
- ◯ Sometimes
- ◯ Fairly often
- ◯ Very often

40 In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

- ◯ Never
- ◯ Almost never
- ◯ Sometimes
- ◯ Fairly often
- ◯ Very often

The effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State

Navorsingstudie
Vraelys 1

Neem asseblief kennis dat u vrywillig deel neem aan hierdie studie as u die vraelys invul. U persoonlike inligting sal anoniem en vertroulik gehou word. U mag enigetyd van hierdie studie onttrek indien u sou wou. Die uitslae van hierdie studie kan gepubliseer word.

BAIE DANKIE VIR U DEELNAME.

Deelnemer nr._____

INSTRUKSIES OM DIE VRAELYS TE VOLTOOI

Meeste vrae is gevolg deur 'n stel antwoorde. Kies **die antwoord wat die meeste van toepassing is op u** en dui u keuse aan in een van die sirkels links.

BYVOORBEELD:

Ek geniet dit om vroeg in die oggend lesings by te woon.

◯ Nooit
⊗ Amper nooit
◯ Soms
◯ Ietwat gereeld
◯ Baie gereeld

Vraag 1-9: Algemene inligting

1 Hoe oud is u? _____

2 Dui u geslag aan.

- ◯ Manlik
- ◯ Vroulik

3 U voorkeur taal vir toekomstige kommunikasie?

- ◯ Engels
- ◯ Afrikaans

4 Dui u ras aan.

- ◯ Swart
- ◯ Kleurling
- ◯ Indiër
- ◯ Blanke
- ◯ Ander

5 Weet u hoe om met 'n selfoon te werk?

- ◯ Ja
- ◯ Nee

6 Besit u 'n selfoon?

- ◯ Ja
- ◯ Nee

7 Hoe gereeld stuur u selfoon boodskappe gedurend 'n fisiologie lesing?

- ◯ Nooit
- ◯ Soms
- ◯ Gedurig
- ◯ Altyd

8 Hoe gereeld ontvang u selfoon boodskappe gedurend 'n fisiologie lesing?

- ◯ Nooit
- ◯ Soms
- ◯ Gedurig
- ◯ Altyd

9 Gebruik u alkohol?

- ◯ Nooit
- ◯ Een keer 'n week
- ◯ Twee keer 'n week
- ◯ Drie keer 'n week
- ◯ Meer as drie keer 'n week

Vraag 10-16: Inligting oor **ONLANGSE** aktiwiteite.

10 Het u in die afgelope 30 minute tande geborsel?

- ◯ Ja
- ◯ Nee

11 Indien u rook, het u in die afgelope 30 minute gerook?

- ◯ Ja
- ◯ Nee

12 Het u in die laaste uur iets geëet?

- ◯ Ja
- ◯ Nee

13 In die afgelope 6 ure, het u enige middel in geneem wat kaffeïen bevat (bv. koffie)?

- ◯ Ja
- ◯ Nee

14 In die afgelope 24 uur, het u enige strawwe fisiese oefeninge gedoen?

- ◯ Ja
- ◯ Nee

15 In die afgelope 24 uur, het u enige medikasie ,nie aan u voorgeskryf, in geneem? Indien ja, spesifiseer asseblief hieronder.

- ◯ Ja
- ◯ Nee

16 In die afgelope 24 uur, het u enige middel wat alkohol bevat in geneem (bv. bier, wyn, hoesstroop ens.)?

- ◯ Ja
- ◯ Nee

Vraag 17-30: Betreffende u gemoed die **AFGELOPE WEEK**, hoe voel u oor die volgende stellings?

17 Ek voel gespanne of opgewerk.

- ◯ Meeste van die tyd
- ◯ Baie van die tyd
- ◯ Van tyd tot tyd
- ◯ Glad nie

18 Ek geniet nog die goed wat ek altyd geniet het.

- ◯ Beslis so baie
- ◯ Nogal nie so baie nie
- ◯ Net 'n bietjie
- ◯ Omtrent nie meer nie

19 Ek raak soort van bang, dit voel of iets aakligs met my gaan gebeur.

- ◯ Baie beslis en nogal erg
- ◯ Ja, maar nie erg nie
- ◯ 'n Bietjie, maar dit pla my nie
- ◯ Glad nie

20 Ek kan lag en die snaakse sy van dinge sien.

- ◯ Soveel as wat ek van tevore kon
- ◯ Nie so baie nou nie
- ◯ Beslis nie so baie nou nie
- ◯ Glad nie

21 Kommerwekkende gedagtes gaan deur my kop.

- ◯ Grotendeels van die tyd
- ◯ Baie van die tyd
- ◯ Van tyd tot tyd maar nie gereeld nie
- ◯ Per geleentheid

22 Ek voel vrolik.

- ◯ Glad nie
- ◯ Nie gereeld nie
- ◯ Soms
- ◯ Meeste van die tyd

23 Ek kan agteroor sit en ontspanne voel.

- ◯ Beslis
- ◯ Gewoonlik
- ◯ Nie gereeld nie
- ◯ Glad nie

24 Dit voel vir my of ek vertraag word.

- ◯ Meeste van die tyd
- ◯ Gereeld
- ◯ Soms
- ◯ Glad nie

25 Ek kry 'n bang gevoel amper soos "skoenlappers" in my maag.

- ◯ Glad nie
- ◯ So nou en dan
- ◯ Ietwat gereeld
- ◯ Baie gereeld

26 Ek het belangstelling verloor in hoe ek lyk.

- ◯ Beslis
- ◯ Ek gee nie soveel aandag as wat ek moet nie
- ◯ Miskien gee ek nie heeltmal soveel aandag as wat ek moet nie
- ◯ Ek gee net so baie aandag as gewoonlik

27 Ek voel rusteloos, asof ek moet beweeg.

- ◯ Baie beslis
- ◯ Nogal baie
- ◯ Nie so baie nie
- ◯ Glad nie

28 Ek sien met genot uit na dinge.

- ◯ Soveel as wat ek van tevore het
- ◯ Ietwat minder as wat ek altyd het
- ◯ Beslis minder as wat ek altyd het
- ◯ Omtrent nie meer nie

29 Ek kry skielike paniek gevoelens.

- ◯ Baie gereeld
- ◯ Ietwat gereeld
- ◯ Nie gereeld nie
- ◯ Glad nie

30 Ek kan 'n goeie boek of TV program geniet.

- ◯ Gereeld
- ◯ Soms
- ◯ Nie gereeld nie
- ◯ Baie seldsaam

Vraag 31-40: vra u oor u gedagtes en gevoelens gedurend die **LAASTE MAAND**.

