Some Physiological Changes in Female Athletes During and After Exercise: Investigating the Use of a New, Low-invasive Sampling Method (Electrosonophoresis)

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ABSTRACT

The purpose of this study was to monitor cardiovascular and endocrine changes in sedentary and training females during a six week period, and to assess the accuracy of a new, low-invasive sampling methodology (electrosonophoresis). Changes in fitness were measured using oxygen consumption (VO_2). The impact on VO_2 of sleep quality, sleep duration and alcohol consumption (recorded in sleep logs) was assessed. Cortisol, testosterone and growth hormone levels in plasma were monitored for acute changes following fitness tests, and chronic changes related to training, oral contraceptive use or alcohol consumption. Hormone concentrations in blood and saliva samples were compared to those in interstitial fluid (obtained using electrosonophoresis) to investigate the accuracy of electrosonophoresis.

Mean VO_2 increased by 3.3 ± 1.3mL/kg/min between Week 1 and Week 5 and the changes detected in heart rate (HR) during the fitness tests suggest that aerobic fitness of the training participants increased across the study. No significant associations between sleep quality, sleep duration or alcohol consumption and VO_2 were detected. No acute changes in plasma hormone concentrations following fitness tests were detected. No chronic changes in plasma cortisol or testosterone concentrations were detected, although a non-significant trend towards increased plasma GH levels in training participants was detected. Resting plasma cortisol levels were significantly lower in oral contraceptive users compared with non-users. Plasma testosterone and growth hormone levels were unaffected by oral contraceptive use. Alcohol consumption had no acute detectable effects on plasma concentrations of the three hormones. Plasma testosterone levels were higher in participants who abstained from alcohol, and higher plasma growth hormone levels were detected in heavy drinkers. These results contrast with published reports. Concentrations of the three hormones in interstitial fluid and plasma exhibited highly significant positive correlations ($r^2 > 0.98$) with an interstitial fluid:plasma concentration ratio of about 1:10 in each case. Equations to predict plasma concentrations of cortisol, testosterone and growth hormone from interstitial fluid concentrations have been derived. The electrosonophoretic method apparently provides an accurate, painless, low-invasive method for prediction of the plasma levels of these three hormones. This technology has far-reaching implications for research in human, animal and biomedical fields.

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LIST OF ABBREVIATIONS

Greek Letters

α	Slope
α_0	Slope for cortisol
α_1	Slope for testosterone
α_2	Slope for growth hormone
α-MSH	Alpha-melanocyte-stimulating hormone
β	y-axis intercept
β_0	y-axis intercept for cortisol
β_1	y-axis intercept for testosterone
β_2	y-axis intercept for growth hormone
μL	Microlitre

English Letters

a.m.	*Ante meridiem*
ACTH	Adrenocorticotropic hormone
ADH^1	Anti-diuretic hormone
ADH^2	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
ANOVA	Analysis of variance
AT	Anaerobic threshold
AVP	Arginine vasopressin
bpm	Beats per minute
BIA	Bioelectric impedance analysis
BMI	Body mass index
BMR	Basal metabolic rate
CO	Cardiac output
CO_2	Carbon dioxide
CRH	Corticotrophin-releasing hormone
CVD	Cardiovascular disease
DHEA	Dehydroepiandrosterone
e.g.	For example
et al.	*et alii*
etc.	*et cetera*
EEG	Electroencephalogram
ELISA	Enzyme-linked immunosorbent assay
EOG	Electrooculogram
ESOP	Electrosonophoresis
FSH	Follicle-stimulating hormone
GABA	Gamma-aminobutyric acid
GH	Growth hormone
GHRH	Growth hormone-releasing hormone
GnRH	Gonadotropin-releasing hormone
GO_x	Glucose oxidase
hr	Hour
HDL	High-density lipoproteins
HPA	Hypothalamic-pituitary-adrenal
HPG	Hypothalamic-pituitary-gonadal
HPLC	High performance liquid chromatography

HR	Heart rate
HR_{max}	Maximum heart rate
i.e	*Id est*
IF	Interstitial fluid
IGF	Insulin-like growth factors (–I or –II)
IGFBP	Insulin-like growth factor binding protein
kD	KiloDalton
kg	Kilogram
km	Kilometre
L	Litre
LDL	Low-density lipoproteins
LH	Luteinizing hormone
max	Maximum
min	Minute
mL	Millilitre
n	Number
n_p	Number of participants
n_s	Number of samples
ng	Nanogram
nmol	Nanomolar
NAD^+	Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
NB	*Nota bene*
NIDDM	Non-insulin-dependant diabetes mellitus
NOC	Not on oral contraceptives
O_2	Oxygen
OC	Oral contraceptives
p	Probability statistic
pg	Picogram
pp	Pages
p.m.	*Post meridiem*
P	Plasma
PGO	Ponto-geniculo-occipital
PCO_2	Partial pressure of carbon dioxide
PO_2	Partial pressure of oxygen
r	Correlation coefficient
r^2	Coefficient of determination
RAS	Reticular activating system
REM	Rapid eye movement
RER	Respiratory exchange ratio
RIA	Radioimmunoassay
RNA	Ribose-nucleic acid
RPE	Ratings of perceived exertion
sd	Standard deviation
S	Saliva
S1	Stage 1
S2	Stage 2
S3	Stage 3
S4	Stage 4
SC	Stratum corneum

SEM	Standard error of the mean
SD	Sleep duration
SCN	Suprachiasmatic nucleus
SG	Sedentary group
SQR	Sleep quality rating
T	Testosterone
TG	Training group
TRH	Thyrotropin-releasing hormone
VO_2	Oxygen consumption
VO_{2max}	Maximal oxygen consumption
V_E	Mean expiratory flow
VCO_2	Carbon dioxide production
X	Concentration of hormone in interstitial fluid
Z_1	Indicator variable for testosterone
Z_2	Indicator variable for growth hormone

Symbols

/	Per
°C	Degrees Celsius
%	Percent
±	Plus or minus sign

Chapter One

Introduction

Provided here is a brief overview of the areas considered in the present thesis. This is followed by detailed consideration of the literature relevant to the areas covered in the thesis work and finally there is a precise statement of the purposes of the work.

Constraints imposed by ethical limits on what human participants can be expected to do during studies and particularly the potential that heavy demands on participants would impede their willingness to comply fully with the study protocol led to the decision to have a broad approach with four main areas (endocrinology, exercise physiology, sleep and alcohol consumption), as opposed to exploring any one of these in great detail. As the present study was conducted in humans, the measures used were less intrusive than what would have been used in animals.

Research into human (and animal) endocrinology has often been conducted using either blood or saliva samples, although both techniques have limitations. Repeated blood sampling currently provides the most accurate method of assessing changes in hormone release and distribution. However, the understandable reluctance of participants to undergo repeated venepuncture has limited interpretation of research findings due to the pulsatile nature of the release of certain hormones. Saliva samples have been used to monitor changes in hormone profiles, but this method has limitations. In particular, the definition of changes in hormone profiles due to acute exercise or to regular exercise training can be limited by the perceived unpleasantness of the methods of sample collection, reducing the willingness of participants to provide the optimal number of samples. Therefore the fewer number of samples restricts the definition of physiological responses to exercise.

The development of a new low-invasive method of monitoring hormone levels in interstitial fluid (electrosonophoresis) has provided a means for painlessly assessing levels of blood constituents. The accuracy of electrosonophoresis to provide an index of

blood constituents was evaluated in the present study through comparison of levels of cortisol, testosterone and growth hormone in plasma, interstitial fluid and saliva.

Research into hormonal changes as a result of acute exercise or chronic training has previously been limited to males, creating a gender bias in the literature. Recently, studies have been conducted focussing on the effects of resistance training or endurance training over long periods of time. The true effect of endurance training on hormone levels over a short time periods in females is still unknown.

Whilst the primary factors that influence performance during fitness tests are prior athletic training and standard of general health on the days surrounding a test, there are many external factors that can influence results. The factors that could affect performance include sleep quality and duration, oral contraceptive use and menstrual cycle phase.

One of the objectives of this research was to investigate possible effects of menstrual cycle phase or oral contraceptive use on the variables measured in this study. As well as an extensive literature review, information was collected from the participants regarding the timing of their cycles and negative symptoms during the different phases of the menstrual cycle. The information was analysed to ascertain if any relationships existed between menstrual cycle phase and either sleep quality ratings or sleep duration, or performance during the treadmill fitness tests. Sleep quality and duration were both determined from sleep logs completed each morning by all participants.

No significant relationships were observed for the phase of the menstrual cycle and the variables described above. This could have been due to lack of data confirming the phase of the menstrual cycle. The background literature and results pertaining to the menstrual cycle were eventually removed, for two reasons. The first reason was that the literature review was already quite long, for the reasons noted above, and to do the topics justice, the section on the menstrual cycle and oral contraceptive use would need to have been lengthy. The second factor was the paucity of significant relationships found in the present study. In addition, no progesterone assays were able to be conducted nor was basal body temperature monitored during the study, so menstrual cycle phase in the participants could not be confirmed.

A review of literature concerning the effects of alcohol and hangovers on performance is presented below. The inclusion of this section followed the observation in the majority of participants of high levels of alcohol consumption. Alcohol is known to affect exercise performance, both acutely and during the presence of a hangover, as well as the structure of sleep and the pattern of hormone release. All three of the above variables were monitored during this study.

1.1. Endocrinology

General characteristics of hormones, their modes of synthesis, secretion and action are presented in this section. Changes in hormone secretion that are induced by exercise are also reviewed.

1.1.1 Testosterone

Testosterone is a cholesterol-derived steroid hormone. In humans it exists in the unbound or free state (2%) or is bound primarily to plasma proteins such as sex-hormone binding globulin (60%) and albumin (38%). However it can also be bound to either corticosteroid binding globulin or red blood cells (Lamb, 1975; Greenspan and Gardner, 2001). Basal total plasma testosterone levels in males are between 2,600 to 10,000 pg/mL while in females the levels are significantly lower, at 150 to 700pg/mL (Greenspan and Gardner, 2001).

1.1.1.1 Synthesis and Secretion

In general, secretory control of testosterone is via the hypothalamic-pituitary-gonadal axis (Figure 1.1). The decapeptide gonadotropin-releasing hormone (GnRH) is released from the hypothalamus in pulses into the hypothalamohypophysial portal blood system, every 90 to 120 minutes (Bergendahl and Veldhuis, 1995; Greenspan and Gardner, 2001). GnRH travels to the anterior pituitary lobe via this portal system and induces secretion of two hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH). In males, testosterone is secreted from the Leydig cells located in the posterior region of the testes. Secretion from the Leydig cells is primarily under influence of LH.

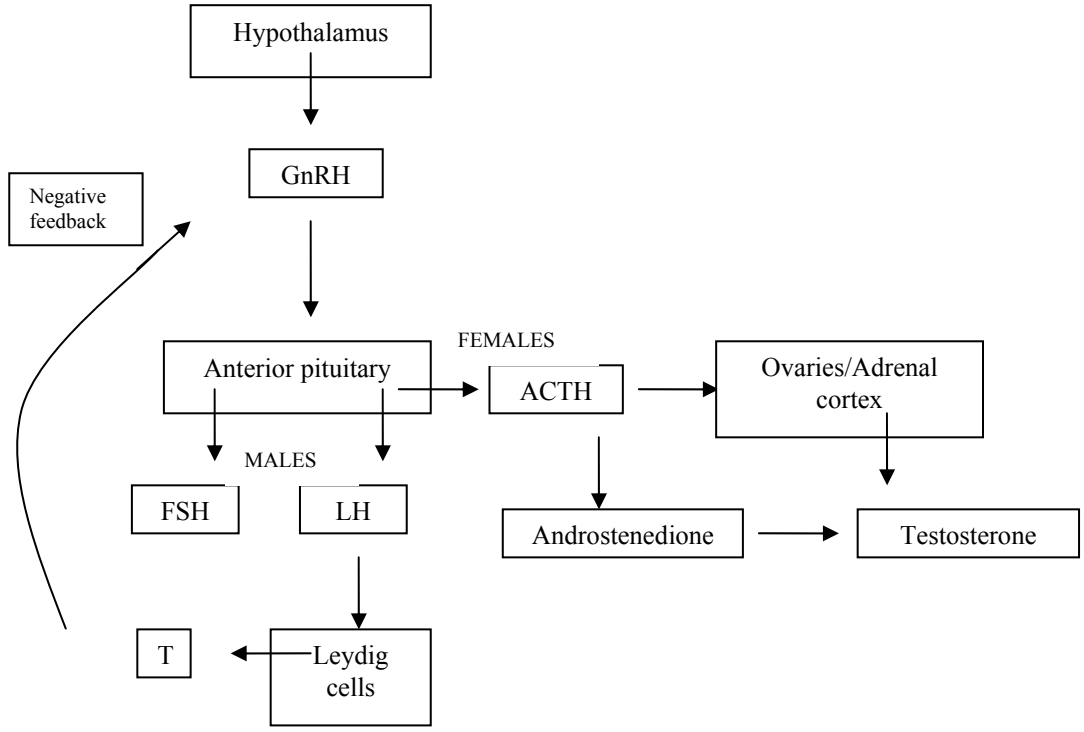


Figure 1.1: Hypothalamic-pituitary-gonadal axis control of testosterone release in males and females. Gonadotropin-releasing hormone =GnRH, Adrenocorticotropic hormone =ACTH, Follicle-stimulating hormone =FSH, Luteinizing hormone =LH.

1.1.1.1.1 Males

In males, FSH acting in conjunction with testosterone has a permissive effect on spermatogenesis within the Sertoli cells, a key process and purpose of the male reproductive system (Hackney, 1996; Nindl *et al.,* 2001). Control of FSH is also achieved via the hormone inhibin, which is released by the Sertoli cells when FSH concentration is high (Guyton, 1991).

1.1.1.1.2 Females

In females, testosterone is produced via three systems (Figure 1.1). The first is conversion from the steroid precursor androstenedione in peripheral tissues (50 to 70%). The second is production in the ovaries (25%) and the third is production in the adrenal cortex (25%). The latter two systems are stimulated by adrenocorticotropic hormone (ACTH), although their relative contributions change during the menstrual cycle (Greenspan and Gardner, 2001; Sowers *et al.,* 2001). The plasma concentrations of testosterone during the menstrual cycle change significantly, with production rates during the luteal phase being on average 2.5 times higher than those in the follicular

phase (Figure 1.2) (Oka *et al.*,1988). Radioimmunoassays have been shown to be less accurate than high-performance liquid chromatography (HPLC) in measuring testosterone concentrations, which may help to explain the lack of significant effects seen in several studies investigating testosterone (Shangold *et al.,* 1981).

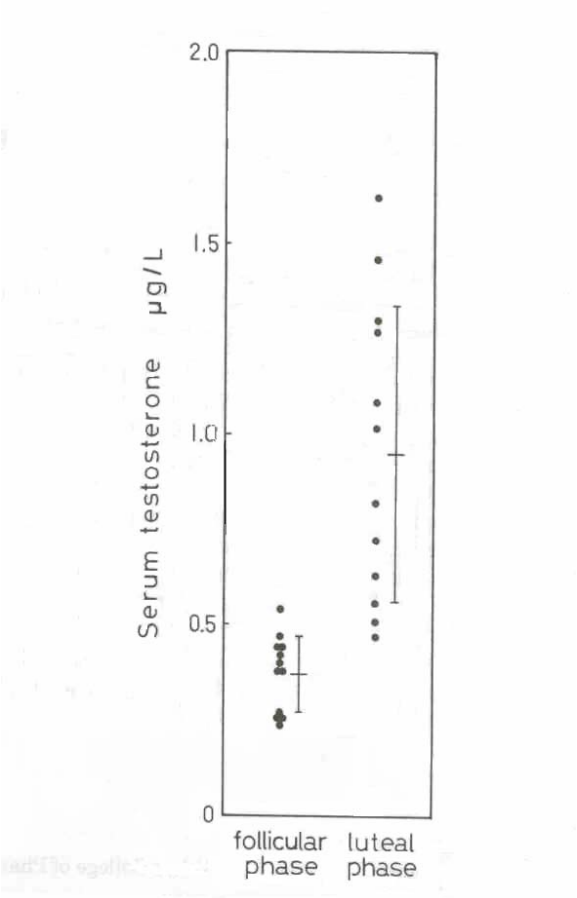


Figure 1.2: Changes in testosterone concentrations during the follicular and luteal phases of the menstrual cycle. Oka, *et al.*, (1988) pp. 558.

Smoking, physical activity, exogenous hormone therapy (such as oral contraceptive or hormone replacement therapy) and body composition indices (weight, body fat percentage and lean body mass) are all significantly and positively related to total plasma testosterone concentrations. In contrast, alcohol consumption, physical activity and dietary intake had no significant effect (Sowers, *et al.*, 2001). The impact of alcohol on the secretion of testosterone and other hormones is covered in detail below.

Control of plasma testosterone concentration (Figure 1.1) occurs by negative-feedback inhibition of GnRH, LH and FSH secretion. This occurs at the level of the hypothalamus and the anterior pituitary gland, once the level of testosterone in circulation has increased (Hackney, 1996; Greenspan and Gardner, 2001).

1.1.1.2 Actions

1.1.1.2.1 Males

In males, testosterone has developmental roles, such as being responsible for embryonic differentiation of male accessory glands and external genitalia. Postnatally, it is responsible for the development of secondary sexual characteristics and reproductive function, and has anabolic actions on skeletal muscle, the larynx, and long bones (Lamb, 1975; Greenspan and Gardner, 2001; Kraemer *et al.,* 2001). Following puberty, testosterone acts in adult males to maintain secondary sexual characteristics. Other actions include anabolic effects on muscle via androgen receptors located on muscle cells, a process facilitated by an increase in growth hormone production. Bone growth is promoted by the action of testosterone through stimulating production of the protein matrix in bone, as well as retention of calcium (Lamb, 1975; Shephard, 2000). The protein synthesis stimulated by testosterone leads to an overall increase in metabolic rate (Nindl, *et al.*, 2001).

1.1.1.2.2 Females

In females, in addition to affecting growth and development, plasma concentrations of testosterone may influence the current bone mass of premenopausal women, and can be used to predict bone mass levels as a woman ages (Consitt, *et al.*, 2001). Testosterone and its precursor dehydroepiandrosterone (DHEA) are important metabolic precursors of oestrogens (Kraemer, *et al.*, 2001). During exercise, the increase seen in plasma testosterone levels (described below) may contribute towards more efficient substrate utilisation (Consitt, *et al.*, 2001), although this can vary between individuals.

1.1.1.3 Changes as a result of exercise

1.1.1.3.1 Males

In males, testosterone displays release patterns specific to different intensities of exercise. After exercise of short duration, plasma testosterone levels increase relative to intensity, duration, muscle mass involved and catecholamine release, whereas endurance exercise of approximately three hours produces a decrease in plasma testosterone levels (Urhausen *et al.,*1995; Wilson, *et al.*, 1998; Lac and Berthon, 2000),

although individuals can display different release patterns. The decrease in basal testosterone levels seen in males can last for hours or days following endurance exercise (Urhausen, *et al.*, 1995; Nindl, *et al.*, 2001). Intensity is a term used throughout the current thesis, and refers to the level of stress experienced during exercise or fitness tests (Robergs and Roberts, 1997). Intensity can be reported in terms of percent of VO_{2max} achieved, and in this thesis, low exercise intensity is ≤45% $VO_{2max,}$ moderate intensity is 50 to 65% VO_{2max} and heavy intensity exercise is ≥65 VO_{2max} (Brooks and Mercier, 1994).

The response of testosterone to challenging situations is not general. In primates, the plasma testosterone response to stress may be related to the position the animal holds within the group hierarchy (Virgin and Sapolsky, 1997). In humans, the nature of the testosterone response to winning a competition may be associated with an individual's power motive, i.e. a disposition for gaining reward from having an impact of others (Schultheiss and Rohde, 2002).

The tissue-building actions of testosterone, combined with the increase seen following resistance exercise, can contribute to increases in fat-free mass (Powers and Howley, 1997). While an acute increase in testosterone is observed following strength training, individuals who resistance-train regularly may not show a chronic rise in plasma testosterone, though increases may occur that are within 'normal' physiological ranges (Zitzmann and Nieschlag, 2001).

In primates, subordinate males display different endocrine patterns from dominant males. Such differences include elevated baseline plasma glucocorticoid levels, an attenuated glucocorticoid response to stress and suppressed plasma testosterone levels during stress. However, these endocrine characteristics can change in relation to social behaviours such as consortship (behaviour leading to increased dominance within a hierarchy) or fighting, and displacement of aggression onto a third party following a stressor (Virgin and Sapolsky, 1997). In humans, mental stress can also decrease plasma testosterone levels. However, whether this phenomenon is mediated through corticotrophin-releasing hormone (CRH) down-regulating Leydig cell synthesis of testosterone or through a blunted anterior pituitary response has not been elucidated (Zitzmann and Nieschlag, 2001).

A chronic decrease in the concentration of testosterone is seen in over-trained endurance athletes. This can have negative effects on both sperm count and motility, possibly due to disruptions to the hypothalamic-pituitary-gonadal (HPG) axis and the balance of energy intake and use, which can alone affect testosterone production (Lamb, 1975; Arce *et al.,* 1993; Hackney, 1996; Lac and Berthon, 2000).

1.1.1.3.2 Females

In females, there may be an anticipatory increase in testosterone levels before exercise, although the mechanism behind this is not fully understood (Cumming *et al.,* 1987). Further information on changes in plasma testosterone levels is considered below in relation to the type of training undertaken.

Resistance training: Current evidence of an immediate increase in plasma testosterone levels following resistance exercise is equivocal. There is some evidence that resistance exercise can induce significant increases in testosterone levels during both the follicular and luteal phases of the menstrual cycle, although a lengthy training period may be required to elicit such a response (Consitt, *et al.*, 2001; Kraemer, *et al.*, 2001; Consitt *et al.,* 2002). Whilst women can significantly increase muscle mass over a period of resistance training, this does not always correlate with increases in plasma testosterone levels, and may be due in part to increased growth hormone levels. The short duration of several studies may have precluded the observation of changes in testosterone levels (Consitt, *et al.*, 2002).

Endurance exercise: Endurance exercise can increase plasma testosterone concentrations, a phenomenon that lasts for at least 30 minutes following the cessation of the exercise. The increase is related to both the exercise duration and intensity (Consitt, *et al.*, 2002). Long-term training effects of endurance exercise on plasma testosterone levels have not been conclusively demonstrated, although it is thought that over time resting concentrations in female athletes are lower than in sedentary controls (Urhausen, *et al.*, 1995; Consitt, *et al.*, 2002). In contrast, some studies have elicited no significant training response (Prior, 1987). As with several other hormones, the impact of exercise on the endocrine system can vary, due to factors such as nutritional status,

stress level, substance use, fitness level and stage or phase of the menstrual cycle (Consitt, *et al.*, 2001).