31 In die laaste maand, hoe gereeld was jy ontsteld oor iets wat onverwags gebeur het?

- ◯ Nooit
- ◯ Amper nooit
- ◯ Soms
- ◯ Ietwat gereeld
- ◯ Baie gereeld

32 In die laaste maand, hoe gereeld het jy gevoel of jy nie beheer oor belangrike dinge in jou lewe het nie?

- ◯ Nooit
- ◯ Amper nooit
- ◯ Soms
- ◯ Ietwat gereeld
- ◯ Baie gereeld

33 In die laaste maand, hoe gereeld het jy al senuweeagtig en "gestres" gevoel?

- ◯ Nooit
- ◯ Amper nooit
- ◯ Soms
- ◯ Ietwat gereeld
- ◯ Baie gereeld

34 In die laaste maand, hoe gereeld het jy al gevoel of jy met vol vertroue persoonlike probleme kan hanteer?

- ◯ Nooit
- ◯ Amper nooit
- ◯ Soms
- ◯ Ietwat gereeld
- ◯ Baie gereeld

35 In die laaste maand, hoe gereeld het jy al gevoel of dinge uitwerk vir jou?

- ◯ Nooit
- ◯ Amper nooit
- ◯ Soms
- ◯ Ietwat gereeld
- ◯ Baie gereeld

36 In die laaste maand, hoe gereeld het jy gevind dat jy nie alles kon hanteer wat jy moes doen nie?

- ◯ Nooit
- ◯ Amper nooit
- ◯ Soms
- ◯ Ietwat gereeld
- ◯ Baie gereeld

37 In die laaste maand, hoe gereeld was jy in staat om irritasies in jou lewe te beheer?

- ◯ Nooit
- ◯ Amper nooit
- ◯ Soms
- ◯ Ietwat gereeld
- ◯ Baie gereeld

38 In die laaste maand, hoe gereeld het jy al gevoel of jy in beheer van sake was?

- ◯ Nooit
- ◯ Amper nooit
- ◯ Soms
- ◯ Ietwat gereeld
- ◯ Baie gereeld

39 In die laaste maand, hoe gereeld was jy kwaad gemaak oor dinge wat buite jou beheer was?

- ◯ Nooit
- ◯ Amper nooit
- ◯ Soms
- ◯ Ietwat gereeld
- ◯ Baie gereeld

40 In die laaste maand, hoe gereeld het jy al gevoel of moeilikhede so op dam, dat jy dit nie sal kan oorkom nie?

- ◯ Nooit
- ◯ Amper nooit
- ◯ Soms
- ◯ Ietwat gereeld
- ◯ Baie gereeld

The effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State

Research Study
Questionnaire 2

Please note that by completing the questionnaire you are voluntarily participating in this research study. Your personal information will be kept anonymous and confidential. You may withdraw from this study at any given moment, should you wish to. The results of this study may be published.

THANK YOU FOR YOUR PARTICIPATION.

Participant nr._____

INSTRUCTIONS FOR COMPLETING THIS QUESTIONNAIRE

Most questions are followed by a list of answers. Please **choose the answer that mostly relates to you** and indicate your choice in one of the circles to the left.

FOR EXAMPLE:

I enjoy attending lectures early in the morning.

◯ Never
⊗ Almost never
◯ Sometimes
◯ Fairly often
◯ Very often

Question 1-5: ask you about the experiment.

1 Did the text messages draw your attention away from the lecture?

- ◯ Strongly disagree
- ◯ Disagree
- ◯ Undecided
- ◯ Agree
- ◯ Strongly agree

2 Did you anxiously wait for the text messages?

- ◯ Strongly disagree
- ◯ Disagree
- ◯ Undecided
- ◯ Agree
- ◯ Strongly agree

3 Did you wish the text messages would stop?

- ◯ Strongly disagree
- ◯ Disagree
- ◯ Undecided
- ◯ Agree
- ◯ Strongly agree

4 Did you find this experiment stressful?

- ◯ Strongly disagree
- ◯ Disagree
- ◯ Undecided
- ◯ Agree
- ◯ Strongly agree

5 Would you say this experience changed the way you feel about texting?

- ◯ Strongly disagree
- ◯ Disagree
- ◯ Undecided
- ◯ Agree
- ◯ Strongly agree

The effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State

Navorsingstudie
Vraelys 2

Neem asseblief kennis dat u vrywillig deel neem aan hierdie studie as u die vraelys invul. U persoonlike inligting sal anoniem en vertroulik gehou word. U mag enigetyd van hierdie studie onttrek indien u sou wou. Die uitslae van hierdie studie kan gepubliseer word.

BAIE DANKIE VIR U DEELNAME.

Deelnemer nr._____

INSTRUKSIES OM DIE VRAELYS TE VOLTOOI

Meeste vrae is gevolg deur 'n stel antwoorde. Kies **die antwoord wat die meeste van toepassing is op u** en dui u keuse aan in een van die sirkels links.

BYVOORBEELD:

Ek geniet dit om vroeg in die oggend lesings by te woon.

◯ Nooit
⊗ Amper nooit
◯ Soms
◯ Ietwat gereeld
◯ Baie gereeld

Vraag 1-5: vrae u oor die eksperiment.

1 Het die boodskappe u aandag van die lesing afgetrek?

- ◯ Stem glad nie saam nie
- ◯ Stem nie saam nie
- ◯ Onbeslis
- ◯ Stem saam
- ◯ Stem baie saam

2 Het u angstig vir die boodskappe gewag?

- ◯ Stem glad nie saam nie
- ◯ Stem nie saam nie
- ◯ Onbeslis
- ◯ Stem saam
- ◯ Stem baie saam

3 Het u gewens die boodskappe wil ophou?

- ◯ Stem glad nie saam nie
- ◯ Stem nie saam nie
- ◯ Onbeslis
- ◯ Stem saam
- ◯ Stem baie saam

4 Het u diè eksperiment stresvol gevind?

- ◯ Stem glad nie saam nie
- ◯ Stem nie saam nie
- ◯ Onbeslis
- ◯ Stem saam
- ◯ Stem baie saam

5 Sal u sê die ondervinding verander hoe u oor "texting" voel?

- ◯ Stem glad nie saam nie
- ◯ Stem nie saam nie
- ◯ Onbeslis
- ◯ Stem saam
- ◯ Stem baie saam

Appendix G: Proofreading Certificate

Dr Annemie Grobler

PhD (English), APEd (SATI) member 1003103

Language practitioner - translation, text editing and proofreading

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This is to certify that the following document has been professionally language edited:

The effect of receiving mobile text messages on salivary cortisol levels in Physiology students at the University of the Free State

Author: Francois Petrus Venter

Nature of document: Master's dissertation

Date of this statement: 15 June 2019

AM Grobler

Appendix H: Raw data

Participant	Age	Gender	Race	Can_operate_phone	Own_mobile_phone	send_texts_during_Lect_Cont	Receive_texts_during_Lect_Cont	Alcohol_Cont	Adherence_Cont
2	21	M	W	Y	Y	3	2	3	Y
3	20	M	W	Y	Y	2	3	4	Y
4	20	M	W	Y	Y	2	3	4	Y
5	25	F	W	Y	Y	1	2	3	N
6	20	F	W	Y	Y	1	2	3	Y
7	20	F	W	Y	Y	2	3	2	Y
8	20	F	W	Y	Y	2	3	2	Y
9	20	M	W	Y	Y	2	2	3	Y
10	22	M	W	Y	Y	2	2	2	Y
11	20	M	W	Y	Y	1	2	2	Y
13	24	F	W	Y	Y	1	2	2	Y
14	20	M	B	Y	Y	1	3	1	N
15	24	F	B	Y	Y	2	2	1	Y
17	20	F	B	Y	Y	2	2	1	Y
18	22	M	B	Y	Y	3	2	2	Y
20	20	M	W	Y	Y	2	2	2	Y
21	22	F	B	Y	Y	3	3	1	Y
22	22	M	B	Y	Y	1	2	2	Y
23	23	F	B	Y	Y	1	2	2	Y

24	22	F	B	Y	Y	1	2	1	Y
25	21	F	B	Y	Y	2	3	1	Y
26	19	F	W	Y	Y	2	3	2	Y
27	21	F	B	Y	Y	2			N
28	20	F	W	Y	Y	3	3	2	Y
29	20	F	W	Y	Y	3	3	2	N
41	20	F	W	Y	Y	1	1	2	N
42	22	M	W	Y	Y	1	2	2	Y
43	21	F	W	Y	Y	1	2	2	Y
50	20	F	W	Y	Y	2	2	1	Y
51	19	F	W	Y	Y	1	2	1	N
52	19	M	W	Y	Y	1	2	2	N
53	21	M	W	Y	Y	3	3	2	N
54	24	M	W	Y	N	2	2	3	N
55	20	F	W	Y	Y	1	2	1	Y
56	20	M	W	Y	Y	1	2	3	Y
57	19	F	W	Y	Y	1	2	2	N
60	19	F	W	Y	Y	2	2	2	N

61	19	F	W	Y	Y	2	2	2	Y
62	21	F	B	Y	Y	2	2	1	N
63	20	M	B	Y	Y	2	2	2	Y
64	19	M	B	Y	Y	1	1	1	Y
65	19	F	B	Y	Y	1	1	1	Y
66	19	M	B	Y	Y	2	3	1	Y
67	20	F	B	Y	Y	1	1	1	N
72	22	M	B	Y	Y	2	2	2	N
73	19	M	B	Y	Y	1	2	2	N
75	19	M	B	Y	Y	2	2	2	N
78	20	M	B	Y	Y	2	3	1	N

Participant	Adherence_details_Cont	HADS-A_score_Cont	HADS-D_score_Cont	PSS_score_Cont	send_texts_during_Lect_Int	Receive_texts_during_Lect_Int	Alcohol_Int	Adherence_Int	Adherence_details_Int
2		11	7	26	2	2	3	Y	
3		3	3	17	2	3	4	Y	
4		3	0	13	2	3	4	Y	
5	CONSUMED SUBSTANCE THAT CONTAINS ALCOHOL	3	2	13	1	2	3	Y	
6		4	2	20	1	2	3	Y	
7		6	1	11	2	3	2	Y	
8		11	6	23	2	3	2	Y	
9		3	1	16	2	2	3	Y	