1.1.1.4. Testosterone:Cortisol Ratio

1.1.1.4.1. Males

In males, the ratio of free plasma testosterone to cortisol (a glucocorticoid hormone) at rest may be a marker of overtraining syndrome. Where there is a decrease in the ratio of more than 30%, the athlete's performance decreases, their mood profile changes often before hormone profile changes, and their time to fatigue decreases (Urhausen, *et al.*, 1995; Urhausen and Kindermann, 2002). Other physiological and psychological measures can be used as an alternative to the testosterone:cortisol ratio. Such alternatives include measurement of mood state, heart rate and immunological parameters, and substances such as ammonia, creatine kinase and lactate can be measured to monitor training responses, predict performance capacity and prevent or diagnose overtraining (Adlercreutz *et al.,* 1986; Urhausen, *et al.*, 1995; Filaire *et al.,* 1998). The validity of many of the parameters mentioned above has been questioned due to large inter-individual variation in many of the responses, and the subjectivity of the psychological measures. In general, the testosterone:cortisol ratio is an effective method for assessing the balance of anabolism and catabolism in individuals.

1.1.1.4.2. Females

In females, the testosterone:cortisol ratio in plasma has also been used as a marker of exercise-induced stress (Urhausen, *et al.*, 1995; Lac and Berthon, 2000). However, the use of this ratio has been questioned, with the dehydroepiandrosterone (DHEA):cortisol ratio being proposed as a better alternative (Filaire *et al.*, 1998). DHEA is a metabolic intermediate in the synthesis of anabolic/catabolic, oestrone or oestradiol. However, while DHEA concentrations are apparently affected by exercise, there being some reports of increases in saliva concentration after a lengthy training schedule (16 weeks) (Filaire *et al.*, 1998), there is a paucity of data on exact responses to different modes of training.

1.1.2 Cortisol

Cortisol is a glucocorticoid hormone, synthesised and secreted from the cortex of the adrenal glands that are located on the superior surface of each kidney. Basal plasma concentrations of cortisol range from 50 to 200ng/mL in the morning and 25 to 100ng/mL in the afternoon, with the unbound or free fraction at approximately 10%. Plasma concentrations of corticotropin releasing hormone (CRH) range from 24 to 40nmol/L and levels of adrenocorticotropic hormone (ACTH) range from 2 to 11pmol/L (Greenspan and Gardner, 2001). The half-life of cortisol in plasma is only 60 to 90 minutes, with the rate of clearance being affected by the extent of binding to the plasma proteins albumin (15%) and corticosteroid-binding protein (75%) (Greenspan and Gardner, 2001).

1.1.2.1 Synthesis and Secretion

Cortisol is an end product of the hypothalamic-pituitary-adrenocortical (HPA) axis hormonal cascade (Figure 1.3). In an unstressed state, the secretion of cortisol throughout the day is episodic and influenced by both time of day and ingestion of food in both sexes (MacLaren *et al.,* 1999). The mean plasma concentration of cortisol is stable throughout the different phases of the menstrual cycle (Galliven *et al.,* 1997; Greenspan and Gardner, 2001).

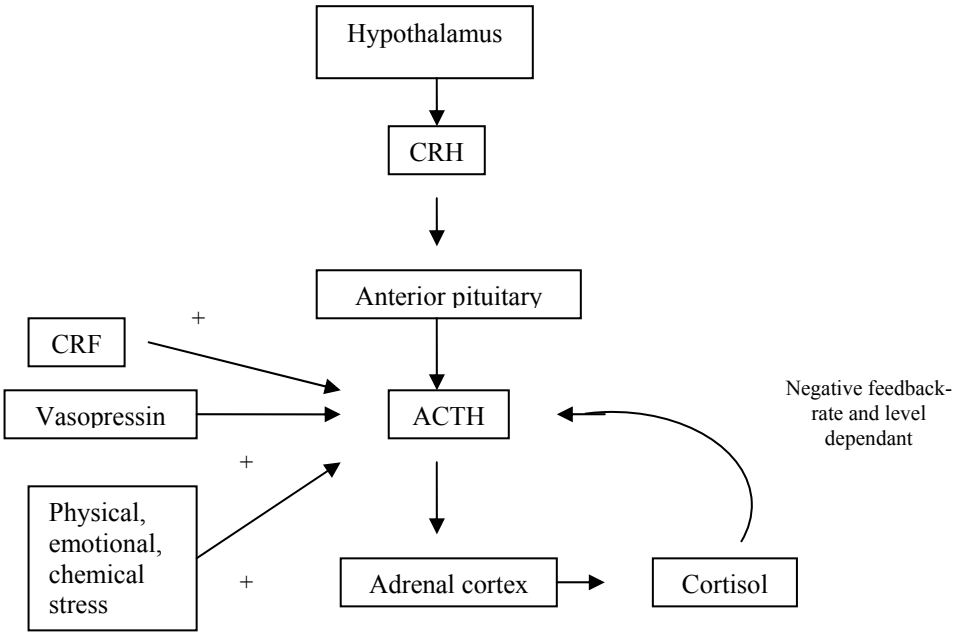


Figure 1.3: Hypothalamic-pituitary-adrenocortical axis control of cortisol release, with some influencing factors.

Cortisol production and release, as assessed by changes in plasma concentration, are sensitive to several stimuli. Physical stress, such as exercise (or restraint in animals), as well as emotional stimuli of sufficient intensity, can increase cortisol release within minutes (Stupnicki *et al.,* 1995; Greenspan and Gardner, 2001). The response of the HPA system to stress in females can vary during the menstrual cycle, with release at its highest at two points in the cycle: following the luteinizing hormone (LH) surge, a time when oestrogen levels are high, and during the luteal phase (McCormick and Teillon, 2001).

Alcohol consumption has been shown to elicit a non-specific stress response. Plasma cortisol levels increase during intoxication and the following morning (Linkola *et al.,* 1979) but not during night-time sleep (Prinz *et al.,* 1980). For further information on the response of different hormones to alcohol, see section 1.5.3 below.

1.1.2.2 Actions

Cortisol has several roles in addition to its characteristic effects on glucose metabolism. This hormone promotes protein catabolism in skeletal muscle by stimulating release of amino acids, which are utilised to repair muscles damaged as a result of exercise. Cortisol also increases glycerol and free fatty acid mobilization, and enhances hepatic glycolysis and gluconeogenesis during exercise (Lac and Berthon, 2000; Greenspan and Gardner, 2001; Kraemer, *et al.*, 2001).

Cortisol has deleterious effects on the body when plasma levels are chronically elevated, either naturally or through exogenous glucocorticoid treatment. Increased levels of cortisol can lead to osteopenia, osteoporosis and bone fracture, due to the actions of glucocorticoids on bone metabolism (Manelli and Giustina, 2000). Glucocorticoids can inhibit bone formation, via direct inhibition of osteoblasts, and resorption, via stimulating apoptosis of osteoclasts. Cortisol also reduces net absorption of calcium from the intestines and stimulates both urinary calcium secretion and parathyroid hormone release (MacLaren, *et al.*, 1999; Manelli and Giustina, 2000; Greenspan and Gardner, 2001).

Prolonged stress resulting in elevated cortisol levels can compromise the functioning of the cells that comprise the immune system, such as lymphocytes (Minton *et al.,* 1992). The suppression of immune system function by cortisol is thought to occur to prevent damage to the human or animal resulting from an 'overshooting' of immune system functions (Minton, 1994).

1.1.2.3 Changes as a result of exercise

In general, there is an anticipatory increase in cortisol concentrations before exercise, but this phenomenon may disappear following training (Hartley *et al.,* 1972). It was originally reported that plasma cortisol concentrations only increased at heavy workloads and would remain elevated for one hour if the person exercised to exhaustion (Hartley *et al.,* 1972). Further studies have revealed that the plasma and salivary cortisol levels increase linearly in proportion to the intensity and duration of exercise, although

this response can be affected by the time of day at which exercise is conducted (Chicharro *et al.,* 1998; Scheen *et al.,* 1998; Lac and Berthon, 2000). The increase in plasma cortisol levels seen during prolonged exercise helps to maintain glucose homeostasis by stimulating hepatic gluconeogenesis and sparing glycolysis through increasing lipolysis, ketogenesis and proteolysis (Del Corral *et al.,* 1998; Utter *et al.,* 1999). Once exercise is completed, levels of cortisol decrease rapidly, reaching basal values within a few hours. Exercise can impact on the release of cortisol during the night, with long-duration exercise of moderate intensity increasing cortisol concentrations in the first, but not second half of the night (Kern *et al.,* 1995).

The effects of training on resting plasma cortisol levels over time are still unclear, but it is thought a decrease may result from both endurance and resistance training, which can favour anabolism (Consitt, *et al.*, 2002).

1.1.2.3.1. Males

In males, cortisol levels increase during endurance exercise, whereas during resistance training cortisol levels decrease. The resultant increase in the testosterone:cortisol ratio favours anabolism and hypertrophy in muscle cells (Shephard, 2000).

1.1.2.3.2. Females

In females, plasma cortisol levels increase as a result of intense exercise in both the follicular and luteal phases of the menstrual cycle (Galliven, *et al.*, 1997). Plasma concentrations of cortisol are apparently higher in elite athletes when compared to sedentary females (Filaire *et al.*, 1998; Shephard, 2000). The same authors also noted a positive correlation between anaerobic threshold (AT)[1] and cortisol concentration.

[1] Anaerobic threshold: the point of a systematic rise in blood lactic acid concentration during exercise. Also known as lactic threshold or the onset of blood lactate accumulation. From Powers and Howley (1997) p.51.

1.1.3 Growth Hormone

Growth hormone (GH) is an anabolic somatomammotropic hormone secreted by the anterior pituitary gland. Concentrations vary throughout life, with maximal secretion occurring at puberty, although levels fluctuate due to its pulsatile release (Hurel *et al.,* 1999). Growth hormone exists in plasma bound to a specific binding protein, and has a plasma concentration of <20,000pg/mL after an overnight fast, and a plasma half-life of 20 to 50 minutes (Greenspan and Gardner, 2001). Reported resting concentrations range from 1,200 to 10,300pg/mL (Välimäki, *et al.*, 1983; Consitt, *et al.*, 2001).

1.1.3.1 Synthesis and Secretion

Growth hormone synthesis and secretion are stimulated by hypothalamic growth hormone releasing-hormone (GHRH) and the newly discovered GH-releasing peptide, Ghrelin, when the inhibitory effect of somatostatin is intermittently removed (Roelfsema *et al.,* 2001). Both GHRH and somatostatin are affected by noradrenergic and cholinergic neurotransmitters. An increase in somatostatin or a decrease in GHRH is stimulated by an increase in GH in a short, negative-feedback loop (Kanaley *et al.,* 1997; Müller *et al.,* 1999). Release from the anterior pituitary gland is pulsatile, with the largest pulses occurring at night during the onset of deep slow-wave sleep, (described in Section 4 below) (Spiegel *et al.,* 2000).

Women secrete more GH than men; baseline concentrations are twice that observed in men, women have larger bursts of secretion, and an overall higher output over 24 hours (Griffin and Ojeda, 2000; Pritzlaff-Roy *et al.,* 2002). The secretory pattern and activity of GH in women is related to ovarian hormones, with administration of oestrogen increasing the magnitude of GH pulses in post-menopausal women. Although mean pulse amplitude, duration and basal concentrations of GH decline with age, the decline is unrelated to menopausal status (Lamberts *et al.,* 1997; Cano *et al.,* 1999; Painson *et al.,* 2000). The decline in GH levels, and the decrease in responsiveness to GHRH, has been associated with increased adiposity, decreased lean body mass and decreased bone

mass leading to osteoporosis (Cano *et al.,* 1999). It should be noted that the pulsatile nature of GH secretion could hinder interpretation of experiments that seek to gauge the level of change in plasma as a result of some stimulatory or inhibitory input.

Stress, be it psychological or physical such as exercise, stimulates GH release, similar to the glucocorticoid cortisol. The impact of exercise on GH release is described below. An increase in body temperature, either actively through physical activity or passively through increased ambient temperature, augments the duration of slow wave sleep, which in turn results in enhanced GH release (Spiegel, *et al.*, 2000).

Insulin-induced hypoglycaemia stimulates the release of GH and, conversely, increased plasma glucose levels inhibit GH release, possibly through hypothalamic-stimulation of somatostatin release (Masuda *et al.,* 1985; Valcavi, 1996). This situation is also seen as a result of alcohol consumption, described in section 1.5.3 below (Redmond, 1981; Frias *et al.,* 2000).

Other substances have a stimulatory effect on GH secretion, such as anti-diuretic hormone (ADH^2), adrenocorticotropic hormone (ACTH), gonadotropin-releasing hormone (GnRH), thyrotropin-releasing hormone (TRH) and alpha-melanocyte-stimulating hormone (α-MSH), although the magnitudes of such effects are much less than that elicited by GHRH and somatostatin (Greenspan and Gardner, 2001).

1.1.3.2 Actions

Growth hormone and its effectors, hepatic insulin-like growth factors I and II (IGF-I and -II), play a significant part in growth and development of the heart, as well as bone, connective, cartilaginous, adipose and muscle tissue (Frias, *et al.*, 2000; Colao *et al.,* 2001; Consitt *et al.,* 2001). The GH-IGF pathway is also responsible for maintaining muscle mass and function in adults (Borst *et al.,* 2001). Growth hormone has acute and chronic metabolic actions within the body; including stimulation of amino acid uptake into cells and protein synthesis, stimulating lipid mobilization and oxidation in adipose tissue (causing the release of both free fatty acids and glycerol) (Redmond, 1981; Berneis and Keller, 1996). The acute actions of GH on carbohydrate metabolism are

transient and insulin-like. Growth hormone decreases plasma concentrations of glucose by stimulating its uptake into skeletal muscle, as well as by increasing the rates of glucose transport into and lipogenesis within adipose tissue (Dominici and Turyn, 2002). However, after a few hours the actions of GH reverse and become antagonistic to those of insulin, by creating a state of insulin resistance, increasing blood glucose levels by inhibiting glucose transport into cells and lipolysis (Dominici and Turyn, 2002).

Both GH and the IGF systems have been linked to reproductive development, as a growth spurt and sexual differentiation occur in synchrony at puberty (Franks, 1998). In younger people, increased GH levels have the capacity to stimulate growth, and in adults they can increase lean body mass and use of stored lipids for energy (Borer, 1995). In the adult female GH has been associated with stimulation of ovarian function such as folliculogenesis and steroidogenesis (Figure 1.4) both directly and indirectly via some actions of IGF-1, which are inhibited by its own insulin-like growth factor binding proteins (IGFBP-1 and -3) (Franks, 1998).

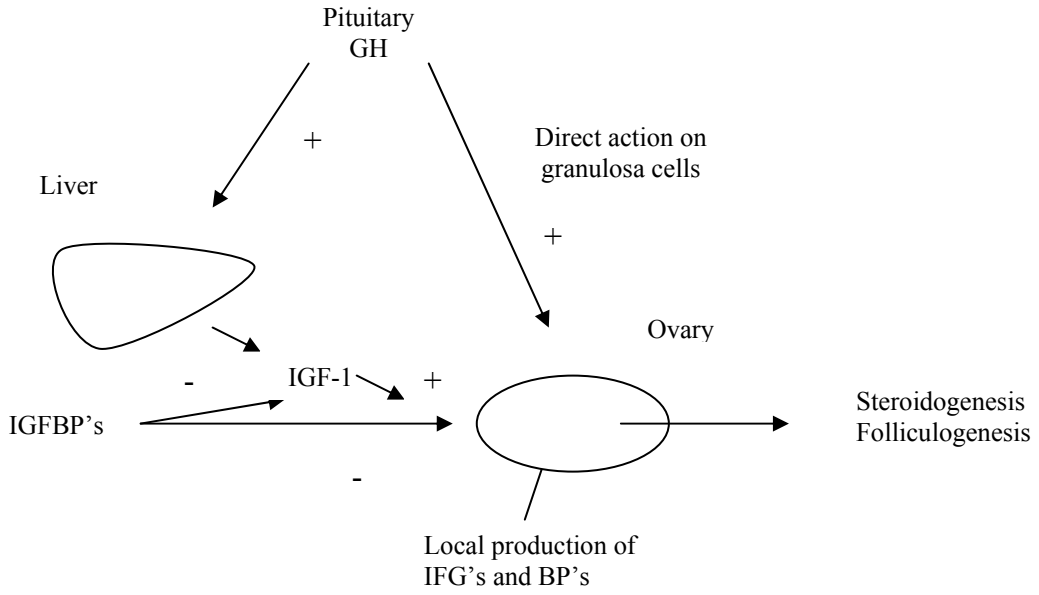


Figure 1.4: Possible role of GH and the IGF system in ovarian function. From Franks, (1998) pp.332.

1.1.3.3 Changes as a result of exercise

In general, exercise increases the height, magnitude and frequency of the circulating GH pulses, proportionally to intensity and duration, but the muscle mass used, fitness level and time of day at which exercise is conducted can influence the magnitude of the change (Felsing *et al.,* 1992; Borer, 1995; Ranallo and Rhodes, 1998; Scheen *et al.,* 1998; MacLaren, *et al.*, 1999; Pritzlaff *et al.,* 1999). This increase can have positive effects, including increasing lean body mass, increasing sensitivity to insulin and producing healthier lipid profiles (Hurel *et al.,* 1999).

A gender difference exists in the magnitude of the response to exercise, with women displaying higher plasma GH concentrations at matched intensities when compared to men (Pritzlaff-Roy, *et al.*, 2002). Both the baseline levels of GH and the response to exercise are increased when oestrogen levels are high, as in the luteal phase, and lower when levels are low, as in the follicular phase of the menstrual cycle (Hornum *et al.,* 1997).

Aerobic exercise stimulates an increase of plasma GH concentrations within 10 to 20 minutes, and GH levels peak during or immediately following exercise cessation, and remain elevated for approximately two hours (Kanaley *et al.,* 1997). The increased concentrations of GH, and therefore IGF-1, observed during and up to 60 minutes following resistance exercise, serve to create hypertrophy by increasing protein synthesis within the muscle, although this process is more efficient during recovery rather than exercise (Borst *et al.,* 2001; Pritzlaff-Roy, *et al.,* 2002).

Growth hormone exerts its metabolic effects during exercise of long duration (above thirty minutes) by changing the predominant fuel source away from carbohydrates towards energy-dense lipid deposits. Increases in the concentrations of several hormones are seen, such as catecholamines, cortisol, glucagon and GH, which act to decrease blood glucose utilisation and stimulate lipid mobilization and oxidation. These hormones do this by increasing lipase activity levels, which is a key step in releasing glycerol and free fatty acids for metabolism (Masuda *et al.,* 1985). The lipases mentioned above work synergistically to increase the release of free fatty acids and to

enhance their use as a fuel, and to increase the availability of glycerol for gluconeogenesis (Utter *et al.*, 1999).

Endurance exercise can increase daytime plasma GH concentrations, and can have variable effects at night. Moderate intensity, long-duration exercise as well as heavy resistance exercise decreases GH concentrations in the first half of sleep and increases levels in the second (Kern *et al.,* 1995; Nindl, *et al.*, 2001).

Circulating GH levels may transiently increase following certain intensities of resistance exercise, although a decrease in circulating levels has been observed 90 and 120 minutes after completion of a resistance exercise session (Kraemer *et al.,* 1993). While acute increases in plasma GH were observed following resistance training in untrained men and women, no chronic changes were observed after 8 weeks of training (Kraemer *et al.,* 1998).

Plasma concentrations of IGF-I and II increase as a result of exercise, although there is a delay in the increase of IGF-I, which can occur up to 16 hours after a bout of resistance exercise (Borst, *et al.,* 2001). Chronic increases in IGF-I concentration occur as a result of both resistance and endurance exercise, but this increase in concentration can take several weeks (Borst, *et al.*, 2001; Jenkins, 1999). Circulating levels of binding proteins (in the IGF system there are six) increase as a result of heavy resistance exercise, however only binding protein IGFBP-2 was increased for a number of hours following the cessation of exercise (Nindl, *et al.*, 2001).

1.2. Exercise Physiology

This section reviews the literature regarding the benefits of exercise and the changes in physiological variables that lead to those benefits. Monitoring change in these variables is achieved through exercise testing. In particular, aspects related to the present study are reviewed, such as gas analysis and the Bruce treadmill protocol, and whether it is possible to elicit changes in fitness over a short time frame.

The long-term benefits of taking regular exercise have long been established. Many countries have adopted recommendations that people engage in 30 minutes of physical activity each day. In New Zealand "Push Play" is one such initiative (Sport and Recreation New Zealand[2]). Benefits of regular exercise such as decreased morbidity and mortality rates, improved functional capacity, psychological well-being and quality of life are widely recognised (Cramer *et al.,* 1991; Lear *et al.,* 1999). As well as the positive benefits of exercise, regular physical activity also prevents the development of factors related to diseases such as obesity, Type 2 diabetes mellitus, hypertension, insulin resistance and many forms of cancers (Booth *et al.,* 2002). Physical inactivity is strongly linked to a poor state of well-being of an individual, and is the third leading cause of death in the United States and the second leading cause of obesity (Booth, *et al.*, 2002).

1.2.1 Physiological changes as a result of exercise

1.2.1.1 Cardiovascular system changes

The cardiovascular changes that occur as a result of endurance exercise are well understood. Adaptations to exercise include an increased rate and depth of respiration, improved perfusion/ventilation ratios in the lungs and an increase in both heart size and rate, and therefore cardiac output (CO). These factors, combined with metabolic and

[2] For more information, visit http://www.pushplay.org.nz/

muscular changes (not discussed in detail in this review) improve the efficiency of responses to exercise, which in turn increases performance (Powers and Howley, 1997). Increases in VO_{2max} can range from 5 to 30% as a result of a specific training programme, although the increase is proportional to the initial level of fitness prior to the intervention (ACSM, 1990).

An increase in fitness resulting from a training program can be seen in changes to cardiovascular measures of fitness. Repeated submaximal or maximal fitness testing allows observation of an increase in VO_{2max} (Xu and Rhodes, 1999). Increased aerobic fitness is also indicated by a lower heart rate at matched submaximal work rates (McInnis and Balady, 1994).

Energy for the aerobic system is derived in part through the oxidation of lipids. Endurance training enhances the oxidation of lipids through a number of adaptations, such as increased mass of the mitochondrial reticulum, increased fatty acid uptake into working muscles, increased β-oxidation and down-regulation of glycogenolysis and glycolysis (Holloszy, 1967; Bergman and Brooks, 1999). Another adaptation to endurance training is a decreased respiratory exchange ratio (RER)[3], which indicates the relative contribution of a substrate to energy production. A value of 0.7 indicates lipid oxidation, with higher values reflecting an increasing reliance on carbohydrate as fuel. A downward shift in RER as a result of training is firm evidence of increased lipid oxidation during endurance exercise in the fasted state (Gollnick, 1985; Bergman and Brooks, 1999).

1.2.1.2. Muscular changes

High-intensity, short duration exercise is commonly associated with large increases in muscle mass. However, the present study focused on increases in aerobic fitness generated by endurance training, so that an account of changes induced by resistance exercise is beyond the scope of this review.

[3] Respiratory exchange ratio is CO_2 output divided by the O_2 uptake (Brooks and Mercier, 1994).