10		7	8	23	2	2	2	N	CONSUMED SUBSTANCE THAT CONTAINS CAFFEINE
11		11	6	19	1	2	2	Y	
13		7	4	21	1	2	2	Y	
14	BRUSHED TEETH	7	2	23	1	3	1	Y	
15	ATE IN LAST HOUR AND PERFORMED STRENOUS EXERCISE	9	1	14	2	2	1	N	BRUSHED TEETH
17		11	7	16	2	2	1	Y	
18		6	7	16	2	2	2	Y	
20		3	1	16	2	3	3	Y	
21		12	7	24	3	2	1	N	ATE IN LAST HOUR
22		9	7	20	1	2	2	Y	
23	BRUSHED TEETH AND ATE IN LAST HOUR AND TOOK UNPRESCRIBED MEDICATION (CORENZA C)	6	0	16	1	3	2	Y	
24	ATE IN LAST HOUR AND PERFORMED STRENOUS EXERCISE AND TOOK PRESCRIBED MEDICATION (FLU MEDICINE)	7	1	14	1	2	1	Y	TOOK UNPRESCRIBED MEDICATION (FLU MEDICINE)

	AND CONSUMED SUBSTANCE THA CONTAINS ALCOHOL								
25	ATE IN LAST HOUR	6	3	15	2	3	1	Y	
26	TOOK UNPRESCRIBED MEDICATION (PROSPAN)	9	3	22	2	3	2	Y	
27	BRUSHED TEETH	5	6	14	1	2	1	N	BRUSHED TEETH
28		7	1	18	3	3	2	Y	
29	ATE IN LAST HOUR	7	1	14	1	1	2	Y	
41	TOOK UNPRESCRIBED MEDICATION (ALLERGEX NON-DROWSY)	5	1	15	1	1	2	Y	
42		4	0	11	1	2	2	Y	
43		1	0	10	1	1	2	Y	
50	CONSUMED SUBSTANCE THAT CONTAINS PARACETAMOL AND UNPRESCRIBED MEDICATION (ECHINAFORCE AND VIRAL GUARD) AND ALCOHOL	5	0	15	2	2	1	N	CONSUMED SUBSTANCE THAT CONTAINS PARACETAMOL AND UNPRESCRIBED MEDICATION (ECHINAFORCE AND VIRAL GUARD) AND ALCOHOL

51	BRUSHED TEETH AND ATE IN LAST HOUR AND TOOK UNPRESCRIBED MEDICATION (BIOPLUS)	7	0	17	1	2	1	Y	
52	BRUSHED TEETH AND ATE IN LAST HOUR AND EXCERCISED	3	0	3	1	2	2	N	BRUSHED TEETH AND CAFFEINE AND EXCERCISED
53	BRUSHED TEETH AND EXCERCISED	4	0	7	2	2	2	N	EXERCISE AND ALCOHOL
54	CAFFEINE	5	2	15	2	2	3	N	CAFFEINE
55		3	0	9	1	2	1	Y	
56		6	5	17	2	2	3	N	ALCOHOL
57	TOOK UNPRESCRIBED MEDICATION (TENSOPYN)	6	1	19	1	2	2	Y	
60	BRUSHED TEETH AND ATE IN LAST HOUR	3	0	13	2	2	2	N	ATE IN LAST HOUR
61		8	5	23	2	2	2	N	UNPRESCRIBED (VIRALGUARD)
62	BRUSHED TEETH	8	7	27	2	2	1	N	BRUSHED TEETH
63		0	0	4	2	2	2	Y	
64		5	0	19	1	1	1	N	EXERCISE
65		9	2	35	1	1	1	N	
66		8	6	18	2	3	1	N	BRUSHED TEETH

67		4	2	18	1	1	1	Y	
72	CAFFEINE	5	6	17	2	2	2	Y	
73	EXERCISE AND UNPRESCRIBED MEDICATION (SUPPLEMENT)	5	4	22	1	2	2	N	EXERCISE AND UNPRESCRIBED MEDICATION (SUPPLEMENT)
75	EXERCISE	6	9	16	2	2	2	Y	
78	ATE IN LAST HOUR AND UNPRESCRIBED MEDICATION (BENYLIN)	16	9	35	2	2	1	Y	

Participant	HADS-A_score_Int	HADS-D_score_Int	PSS_score_Int	Text_distract_from_lect	Anxiously_wait_for_text	Wish_texts_would_stop	Find_experience_stressful	Feel_different_about_texting	Text_emotion_CONT	Text_Frequency_CONT	Text_emotion_Int	Text_Frequency_Int
2	9	7	25	4	4	4	3	3	Neutral	10	Negative	10
3	3	2	16	3	4	2	2	1	Neutral	10	Negative	10
4	2	2	11	4	3	2	3	4	Neutral	10	Negative	10
5	3	1	13	4	4	5	2	4	Negative	10	Neutral	10
6	3	1	17	4	2	4	1	4	Negative	10	Neutral	10
7	7	0	14	4	4	1	1	1	Negative	10	Neutral	10
8	13	8	23	3	4	4	3	4	Negative	10	Neutral	10
9	3	2	18	4	4	4	3	4	Negative	20	Positive	15
10	7	7	21	4	4	2	2	2	Negative	20	Positive	15
11	12	7	23	5	2	5	3	4	Negative	20	Positive	15

13	6	2	21	5	3	4	1	3	Positive	15	Negative	20
14	2	1	14	2	2	3	2	2	Neutral	10	Positive	10
15	10	2	13	4	3	4	4	5	Neutral	10	Positive	10
17	10	7	19	4	5	5	4	3	Neutral	10	Positive	10
18	4	6	12	4	3	3	2	4	Positive	10	Neutral	10
20	2	0	17	1	1	1	1	1	Positive	10	Neutral	10
21	7	5	25	5	5	4	1	4	Negative	20	Negative	20
22	9	6	24	4	4	4	2	4	Negative	20	Negative	20
23	5	0	14	4	2	3	3	1	Negative	20	Negative	20
24	7	5	14	5	3	5	4	5	Negative	20	Negative	20
25	6	3	18	5	5	5	3	5	Negative	20	Negative	20
26	7	4	22	5	5	4	3	4	Negative	20	Negative	20
27	5	6	14	2	2	4	2	3	Negative	20	Negative	20
28	7	1	16	4	3	4	2	2	Negative	20	Negative	20
29	8	1	13	3	3	5	3	2	Negative	20	Negative	20
41	9	1	12	4	4	5	4	4	Positive	15	Negative	20
42	0	0	6	5	4	4	2	3	Negative	20	Negative	20
43	2	0	12	5	4	4	3	3	Negative	20	Negative	20
50	6	0	14	4	4	4	2	3	Positive	10	Neutral	15

51	9	0	18						Positive	10	Neutral	15
52	4	2	7	5	3	5	3	2	Positive	10	Neutral	15
53	4	1	6	1	4	2	1	4	Positive	10	Neutral	15
54	4	1	13	4	4	2	1	3	Positive	10	Neutral	15
55	2	0	9	4	2	4	2	4	Positive	10	Neutral	15
56	5	5	15	4	4	3	3	4	Neutral	15	Positive	10
57	4	1	14	2	3	3	1	3	Neutral	15	Positive	10
60	2	0	10	5	4	4	3	3	Neutral	15	Positive	10
61	7	4	20	5	3	4	2	3	Neutral	15	Positive	10
62	5	6	25	2	2	4	1	4	Positive	20		
63	1	0	5	4	4	5	1	1	Positive	20		
64	6	0	11	4	2	2	2	2	Positive	20		
65	8	2	32	5	4	4	2	4	Positive	20		
66	8	4	26	5	4	4	3	2	Positive	20		
67	7	4	22	4	3	5	3	4	Positive	20		
72	6	7	23	4	3	5	4	4			Positive	20
73	6	7	21	4	2	4	2	4			Positive	20
75	6	8	18	4	4	5	2	4			Positive	20
78	14	10	33	4	4	5	3	3			Positive	20

Participant	Cortisol_Cont_Bef	Cortisol_Cont_Aft	Cortisol_Int_Bef	Cortisol_Int_Aft
2	0.58107070	1.00553900	0.30738570	0.64949470
3	0.40285070	0.40017090	0.55166100	0.88108630
4	0.39483700	0.90159740	0.68829640	1.15851700
5	0.28396450	0.49503400	0.24165930	0.27937310
6	0.34146660	0.33034700	0.14656760	0.38492880
7	0.39816660	0.50181090	0.39683320	0.48980070
8	0.96239450	1.13880100	0.94163200	0.96239450
9	0.34583360	0.43500870	0.43710450	0.53970700
10	0.13834730	0.21155610	0.44342350	0.38558570
11	0.47381240	0.66587380	0.64645650	1.04773000
13	0.18144190	0.26777440	0.17469930	0.17298530
14	0.46558720	0.72927850	0.48299010	0.85640300
15	0.17672570	0.28376620	0.21483070	0.30800980
17	0.47773280	0.51611360	1.00624200	1.10965000
18	0.61718260	0.51520230	0.38369630	0.21916060
20	0.19726930	0.38680750	0.18660590	0.40576650
21	0.22712010	0.17527190	0.35782400	0.28123980
22	0.14922070	0.16962520	0.26054770	0.23807870
23	0.57932880	0.84212440	0.45701510	0.59111890

24	0.42112520	0.57835320	0.43595820	0.53826450
25	0.13740220	0.36759370	0.34601450	0.44180050
26	0.49685950	0.48825490	0.19936810	0.25467350
27	0.73318470	0.69521610	0.77500140	1.07877400
28	0.40892380	0.48398310	0.30678140	0.46209240
29	0.74767330	1.24145300	0.54840990	0.96713230
41	0.47718990	0.65765070	0.40658870	0.47044800
42	0.28333580	0.65254650	0.15624320	0.07242133
43	0.23752240	0.68358270	0.12664790	0.43669210
50	0.47579340	0.77354540	0.55509070	0.77130040
51	0.71733240	1.18551900	0.51552710	0.48176520
52	0.62111100	0.76349680	0.77354540	1.11894800
53	0.29334120	0.53786780	0.59695690	1.16975100
54	0.33281120	0.59519960	0.09450863	0.33463510
55	0.32797160	0.66422640	0.78831250	0.85689440
56	0.31484120	0.38448980	0.22519990	0.67297270
57	0.24567770	0.30545460	0.33707460	0.46472870
60	0.581293	0.98807850	0.53544070	1.08870800

61	1.09220100	0.29850410	0.46326600	1.09923700
62	0.58069050	0.64568430	0.74424510	0.96981310
63	0.79176530	0.62345670	0.68784930	0.84210870
64	0.19546650	0.26195890	0.16419910	0.22455810
65	0.43346330	0.47612520	0.32834290	0.43800220
66	0.29875300	0.40535630	0.45178720	0.66449880
67	0.08287639	0.10946520	0.15295410	0.18813310
72	0.45877470	0.53484610	0.34781110	0.52461830
73	0.47373660	0.66550060	0.43421770	0.63691670
75	0.16136820	0.53800000	0.29994620	0.44409770
78	0.29994620	0.51451650	0.68374170	0.66249830

Plate 1 OD (450-630)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.761	1.883	0.016	0.503	0.511	0.643	0.51	0.484	0.671	0.723	0.804	0.833
B	1.56	1.54	0.8	0.833	0.528	0.546	0.844	0.801	0.702	0.702	0.924	0.9
C	1.438	1.358	0.894	0.852	0.861	0.867	0.763	0.731	0.824	0.816	0.448	0.457
D	1.029	1.001	0.52	0.506	0.79	0.796	0.887	0.834	0.998	0.96	0.927	1.202
E	0.639	0.626	0.475	0.431	0.282	0.256	0.626	0.689	0.982	0.802	0.534	0.557
F	0.228	0.218	0.73	0.7	0.441	0.452	0.66	0.613	0.928	0.904	0.979	0.921
G	1.26	1.228	0.83	0.009	1.057	1.049	0.764	0.746	0.81	0.832	0.516	0.51
H	0.007	0.794	0.521	0.009	0.751	0.694	0.85	0.733	0.802	0.857	0.795	0.779