1.2.1.3. Body composition and cardiovascular disease risk factors

The body composition of an individual (total body weight, fat mass and fat-free mass) is a reflection of a combination of their activity level, energy intake and hormonal profile. As mentioned earlier, physical inactivity is a significant factor in the pathogenesis of many diseases. A person's fat-free mass is the primary determinant of their basal metabolic rate (BMR) (Poehlman, 2002). Endurance training of sufficient duration and intensity generally increases fat-free mass (although it may remain constant) and decreases fat weight and total body mass. More rapid changes in body composition can be achieved through the addition of dietary manipulations to the training schedule. Thresholds of exercise intensity, duration and volume are outlined elsewhere (ACSM, 1990). These changes can help to decrease an individual's body mass index (BMI, measured in kg/m^2) which despite its limitations, is widely used to indicate at-risk individuals for many disease states, including diabetes mellitus, cardiovascular disease and hypertension.

The development of coronary heart disease is highly correlated with the appearance of several factors that are collectively called 'Syndrome X' or the 'Metabolic Syndrome'. These factors (Figure 1.5) include insulin resistance, increased blood pressure (hypertension) and blood lipid profiles, obesity (a BMI of over $27kg/m^2$), high levels of triglycerides and low-density lipoprotein (LDL) and low levels of high-density lipoprotein (HDL) (Krone and Muller-Wieland, 1990; MacMahon *et al.,* 1990; Barnard and Wen, 1994; Barth, 1995; Bray, 1996).

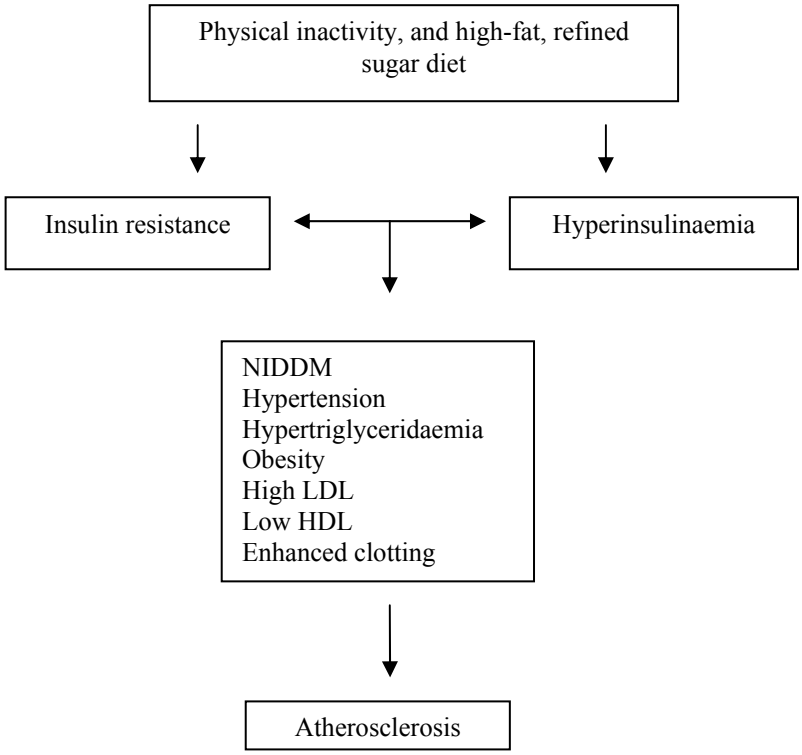


Figure 1.5: Proposed scheme for the development of risk factors associated with the metabolic syndrome. NIDDM: Non-insulin-dependent diabetes mellitus. From Barnard and Wen (1994) pp.219.

Exercise, in conjunction with nutritional (and pharmacological) modifications can prevent the development of risk factors and can aid in the treatment of coronary artery disease. Altering the diet of individuals with these risk factors has been shown to have significant effects, but this field of research, as well as that of pharmacological interventions, is beyond the scope of this review.

A single bout of exercise initiates a short-term (several hours) and longer-term (up to 24 hours) effect on glucose metabolism, by inducing insulin-like effects and increasing insulin sensitivity. This effect has been noted in insulin-resistant obese Zucker rats and in patients with obesity or non-insulin dependant diabetes mellitus (Barnard and Wen, 1994).

Exercise training has been shown to decrease weight, blood pressure and triglyceride levels, increase HDL levels, and to cause coronary disease to regress, changes that can occur without an increase in VO_{2max} (Barnard and Wen, 1994; Barth, 1995; Kokkinos and Fernhall, 1999; Booth, *et al.*, 2002). The exact effects of exercise on pre- and post-menopausal women have yet to be clarified, as in premenopausal women the cyclical variation in sex steroids markedly affects both cholesterol and triglyceride levels, while in post-menopausal women the age and the use of hormone replacement therapy may affect results (Krummel *et al.,* 1993).

There is a wide body of literature available on different aspects of obesity, hypertension, hypercholesterolaemia and cardiovascular disease, but it is not reviewed further in the present study.

1.2.2. Exercise testing

Exercise testing has been conducted since the early 1900's. While the methods used then were simplistic by today's standards, they began a field of exercise science that has produced many tests that are applicable in the medical arena to diagnose and manage cardiopulmonary disease (Bjarnason-Wehrens, 1999; Lear, *et al.*, 1999). In the exercise science area, these tests are used to assess fitness and monitor the effect of training in order to maximise performance (Mahler, *et al.*, 1999). There are many exercise tests that have been designed to test either a participant's aerobic fitness, defined as their maximal (or submaximal) oxygen consumption, to diagnose coronary artery disease, or to monitor the effects of exercise training (Powers and Howley, 1997; Heyward, 1998). Measurements of VO_{2max} are widely used, as it is a reproducible measure of the efficiency of an athlete's cardio-pulmonary system and aerobic metabolism. Tests to assess an individual's anaerobic fitness have also been developed, but a review of the literature on theoretical foundations, and the validity and accuracy of these tests is not conducted in this review.

1.2.2.1 Physiological variables during exercise

Several physiological variables change as a result of exercise to maintain homeostasis and muscular work. As an individual increases in fitness, the responses of several variables change and exercise tests can track these changes. Linear increases in ventilation, cardiac output (CO) and carbon dioxide output and oxygen uptake are normally observed during dynamic exercise with increasing intensity (Hill *et al.,* 1927; Åstrand and Saltin, 1961). These changes are associated with increasing heart rate, stroke volume and respiratory frequency, which combine to increase both oxygen delivery to working muscles and transport of metabolites to the liver to be catabolised. The point at which a participant's cardiovascular system cannot respond adequately to the demands placed on it by the exercise defines their maximal aerobic capacity, or VO_{2max}.

Originally, Hill, *et al.*, (1927) reported that VO_{2max} is limited only by the oxygen saturation of the blood, the oxygen capacity of the blood and the circulation rate, without mention of the oxidative capacity of the muscles. It is now accepted that VO_{2max} is limited both centrally and peripherally by the efficiency of the heart (CO), the ability of the lungs to oxygenate the blood and the capability of the working muscle to utilise the oxygen. During an exercise test, attainment of VO_{2max} is determined by the appearance of one or more of the following: a plateau in VO_2 despite increases in work intensity, onset of extreme exhaustion, a blood lactate level of >10nmol/L, a rating of perceived exertion (RPE) of 9 or 10, or if the participant signals to end the test (McConnell, 1988).

1.2.2.2. Submaximal tests

Submaximal tests are used in cases when a maximal test is not appropriate, such as in previously untrained sedentary individuals or persons with respiratory or cardiovascular disease (Mahler, *et al.*, 1995). A submaximal test is based on the assumption that heart rate increases linearly with work of increasing intensity to a maximum value, so that heart rate can be used to predict an individual's VO_{2max}. Protocols such as Åstrand and modified versions of other popular tests, such as the Balke protocol, are commonly used

as they activate large muscle groups and are of an appropriate duration and intensity (Åstrand and Ryhming, 1954).

In these instances, a submaximal test is conducted and the participant's VO_{2max} is predicted by nomogram-based algorithms or extrapolation of the linear relationship between heart rate and VO_2, up to a persons predicted maximum heart rate. Whilst the Åstrand nomogram has been widely used, linear extrapolation methods result in less bias and tighter limits and are therefore preferable (Macsween, 2001). Problems with the linear extrapolation method occur due to assumptions that are not always met. For example, an individual's maximum heart rate (HR_{max}) is commonly derived from the equation by Mahler, *et al.*, (1995):

$$HR_{max} = 220 - \text{participant's age}$$

HR_{max} has a standard deviation of approximately 11 beats/min. This standard deviation can lead to one of two problems: an individual could be over exerted by exercising at heart rates higher than their true maximum (which could pose risks to the participant), or under exerted by exercising at heart rates below their true maximum. Both situations could occur without the knowledge of either the participant and tester, and could lead to inaccurate results (Londeree and Moeschberger, 1984).

1.2.2.3. Gas analysis

The recent development of on-line gas analysis techniques has provided the opportunity to more accurately determine exercise capacity, exercise intensity, energy expenditure and substrate utilisation during exercise. In patients with cardiorespiratory illnesses, gas analysis can help to differentiate between cardiac or pulmonary causes, due to disease-specific changes in cardiovascular variables during exercise, and thereby help to determine the prognosis for the individual (Lear, *et al.*, 1999; Singh, 2001). The Douglas bag method is considered to be the "gold standard", and can be used to assess both maximal and submaximal VO_2 and the anaerobic threshold (Yoshida *et al.,* 1981). However, the recent development of portable and automated on-line breath-by-breath

analysis systems has provided the opportunity to monitor rapid changes in gas volume, ventilation and concentrations of both O_2 and CO_2 (Carter and Jeukendrup, 2002).

1.2.2.3.1. The Pulmolab EX670 System

The efficacies of three mass spectrometry systems including the Pulmolab EX670 system (used in this study) have been compared to the Douglas bag method. Results from the Douglas bag method and the three systems tested on a portable metabolic simulator and from 10 healthy active participants undergoing fitness tests on a cycle ergometer were compared. Carter and Jeukendrup (2002) reported results that question both the validity and reliability of the Pulmolab at workloads up to 150 watts.

Compared to the other systems, the Pulmolab underestimated both mean expiratory flow (V_E) and VO_2 and overestimated carbon dioxide production (VCO_2) in the metabolic simulator test. The results from the test using human participants showed that the Pulmolab produced similar V_E results to the Douglas bag method, but again underestimated VO_2, while VCO_2 and RER results exceeded physiological limits (Carter and Jeukendrup, 2002). The coefficients of variation were also reported, with the Pulmolab producing the highest values, as seen in Figure 1.6 below.

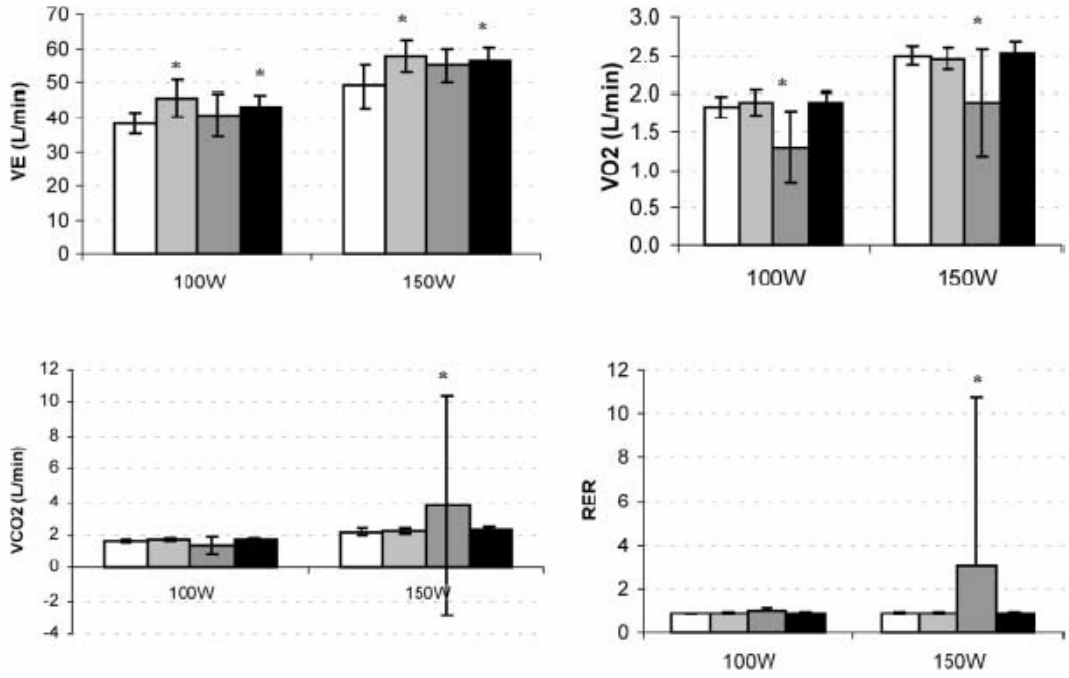


Figure 1.6: Mean V_E, VO_2, VCO_2, and RER for four different gas analysis systems at 100W and 150W workloads. NB. $V_{E=}$ Mean expiratory flow, $VO_{2=}$ Oxygen uptake, $VCO_{2=}$ Carbon dioxide production, RER= Respiratory exchange ratio. *White bars* =Douglas bags, *light grey bars* =Oxycon Alpha, *dark grey bars* =Pulmolab, black bars =Oxycon Pro. From Carter and Jeukendrup (2002) pp.439.

Some of the variation in results can be attributed to the design of the mouthpiece. The close proximity of the saliva collection container to the capillary tube that samples the inspired and expired air could lead to blockage of the tube by saliva, resulting in inconsistent gas samples, affecting the results (Carter and Jeukendrup, 2002).

In general, such problems were not encountered in the present study, which employed the Pulmolab EX670, except perhaps during the final week as explained in detail in the Results section.

1.2.2.4. Validation of Bruce protocol

The Bruce protocol (described in the Materials and Methods section) was developed in the early 1970's and is used extensively by both cardiology and exercise physiology laboratories. It was the test used in large scale studies such as the Coronary Artery

Surgery Study (Sami *et al.,* 1984) and the Duke Treadmill Score (Fielding *et al.,* 1997; Myers and Bellin, 2000). The Bruce protocol is considered to be suitable for both trained and healthy untrained individuals, both young and old, as well as patients recovering from acute myocardial infarction (Fielding, *et al.*, 1997; Lear, *et al.*, 1999; Senaratne *et al.,* 2000). However, Myers and Bellin (2000) have shown that the Bruce protocol overestimates exercise capacity in untrained people and has a lowered sensitivity for detecting coronary disease, due to the large and unequal increases in exercise intensity during the test. Accordingly, the Bruce protocol was modified (Section 2.5.1) to produce equal increases in intensity before use in the study described here.

The Bruce protocol provides the more familiar exercise modality of running compared with tests using a bicycle ergometer and is within the test duration (8 to 12 minutes) recommended to minimise discomfort and the perception of difficulty, while obtaining the highest VO_{2max} result (Kang *et al.,* 2001; Lear, *et al.*, 1999). However, Kang and Chaloupka (2001) report that the Bruce protocol may underestimate VO_{2max} in highly trained people as the large increments in work intensity during the latter stages of the test can precipitate muscle fatigue and increase anaerobic metabolism. Compared to similar exercise tests such as the Costill/Fox protocol and the Åstrand protocol, the Bruce protocol elicits the anaerobic threshold at lower VO_2 measurements, a phenomenon thought to be due to increased anaerobic metabolism and hyperventilation resulting from the large increment in work intensity between stages (Kang and Chaloupka, 2001). The Bruce protocol has shown high test-retest reliability, with a coefficient of variation of 3% (Fielding, *et al.*, 1997).

1.2.2.5. Is it possible to elicit changes in VO_{2max} over a short period of time?

The present study was designed to assess increases in aerobic fitness of the participants over the course of a six-week study. A review of the literature focusing on increases in fitness over short-time periods was therefore conducted.

Female army recruits undergoing a six-week period of exercise training displayed increases in mean body weight, fat free mass, maximal cycle ergometer test time, estimated VO_{2max}, and strength variables, in both British and American recruits (Brock and Legg, 1997; Daniels *et al.,* 1979). However, it should be noted that the physical training undertaken by recruits was more extensive than the training regime undertaken by the participants in the present study. A six-week training period can result in increases in VO_{2max} in participants undergoing high intensity (Hickson *et al.,* 1981), lower intensity (Cunningham and Cantu, 1990) and endurance training (Carter *et al.,* 1999).

Aerobic endurance capacity and work rate at predefined heart rates increased after a four-week exercise programme in patients with coronary artery disease, independent of motivational factors (Bjarnason-Wehrens *et al.,* 1999). Increased aerobic capacity was also observed in control participants and heart transplant recipients following a six-week training programme (Lampert *et al.,* 1998).

1.2.3. Ratings of perceived exertion

Physical exertion is the subjective intensity of effort, strain and/or discomfort that is experienced during exercise (Robertson, *et al.,* 1998). This definition has provided a basis for a sliding scale of perceived exertion to be formulated, a psychophysical link between physiological changes that occur during exercise and any consciously monitored sensory responses. Each facet of physiological change exerts an influence on the perception of well-being during exercise. One of the leading scholars in this field

was Professor Gunnar A. Borg, who developed the 15-category Borg Scale and a 10-unit category-ratio scale (Table 1.1) (Borg, 1982).

Table 1.1: Borg's rating of perceived exertion scales. From Borg (1982) pp.378-380.

Borg RPE scale	Verbal phrase	Category-Ratio RPE scale	Verbal phrase
6		0	Nothing at all
7	Very, very light	0.5	Very, very weak
8		1	Very weak
9	Very light	2	Weak
10		3	Moderate
11	Fairly light	4	Somewhat strong
12		5	Strong
13	Somewhat hard	6	
14		7	Very strong
15	Hard	8	
16		9	
17	Very hard	10	Very, very strong
18		.	Maximal
19	Very, very hard		
20			

The scales above are based upon Borg's Range Model that links human sensory responses to external physical stimuli. It is based on the assumption that for cognitively and physiologically 'normal' individuals, stimulus-response agreement exists throughout the entire relative metabolic range during dynamic exercise (Noble and Robertson, 1996; Robertson, *et al.*, 1998). That is, increasing exercise intensity induces changes in both physiological state and perception of that state. This awareness can be used to assess exercise tolerance, adaptation to training and to prescribe and monitor training intensity. The second method (category method) uses the reference intensity of maximum exertion, which allows for inter-individual comparisons.

The category ratio scale and the Borg Rating of Perceived Exertion (RPE) Scale (Table 1.1) are the most commonly used methods today, as they have many practical applications (Russell, 1997). The RPE scale uses values from 6 to 20, matching increased heart rates from 60 to 200 beats, although RPE has been shown to be more highly correlated to VO_{2max} than heart rate (Dishman, 1994).

Validation of this perceptual measure has been achieved using such measures as the Perceptually Modified Sjöstrand Cycle Ergometer Test, the Simple Walk/Run Test, treadmill running, field running and swimming (Noble and Robertson, 1996; Robertson, *et al.*, 1998; Russell, 1997). The run test is a submaximal test that can be incorporated into daily training to track progress or to assess aerobic fitness. Unlike the Sjöstrand protocol, the intensity (velocity) is self-selected over each of the three trials, each increasing in intensity. The benefit of this test is that training sessions can be compared easily and progress can be tracked, as the faster the running velocity, the higher the aerobic fitness (Robertson, *et al.*, 1998).

The physiological responses to stress such as exercise can be measured and correlated to changes in perceived exertion, as multiple sensory inputs (both central and local) are integrated and weighted by the individual to arrive at an overall rating (Russell, 1997). However, there is still some 30% of variation between RPE and physiological parameters, which has not yet been explained. These responses can be divided into respiratory/metabolic mediators and peripheral mediators. Pulmonary ventilation, oxygen uptake and heart rate are the (central) primary respiratory/metabolic signals of physical exertion, however each has its limitations. Ventilatory function appears to exert influence over perception only once the anaerobic threshold is reached, and under experimental conditions, heart rate did not change in parallel with perceived exertion (Robertson, *et al.,* 1998).

The peripheral or local signs of exertion such as muscle discomfort are mediated by metabolic changes during exercise, such as blood pH, lactic acid concentration and energy substrate availability (Noble and Robertson, 1996; Russell, 1997). Several hormones such as catecholamines and beta-endorphins, as well as metabolites such as blood glucose and blood pH are correlated with perception, especially at exercise intensities exceeding that of the anaerobic threshold (Robertson, *et al.*, 1998; Utter *et al.*, 1999). Inducing lactic acid production by exogenous application of alkali does not influence perceived exertion, questioning its validity as a mediator (Noble and Robertson, 1996). Local factors dominate perceived exertion during low to moderate intensity exercise, while central factors have more of an influence during high intensity exercise (Russell, 1997).

1.3. Sample collection: Past, present and future

Monitoring of substrates has previously centred on blood and saliva samples, which whilst having many advantages, have posed problems for researchers due to their limitations. An introduction to iontophoresis is presented here, as a background to the developing field of sample collection by reverse iontophoresis and electrosonophoresis.

1.3.1 Blood

Blood is the body's transport medium for many constituents, including cells, immune system components, hormones, blood-clotting agents, buffering systems, gases, metabolites, wastes and nutrients. It is a medium within the vascular system that is constantly changing due to the simultaneous occurrence of endogenous and exogenous events. Endogenous events include the pulsatile release of hormones throughout a day or the changing baseline concentrations throughout the menstrual cycle. Exogenous events include the consumption of a meal, which itself has downstream effects on the concentrations of other constituents.

The variable nature of blood concentrations is a reflection of the process of homeostasis, as the body seeks to keep its systems in balance in the face of varying demands, both internal and external. The constituents carried in the blood may be compounds en route to a target site to initiate a pathway or secretion of a hormone. They may also be metabolites and other compounds resulting from breakdown of a cell or substrate, which are being transported to the liver or kidney to be recycled or excreted.

Measuring levels of blood constituents in plasma is considered to be the "gold standard" within medical and exercise science fields, as it gives the most accurate indication of, for instance, the hormonal milieu at that particular site at that time. Taking repeated samples following a particular treatment or exercise allows researchers to evaluate responses and/or detect patterns of hormonal release as a result of that treatment. The composition of the blood can be dependant on the site of sampling which affects the type of blood (arterial/mixed/venous) obtained.

An example of this is the difference in partial pressures of oxygen (PO_2) and carbon dioxide (PCO_2) in arterial and venous blood. Arterial blood has PO_2 of 95mmHg before it enters capillaries, while the PO_2 of venous blood is only 40mmHg, a concentration gradient favouring net diffusion of O_2 into cells. There is a 5mmHg difference between PCO_2 in the venous blood (45mmHg) and that of arterial blood (40mmHg), although this difference is sufficient to drive the movement of the CO_2 molecule from the pulmonary capillaries into the alveoli (Guyton, 1991).