Plate 1 sample loading

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL A	CAL A	1AC	1AC	1BC	1BC	2AC	2AC	2BC	2BC	3AC	3AC
B	CAL B	CAL B	3BC	3BC	4AC	4AC	4BC	4BC	5AC	5AC	5BC	5BC
C	CAL C	CAL C	6AC	6AC	6BC	6BC	7AC	7AC	7BC	7BC	8AC	8AC
D	CAL D	CAL D	8BC	8BC	9AC	9AC	9BC	9BC	10AC	10AC	10BC	10BC
E	CAL E	CAL E	1AI	1AI	1BI	1BI	2AI	2AI	2BI	2BI	3AI	3AI
F	CAL F	CAL F	3BI	3BI	4AI	4AI	4BI	4BI	5AI	5AI	5BI	5BI
G	CONTROL 1	CONTROL 1	6AI	6AI	6BI	6BI	7AI	7AI	7BI	7BI	8AI	8AI
H	CONTROL 2	CONTROL 2	8BI	8BI	9AI	9AI	9BI	9BI	10AI	10AI	10BI	10BI

Key:

Numeric value indicates participant

"A"- indicates that the sample was obtained after the lecture

"B"- Indicates that the sample was obtained before the lecture

"C"- indicates Control day

"I"- indicates Intervention day

Red highlight- indicates pipetting error

Example: 1AC- indicates a sample collected from participant 1 on the Control day after the lecture

Plate 2 OD (450-630)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.814	1.79	0.566	0.586	0.693	0.725	0.857	0.802	1.001	0.873	0.734	0.627
B	1.602	1.554	0.724	0.68	0.713	0.649	0.645	0.611	0.903	0.929	0.949	0.873
C	1.417	1.385	0.961	0.917	0.866	0.892	0.885	1.009	0.999	0.96	0.561	0.498
D	0.966	0.921	0.682	0.612	0.648	0.647	0.757	0.714	0.776	0.763	1.05	0.953
E	0.596	0.583	0.529	0.519	0.714	0.684	0.822	0.8	0.894	0.889	0.458	0.42
F	0.2	0.197	0.475	0.467	0.908	0.866	0.79	0.728	0.762	0.728	0.959	0.889
G	1.266	1.23	0.842	0.821	0.799	0.753	0.896	0.841	0.876	0.821	0.654	0.628
H	0.635	0.567	0.728	0.7	0.666	0.671	0.75	0.703	0.758	0.688	0.01	0.784

Plate 2 sample loading

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL A	CAL A	14AC	14AC	14BC	14BC	15AC	15AC	15BC	15BC	17AC	17AC
B	CAL B	CAL B	17BC	17BC	18AC	18AC	18BC	18BC	20AC	20AC	20BC	20BC
C	CAL C	CAL C	21AC	21AC	21BC	21BC	22AC	22AC	22BC	22BC	23AC	23AC
D	CAL D	CAL D	23BC	23BC	24AC	24AC	24BC	24BC	25AC	25AC	25BC	25BC
E	CAL E	CAL E	14AI	14AI	14BI	14BI	15AI	15AI	15BI	15BI	17AI	17AI
F	CAL F	CAL F	17BI	17BI	18AI	18AI	18AI	18AI	20AI	20AI	20BI	20BI
G	CONTROL 1	CONTROL 1	21AI	21AI	21BI	21BI	22AI	22AI	22BI	22BI	23AI	23AI
H	CONTROL 2	CONTROL 2	23BI	23BI	24AI	24AI	24BI	24BI	25AI	25AI	25BI	25BI

Key:

Numeric value indicates participant

"A"- indicates that the sample was obtained after the lecture

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"I"- indicates Intervention day

Red highlight- indicates pipetting error

Example: 1AC- indicates a sample collected from participant 1 on the Control day after the lecture

Plate 3 OD (450-630)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.856	1.914	0.667	0.62	0.743	0.75	0.849	0.791	0.866	0.842	0.897	0.881
B	1.668	1.679	1.012	0.949	0.752	0.724	0.774	0.692	0.567	0.527	0.633	0.591
C	1.477	1.449	0.812	0.669	0.823	0.75	0.459	0.407	0.626	0.585	0.682	0.613
D	0.99	1.001	0.785	0.704	0.65	2.132	0.858	0.894	0.669	0.601	0.926	0.907
E	0.642	0.613	0.521	0.46	0.684	0.622	1.008	0.969	0.872	0.855	0.961	1.024
F	0.216	0.213	1.022	0.958	0.941	0.86	1.003	0.912	0.503	0.458	0.607	0.58
G	1.33	1.283	0.763	0.744	0.875	0.84	0.541	0.495	0.737	0.671	0.774	0.723
H	0.696	0.64	0.813	0.763	1.259	2.035	1.101	0.937	0.687	0.851	1.078	2.166

Plate 3 sample loading

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL A	CAL A	11AC	11AC	11BC	11BC	12AC	12AC	12BC	12BC	13AC	13AC
B	CAL B	CAL B	13BC	13BC	26AC	26AC	26BC	26BC	27AC	27AC	27BC	27BC
C	CAL C	CAL C	28AC	28AC	28BC	28BC	29AC	29AC	29BC	29BC	41AC	41AC
D	CAL D	CAL D	41BC	41BC	42AC	42AC	42BC	42BC	43AC	43AC	43BC	43BC
E	CAL E	CAL E	11AI	11AI	11BI	11BI	12AI	12AI	12BI	12BI	13AI	13AI
F	CAL F	CAL F	13BI	13BI	26AI	26AI	26BI	26BI	27AI	27AI	27BI	27BI
G	CONTROL 1	CONTROL 1	28AI	28AI	28BI	28BI	29AI	29AI	29BI	29BI	41AI	41AI
H	CONTROL 2	CONTROL 2	41BI	41BI	42AI	42AI	42BI	42BI	43AI	43AI	43BI	43BI