In contrast to the changes in gas concentrations in different blood vessels, the concentrations of glucose in venous blood, capillary blood and interstitial fluid are highly correlated. Changes in glucose concentrations display no measurable lag time between each fluid (Thennadil *et al.,* 2001). There is also congruence between glucose concentrations sampled from veins in both the hand and foot (Seaquist, 1997). A detailed search of the literature has revealed a lack of information regarding arterial-venous differences in hormone concentrations. One of the reasons for this paucity is that studies of hormonal changes and effects are focused on the site of action and do not require a comparison of the concentrations at different places in the vascular system. However, one study reported different concentrations of catecholamines in arterial and venous blood, with concentrations of both adrenaline and noradrenaline being higher in arterial blood compared to venous blood during marked hypoglycaemia (Liu *et al.,* 1993). Tamm *et al.,* (1982) reported concentration gradients in plasma of six steroid hormones across the testes, with the largest gradient being reported for testosterone.

Repeated blood sampling is often required of hospital patients or research participants for monitoring acute changes in blood constituents. Repeated venepuncture can cause significant pain to the individual and compromise the integrity of the blood vessels being used. An alternative to this is the insertion of a short- or long-term indwelling catheter. The incidence of catheter use in hospitals is increasing, with above 50% of all hospitalised patients undergoing insertion of such a device (Bouza, 2002). The presence of a catheter may expose the individual to micro-organisms present in the external environment, by providing a pathway into the body along the external wall of the catheter. The most common infections resulting from the presence of a catheter include staphylococcal, enterococcal and candida blood stream infections (Schierholz *et al.,*

2001). The type of bacteria causing the infection is related to the length of time the catheter is inserted. Short-dwelling catheters (<8 days) are linked primarily to infection from skin micro-organisms (70 to 90%), followed by bacteria from the hub/lumen of the catheter (10 to 50%), the bloodstream (3 to 10%) and infusate (<3%). Catheters present for over 8 days are primarily infected from bacteria within the hub of the catheter, followed by skin microorganisms (Pascual, 2002). Research continues into altering the properties of the catheter material, including the addition of anti-microbial drugs within the polymer matrices. However, the use of catheters is still associated with increased morbidity, mortality and negative economic consequences (Goldmann and Pier, 1993; Schierholz *et al.,* 2001; Eggimann and Pittet, 2002; Pascual, 2002).

The problems and limitations associated with blood sampling have led to research into collecting alternative fluids, such as saliva and interstitial fluid, so that an index of the plasma concentration of a constituent can be determined as an alternative to the true plasma concentration.

1.3.2 Saliva

Saliva samples have been used for over 40 years in order to monitor the levels of hormones and other blood constituents. Collection of saliva is straightforward, non-invasive and painless in humans, ruminant livestock, and mid-sized and small animals (Fenske, 1996; Cook, 2002). Levels of hormones in saliva represent only 3 to 10% of plasma levels (Cook, 2002). Saliva concentrations of hormones correlate well with plasma concentrations, when the 20 to 30 minute time lag for constituents to partition into saliva is allowed for (Cook, 2002). The ease of use of saliva sampling in laboratory and field settings, especially in human studies, has previously been unparalleled. This is due to the low level of risk for both the provider and the collector of the saliva, the ability to take repeated samples at the most beneficial times and the stability of salivary constituents at ambient temperatures if treated or preserved correctly (Hofman, 2001). Many substances can be tested for in saliva, including steroid and non-peptide hormones, antibodies and therapeutic and illegal drugs (Hofman, 2001). Previously, it was assumed that only free, rather than protein-bound hormones were present in saliva.

However, it is now thought that complex partitioning from blood confers the ability of saliva under certain circumstances to reflect total hormone levels (Cook, 2002).

Partitioning of constituents from plasma into saliva is generally not well understood. Concentrations of non-polar constituents and unconjugated steroids in saliva directly reflect that in blood, as their release is not flow-dependant. This is in comparison to some polar hormones (e.g. thyroxine and peptide or conjugated hormones) which are dependant on flow rate, making accurate assessment of their levels in saliva difficult (Hofman, 2001). Additional problems can occur when collecting from animals. Mid-sized to large animals require restraint, which may be a stressor for the animal, whilst small animals may not provide enough volume of saliva to make assays possible, and in general repeated sampling over a short time-span may be difficult (Minton, 1994; Cook, 2002).

The analysis of saliva constituents can be difficult, due to the wide range in concentrations that each hormone can display, including both diurnal and monthly patterns (Hofman, 2001). Concentrations of hormones and other constituents may be affected by blood, which can be present following teeth cleaning (Hofman, 2001). Salivary flow rate and therefore the salivary concentration of constituents can be increased by consumption of food and drink and decreased by alcohol consumption. Alcohol consumption can decrease the flow rate and inhibits the release of protein, amylase and electrolytes (Enberg *et al.,* 2001). The flow rate and composition of saliva from each of the three glands can also affect hormone analysis is. Flow rate of saliva from each of the three glands (labial, parotid and submandibular) is affected by the activity of the autonomic nervous system (which can be stimulated during exercise), but is not affected by gender or age before the age of 60 (Ferguson, 1999). Saliva composition from the labial glands differs from that from the parotid and submandibular glands, in that saliva from the labial gland has higher (and more variable) sodium, protein, IgA and blood group substance concentrations, and lower phosphate and bicarbonate concentrations (Ferguson, 1999).

The monitoring of biological rhythms such as menstrual cycle changes in progesterone, responses to stressful situations in animals and humans, including exercise, and prediction of the onset of pre-term labour can be achieved using analysis of saliva

samples (Hofman, 2001; Cook, 2002). In humans and small animals, changes in salivary cortisol can indicate the level of function of the hypothalamic-pituitary-adrenal (HPA) axis, due to high correlations between plasma and salivary cortisol. Additionally, less restraint is required to obtain saliva compared to blood sampling (Fenske, 1996; Putignano *et al.,* 2001).

Advances in medical technology have led to the development of techniques such as iontophoresis and reverse iontophoresis. These techniques have opened up new avenues in the pursuit of better, more efficient monitoring of human and animal physiology.

1.3.3. Iontophoresis

The use of iontophoresis can be traced back to ancient Greece. Iontophoresis was first reported in the literature in 1748, but initial experiments using iontophoresis did not occur until the early 1900's (Roberts, 1999). Iontophoresis refers to the administration of an electrical current to the skin to aid the absorption of molecules. Increased absorption is achieved through altering the structure of the lipid bilayers, hair follicles and sweat glands to facilitate movement of charged and polar species (Curdy, *et al.*, 2001). It has been used for years to aid in transdermal administration of low molecular-weight drugs.

The structure of the human skin prevents the transdermal delivery of drugs of differing molecular weight and charge into the human body. The outermost layer of the skin is the stratum corneum, which is comprised of dead keratinocytes (skin cells filled with keratin fibres), sweat glands and hair follicles surrounded by impermeable lipid bilayers. The composition of the stratum corneum limits water loss from the body, is an impermeable barrier to molecules entering the body and provides resistance to the passage of an electrical current (Mitragotri *et al.,* 1995; Curdy *et al.,* 2001). The properties of the stratum corneum affect the movement of water and ions. Water displays diffusion characteristics independent of the thickness of the stratum corneum, while ion mobility (created and directed by the application of an alternating electric current) is affected by its depth and the structure (Kalia *et al.,* 1998). Increasing the

magnitude of the electrical current may sufficiently disrupt the stratum corneum so that passage of ions through the skin is uniform.

When a drug is dissolved in water, it separates into anions and cations (ions that have negative or positive charge, respectively). When an electrical current is passed through the solution the ions move towards the electrode with the opposing charge (Roberts, 1999). Iontophoresis alters the nature of the stratum corneum so that the molecules are 'pushed' or repelled through the skin when the electrical current is applied, often using an electrode that contains a 'patch' containing the drug to be administered.

The advantages of iontophoresis compared to traditional drug administration include the elimination of first-pass metabolism of the drug, either in the gastrointestinal system or by the liver, before it has reached its site of action. Other advantages include reduced frequency of drug dosing and increased compliance, and it also introduces the possibility of sustained drug administration over a lengthy time period without the use of needles or intravenous drips that can invoke anxiety (Curdy, *et al.*, 2001). Other benefits are a more controlled site of action of the drug, more rapid termination of administration, maintenance of the histological and barrier properties of the skin once the iontophoresis is complete, and reduction of the use of needles and the associated risk of infection (Mitragotri, *et al.*, 1995; Roberts, 1999).

Measurements of trans-epidermal water loss by infrared spectroscopy, impedance spectroscopy and laser Doppler flowimetry have shown that there are no lasting changes to variables of skin hydration, lipid structure or ionic concentration, and that the mild oedema and/or erythema occasionally experienced is only transient (Curdy, *et al.*, 2001). Drugs that can be administered in this fashion include topical non-steroidal anti-inflammatories, corticosteroids, local anaesthetics and antibiotics (Roberts, 1999).

The disadvantages that accompany iontophoresis are considered to be solvable with further research, which will aim to reduce skin irritation, tingling and burning, and improve transferral rates of non-polar drugs (Roberts, 1999).

An example of the use of iontophoresis in clinical practice is the transdermal administration of anaesthesia such as lidocaine by dermatologists. The advantages of

using the iontophoretic technique with local anaesthetics include minimal absorption into the blood, an 80 to 100% reduction in pain in performing several dermatological techniques, a lack of bolus distortion of the area (seen with injectable lidocaine) and a reduction in anxiety in patients of all ages with needle phobias (Greenbaum, 2001).

Low-frequency ultrasound (sonophoresis) has been used to increase the rate of transdermal delivery. Ultrasound waves have frequencies beyond the range of human ears, at 800 to 1000 kHz, and cause the air pockets within the keratinocytes to increase in size and oscillations (Roberts, 1999). This phenomenon disrupts the lipid bilayer, increasing the permeability of the skin and making transportation of high molecular weight proteins such as insulin, γ-interferon and erythropoietin possible (Mitragotri, *et al.*, 1995). Ultrasound also increases blood circulation at the area of administration, which increases diffusion of the drug through the layers of the skin and into the capillaries for clearance (Roberts, 1999).

The area of skin, the concentration of the drug and the frequency, pulse length and intensity of the ultrasound application all influence the efficacy of drug administration (Mitragotri, *et al.*, 1995). Therefore, specific techniques need to be determined for each individual drug. As ultrasound can penetrate deeper into tissue (up to 5cm below the skin), it can be used to treat non-superficial inflammation, however care needs to be taken to avoid periosteal burns and tissue necrosis (Roberts, 1999). The beneficial use of ultrasound to alter the permeability characteristics of the skin is now widely applied in both iontophoresis and reverse iontophoresis (Kost, *et al.,* 2000).

1.3.4 Reverse iontophoresis

The development of iontophoresis provided an opportunity to utilise the knowledge from that field to develop a technique to sample bodily fluids in animals and humans. Reverse iontophoresis uses ultrasound and low-voltage electric current to alter the permeability characteristics of the skin. This facilitates the migration of interstitial fluid towards the surface of the skin for collection and analysis. Interstitial fluid contains molecules of various molecular weight, charge and hydrophilic or hydrophobic characteristics.

Once the sample has been collected, it can either be analysed off-line for a variety of substrates, or measured *in situ* by selective biosensors. However, the former option must take into account the time lag created by diffusion of substances into the capillaries supplying the epidermis and across the stratum corneum (Kost, *et al.*, 2000; Cook, 2002).

Ultrasound is used to increase the rate of flux of substances toward the surface of the skin. The rate of flux can be increased by 25-fold when compared to reverse iontophoresis without ultrasound (Kost, *et al.*, 2000). Added advantages of using ultrasound include a decrease in the duration of exudate collection, the surface area of skin used, and the interference experienced. Human cadaver skin tested *in vitro* showed that ultrasound application significantly increased the permeability of the skin and therefore the extraction of molecules by as much as 570-fold, a finding that was repeated *in vivo* in rats (Kost, *et al.*, 2000). The levels of ultrasound used to initiate the transdermal flux have not resulted in damage to the epidermis or surrounding tissue, or in participants reporting discomfort, although mild oedema was sometimes noticeable at the site of application (Tamada *et al.,* 1995; Kost, *et al.*, 2000).

Reverse iontophoresis provides a method for tracking trends in plasma concentrations of constituents such as glucose, as the concentrations in transdermal exudate closely follow those of plasma glucose, at both hypoglycaemic and hyperglycaemic plasma levels (Kost, *et al.*, 2000; Mitragotri *et al.,* 2000). The reverse iontophoresis method has been applied in both human and animal settings, as described below.

The GlucoWatch® (Cygnus, Inc., Redwood City, CA, USA) was designed for diabetics, and is an example of reverse iontophoresis put into clinical practice. It is a battery-powered device, containing a glucose biosensor (described below) that can monitor changes in glucose concentration in interstitial fluid over a 12-hour period. Technology such as this can improve control of blood glucose levels through higher compliance rates, which reduces the incidence of the negative complications of diabetes mellitus, as well as eliminating the pain and inconvenience of blood sampling (Tamada *et al.,* 1995; Garg *et al.,* 1999). Blood glucose concentrations ranging between 2.2 and 22.2mmol/L are displayed on a digital screen every 20 minutes during a 12-hour time period, requiring only one glucose calibration per 12 hours (Potts *et al.,* 2002). The

GlucoWatch® is attached to the skin with adhesive pads and is worn on the anterior surface of the lower forearm. The low voltage electric current causes a small amount of fluid (<1μl) containing glucose to pass through the skin. The movement of glucose is achieved via a solvent flow created by the movement of both sodium ions and water from interstitial fluid through hydrogel pads towards the anode and cathode within the GlucoWatch® (Lenzen *et al.,* 2002; Potts, *et al.*, 2002).

Selectivity of the biosensor to glucose is achieved in several different ways. A low potential current (0.42V) is used, which ensures that species that could interfere with glucose measurement do not react at the same electrode (Tierney *et al.,* 1999). Molecules under a molecular weight of ~500 are excluded by manipulating the variables of ultrasound and electric current, and hence the level of permeability of the skin (Garg, *et al.*, 1999). The skin provides the third level of exclusion, as the net negative charge that the skin has at physiological pH prevents negatively charged molecules from passing through the skin. The sample is analysed electrochemically and/or enzymically, when glucose is metabolised by the enzyme glucose oxidase (GOx) to form hydrogen peroxide in the reaction:

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconic acid} + H_2O_2$$

The hydrogen peroxide produced in the above reaction reacts at the platinum-containing electrode, reforming O_2 in the oxidation reaction:

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

An electrochemical signal generated by the H_2O_2 is processed by an algorithm to give a reading equivalent to the blood glucose level (Lenzen *et al.,* 2002).

There are several benefits to using devices like the GlucoWatch® compared to conventional measurement techniques. The first and most intuitive is the reduction in need for invasive blood sampling, a situation of great benefit to those who suffer from needle phobias. Correlation analyses performed on glucose concentrations determined by the GlucoWatch® and by 'fingerstick' measurements show high correlation. High

correlations were also observed between readings from two GlucoWatches® sampling at the same time on the same person (Garg, *et al.*, 1999). However, there is a 5-minute physiological time lag between plasma glucose and the concentration determined from a sample taken by the GlucoWatch® (Chan and Hurel, 2002). Glucose concentrations below and above physiological limits can be detected by the GlucoWatch®, ensuring that extreme glucose levels are reported correctly. Set limits for high and low blood glucose levels can be programmed into the device, and if the individual's plasma levels reach the set point, an alarm is triggered (Garg *et al.*, 1999). This can be of particular benefit for people who suffer frequent hypoglycaemic attacks without any prior warning (Chan and Hurel, 2002). Interstitial fluid samples can also be used to track changes in plasma glucose levels (Figure 1.7).

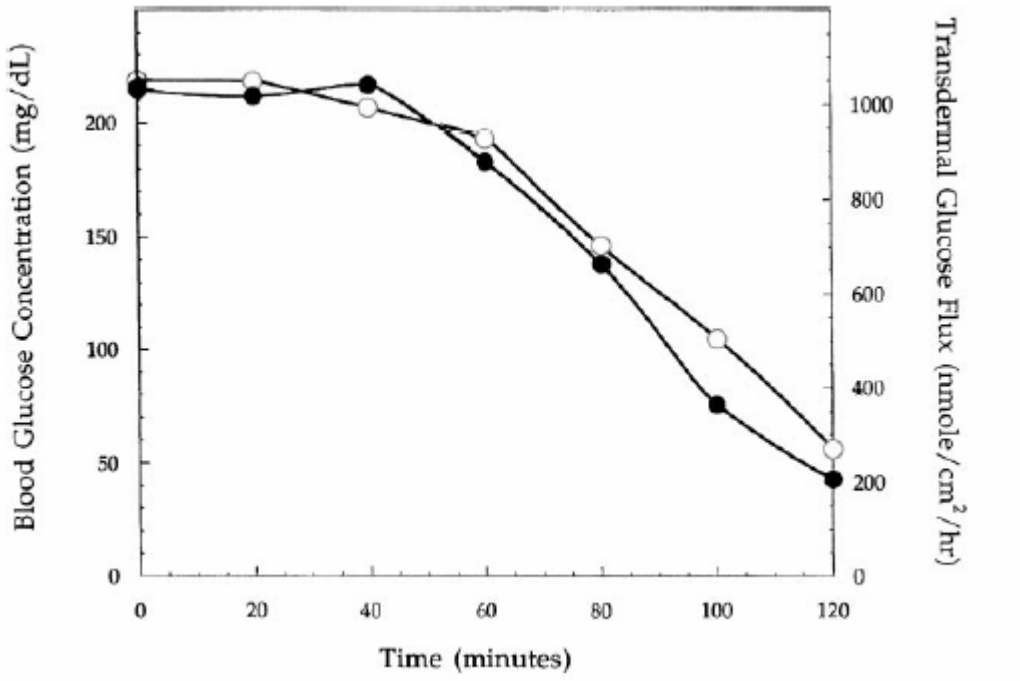


Figure 1.7: Variation of blood glucose levels in mg/dL (●) and transdermal glucose flux in nmol/cm^2/hr (○) over time (minutes). Typical data for one rat are shown. From Mitragotri, *et al.*, (2000) pp.962.

This system is not without problems. The time lag involved in using the biographer is approximately 20 minutes, due to the sample collection and analysis process. Extraction of glucose takes 3 minutes, using an electric current of 0.3mA, followed by integration of the glucose-derived biosensor current. Following this, the polarity of the current is reversed and integration of the signal from the opposite cathode occurs (Chan and Hurel, 2002). An average glucose reading is reported from the two readings taken every

20 minutes. When the data are plotted on a graph with actual plasma glucose levels, the GlucoWatch® values consistently lag behind that of actual plasma glucose (Potts *et al.,* 2002).

Another negative aspect of using this device is that it is sensitive to temperature changes and the amount of sweat on the skin (sweat interferes with conductance and may contain glucose). Both temperature and sweat are monitored by the device, which can skip a cycle or shut down the device if the conditions are sub-optimal (Garg, *et al.*, 1999). Reports from individuals who tested the GlucoWatch® revealed two significant problems. The 3-hour calibration period was too long to perform every day without interfering with normal daily life, and sustained application of the device on the skin can result in irritation, itching, tingling, mild oedema and erythema, although these reactions subsided after the calibration period (Garg, *et al.*, 1999; Lenzen *et al.,* 2002). With further development and modification to resolve the problems mentioned above, technologies similar to the GlucoWatch® could be invaluable to diabetics, health professionals and caregivers worldwide.

Other research groups have created devices based upon similar theory but with different application from the GlucoWatch® biographer. All are aimed at creating a non-invasive, painless, convenient method of measuring blood constituents. Examples include implantable sensors, near-infrared spectroscopy and transdermal permeation enhancers, although regular clinical use is rare (Kost *et al.,* 2000). Minimal-invasion techniques have also been developed, that use either laser or lancets that microscopically penetrate the skin to aid removal of blood or interstitial fluid for analysis (Mitragotri *et al.,* 2000). An electrosonophoretic device (Figure 1.8) has been developed that harvests transdermal exudate and has the ability to collect and store a sample, or analyse the sample *in situ*, using immunosensors to provide an instant read-out (Cook, 2002). This technology was the type used in the present study.

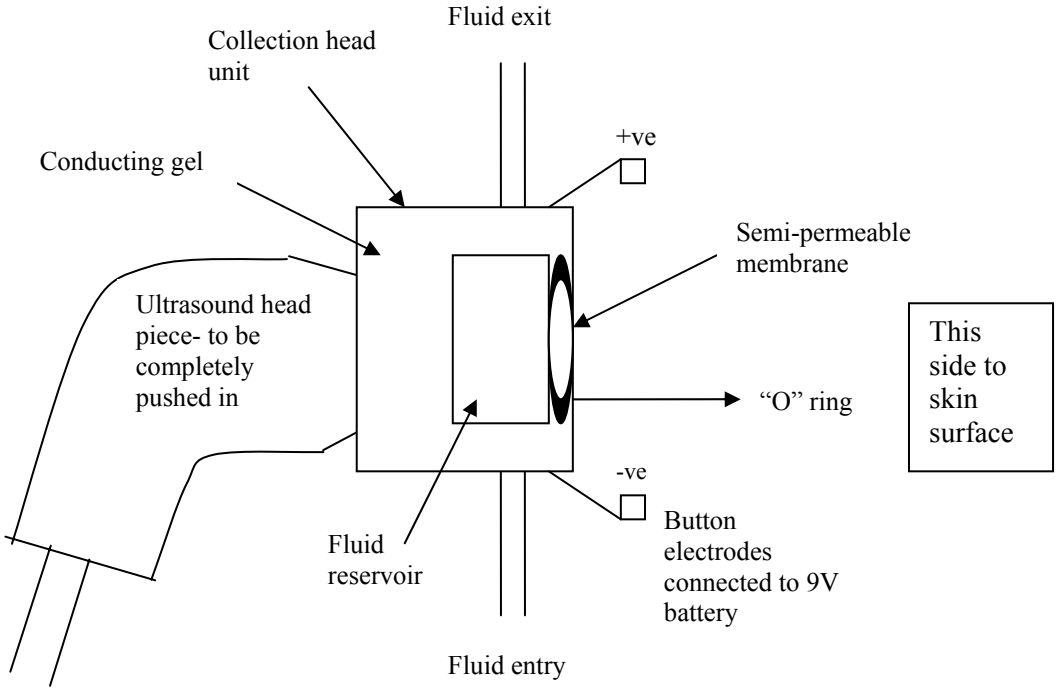


Figure 1.8: Diagram of the electrosonophoretic device. Adapted from Figure 1 in Cook (2002) pp. 172.

Another application of technology such as that described above is the monitoring of welfare in livestock. There are several situations common to farm practices which are potent stressors to animals, such as restraint, isolation, transport, weaning, branding, vaccination and dehorning (Minton and Blecha, 1990; Minton *et al.,* 1992; Minton, 1994; Mellor *et al.,* 2000). As well as observed behavioural responses to stress, these situations activate the hypothalamic-pituitary-adrenal (HPA) axis, which leads to release of cortisol (refer to Section 1.2). The sympathetic-adrenomedullary axis is also activated, causing release of the catecholamines adrenaline and noradrenaline.

A confounding factor in experiments on stress in animals is the requirement for a sample of blood or saliva to monitor changes in hormone levels, to give an indication of the level of stress experienced when an animal undergoes certain procedures. The collection of fluid itself has been shown to be a stressor in animals due to the catching and restraint of the animal and the pain inflicted by blood sampling. Therefore separating the impact of stress caused by the farm practice, from the stress caused by sampling can be problematic.