Key:

Numeric value indicates participant

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"I"- indicates Intervention day

Red highlight- indicates pipetting error

Example: 1AC- indicates a sample collected from participant 1 on the Control day after the lecture

Plate 4 OD (450-630)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.726	1.636	0.524	0.497	0.678	0.671	0.371	0.879	0.525	0.548	0.555	0.475
B	1.397	1.476	0.637	0.535	0.663	0.606	0.823	0.806	0.61	0.591	0.799	0.763
C	1.3	1.308	0.563	1.215	0.785	2.007	0.739	0.741	0.792	0.8	0.855	0.753
D	0.912	0.949	0.831	0.885	0.431	0.425	0.611	0.606	0.726	0.894	0.41	0.382
E	0.533	0.561	0.517	0.506	0.636	0.612	0.663	0.678	0.675	0.622	0.4	0.377
F	0.204	0.205	0.54	0.481	0.387	0.363	0.619	0.58	0.792	0.767	0.035	1.063
G	1.197	1.289	0.542	0.409	0.515	0.493	0.573	0.544	0.878	0.039	0.035	0.682
H	0.585	0.597	0.801	0.754	0.004	0.397	0.658	0.614	0.397	0.391	0.002	0.683

Plate 4 sample loading

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL A	CAL A	50AC	50AC	50BC	50BC	51AC	51AC	51BC	51BC	52AC	52AC
B	CAL B	CAL B	52BC	52BC	53AC	53AC	53BC	53BC	54AC	54AC	54BC	54BC
C	CAL C	CAL C	55AC	55AC	55BC	55BC	56AC	56AC	56AC	56AC	57AC	57AC
D	CAL D	CAL D	57BC	57BC	60AC	60AC	60BC	60BC	61AC	61AC	61BC	61BC
E	CAL E	CAL E	50AI	50AI	50BI	50BI	51AI	51AI	51BI	51BI	52AI	52AI
F	CAL F	CAL F	52BI	52BI	53AI	53AI	53BI	53BI	54AI	54AI	54BI	54BI
G	CONTROL 1	CONTROL 1	55AI	55AI	55BI	55BI	56AI	56AI	56BI	56BI	57AI	57AI
H	CONTROL 2	CONTROL 2	57BI	57BI	60AI	60AI	60BI	60BI	61AI	61AI	61BI	61BI

Key:

Numeric value indicates participant

"A"- indicates that the sample was obtained after the lecture

"B"- Indicates that the sample was obtained before the lecture

"C"- indicates Control day

"I"- indicates Intervention day

Red highlight- indicates pipetting error

Example: 1AC- indicates a sample collected from participant 1 on the Control day after the lecture

Plate 5 OD (450-630)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.787	1.777	0.66	0.649	0.71	0.668	0.532	0.502	0.619	0.595	0.629	0.703
B	1.645	1.549	0.586	0.586	0.589	0.541	0.637	0.63	0.79	0.711	0.802	0.754
C	1.446	1.431	0.779	0.771	0.894	0.813	0.781	0.813	0.875	0.88	0.669	0.621
D	1.014	1.015	0.741	0.791	1.237	1.036	1.207	1.23	1.027	0.955	1.079	1.009
E	0.631	0.625	0.932	0.888	1.045	0.918	0.95	0.945	1.075	0.976	0.73	0.7
F	0.253	0.244	0.75	0.773	0.757	0.685	0.923	0.754	0.693	0.596	0.786	0.718
G	1.203	1.261	0.664	0.654	0.782	0.773	0.713	0.025	1.036	1.024	0.784	0.758
H	0.674	0.673	0.9	0.853	0.725	0.729	0.913	0.84	0.649	0.643	0.658	0.613

Plate 5 sample loading

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL A	CAL A	62AC	62AC	62BC	62BC	62AI	62AI	62BI	62BI	63AC	63AC
B	CAL B	CAL B	63BC	63BC	63AI	63AI	63BI	63BI	65AC	65AC	65BC	65BC
C	CAL C	CAL C	65AI	65AI	65BI	65BI	66AC	66AC	66BC	66BC	66AI	66AI
D	CAL D	CAL D	66BI	66BI	67AC	67AC	67BC	67BC	67AI	67AI	67BI	67BI
E	CAL E	CAL E	64AC	64AC	64BC	64BC	64AI	64AI	64BI	64BI	72AC	72AC
F	CAL F	CAL F	72BC	72BC	72AI	72AI	72BI	72BI	73AC	73AC	73BC	73BC
G	CONTROL 1	CONTROL 1	73AI	73AI	73BI	73BI	75AC	75AC	75BC	75BC	75AI	75AI
H	CONTROL 2	CONTROL 2	75BI	75BI	78AC	78AC	78BC	78BC	78AI	78AI	78BI	78BI

Key:

Numeric value indicates participant

"A"- indicates that the sample was obtained after the lecture

"B"- Indicates that the sample was obtained before the lecture

"C"- indicates Control day

"I"- indicates Intervention day

Red highlight- indicates pipetting error

Example: 1AC- indicates a sample collected from participant 1 on the Control day after the lecture

Appendix I:Elisa plate data

Table 5.1 Cortisol ELISA Plate 1.