A device has been developed that can be mounted onto the back of an animal, that can be remotely activated to take a blood sample which can either be analysed *in situ* or collected for later analysis (Cook *et al.,* 2000). This technology has been shown to reduce the stress experienced by the animal, leading to more accurate assessment of basal hormone profiles, and to reduce welfare compromise.

Electrosonophoresis has also been utilised in human experiments, to monitor testosterone, cortisol and growth hormone levels over a short time period in young male rugby players (Blackmore, L. Unpublished data, August 2001). Correlation analyses were conducted on the hormone levels obtained in interstitial fluid compared to plasma or saliva. The resultant correlation coefficients for the relationships ranged from 0.965 to 0.977 (Blackmore, L. Unpublished data, August 2001). Regression analysis produced a conversion factor that was applicable to all three hormones, with plasma concentrations being 9.86 times the value obtained using electrosonophoresis of the concentration ranges observed.

The practical implications of this technology are far-reaching as it provides a safe, non-invasive and painless sampling technique that reduces the risk to welfare in both animals and humans. It provides significant benefits to individuals wanting or requiring intermittent or continuous sampling of blood constituents. An evaluation of electrosonophoresis use in female athletes is therefore included in the present study.

1.4. Sleep

The outline below is relevant to the interpretation of sleep logs, which were completed by the participants in the present study.

Consciousness is "the complete set of mental facilities of an individual, expressed in awareness of what he is doing and what is happening around him, the level of consciousness indicates the degree of critical reactivity of an individual, analysing and evaluating incoming information in light of previous experiences and preparing covert or overt responses directed toward certain goals" (Coenen, 1998). Each level of consciousness (maximal alertness, wakefulness, sleep and coma) has its own unique characteristic, including specific electroencephalogram readings (Zeman, 2001). For example, during the sleep-wake cycle the awareness of external stimuli displays a circadian rhythm. Consciousness does not cease during sleep, as the individual experiences dreams, which are an example of inward consciousness. Previously, sleep was viewed as a passive act, with little purpose, whereas now it is known that global average cerebral blood flow and global average cerebral metabolic rate of oxygen increase during rapid eye movement (REM) sleep, although fluctuations in physiological variables may account for this phenomenon (Madsen, 1993).

The sleep-wake cycle is regulated by three neural systems, which interact to produce the different states of consciousness. The arousal system works together with the slow-wave sleep centre and the REM sleep centre to generate the rhythmic cycling of consciousness. Predictably, the arousal system can override the slow-wave sleep and REM sleep centres. The arousal system can be stimulated by sensory input, inputs descending from higher brain centres, intense concentration, strong emotion and motor activity (Sherwood, 2001).

Sleep can be divided into the sub-states of REM and non-REM sleep, which are determined by electroencephalogram (EEG) readings, electrooculogram (EOG) readings and physical movement and muscle tone. The smooth and symmetrical rhythm of human body temperature follows a circadian rhythm, and is intrinsically linked with

sleep and sleep states. The nadir of core temperature occurs between 0400 and 0600 hours, with a peak at approximately 1700 hours and an overall mean temperature of 37°C (Refinetti, 2000). The peak of the deepest non-REM sleep coincides with a decrease in brain temperature, approximately two to three hours after sleep onset, due to a decrease in the sensitivity of thermoregulatory mechanisms (Morrissette and Heller, 1998). An increase in body temperature, either passively via increased ambient temperature or actively through exercise, increases the amount of non-REM sleep experienced. This response is thought to offset hyperthermia, as non-REM sleep is linked to a decrease in brain temperature, as mentioned above (Morrissette and Heller, 1998; Kräuchi *et al.*, 2000). This free-running cycle is quite independent of light-dark oscillations, day-night differences in ambient temperature or social schedules (Refinetti, 2000).

Non-REM sleep, or "slow-wave" sleep, and REM sleep occur in a 90 to 100 minute ultradian cycle that occurs three to five times throughout the night, and is superimposed on the circadian sleep-wake cycle (Endo *et al.,* 1998). Each cycle contains variable amounts of REM sleep, with proportionally more REM sleep occurring as the sleep progresses, from five to ten minutes initially through to 50 minutes by the last cycle. The last cycle occurs in synchrony with the nadir of human body temperature (Endo *et al.,* 1998).

The duration of sleep is dependant on both the time of day that the individual retires to bed to sleep, and the time spent awake prior to sleep (as increased time spent awake increases duration of sleep), while the amount of REM sleep is dependant on the amount of prior non-REM sleep (Åkerstedt *et al.,* 1998; Endo *et al.,* 1998).

1.4.1 Slow Wave or Non-REM sleep

This type of sleep is characterised by minor decreases in respiratory rate, blood pressure and heart rate, together with infrequent respiratory sinus arrhythmia, increased muscle tone, frequent changes in body position and a lack of rapid eye movements (Verrier *et al.,* 1998; Landolt *et al.,* 2001). The activity of the cardiac parasympathetic nervous system increases across the four stages of non-REM sleep (Burgess *et al.,* 1997). The preoptic area of the brain has been implicated in causing non-REM sleep, while gamma-aminobutyric acid (GABA)-containing neurons have been implicated in generating slow-wave brain activity (Santaigo *et al.,* 2001).

It is during this type of sleep that sleep walking and talking may occur. While dreams do not occur or are very infrequent, nightmares may occur in deep slow-wave sleep (Stage Three or Four of non-REM sleep). People in non-REM sleep are easily awakened, brain activity is more conceptual than visual, and recollections of dreams are less likely (Sherwood, 2001). Empson (1993) and Tortora and Grabowski (1996) describe non-REM sleep as having four distinct phases with unique characteristics, such as increasing arousal thresholds. A full description of these stages can be found in the aforementioned publications, but is beyond the scope of this review, as the structure of sleep was not investigated in the present study.

Deep slow wave sleep predominates in the first half of the night, and determines the proportion of REM sleep experienced later in the night. As the proportions of deep slow wave sleep decrease, the amounts of light slow-wave sleep, along with REM sleep increase (Empson, 1993). The transition from non-REM sleep to REM sleep is characterised by the appearance of ponto-geniculo-occipital (PGO) waves, which activate visual and motor areas of the brain, and may play an important role in memory consolidation (Stickgold *et al.,* 2001).

1.4.2 REM Sleep

Heightened brain activity occurs during REM sleep, especially in high-level visual processing areas. In contrast, activity is decreased in the dorsolateral prefrontal cortex, the area involved with reasoning (Stickgold *et al.,* 2001). In contrast to non-REM sleep, REM sleep is characterised by strong inhibition of muscle tone, due to hyperpolarized motorneurons. Oculomotorneurons and most respiratory motorneurons remain unaffected, allowing breathing and rapid eye movements to occur (Empson, 1993; Fenick *et al.,* 1998). Heart rate, respiratory rate and blood pressure, which were depressed during non-REM sleep, now fluctuate. This is due to reduced responsiveness to stimuli that normally elicit breathing or cardiovascular responses, as well as surges in heart rate due to sleep state, hippocampal theta activity, PGO wave activity and eye movement clusters (Verrier *et al.,* 1998; Rowe *et al.,* 1999; Rector *et al.,* 2000). Most dreaming occurs in REM sleep and men commonly experience erections during this time. Dreams are described as "internally generated visual imagery, reflecting activity of a person's memory bank without guidance or interpretation, and often involve intense emotion, a distorted sense of time with bizarre content which is accepted as real and with little reflection" (Sherwood, 2001).

The onset of REM sleep is initiated by the cholinergic neurons of the dorsolateral pons, and the concomitant decrease in activity by the noradrenergic and serotonergic neurons in the brainstem and basal forebrain (Santaigo *et al.,* 2001). Before EEG recordings indicate that REM sleep has begun, PGO waves are observed, which occur in bursts preceding both eye movements and characteristic REM wave recordings, and may be responsible for the dreaming experience (Empson, 1993). EEG recordings during REM sleep show low-amplitude, mixed frequency features, similar to Stage 1 of non-REM sleep, with a prominent theta rhythm originating from the hippocampus (Inoué and Borbély, 1985; Roehers and Roth, 2001).

1.4.3 Sleep and Hormone Secretion

The suprachiasmatic nucleus (SCN), located in the anterior hypothalamus, works in conjunction with zeitgebers[4] to entrain many physiological variables to circadian rhythms. Hormone secretion is an example of such a variable, and hormones affected include melatonin, cortisol, growth hormone, aldosterone, prolactin, gonadotropin-releasing hormone (GnRH), thyroid-stimulating hormone, oxytocin, testosterone and placental hormones during pregnancy (Santaigo *et al.,* 2001). Only the circadian rhythms of cortisol, testosterone and growth hormone are discussed in detail here, as they were the hormones investigated in the present study. For further information on melatonin, reviews can be found by Refinetti (2000), Cajochen *et al.,* (1999) and Wyatt (2001), while information on aldosterone is presented by Charloux *et al.*, (1999), and prolactin by Linkowski (1998) and Freeman *et al.*, (2000).

Cortisol release displays a circadian rhythm, with two peaks in secretion, one occurring in the early morning, coinciding with an increase in light levels and another peak at noon (Leproult *et al.,* 1997; Linkowski *et al.,* 1998; Santaigo *et al.,* 2001).

In males, testosterone release has been associated with the sleep-wake cycle, with an increase in testosterone secretion following the onset of sleep. The rise in its plasma levels precedes the first REM sleep episode by approximately 90 minutes and levels remain elevated until awakening (Luboshitzky *et al.,* 2001). The number of REM sleep episodes appears to be unrelated to the mean plasma concentration of testosterone during the night (Luboshitzky, 2001).

Growth hormone (GH) also displays circadian rhythmicity, with increases in the pulsatile release seen in synchrony with deep, uninterrupted non-REM sleep (Fishbein, 1981; Empson, 1993; Gardi *et al.,* 1999). Ten to twenty bursts of GH secretion occur over a 24-hour period, the largest occurring with the first phase of deep non-REM sleep, although levels are generally low throughout the rest of the night (Spiegel, *et al.*, 2000).

[4] Zeitgebers: An exogenous time signal, like light or darkness, which helps to entrain the circadian pacemaker to a 24-hour rhythm. From Vitaterna *et al.*, (2001) pp.86.

The hormones that help to regulate GH release, somatostatin and growth hormone-releasing hormone (GHRH), display diurnal variation. GHRH promotes non-REM sleep via neurotransmitter-like activity in the anterior hypothalamus/preoptic region (Beranek, *et al.*, 1997; Gardi, *et al.,* 1999). This effect is independent of both the non-REM and REM sleep-inducing effects of GH in both humans and animals, which possibly occur via some metabolic actions (Lachmansingh and Rollo, 1994; Moreno-Reyes *et al.,* 1998).

1.4.3.1 Sleep and the menstrual cycle

The fluctuating levels of oestrogens and progesterone that occur during the menstrual cycle in women can have an effect on the composition of sleep. A decrease in REM sleep is noted in women with and without menstrual irregularities, due to the progesterone-induced changes in body temperature during the luteal and menstrual phases of the cycle (Baker *et al.,* 1999). Women who do not experience negative symptoms related to their menstrual cycle do not exhibit changes in the subjective quality of their sleep across the menstrual cycle.

In contrast, women who suffer from primary dysmenorrhoea[5] encounter reduced sleep efficiency, decreased subjective sleep quality and decreased REM sleep (Baker *et al.,* 1999). In these women, the structure of sleep across the menstrual cycle changes dramatically. An increase in the percentage of non-REM sleep and the power density of sleep spindles occurring in the luteal phase is observed, in concert with the changes in rectal temperature observed during a menstrual cycle (Driver *et al.,* 1996).

1.4.4 Functions of Sleep

One of the generally accepted theories of the function of sleep is that it is "catch-up" time; the anatomical, biochemical and physiological processes that have been progressively degraded during wakefulness have time to regenerate and restore full functional capacity (Fishbein, 1981; Inoué and Borbély, 1985; Empson, 1993). The

[5] Primary dysmenorrhea is a condition where the individual experiences painful uterine cramps so severe that normal daily functioning is impaired (Baker, *et al.*, 1999).

decrease in cerebral metabolism that occurs during sleep may be linked to such activities as neuronal restitution and detoxification, learning and dreaming (Gambelunghe *et al.,* 2001; Stickgold *et al.,* 2001). Accompanying this is evidence of increases in plasma GH levels and tissue mitosis; but the latter phenomenon occurs with or without light-dark cycles (Inoué and Borbély, 1985).

Sleep in humans is a primarily fasting state, as the last meal of the day usually occurs before 2000 hours, with sleep usually occurring after 2200 hours. Therefore, the increased GH secretion early in the sleep period mentioned above may be an anticipatory response, to protect tissue protein from catabolism and to increase lipid oxidation as the primary fuel until the next meal, rather than being involved in anabolic pathways (Inoué and Borbély, 1985). However, as the main burst of GH secretion occurs in the period immediately following sleep onset, the protective actions of GH may be lost, leading to the possible degradation of protein in the latter half of the night. Results from metabolic studies in animals indicate increased rates of protein synthesis (not degradation) during sleep, so the net protein turnover rates during sleep are not known (Inoué and Borbély, 1985).

Non-REM sleep is associated with energy conservation and with synthesis of ribose nucleic acid (RNA) and protein, products that are utilised during REM sleep (Gambelunghe *et al.,* 2001). Deep slow-wave sleep prevents lethargy and physical tiredness, and facilitates restoration of damage to the body from exercise, pain, injury and excess catabolism (Fishbein, 1981; Empson, 1993). REM sleep in particular (although non-REM sleep has been implicated) has been suggested as a means for the brain to "shift gears" in order to establish long-term structures and chemical adjustments such as protein synthesis. Protein synthesis may be necessary for learning and consolidating memories, although the evidence is equivocal (Fishbein, 1981). REM sleep is effective in maintaining attention and ego integrity and in regulating emotion and a sense of self (Fishbein, 1981). However, there is no direct evidence linking sleep with restorative processes.

1.4.5. Sleep and Exercise

There are conflicting results in the literature regarding the true effect of exercise on the duration and composition of sleep. Moderate intensity exercise has been reported to increase the amount of non-REM sleep and decrease the proportion of REM sleep during the night following exercise (Kern *et al.,* 1995). However, similar results have also been observed due to passive increases in body temperature (Horne and Staff, 1983). Exercise performed in the evening or prior to bed time does not appear to affect sleep onset, continuity of sleep or duration of slow-wave sleep (O'Connor *et al.,* 1998; Garcia-Garcia and Drucker-Colin, 1999). However, none of the above authors have investigated the possible impact of increased cortisol (as a consequence of exercise) on sleep onset latency.

1.4.6. Sleep deprivation and performance

Athletes, in the time period leading up to competitions and performances, can often experience a lack of sleep due to nerves or as a consequence of travelling into different time zones. For either reason, the lack of sleep or change in normal sleep patterns can affect variables related to performance. Another factor that can result in a disruption of sleep patterns is alcohol consumption. Consumption often occurs in the evening at social events and may continue for several hours, thus competing with sleep time. This may create a state of sleep deprivation, effects of which could be superimposed on any hangover symptoms that could result from the night before.

The initial effects of sleep deprivation include changes in neurobehaviour, such as difficulty in focussing and maintaining attention. Further deprivation can lead to a change in endocrine secretions, thermoregulation, psychomotor abilities and gross cognitive impairment including hallucinations, illusions and delusional psychosis, which could all significantly impair performance (Endo *et al.,* 1998; Spiegel, *et al.*, 2000; Wyatt, 2001). Interestingly, performance in cognitive and motor performance tests following a period of sleep deprivation can be as poor as or worse than the same

tests performed following moderate alcohol consumption (Williamson and Feyer, 2000).

Much of the information regarding sleep deprivation and performance refers to periods of sleeplessness far in excess of what an average athlete would encounter. Prior to a competition, sleep duration may decrease because of nervousness, anxiety or from jetlag. Decreased sleep duration may have different effects when compared to severe restrictions on sleep time. Partial sleep loss may not affect the level of exercise intensity achieved during a test the following day, but may affect motivation, increase heart rate, lactate and ventilation variables and decrease peak VO_2 achieved during a fitness test (Bond *et al.,* 1986; Mougin *et al.,* 1991). Sleep deprivation causes bradycardia, both at rest and during exercise, and significantly decreases peak heart rate achieved during a submaximal fitness test, a phenomenon possibly due to increased plasma volume (Bond, *et al.*, 1986).

Sleep deprivation affects thermoregulation, causing a decrease in rectal temperature and an increased temperature response to exercise, although the circadian rhythm of body temperature is unaffected (VanHelder and Radomski, 1989). However, the mechanism behind these effects is not known. A change in temperature may or may not negatively affect performance, depending on the mechanism behind this change. A decreased hypothalamic set point would delay heat stress (a factor in voluntary cessation of exercise), thus increasing performance, while a decrease in metabolism could decrease performance (VanHelder and Radomski, 1989).

In general, mild sleep deprivation does not significantly or consistently affect performance, as performances can improve, worsen or remain the same following a night of sleep deprivation (VanHelder and Radomski, 1989).

1.5. Alcohol

The section below is a series of descriptions of the physiological effects of alcohol, the effects of both alcohol and the hangover it can produce on physical performance, and the incidence of alcohol consumption in young adults in New Zealand. The high incidence of alcohol consumption in young adults, and in young athletes in particular (O'Brien and Lyons, 2000) makes it worthy of review, due to the significant impact it can have on both physiology and performance. Moreover, high levels of alcohol consumption were observed in the present study.

Alcohol has many effects on the human body, some of which can contribute to the appearance of a hangover. Swift and Davidson (1998) summarised the effects of alcohol on different systems as follows:

Dehydration and Electrolyte Imbalance: Dehydration and electrolyte imbalance results from alcohol stimulating both urinary production and output. This is achieved by inhibiting vasopressin, which stimulates the tubules within the kidneys to reabsorb water. Alcohol can also increase the rate of nutrient loss from the body by causing excretion of nutrients in the urine (Stowell, 1989). Common symptoms of a hangover, such as diarrhoea, vomiting and sweating can all contribute to dehydration, which can independently cause some symptoms seen in hangovers.

Gastrointestinal Disturbance: Alcohol can directly inflame the mucous lining of both the stomach and intestines, leading to a range of negative symptoms such as pain, nausea and vomiting, as well as affecting nutrient absorption (Stowell, 1989). Gastric acid, pancreatic enzyme and intestinal secretions all increase as a result of alcohol consumption. Fatty liver is a condition caused by excess alcohol consumption, in which triacylglycerols and fatty acids are deposited in liver cells.

Metabolic effects: Alcohol can cause hypoglycaemia through inhibition of glucose production. This is achieved through the development of fatty liver and a build-up of metabolites such as lactic acid, which inhibit hepatic glucose production. This effect can be compounded by no or low food intake, which is often associated with binge drinking,

discussed below. The brain is reliant on glucose as its primary energy source, and a reduction in glucose supply to the brain can result in symptoms often seen in hangovers, such as mood disturbances, weakness and fatigue.

Effects on bone remodelling: Bone tissue is constantly resorbed and reformed throughout life. Osteoclasts and osteoblasts work in synchrony to improve the quality of the bone structure, a process that helps to prevent fractures from occurring. Oestrogens play an important role in bone tissue turnover, an effect that is lost after menopause in women. Moderate alcohol consumption can increase levels of oestrogens in both males and females (described below). This may be of benefit to women in preventing the loss of bone mass, although the evidence is equivocal at this stage (Turner and Sibonga, 2001). However, alcoholics of both sexes often display signs of osteoporosis, and any potential benefit of increased bone mass must be weighed against the many negative effects of alcohol consumption.

1.5.1 Metabolism of alcohol

Alcohol is absorbed into the bloodstream through the stomach and small intestines, and passes through the hepatic portal blood system to the liver, where it undergoes first-pass metabolism. This occurs until a point when the metabolising rate of the enzyme is exceeded, and alcohol is released into the blood stream, increasing blood alcohol concentrations (Mumenthaler *et al.,* 1999). Alcohol is cleared from the body by the liver in a two-step process that initially converts alcohol to acetaldehyde, using the enzyme alcohol dehydrogenase (ADH^2). Acetaldehyde is then metabolised to form acetate, via the enzyme aldehyde dehydrogenase (ALDH), as shown in Figure 1.9 below.

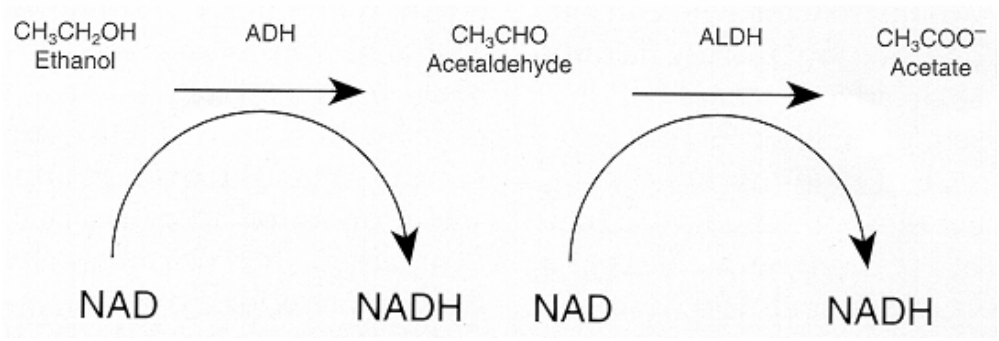


Figure 1.9: Metabolism of alcohol in the liver. NAD= Nicotinamide adenine dinucleotide (oxidized form) NADH= Nicotinamide adenine dinucleotide (reduced form) ADH[2]= alcohol dehydrogenase, ALDH= aldehyde dehydrogenase From Swift, *et al.* (1998) pp. 60.

At high blood alcohol concentrations, a metabolising system in the liver is activated, called the microsomal ethanol oxidising system, which explains the increase in clearance rates of alcohol observed with high blood alcohol concentrations (Mumenthaler *et al.,* 1999). The microsomal ethanol oxidising system involves a cytochrome protein that oxidises ethanol and generating oxygen radicals although this system can be utilised to metabolise other substances (Lieber, 1994). The microsomal ethanol oxidising system can help to treat methanol poisoning by metabolising methanol to formaldehyde, once the system has been induced by alcohol (Lieber, 1994).

1.5.1.1 Alcohol pharmacokinetics

When men and women consume an equivalent dose of alcohol, women have a higher blood alcohol concentration. This is due to higher body fat and lower body water levels in females, who are more susceptible to the negative health effects of alcohol (Mumenthaler *et al.,* 1999). The rate that alcohol disappears from the body is also affected by gender, as women have faster elimination rates (grams/litre of blood/hour). This phenomenon is possibly due to increased liver volumes per kg of lean body mass (Eckardt *et al.,* 1998).