IBL International kit validation results (Lot: ECO138)

		Manual		
	Concentration µg/dL	Measured Value OD	Range OD	OD/OD max
CAL A	0.00	2.033	> 1.000	100%
CAL B	0.015	1.606	-	79%
CAL C	0.04	1.445	-	71%
CAL D	0.17	1.001	-	49%
CAL E	0.70	0.592	-	29%
CAL F	3.00	0.234	-	12%

		Acceptable range		
	Concentration found µg/dL	Lower limit µg/dL	Target µg/dL	Upper limit µg/dL
CONTROL 1	0.074	0.053	0.082	0.111
CONTROL 2	0.57	0.42	0.60	0.78

R square	0.9983

Study results

	Samples run in duplicate			
	Measurement 1	Measurement 2	Mean Measured Value OD (450-630)	SD (Error bar)
CAL A	1.761	1.883	1.822	0.08627
CAL B	1.56	1.54	1.55	0.01414
CAL C	1.438	1.358	1.398	0.05657
CAL D	1.029	1.001	1.015	0.01980
CAL E	0.639	0.626	0.6325	0.00919
CAL F	0.228	0.218	0.223	0.00707

	Concentration found µg/dL
CONTROL 1	0.0633
CONTROL 2	0.4336

Table 5.2 Cortisol ELISA Plate 2.

IBL International kit validation results (Lot: ECO139)

		Manual		
	Concentration µg/dL	Measured Value OD	Range OD	OD/OD max
CAL A	0.00	1.845	> 1.000	100%
CAL B	0.015	1.559	-	84%
CAL C	0.04	1.279	-	69%
CAL D	0.17	0.859	-	47%
CAL E	0.70	0.501	-	27%
CAL F	3.00	0.193	-	10%

		Acceptable range		
	Concentration found µg/dL	Lower limit µg/dL	Target µg/dL	Upper limit µg/dL
CONTROL 1	0.071	0.047	0.073	0.098
CONTROL 2	0.54	0.37	0.53	0.69

R square	0.9993

Study results

	Samples run in duplicate			
	Measurement 1	Measurement 2	Measured Value OD (450-630)	SD (Error bar)
CAL A	1.814	1.79	1.802	0.01697
CAL B	1.602	1.554	1.578	0.03394
CAL C	1.417	1.385	1.401	0.02263
CAL D	0.966	0.921	0.9435	0.03182
CAL E	0.596	0.583	0.5895	0.00919
CAL F	0.2	0.197	0.1985	0.00212

	Concentration found µg/dL
CONTROL 1	0.0619
CONTROL 2	0.6737

Table 5.3 Cortisol ELISA Plate 3.

IBL International kit validation results (Lot: ECO139)

		Manual		
	Concentration µg/dL	Measured Value OD	Range OD	OD/OD max
CAL A	0.00	1.845	> 1.000	100%
CAL B	0.015	1.559	-	84%
CAL C	0.04	1.279	-	69%
CAL D	0.17	0.859	-	47%
CAL E	0.70	0.501	-	27%
CAL F	3.00	0.193	-	10%

		Acceptable range		
	Concentration found µg/dL	Lower limit µg/dL	Target µg/dL	Upper limit µg/dL
CONTROL 1	0.071	0.047	0.073	0.098
CONTROL 2	0.54	0.37	0.53	0.69

R square	0.9999

Study results

	Samples run in duplicate			
	Measurement 1	Measurement 2	Measured Value OD (450-630)	SD (Error bar)
CAL A	1.856	1.914	1.885	0.04101
CAL B	1.668	1.679	1.6735	0.00778
CAL C	1.477	1.449	1.463	0.01980
CAL D	0.99	1.001	0.9955	0.00778
CAL E	0.642	0.613	0.6275	0.02051
CAL F	0.216	0.213	0.2145	0.00212

	Concentration found µg/dL
CONTROL 1	0.063
CONTROL 2	0.6166

Table 5.4 Cortisol ELISA Plate 4.

IBL International kit validation results (Lot: ECO 138)

		Manual		
	Concentration µg/dL	Measured Value OD	Range OD	OD/OD max
CAL A	0.00	2.033	> 1.000	100%
CAL B	0.015	1.606	-	79%
CAL C	0.04	1.445	-	71%
CAL D	0.17	1.001	-	49%
CAL E	0.70	0.592	-	29%
CAL F	3.00	0.234	-	12%

		Acceptable range		
	Concentration found µg/dL	Lower limit µg/dL	Target µg/dL	Upper limit µg/dL
CONTROL 1	0.074	0.053	0.082	0.111
CONTROL 2	0.57	0.42	0.60	0.78

R square	0.9982

Study results

	Samples run in duplicate			
	Measurement 1	Measurement 2	Measured Value OD (450-630)	SD (Error bar)
CAL A	1.726	1.636	1.681	0.06364
CAL B	1.397	1.476	1.4365	0.05586
CAL C	1.3	1.308	1.304	0.00566
CAL D	0.912	0.949	0.9305	0.02616
CAL E	0.533	0.561	0.547	0.01980
CAL F	0.204	0.205	0.2045	0.00071

	Concentration found µg/dL
CONTROL 1	0.0454
CONTROL 2	0.6121

Table 5.5 Cortisol ELISA Plate 5

IBL International kit validation results (Lot: ECO141)

		Manual		
	Concentration µg/dL	Measured Value OD	Range OD	OD/OD max
CAL A	0.00	2.071	> 1.000	100%
CAL B	0.015	1.765	-	85%
CAL C	0.04	1.514	-	73%
CAL D	0.17	1.041	-	50%
CAL E	0.7	0.647	-	31%
CAL F	3.0	0.254	-	12%

		Acceptable range		
	Concentration found µg/dL	Lower limit µg/dL	Target µg/dL	Upper limit µg/dL
CONTROL 1	0.079	0.055	0.085	0.115
CONTROL 2	0.55	0.41	0.59	0.76

R square	0.9996

Study results

	Samples run in duplicate			
	Measurement 1	Measurement 2	Measured Value OD (450-630)	SD (Error bar)
CAL A	1.787	1.777	1.782	0.00707
CAL B	1.645	1.549	1.597	0.06788
CAL C	1.446	1.431	1.4385	0.01061
CAL D	1.014	1.015	1.0145	0.00071
CAL E	0.631	0.625	0.628	0.00424
CAL F	0.253	0.244	0.2485	0.00636

	Concentration found µg/dL
CONTROL 1	0.0792
CONTROL 2	0.6093