1.5.2 Alcohol and Cardiovascular Disease

Whilst a review of the literature regarding cardiovascular disease (CVD) and its genetic and nutritional origins is outside the scope of this section, it is worth referring to the effect that alcohol has in the multifactorial development of this disease. The primary risk factors include cigarette smoking, heredity, high blood pressure, male gender, obesity and a sedentary lifestyle. Another important risk factor is the cholesterol profile, which includes high levels of both plasma triglycerides and plasma low-density lipoproteins (LDL) and low levels of high-density lipoproteins (HDL) (Brody, 1999). High levels of LDL are significantly and positively correlated to the progression of CVD. LDL's lead to the formation of foam cells, previously macrophages, which can develop into fatty streaks and eventually into atherosclerotic lesions (Brody, 1999). Epidemiological studies have begun to assess the impact that alcohol has on the risk factors associated with CVD, and although red wine especially is investigated in the literature, beer and spirits also have protective effects (Figure 1.10).

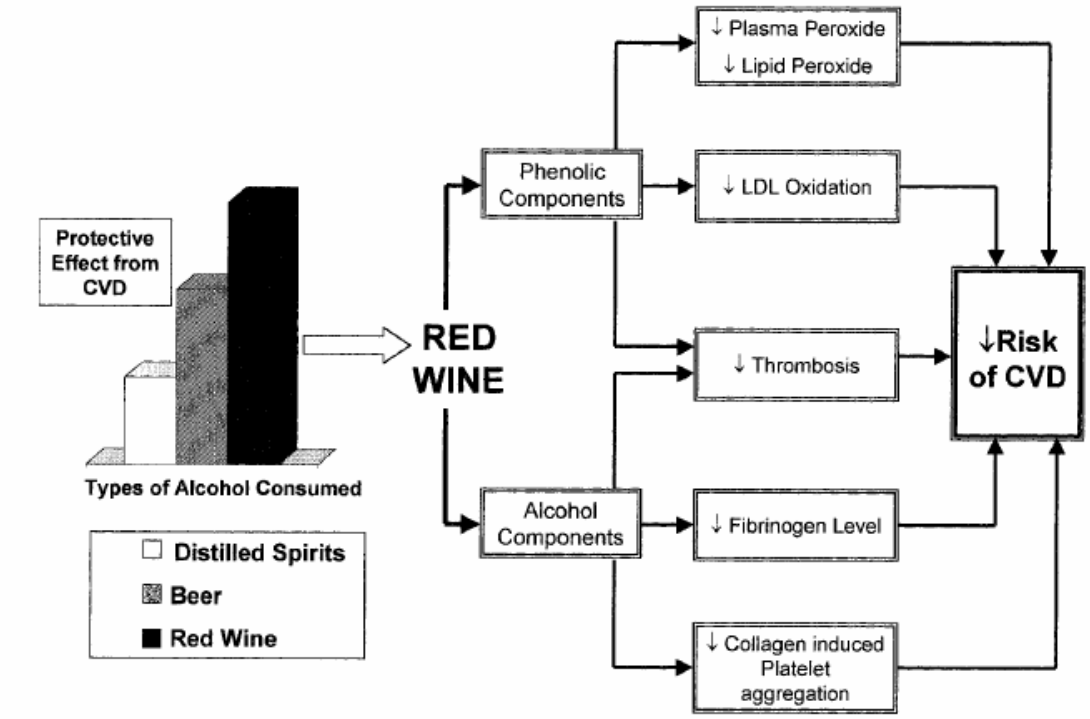


Figure 1.10: Schematic representation of the effects of alcohol on risk factors for cardiovascular disease. From Wollin and Jones (2001) pp.1404.

Moderate consumption of red wine has been proposed as having protective effects against the development of CVD. Large-scale studies have reported a J- or U-shaped association between the two variables, but in some cases when results were adjusted for social class, the relationship no longer existed (Vliegenthart, *et al.,* 2002). The protective effects from red wine can be divided into benefits derived from the alcoholic component of the wine and benefits derived from the non-alcoholic component. Many of the studies investigating the effects of ethanol and/or non-alcoholic components of red wine on the development of CVD have results that could be confounded by other variables, or have not fully reported participant details or alcohol consumption figures, making extensive generalisations difficult. However, ethanol appears to increase HDL concentrations in the blood, and cause a decrease in both platelet aggregation and fibrinogen levels in the blood. This can lead to an overall decrease in the incidence of thrombosis, but some of these effects may also be generated by non-alcoholic purple grape juice (Wollin and Jones, 2001).

The non-alcoholic phenol compounds in red wine contribute to the protective effects by increasing endothelial function and inhibiting the oxidation of LDL-cholesterol. LDL-cholesterol is the form taken up by the macrophages to form foam cells. Inhibition of oxidation occurs through the reduction of the copper catalyst involved in the oxidation reaction (Wollin and Jones, 2001).

However, the protective effect of red wine may be negated (especially in males), by cigarette smoking. The association between red wine consumption and protection against CVD was strongest in women who had never smoked (Vliegenthart *et al.,* 2002).

1.5.3 Alcohol and Endocrinology

The responses of the endocrine system to alcohol consumption are similar to those that occur when a person experiences physiological or psychological stress. Cortisol, adrenocorticotropic hormone (ACTH), β-endorphins and prolactin are all secreted in increasing amounts during periods of alcohol intoxication (Karila *et al.,* 1996; Frias *et al.,* 2000). As well as instigating the "stress response" (Section 1.2), alcohol can directly

affect hormonal pathways and indirectly affect hormone secretion by affecting variables required for maximal secretion of a hormone. Alcohol can also exert significant effects on both the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axes.

While some hormones are released during the night independently of sleep patterns, others are reliant on timing and duration of sleep. GH is an example of such a hormone. It is released from the anterior pituitary gland in a pulsatile fashion, with peak secretion seen at the onset of slow wave sleep. Secretion of GH is inhibited by alcohol in a dose-dependant manner, despite alcohol increasing the amount of slow wave sleep (Plotnick *et al.,* 1975; Redmond, 1981; Välimäki *et al.,* 1983; Roehers and Roth, 2001). In both adolescents and adults, alcohol inhibits GH messenger RNA, increases blood glucose levels (which inhibit GH release) and inhibits secretion of growth-hormone releasing hormone (Frias, *et al.*, 2000).

Alcohol administration in rats induces a decrease in plasma testosterone levels, an observation thought to result from inhibition of the testicular response at some level of a proposed neural pathway linking the brain and the testes (Rivier, 1999). In human males, alcohol consumption at levels high enough to induce a hangover has been shown to impair sex steroid production, both acutely and chronically, by damaging Leydig cells (Gavaler *et al.,* 1983; Karila *et al.,* 1996).

Plasma levels of testosterone decrease during acute intoxication and are chronically depressed in alcoholics, as both ethanol and its metabolites act as toxins to gonadal tissue and interfere with the function of the HPG axis (Välimäki *et al.,* 1983; Frias *et al.,* 2000). Testosterone levels decrease and oestrogen levels increase because of conversion in peripheral tissues. The conversion reaction is catalysed by the enzyme aromatase, the release of which is stimulated by alcohol (Purohit, 2000; Turner and Sibonga, 2001). Male chronic alcoholics can have half the normal testosterone concentration as well as increased oestrogen levels. In addition, they may experience a range of negative symptoms due to prolonged alcohol abuse, such as testicular atrophy, impotence, little or no libido and feminization (Gavaler *et al.,* 1983).

Alcohol has a significant effect on the HPG axis in both sexes. Both testosterone and dehydroepiandrosterone (DHEA) levels increase in adolescent and premenopausal females in response to acute alcohol consumption, although this effect is more pronounced in women taking oral contraceptives (Välimäki, *et al.*, 1983; Frias, *et al.*, 2000; Sarkola *et al.,* 2001). Rates of alcohol consumption have been shown to correlate with plasma testosterone levels in premenopausal women (Martin *et al.,* 1999). The consequences of increased testosterone levels such as hirsutism and polycystic ovary syndrome have been well documented, whereas links to chronic diseases such as breast cancer are more tenuous (Sowers, *et al.*, 2001).

In females, plasma levels of testosterone are affected at several sites along the HPG axis, as well as the liver. Hepatic catabolism is reduced by changes in the ratio of NADH to NAD^+, which inhibits the oxidation of testosterone to androstenedione (Figure 1.11).

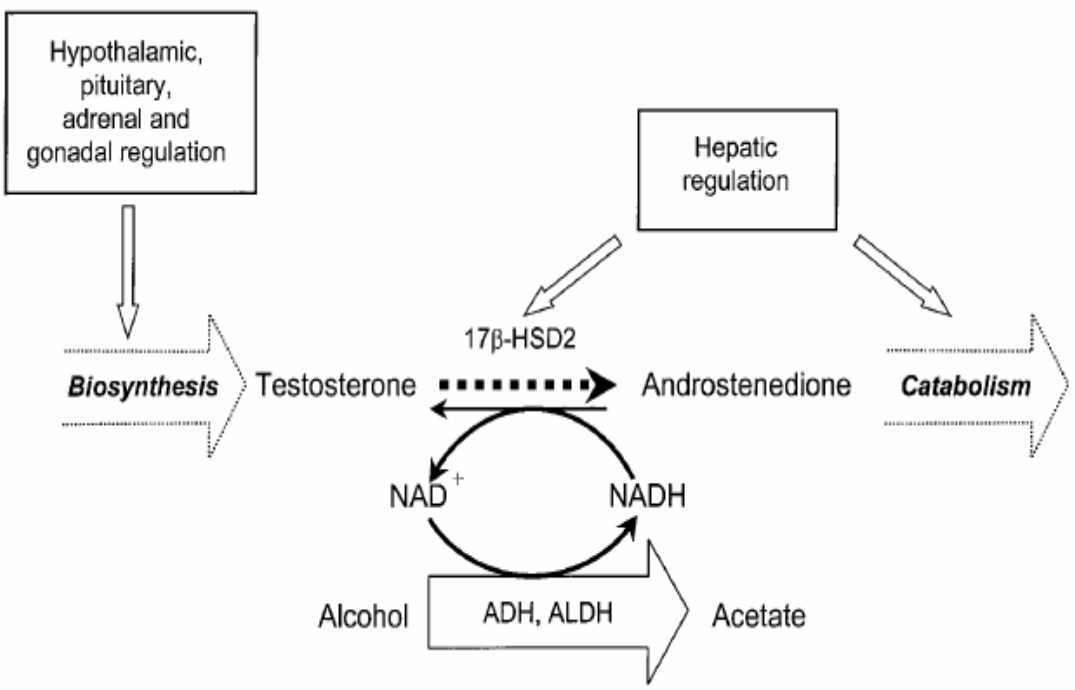


Figure 1.11: The coupling of ethanol and sex steroid metabolism in the liver. From Sarkola *et al.*, (2001) pp.981. ADH: Alcohol dehydrogenase, ALDH: Aldehyde dehydrogenase. Dashed line indicates a reduced reaction rate.

Administration of alcohol to female rats causes a decrease in both the gonadotropin luteinizing hormone (LH) and in sex steroid production, an observation linked to inhibition of LH-releasing hormone (Ogilvie and Rivier, 1997). In contrast to rats,

human oestradiol levels show a significant increase in both adolescents and premenopausal women who consume alcohol. This result is possibly due to either increased induction of aromatases that convert androgens to oestrogen or inhibited metabolism of oestrogen in the liver (Martin *et al.,* 1999; Sarkola *et al.,* 1999; Purohit, 2000; Sarkola *et al.,* 2001). Acute increases in plasma oestradiol and decreases in oestrone are also observed during alcohol metabolism (Välimäki, *et al.*, 1983; Karila, *et al.*, 1996; Frias *et al.,* 2000). Plasma progesterone concentrations increase as a result of alcohol consumption in women who take oral contraceptives and in those who do not (Figure 1.12) (Välimäki, *et al.*, 1983; Sarkola, *et al.*, 1999; Sarkola *et al.*, 2001).

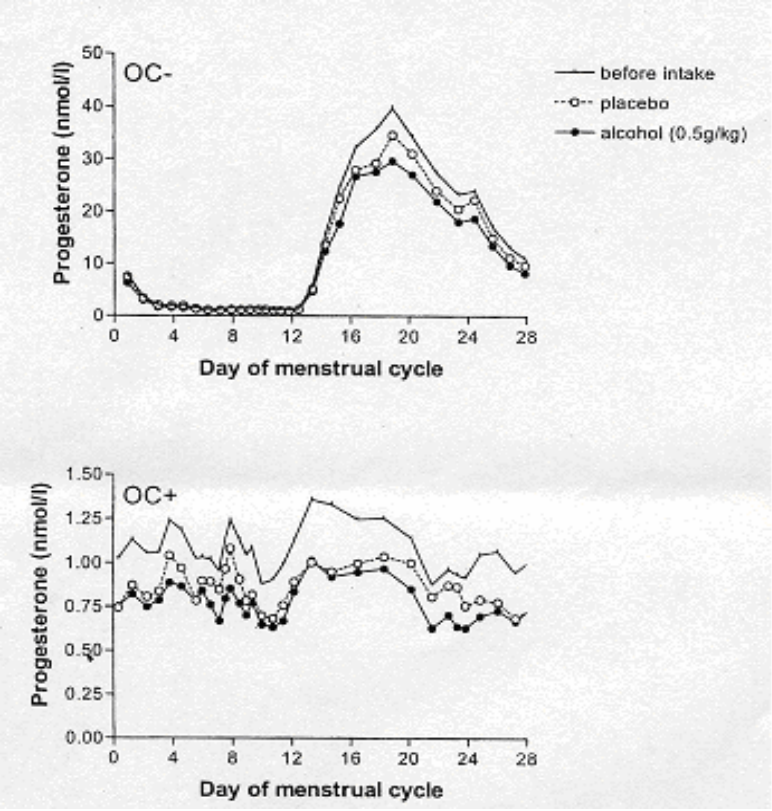


Figure 1.12: Plasma progesterone levels before and after placebo or alcohol consumption in subjects not taking oral contraceptives (OC-) and subjects taking oral contraceptives (OC+). From Sarkola, *et al.*, (1999) pp.979.

The reproductive anatomy of the alcoholic female can be affected, with decreased mass of ovarian and uterine structures (Gavaler *et al.,* 1983). Whilst oestrogen and progesterone have the capacity to affect the neurotransmitters gamma–aminobutyric acid (GABA) and dopamine (substances that are implicated in mediating the effects of ethanol), the fluctuating levels of the sex steroids during the menstrual cycle do not

apparently affect the direct subjective, behavioural or physiological effects of alcohol (Holdstock and de Wit, 2000).

Alcohol has been shown to stimulate the activity of the HPA axis in rats, and in both adolescent and adult humans. Female rats have shown larger increases of both ACTH and corticosterone than their male counterparts at the same dose of alcohol. This response is thought to be mediated by oestrogens, although the mechanism by which this happens is not clear (Ogilvie and Rivier, 1997). ACTH levels are also increased by alcohol stimulating secretion of both CRH and vasopressin (Rivier and Lee, 1996). In humans, indirect stimulation of the adrenal cortex also occurs, with increased ACTH and cortisol levels accompanying acute intoxication (Plotnick *et al.,* 1975; Sturtevant and Sturtevant, 1989; Frias *et al.,* 2000).

1.5.4 Alcohol, Sleep and Circadian Rhythms

Alcohol acts as a nervous system depressant by affecting the behaviour of the neurotransmitters GABA and glutamate. The time to sleep onset can be shortened by alcohol, through enhancement of the inhibitory neurotransmitter GABA and inhibition of the excitatory neurotransmitter glutamate (Roehers and Roth, 2001). In non-alcoholics, high and low doses of alcohol can initially improve sleep. However, high doses of alcohol prevent the usual increase in REM sleep and cause an increase the amount of light slow wave sleep in the first half of the night (Roehers and Roth, 2001). However, chronic consumption of alcohol can lead to a desensitization of the sedative effects of alcohol after only three nights (Roehers and Roth, 2001).

The normal circadian rhythm of body temperature is affected by alcohol consumption in that during intoxication body temperature is abnormally low, while during hangover the body temperature is unusually high when compared to normal values (Swift and Davidson, 1998).

1.5.5 Consumption amongst young adults

This section on alcohol consumption in young adults, both internationally and nationally, is included due to the unexpectedly high levels of alcohol consumption observed during the present study. Current literature was reviewed in order to determine if any effects on health and performance could be expected among the participants who consumed alcohol.

There exists a wealth of data regarding alcohol consumption in the Unites States of America, although differences in methodology have led to wide-ranging conclusions about the levels of alcohol consumption of university students (Meilman *et al.,* 1990). Alcohol continues to be linked with mortality, as in North America alcohol is the major factor related to deaths of teenagers and young adults (O'Brien and Lyons, 2000). Industrialised countries have a high incidence of alcohol consumption amongst young adults, with 35% of habitual drinkers aged 16 years or younger (Frias *et al.,* 2000).

"Binge drinking" or heavy drinking is generally defined as five or more alcoholic drinks in a row in one session, and is becoming increasingly prevalent in New Zealand (Alcohol Advisory Council of New Zealand, 2002; O'Malley and Johnston, 2002). The incidence of binge drinking is increasing, with 40% of American college students, equivalent to University students in New Zealand, reporting binge drinking sessions (O'Malley and Johnston, 2002). Gender differences in levels of alcohol consumption are observed, with proportionally more males binge drinking than females (Baer, 2002). In addition, athletes have been found to drink more than non-athletes (O'Malley and Johnston, 2002).

Statistics concerning trends in alcohol consumption in New Zealand have shown that during the decade 1990 to 1999 there was an increase in the number of people drinking heavily. During this time period approximately 1 in 8 women consumed 4 or more alcoholic drinks at least once per week, and by 2002 it was stated that "young people who drink are drinking more heavily, more often and are beginning to drink at an earlier age" (Casswell and Bhatta, 2001; Alcohol Advisory Council of New Zealand, 2002). The increasing trend in alcohol consumption is linked to an increase in adverse health

outcomes, such as falls, suicide and alcohol-related motor vehicle accidents (Alcohol Advisory Council of New Zealand, 2002). Another impact related to alcohol consumption in young adults is the possible impact on their growth. Animal studies have shown that alcohol can limit peak bone mass achieved, therefore putting the skeleton at risk for osteoporosis and fractures later in life (Turner and Sibonga, 2001).

1.5.5.1 Binge drinking effects

The incidence of individuals, especially young adults, concentrating their drinking episodes into weekends or holidays is on the increase. The physiological effects accompanying binge drinking is different when compared to moderate drinking (Frias *et al.,* 2000). Chronic drinking can result in increased blood pressure (discussed earlier), while binge drinkers display a transient increase in blood pressure, which is reversed by the time the alcohol is fully metabolised (Seppä and Sillanaukee, 1999). Binge drinking is also purported to be a risk factor for both intracerebral haemorrhage and brain infarction (Seppä and Sillanaukee, 1999).

1.5.6 Hangovers

Hangovers are a set of unpleasant physiological and psychological reactions from drinking to intoxication. The severity of the hangover is related to the amount and type of alcohol consumed. There is a range of symptoms (Table 1.2) that are commonly experienced, although the combination and intensity of symptoms vary widely between people. The symptoms of a hangover begin to occur as the blood alcohol concentration starts to fall, with the most intense symptoms experienced as the blood alcohol concentration falls to zero (Swift and Davidson, 1998).

Table 1.2: Hangover symptoms. Collated from Harburg, *et al.*, (1981) and Roehers and Roth (2001).

Physical symptoms	Mental symptoms
Diarrhoea	Anxiety
Fatigue	Depression
Gastritis	Dizziness
Headache	Irritability
Increased blood pressure	Memory loss (partial or total)
Increased sensitivity to light and sound	Vertigo
Loss of appetite	
Muscular aches and tremor	
Nausea and vomiting	
Rapid heartbeat (tachycardia)	
Redness of the eyes	
Sweating	
Thirst	

The headache experienced during a hangover can be a result of vasodilatation combined with the increased release of substances such as serotonin, histamines and prostaglandins (Swift and Davidson, 1998).

There is continuing debate about whether a hangover is merely mild alcohol withdrawal, although hangovers do not include symptoms such as seizures or hallucinations, and the period of negative symptoms is much shorter (Swift and Davidson, 1998). Alcohol is not the only contributing factor to the appearance of a hangover, as drinking behaviour is often associated with disrupted sleep patterns. In addition, a person's mental attitude towards drinking can increase or decrease the severity of the hangover experienced (Harburg *et al.,* 1981). Also, biologically active metabolites of alcohol and components of alcoholic drinks (congeners) can contribute to the severity of a hangover (Swift and Davidson, 1998). Alcoholic beverages such as wine can also cause the release of other substances that can contribute to the hangover experience, such as serotonin and histamine (Swift and Davidson, 1998).

Metabolites

As mentioned earlier, acetaldehyde is the primary product of alcohol metabolism, and it can affect the body independently of alcohol. Acetaldehyde has the ability to bind to proteins and other biological compounds, and can induce the following symptoms at

toxic levels: rapid pulse, sweating, skin flushing, nausea and vomiting. The effects are short-lived, as the enzyme aldehyde dehydrogenase metabolises acetaldehyde to acetate (Swift and Davidson, 1998).

Congeners

Congeners are substances that are added to alcoholic drinks to alter their taste, appearance and smell. Such substances can include methanol, propanol, butanol, ketones, aldehydes, esters and other compounds (Jones, 1989). There are different stages during the process of producing alcoholic beverages that congeners can be added, however their presence is thought to contribute both to intoxication and hangovers (Swift and Davidson, 1998). Methanol is a congener that also exhibits toxic effects at high concentrations, and is found in beverages such as brandies and whiskeys (Swift and Davidson, 1998).

1.5.6.1 Alcohol, Hangovers and Performance

Athletes in general participate in more 'risky behaviour' such as binge drinking than their non-athletic peers (O'Brien and Lyons, 2000). In addition, the type of sport one participates in appears to be a determinant of alcohol consumption, with heavier drinkers playing rugby, cricket, soccer and Gaelic football. This trend appears to be related to the social aspects of sporting clubs rather than related to behaviour during intense training or competition (Economos *et al.,* 1993; O'Brien, 1993).

When alcohol is consumed prior to exercise, the effects produced are different from those experienced the morning after a drinking session, although the result of a poorer performance may be the same. The acute effects on performance while intoxicated include a decrease in psychomotor skill capacity and impaired temperature regulation, which may negatively affect performance. In contrast, indices of performance such as VO_{2max}, cardiac function, muscular work capacity and respiratory dynamics are apparently not affected (O'Brien and Lyons, 2000). Measures related to performance such as time to exhaustion, aerobic performance and the pumping force of the heart are decreased, while performance time in a 5-mile run is increased following alcohol consumption (Bond *et al.,* 1983).

Performance in flight simulators under the influence of alcohol or during hangovers has been extensively studied. Pilots' performance using Link GAT-1 stimulator was significantly impaired during a hangover condition (Yesavage and Leirer, 1986). Although different phases of the menstrual cycle can affect alcohol pharmacokinetics, alcohol-impaired performance in a flight simulator, (both acutely and after 8 hours following the last alcoholic drink) is similar in each phase of the menstrual cycle (Mumenthaler *et al.,* 1999; Mumenthaler *et al.,* 2001).

It is a logical assumption that the presence of a hangover would negatively affect indices of psychological and physical performance, due to its negative effects upon the functioning of synaptic transmission within the central nervous system (Ekman *et al.,* 1996). There is a wide range of literature on the deleterious effects of alcohol on different indices of mental performance [for more information see Seppala, *et al.*, (1976), Misawa *et al.*, (1983), Lemon *et al.*, (1983)].

Heart rate (HR) at rest and following exercise during a hangover was shown to be significantly higher than at rest and following exercise when sober (Karvinen *et al.,* 1962). Aerobic (but not anaerobic) performance also suffers as a result of a hangover, with decrements in performance noted at quantities ranging from between 1 and 38 units of alcohol (O'Brien, 1993). The decrease in aerobic performance can be explained by describing the metabolic and psychological changes induced by alcohol, some of which are mentioned earlier. When alcohol is metabolised (described above) there is a change in the ratio of NADH to NAD. More specifically, the increase in NAD (an electron carrier) causes the citric acid cycle (part of the aerobic metabolic pathway) to slow down, decreasing the maximal rate at which aerobic metabolism can occur (O'Brien and Lyons, 2000). Combined with the slowing of the citric acid cycle, increased NAD concentrations also result in hyperlactacidemia and hypoglycaemia, both of which can have a negative effect on aerobic performance (O'Brien and Lyons, 2000).

Aside from the acute and delayed effects of alcohol, there is a significant difference in the injury rates between drinkers and non-drinkers. This trend was apparent in an athletic population, when in 1993 the injury rate for drinkers was about 55% compared to about 24% in the non-drinking athletic population (O'Brien and Lyons, 2000).

Outline of thesis

The following chapters outline the steps taken to answer the research questions described below. The second chapter outlines the experimental design of the study and the materials and methods used, the third chapter reports the results obtained during the study, and the fourth and final chapter consists of a discussion of the findings of the study and their implications, a general discussion of the lessons learnt during the study and some wider implications of the findings.

Research Objectives

The first research objective was to assess whether changes in aerobic fitness occurred between the first and sixth weeks of the study in a group of sedentary participants as a result of little or no activity, and in a group of training participants after six weeks of rugby training. Changes in fitness were assessed by references to changes in heart rate, rating of perceived exertion and oxygen consumption (VO_2) during treadmill fitness tests. A comparison of VO_2 results between the sedentary control participants and the training participants was conducted in the first and final weeks of the experiment.

The second research objective was to investigate effects of variables such as sleep quality, sleep duration, stage of the menstrual cycle, oral contraceptive use and alcohol consumption on VO_2 in both sedentary and training participants.

The third objective was to investigate acute or chronic changes in the plasma concentrations of cortisol, testosterone and growth hormone during the six-week training period in training participants. In addition, any relationships between either oral contraceptive use or alcohol consumption on the plasma concentrations of the three hormones were to be examined.

The fourth objective was to determine the accuracy of the electrosonophoretic method in predicting plasma concentrations of cortisol, testosterone and growth hormone, by using correlation analysis. In addition, the derivation of equation that would allow the concentrations of these three hormones in plasma to be estimated from those in

interstitial fluid obtained by electrosonophoresis was investigated. The relationships between concentrations of cortisol and testosterone in plasma or saliva and those in interstitial fluid were also to be evaluated.

Chapter 2

Materials and Methods

2.1. Ethical Approval

Approval for the study was obtained prior to its commencement from the Massey University Human Ethics Committee (Number 14/02).

2.2. Recruitment of Participants

In total, thirty-one female participants were recruited for the study. Sixteen sedentary control participants were recruited from the student population at Massey University and fifteen training participants from a university-based winter sports club. Sedentary participants were recruited through advertisements around the university campus and by direct communication to groups of students prior to lectures commencing. Recruitment of training participants was on a whole-team basis, due to uniformity of training schedules.

Approval was sought and provided by management within the winter sports club to approach the coaches of the team. When consent was granted from the coaches, the players were approached as a team and after an explanation of the project and provision of written details, volunteers were sought.

Consultation occurred at the clubrooms of the sports team for the training participants and at the Human Performance Laboratory (see below) for the sedentary participants. All participants met with the researcher individually prior to the study, to ensure a good understanding of the requirements. When verbal consent was obtained, a time schedule was drawn up by both the researcher and the participant to incorporate the sampling schedule and fitness testing. Written consent was then obtained on the first morning of the study.

2.3. Inclusion/Exclusion Criteria

The inclusion and exclusion criteria for the sedentary and training participants are reported below.

2.3.1 Sedentary participants

Inclusion criteria for each sedentary participant included the following:

- Passed a routine medical examination by a general practitioner before participation in the study to ensure that their health was of a sufficient standard to be able to undergo treadmill fitness tests (e.g. normal blood pressure, no prior illnesses that would compromise fitness testing)
- Not engaged in regular sports training
- Healthy, 18 to 25 year old female who is non-injured (i.e. not currently carrying any injuries that would restrict their participation in the fitness tests).

Exclusion criteria for each sedentary participant included the following:

- Performance enhancing or recreational drug use before or during the study, not including alcohol, caffeine or nicotine
- Injury during trial
- Health conditions incompatible with regular training and exercise
- Pregnant or lactating
- Unease with any aspect of the study.

2.3.2 Training Participants

Inclusion criteria of each training participant included the following:

- Passed a routine medical examination by a general practitioner before participation in the study to ensure that their health was of a sufficient standard to be able to

partake in treadmill fitness tests (e.g. normal blood pressure, no prior illnesses that would compromise fitness testing)

- Engaged in twice weekly rugby training sessions and weekly games
- Healthy, 18 to 25 year old female who is non-injured (i.e. not currently carrying any injuries that would restrict their participation in the fitness tests).

Exclusion criteria of each training participant included the following:

- Performance enhancing or recreational drug use, not including alcohol, caffeine or nicotine
- Injury during trial
- Health conditions incompatible with regular training and exercise
- Pregnant or lactating
- Unease with any aspect of the study.

Thirty-two participants applied and were accepted into the trial. One sedentary participant on the first morning of the study withdrew due to the degree of time commitment required in the study. One training participant withdrew from the study at the end of the fourth week due to the need to travel to meet a prior overseas rugby commitment.

2.4. Venue

All sample collections and fitness tests were conducted at the Human Performance Laboratory, Massey University, Palmerston North, New Zealand.

2.5. Study methodology

Blood, saliva and interstitial fluid samples (discussed below) were collected on three weekday mornings for six weeks to establish mean concentrations of cortisol, testosterone and growth hormone for each participant. Sample collection took a maximum of 15 minutes, and for each participant collection occurred at the same time (within thirty minutes) each day throughout the study. This was done to increase the

precision of mean hormone concentrations for each participant over the duration of the study and to avoid large variation in concentrations, as all three hormones display circadian rhythmicity, as noted in the Introduction (Section 1.4.3). Saliva and interstitial fluid samples were also collected within two minutes of the treadmill fitness test being completed. This occurred in order to assess any acute changes in the concentrations of cortisol, testosterone or growth hormone from the mean values determined by the samples taken in the morning.

Fitness tests were conducted weekly for the training participants and in the first and final weeks for the sedentary participants. Testing was carried out to monitor changes in the indices of fitness due to training (in the training participants) or a sedentary lifestyle (in the sedentary participants). The indices of fitness measured were the maximal oxygen consumption (VO_{2max}) for the training participants and 85% of VO_{2max} for the sedentary participants as described below (Section 2.5.1.3 and 2.5.1.4).

The Pulmolab EX670 mass spectrometer (Morgan Medical Ltd., Kent, England) which has a reported error of ± 2% was used in the present study to analyse the volume and gas concentration of each breath. These data provide an accurate estimation of O_2 consumption and CO_2 production which in turn allows for VO_2 and an estimation of substrate utilisation or respiratory exchange ratio (RER) to be determined. The RER is determined by the ratio of carbon dioxide produced to oxygen consumed (VCO_2/VO_2).

The Pulmolab EX670 uses mass spectrometry to quantitatively determine the different proportions of gases in the samples of expired air. Like all mass spectrometers it is based on a series of processes. Initially, the sample is converted into a gas phase and then ionised using an ion source or 'ioniser'. This ionised gas undergoes a series of steps to decrease its pressure to equal that inside the vacuum. Once inside the analyser, it is subjected to either electromagnetic or electrostatic fields. In the case of the Pulmolab EX670, the gas is exposed to both a direct current and a combined radio frequency. The specific frequencies of the two electrostatic fields can be selected so that only one species of gas at a time moves through to be measured. The stream of ions that has passed through the electrostatic fields is equivalent to a very small electrical signal, which is amplified many times and the final current is measured. The mass spectrometer

can measure only one species of gas at a time, and so the process or cycle described above can be repeated up to eight times nearly simultaneously (Holtzclaw, *et al.*, 1984). Before each series of fitness tests the mass spectrometer system was calibrated twice (a turbine calibration and a gas analyser calibration) according to the manufacturer's instructions. The vacuum pumps within the mass spectrometer system remained running for the duration of the fitness test to maintain consistency. The treadmill fitness test protocol that was used was the Bruce protocol, described below.

2.5.1 Bruce Exercise Test Protocol

The Bruce exercise protocol for the treadmill fitness tests was used as it is of an appropriate duration (8 to 12 minutes) for both sedentary and trained individuals as a maximal or submaximal test (Fielding, *et al.*, 1997). A more thorough investigation into the validity of the Bruce protocol is presented in Section 1.2.2.4 of the Introduction. It is a fitness test that begins at walking pace, with even increments in speed and angle of incline at three-minute intervals. The increase in both speed and angle of incline occurred within 5 seconds of the beginning of each stage. The traditional protocol is described below as well as a modified version used in the present study.

The warm-up for the fitness test was 5 minutes in duration at 0% incline, with speed at 6km/hr. The test was conducted on the Payne TM4-NZ treadmill (Stanton Engineering Pty Ltd., Blacktown, New South Wales, Australia).

2.5.1.1 Standard Bruce Protocol

The standard Bruce protocol outlined below (Table 2.1) is as reported by Mahler *et al.*, (1995).

Table 2.1: Stage, time (min), grade (%), speed (km/hr), change in grade (%) and change in speed (km/hr) for the Bruce protocol. NB: assuming 1 mile = 1609 metres.

Stage	Time (min)	Grade (%)	Speed (km/hr)	Change in Grade (%)	Change in Speed (km/hr)
1	0-	10	2.74		
2	3-	12	4.02	2	1.28
3	6-	14	5.47	2	1.45
4	9-	16	6.76	2	1.29
5	12-	18	8.05	2	1.29
6	15-	20	8.85	2	0.8

2.5.1.2 Modified Bruce Protocol

The changes observed in the modified protocol (Table 2.2) were to standardise the increment in speed at each phase to 1.3km/hr, with the difference between the standard protocol and the modified protocol becoming greater towards the later stages of the fitness test. Few participants were expected to reach Stage 5 and few if any to reach Stage 6 so the impact of this modification is expected to be negligible.

Table 2.2: Stage, time (min), grade (%), speed (km/hr), change in grade (%) and change in speed (km/hr) for the Modified Bruce protocol.

Stage	Time (min)	Grade (%)	Speed (km/hr)	Change in Grade (%)	Change in speed (km/hr)
1	0-	10	2.7	2	1.3
2	3-	12	4.0	2	1.3
3	6-	14	5.3	2	1.3
4	9-	16	6.6	2	1.3
5	12-	18	7.9	2	1.3
6	15-	20	9.2	2	1.3

2.5.1.3 Submaximal tests

Submaximal tests were conducted on the sedentary participants and were stopped when the participant reached 85% of their predicted maximal heart rate reserve (85% HR_{max}) (Mahler *et al.*, 1995), which was determined using the equation:

$$85\ \%\ HR_{max} = \text{Maximal HR} * 0.85$$

Where: Maximal HR = 220bpm – age of participant

The submaximal tests could also be terminated by the participant if they raised their hand to signal the end of the test, or activated one of the three personal stop mechanisms on the treadmill.

2.5.1.4 Maximal tests

The maximal tests conducted on the training participants could be terminated by the participant when they raised their hand to signal the end of the test or activated one of the three personal stop mechanisms on the treadmill. The test could also be stopped by the researcher when the predicted maximal HR was reached, when the RER (defined in Section 5 above) increased above 1.1 or when VO_2 reached a plateau or did not increase with further increases in exercise intensity.

2.5.1.5 Measurements of heart rate, rating of perceived exertion and sample collection

During each fitness test, each participant's heart rate was monitored using the Polar Vantage® NV™ Heart Rate Monitor, (Polar Electro Oy, Kempele, Finland). Heart rate and rating of perceived exertion (RPE) were noted two minutes into every three-minute stage or at the completion of the test if the test ended before or after the two-minute point of the stage. RPE recordings gave an indication, on a scale of 0 to 10, of exercise tolerance, and are apparently strongly correlated with both heart rate and oxygen consumption (Mahler, *et al.*, 1995).

Saliva and interstitial fluid samples, for later hormone analysis, were collected within two minutes of the end of each fitness test.

2.5.2 Training Schedule

The training participants trained twice-weekly, on Monday and Wednesday evenings, at the Massey University rugby fields. The training sessions had the features indicated in Table 2.3. The Monday sessions had relatively more aerobic fitness training and less short-distance speed work than did the Wednesday sessions. There was no scheduled warm-down component, as participants were expected to design their own warm-down procedure. During the first week of the study, one of the training participants noted in her sleep log when extra training was undertaken but this reporting was for the first

week only. No other training participant reported any extra exercise outside of the twice-weekly rugby training and once-weekly rugby game.

Table 2.3: Description of the nature of the twice weekly training sessions for training participants over the six-week study.

Training	Duration (minutes)	Notes
Warm-up and stretch	10	
Intense warm-up	10	Aerobic fitness component
Technical drills	40	High fitness component and relatively intense e.g. Contact drills
Technical work (Forwards only)	15	High fitness and strength component* e.g. Line-out jumping, scrum work
Team run	15	Aerobic fitness component

* = this section of the training session incorporated both aerobic and anaerobic fitness elements.

2.6. Sleep Logs

Upon waking every morning of the six-week study, each participant initially noted in their sleep log the date, the time that the log was being completed and the time of awakening. The sleep log consisted of eight simple questions regarding sleep length, sleep quality and spontaneous disturbances. In addition to the questions on sleep, alcohol consumption during the previous 24 hours was noted, as well the stage in the menstrual cycle and any medication consumed in the previous 24 hours. Any spontaneous sleep disturbances recorded in the sleep logs were to be assessed in relation to hormone concentrations measured on the following day to see if any correlations were evident. The effect of sleep disturbances or sleep quality on performance (as determined by VO_{2max} or 85% VO_{2max} values) were investigated. Also, relationships between VO_2 results and amount of alcohol consumed (defined as the number of standard drinks) during the previous 24 hours, or the day of the menstrual cycle were examined.

The researcher recommended to all participants that they note any extra physical activity that was undertaken during the study. No sedentary participants recorded any physical exercise during the study and as mentioned above, one training participant for a brief time recorded extra physical training but this was for the first week only.

2.7. Sample collection

On the first morning of the study, prior to sample collection, each participant was weighed on a Jadever JPS-2030 Scale (Jadever Scale Co. Ltd., Taipei County, Republic of China). Additionally, each participant had their height measured and underwent bioelectric impedance analysis (BIA) to give an index of body composition. The device used was the Body Composition Analyzer, Model 310, (Biodynamics Corporation, Seattle, Washington, United States of America). This technique gave an indication of the percentage of fat in each person by passing a small electric current between the wrist and the ankle and measuring the resistance to the flow of the current. The BIA method was chosen for its ease of use in a clinical setting and for its convenience, although it may overestimate the percentage of fat in very lean individuals (Mahler *et al.*, 1995).

The number of samples taken in the morning was dependent on the day, as on Mondays and Fridays, saliva, blood and an interstitial fluid sample were taken, whereas on Wednesdays only saliva and interstitial fluid samples were collected. No attempt was made to standardise the participants' sleep, or their alcohol, nicotine or medicine consumption or their prandial state, due to the relatively high demands of the study protocol. However, environmental temperature, humidity and sample collection procedures were standardised in the Human Performance Laboratory to minimise variation in results due to fluctuations or changes in these factors. The blood, saliva and interstitial fluid samples were frozen at -20°C shortly after collection and were stored until hormone analysis was conducted.

2.7.1 Blood

Approximately 200 μL of blood were taken on Monday and Friday mornings, between 7:30a.m. and 12:00p.m. Fingers were initially wiped with a Sterile Alcohol Prep Pad (Professional Disposals Inc., New York, United States of America). The participant was then instructed to rotate her arm at the shoulder joint to increase peripheral blood flow. Samples were collected by a finger-tip prick, using a sterile Accucheck lancet (Roche Diagnostics, Auckland, New Zealand). The blood sample was collected into a heparinized capillary tube (Chase Scientific Glass, Inc., Rockwood, Tennessee, United

States of America). The blood in the capillary tubes was separated into plasma and cells using the Microcapillary Centrifuge MB (International Equipment Company, Massachusetts, United States of America). The plasma was harvested and stored in labelled Eppendorf tubes (Biolab Scientific, Auckland, New Zealand). Members of both the training group and the sedentary group provided up to fourteen finger tip 200μL blood samples each (total 2800μL per participant).

2.7.2 Saliva

Saliva samples were voided directly into labelled sample containers, on Monday, Wednesday and Friday mornings between 7:30a.m.-12:00p.m. Samples were also collected within two minutes of the participant completing either a submaximal or maximal fitness test. The training participants provided up to thirty-five 5mL saliva samples each (total 175mL each) while the sedentary participants provided up to twenty-eight 5mL saliva samples each (total 140mL each).

2.7.3 Interstitial fluid

Samples of interstitial fluid were collected into labelled Eppendorf tubes using the electrosonophoretic device (Figure 2.1) on Monday, Wednesday and Friday mornings and within two minutes of participants completing a submaximal or maximal fitness test. The training participants each provided up to 35 transcutaneous harvests from interstitial fluid, while the sedentary participants each provided up to 28 transcutaneous harvests from interstitial fluid. The semi-permeable membrane used during this study had a molecular weight cut-off point of 40kD.

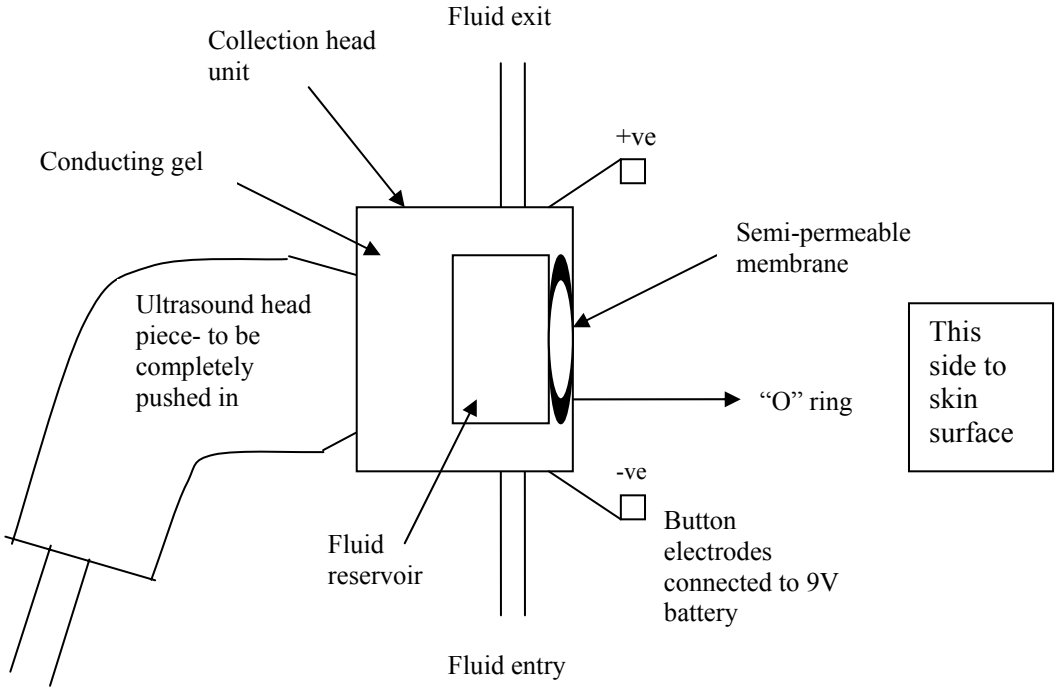


Figure 2.1: Diagram of electrosonophoretic (ESOP) device. Adapted from Figure 1 in Cook, (2002) pp. 172.

2.8. Sample processing

Plasma, saliva and interstitial fluid samples were analysed for concentrations of cortisol, testosterone and growth hormone, except that growth hormone was not measured in saliva. Enzyme-linked immunosorbent assays (ELISA) were conducted on plasma, saliva and interstitial fluid samples for both cortisol and testosterone using standardised kits (Salimetrics, United States of America and DRG Diagnostics, Germany), whereas a radioimmunoassay (RIA) using an iodinated label (Sigma Radiochemical, United States of America) was used to test plasma and interstitial fluid samples for growth hormone.

Immunoassays such as ELISA and RIA exploit the reactions that occur between antigens and antibodies in order to determine the amount of a substance in a solution (Kemeny, 1991). There are several methods for measuring the amount of a substance in a sample. For example, detection of the antigen-antibody complex can be achieved by using an enzyme-labelled or radioactive isotope-labelled antibody. In the present study, the hormone molecules in the plasma, interstitial fluid or saliva sample acted as the

antigen to which the antibody binds. The amount of hormone in the sample is determined by the competition between the labelled hormone and the hormone in the sample in binding to the antibody which coats a microtitre plate. In the case of cortisol and testosterone, enzyme labels were used, and the activity of the enzyme and how activity changes following binding is measured to give an index of hormone concentrations. In the case of growth hormone, radioactive iodine labels were used and the radioactivity levels measured to give an index of growth hormone concentration.

The accuracy of the hormone assays for cortisol, testosterone and growth hormone was ± 3ng/mxL, ± 2pg/mL and ± 1pg/mL, respectively, while the mean inter- and intra-assay coefficients of variation were 4.3%, 3.1% and 2.7%, respectively. All the hormone assays were conducted in series to avoid any variations between assays. Following the analysis, all samples were incinerated. The option was presented to participants of other cultures to have their samples sterilised and then buried, but no participant chose to have their samples disposed of in this way.

The collection of interstitial fluid samples at the same time as the blood and/or saliva samples provided the opportunity to investigate the use of the electrosonophoretic device as a non-invasive blood sampling methodology.

2.9. Statistical tests

Data were analysed using SPSS 11.0 software (SPSS Inc. Chicago, Illinois, United States of America) and graphs were created using Prism 3.0 software (Graphpad Software, United States of America). Values in the text and in tables are expressed as mean ± standard error of the mean (SEM). Participants had several groupings, according to whether they were sedentary or training, whether they used oral contraceptive or not, and according to whether their alcohol consumption was high or moderate or if they abstained from alcohol.

Pearson correlations (two-tailed) were performed to examine the relationship between heart rates at the same stage of the treadmill fitness test between each week. Pearson correlations (two-tailed) were also conducted to examine the relationship between the

rating of perceived exertion at the same stage of the treadmill fitness test between each week.

Paired t-tests were used to determine the significance of any differences in body composition values, change in heart rate at a low intensity of exercise, change in the rating of perceived exertion at a low intensity of exercise and VO_{2max} results in training participants, before and after the six-week training period. For sedentary and training groups, paired t-tests were utilised to determine the significance of any differences between measured and calculated VO_2 results, and to test the significance of any differences between mean VO_2 results for the sedentary and training groups.

The relative contribution of training to the observed change in VO_{2max} for the training group was assessed using covariate analysis, where x and y were the covariates and z the factor tested.

Sleep quality ratings were investigated using a one-way ANOVA with sleep duration, group (sedentary or training), oral contraceptive use, degree of alcohol consumption, day of the menstrual cycle and time as factors. The same analysis was conducted for sleep duration. The significance of week × group interactions was also determined using repeated measures ANOVA. Pearson correlations (two-tailed) were used to examine the relationships between sleep quality rating and VO_2, sleep quality rating and alcohol consumption, sleep quality rating and day of the menstrual cycle, sleep duration and VO_2, sleep duration and alcohol consumption and sleep duration and day of the menstrual cycle.

The concentrations of cortisol in plasma were investigated using one way ANOVA with participants as a within subjects factor, and then with groups (sedentary or training), oral contraceptive use, degree of alcohol consumption and time as factors. Interactions between these factors were also investigated, where appropriate. The significance of time × group interactions was also determined using ANOVA with repeated measures. The same analysis as described above was conducted for plasma concentrations of both testosterone and growth hormone.

Pearson correlations (two-tailed) were conducted to examine the relationship between hormone concentrations in plasma and saliva, plasma and interstitial fluid, and saliva and interstitial fluid.

Multivariate analysis, in which cortisol, testosterone and growth hormone were the variates, and plasma and interstitial fluid were the factors, was conducted to see if the ratios of the three hormones in plasma and interstitial fluid were similar.

For all tests, a p value less than 0.05 was considered to be statistically significant.

Chapter Three

Results

Numerous parameters were measured and the results are reported below in the following order: characteristics of the participants are described first, then data relating to the fitness tests and sleep logs are presented, after which results from the hormone analysis of samples collected from the participants are reported, and finally data allowing assessment of the accuracy of the electrosonophoretic method for predicting plasma hormone concentration are outlined.

3.1. Participants

Thirty-two female participants volunteered for the present study. During the course of the six-week study one sedentary participant withdrew due to the time commitments required and after four weeks, one training participants left because of overseas rugby commitments. Once the study had commenced, no participants experienced untoward health complications as a result of their participation in it.

Most of the participants (29 of 31) reported their ethnicity as Caucasian, while the remaining two were from China and Africa, respectively.

3.1.1 Physical characteristics

3.1.1.1 Age and Height

The participants were aged from 18 to 25 years, their heights ranged from 1.54 to 1.74m, and both parameters exhibited similar ranges within the sedentary and training groups (see Table 3.1).

3.1.1.2 Weight and Body composition

There were no significant differences in the mean values for weight, body mass index (BMI), lean body mass or body fat content between the first and sixth weeks for either the sedentary or training participants (Table 3.1). However, the changes in weight and lean body mass in the sedentary group approached significance (p= 0.087 and 0.068, respectively; paired t-test). The mean decrement in weight was 0.6kg for the sedentary group and 0.2kg for the training group.

Table 3.1: Physical parameters: Mean ± SEM for age, height, body mass index (BMI), lean body mass, and body fat in sedentary and training participants at the beginning and end of a six-week study.

Parameter	Week	Sedentary Group	Training Group
Age (years)		19.6 ± 2.07	20 ± 1.7
Height (m)		1.7 ± 0.01	1. 7 ± 0.01
Weight (kg)	1	69.0 ± 2.9	73.5 ± 2.6
Weight (kg)	6	70.3 ± 3.1	73.7 ± 2.7
BMI (kg/m^2)	1	24.9 ± 1.0	26.4 ± 0.8
BMI (kg/m^2)	6	25.3 ± 1.1	26.5 ± 0.8
Lean body mass (% total weight)	1	53.2 ± 1.7	57.4 ± 1.4
Lean body mass (% total weight)	6	54.1 ± 1.8	57.3 ± 1.4
Body fat (% total weight)	1	22.2 ± 1.2	21.4 ± 1.1
Body fat (% total weight)	6	22.4 ± 1.3	21.7 ± 1.1

3.1.1.3. Menstrual Cycle Length and Oral Contraceptive (OC) Usage

The mean cycle length for the sedentary group and for the training group was 30 ± 1.5 and 28 ± 2.5 days, respectively (Table 3.2). There were no significant differences between the mean menstrual cycle lengths when participants were assessed according to group and OC usage ($p>0.05$; ANOVA). Whilst normally distributed, the length of the menstrual cycle varied greatly, ranging from 19 days to 61 days. Reasons for the extreme length of some participants' cycles included irregular cycling, or not taking the inactive pills contained in a sheet of OC tablets (which initiates the onset of menstrual bleeding).

Table 3.2: Mean ± SEM (n) of menstrual cycle length in sedentary and training participants, who did or did not use oral contraceptives (OC).

Oral contraceptive use	Sedentary Group	Training Group
Not on OC	31 ± 3.5 (n=9)	28 ± 2.2 (n=6)
On OC	31 ± 3.1 (n=7)	29 ± 1.9 (n=9)

Oral contraceptives: The brand names and chemical composition of each brand of oral contraceptive used during the study are shown in Table 3.3 below.

Table 3.3: Brand names and chemical composition of types of oral contraceptives used during the six-week study. NB. mg= milligram, µg=microgram.

Brand name	Active Ingredients
Estelle 35™	Ethinyloestradiol 35µg and Cyproterone acetate 2mg
Femodene 28®	Ethinyloestradiol 30µg and Gestodene 75µg
Loette®	Ethinyloestradiol 20µg and Levonorgestrel 100µg
Mercilon 28®	Ethinyloestradiol 20µg and Desogestrel 150µg
Microgynon 30ED®	Ethinyloestradiol 30µg and Levonorgestrel 150µg
Monofeme 28®	Ethinyloestradiol 30µg and Levonorgestrel 150µg

3.1.1.4 Medication

A variety of other medications were taken over the six-week study, as listed in Table 3.4.

Table 3.4: Medications excluding oral contraceptives taken by participants during the six-week study.

Pharmaceuticals	**Pharmaceuticals continued**
Alanase nasal spray™	Paracetamol
Aropax™	Phemogen
Aspamox	Polaramine®
Cataflam®	Postinor-2®
Cepacol cough disks™	Voltaren®
Claratyne®	Ventolin™
Diazepam	
Dispirin®	**Herbal remedies**
Dimetapp Day-Night®	Arnica cream*
Doxycycline 100mg	Echinacea 6000mg*
Fluoxetine 20mg	Herbal sleeping pill*
Hayfever injection*	
Ibuprofen	**Topical applications**
Influenza injection*	Deep Heat®
Local anaesthesia*	Warm-Up®
Naprosyn®	
Naproxen	

*NB. Indicates no brand name reported

3.1.1.5 Compliance

The compliance rates for the sleep logs were very high, with 97% of the pages completed. All of the sedentary participants completed their two scheduled fitness tests (total = 32), while 86% of the fitness tests scheduled for the training participants were completed (total = 76). Sample collection compliance was similarly high, with 89.5% of scheduled samples acquired.

3.2. Treadmill Fitness Tests

Validation of the use of measures such as heart rate and ratings of perceived exertion were carried out before examining whether any significant changes in oxygen consumption (VO_2) occurred. This was determined by observing if any changes in VO_2 at maximal effort (VO_{2max}) occurred in the participants during the study. When performance is discussed in the following sections, it refers to the VO_2 at max result the training participant achieved in their fitness test, the VO_2 at 85% of maximum HR for sedentary participants, or to changes in VO_2 at max across several tests. It should be noted here that in the sixth and final week of the study, the on-line gas analysis system began to report erroneous values (including one of oxygen *production*) which resulted in all of the fitness test data from that week being discarded. This prevented analysis of whether any changes in fitness had occurred in the sedentary participants, or the true effect of six weeks of training on aerobic fitness in the training participants.

3.2.1 Heart Rate

Heart rate (HR) recordings were taken from each participant two minutes into each three-minute stage of the treadmill fitness test described in Table 2.2 (Materials and Methods), with the mean HR increasing in a step-wise manner with each successive stage, in response to the increased speed and angle of the treadmill. The mean HR at each stage of the treadmill fitness test was calculated for each group (Table 3.5).

Over the course of the study, the mean HR for Stages 2 to 5 changed little in sedentary participants (Weeks 1 and 6 compared) and training participants (Weeks 1 and 6 compared). For the sedentary participants, the mean HR during Stage 1 was similar in Weeks 1 and 6 ($p= 0.115$; paired t-test), whereas for the training participants, the mean HR in Stage 1 tended to be higher in Week 1 than in Week 6, with means of 116 ± 2.7 and 111 ± 2.7bpm respectively, a difference that approached significance ($p=0.077$; paired t-test). An assessment of the changes in HR from resting values during the different stages of the treadmill fitness test was not conducted due to the absence of resting HR data.

The mean HR during Stage 1 of the treadmill fitness test in Week 1 for the sedentary participants was numerically but not significantly higher (p= 0.114; independent samples t-test) than for the training participants in Week 1 (125 and 116bpm, respectively) and significantly higher (p= 0.000; independent samples t-test) during Week 6 (129 and 111bpm, respectively).

The difference in mean HR during Stage 1 between the sedentary and training group was lower in Week 1 than in Week 6 (9 and 18bpm, respectively).

Table 3.5: Range and mean ± SEM (n) of heart rates taken at each stage of the treadmill fitness test undertaken by sedentary and training participants.

Group	Range (bpm)	Mean ± SEM	n
Sedentary			
Stage 1	94 – 161	127 ± 2.9	31
Stage 2	112 – 182	151 ± 3.1	30
Stage 3	149 – 188	174 ± 1.5	27
Stage 4	171 – 174	172 ± 1.5	2
Training			
Stage 1	86 – 144	112 ± 1.2	75
Stage 2	116 – 156	136 ± 1.1	75
Stage 3	150 – 183	166 ± 1.0	74
Stage 4	161 – 217	185 ± 1.0	75
Stage 5	173 – 202	191 ± 1.4	25

3.2.1.1. Sedentary group

For the sedentary participants, the duration of the treadmill fitness test was determined by the point at which their HR reached 85% of their calculated maximal HR (HR_{max}) determined using the equation in 2.5.1.3 (Materials and Methods). Of the 32 treadmill fitness tests performed in the first and sixth weeks, the tests were terminated during Stage 2 on 5 occasions, during Stage 3 on 25 occasions and during Stage 4 on 2. There were very high correlations between mean HR recorded during Stage 1, 2 and 3 in Week 1 and the HR recorded during the corresponding stages in Week 6 (Table 3.6). High correlations during Stages 2 and 3 were expected due to the termination of the fitness test when the participant's HR reached 85% of HR_{max}. All correlations reached the 0.01 level of significance, supporting the consistency of heart rate recordings at each stage of the treadmill fitness test between Weeks 1 and 6. As mentioned above, the

mean HR during Stage 1 did not change significantly between the initial and final fitness tests, which suggests that there was no changes in fitness over the study.

Table 3.6: Correlation analysis of heart rates (HR) obtained at each stage of treadmill fitness tests conducted on sedentary participants during Week 1 and Week 6 of a six-week study. NB: No correlation coefficient (r) was determined for heart rates during Stage 4 as only one participant achieved that level. ** denotes significance at the 0.01 level (2-tailed).

Relationship	Correlation coefficients (r)
HR Stg1 Wk1 vs. Wk6	0.776**
HR Stg2 Wk1 vs. Wk6	0.909**
HR Stg3 Wk1 vs. Wk6	0.844**

3.2.1.2. Training group

The 15 participants within the training group underwent a maximum of six fitness tests that were longer in duration than those for the sedentary group as the training group ran to exhaustion. Of the 76 tests performed over the six weeks, 25 tests were terminated during Stage 5, 50 during Stage 4, and one during Stage 3, although this last test was terminated due to a pre-existing injury causing pain. To determine the stability of HR at each stage of the treadmill fitness tests, correlation analyses were conducted. Mean HR values were similar at each stage of the fitness tests across the six weeks (Figure 3.1). When each possible combination was analysed, 90% had correlation coefficients between 0.5 and 1, indicating strong positive relationships, and 78.6% of these correlations reached a 0.05 level of significance or better. The high correlation coefficients, but low significance of some values observed for the relationship of HR at Stage 4: Wk2 vs. Wk3 and Wk6, as well as Wk3 vs. Wk4, and Wk5 vs. Wk6 arose from low numbers (n=3 or n=4).

In general the correlation analysis suggested a high degree of consistency of HR at particular stages of the treadmill fitness test within individuals.

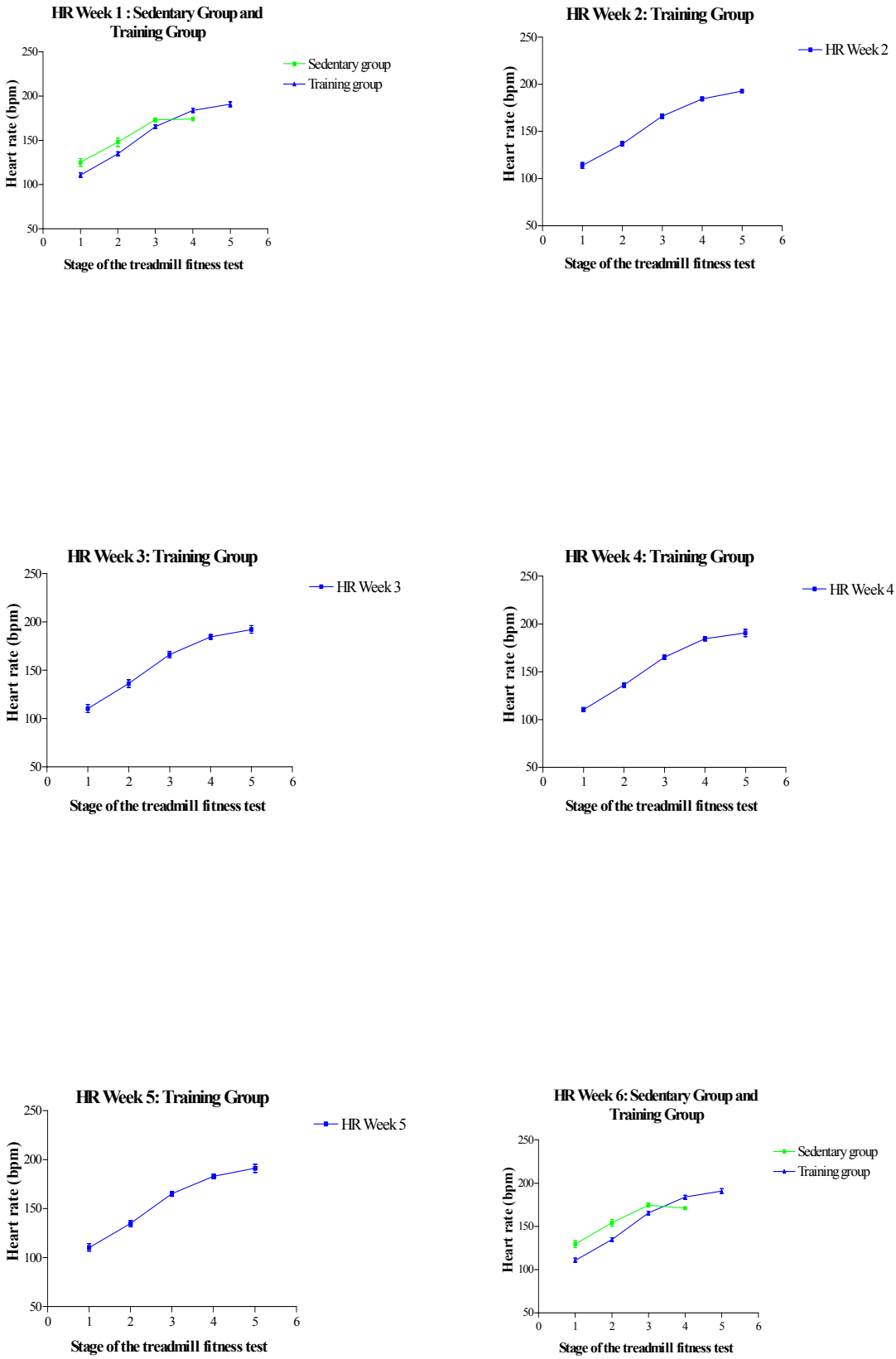


Figure 3.1: Graphs of mean ± SEM of heart rate (HR) at each stage of the treadmill fitness test each week, in both sedentary and training participants during a six-week study.

3.2.2 Ratings of Perceived Exertion

Ratings of perceived exertion (RPE) were recorded two minutes into every three-minute stage during the fitness test, and, as with HR, also increased with each successive stage of the treadmill fitness test (Figure 3.2). Overall mean RPE values for each stage of the test were calculated (Table 3.7). In general, RPE recordings were less consistent when compared to HR recordings.

Table 3.7: Range and mean ± SEM (n) of rating of perceived exertion (RPE) taken at each stage of the treadmill fitness test undertaken by sedentary and training participants.

Group	Range (bpm)	Mean ± SEM	n
Sedentary			
Stage 1	0.5 – 3	2.0 ± 0.1	32
Stage 2	2 – 6	3.6 ± 0.2	32
Stage 3	4 – 9	4.9 ± 0.3	14
Stage 4	5	5 ± 0.0	1
Training			
Stage 1	0.5 – 3	1.2 ± 0.1	76
Stage 2	1 – 4	2.6 ± 0.1	76
Stage 3	3 – 8	4.6 ± 0.1	76
Stage 4	4 – 10	7.1 ± 0.2	71
Stage 5	7 – 10	8.8 ± 0.2	26

3.2.2.1. Sedentary group

The consistency of RPE recordings at each stage of the treadmill fitness test was investigated. The RPE values during the fitness tests were highly correlated between Week 1 and Week 6, with correlation coefficients (r) values of 0.705, 0.718 and 0.607 ($p < 0.05$; t-test) for Stage 1, 2 and 3, respectively. The minimal increase noted between the mean RPE in Stage 3 and Stage 4 is due to the higher baseline fitness level of the one participant who achieved Stage 4.

3.2.2.2. Training group

The consistency of RPE recordings was also determined for training participants. The majority (70%) of the correlations were strongly positive (greater than 0.5) although one was a negative, and 65% of these correlations reached at least a 0.05 level of significance. In general, this result suggests that there was a reasonable degree of stability of RPE at each stage over several fitness tests.

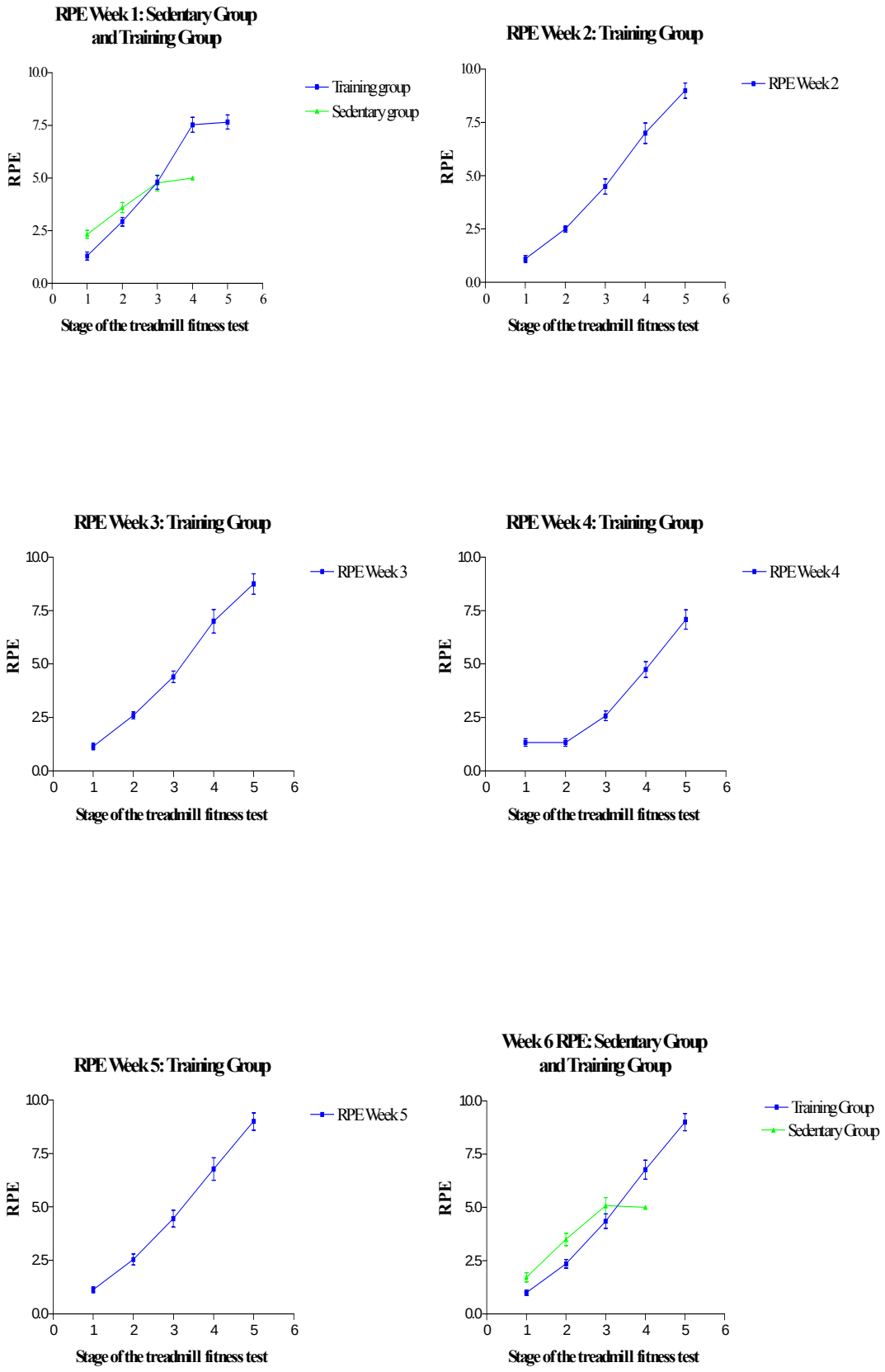


Figure 3.2: Graphs of mean ± SEM rating of perceived exertion (RPE) at each stage of the treadmill fitness test during a six-week study in both sedentary and training participants.

3.2.3 Heart Rate vs. Ratings of Perceived Exertion

When the relationship between HR and RPE recordings within the same stage of the fitness test were examined, the correlation coefficients for each stage were similar for each week, and accordingly the data were combined (Table 3.8).

Table 3.8: Heart rate (HR) and rating of perceived exertion (RPE) ranges and correlation coefficients (r) in the sedentary and training groups at each stage of a treadmill fitness test. NB. **denotes significance at the 0.01 level (2-tailed).

Group	n	HR range (bpm)	RPE range (0 – 10)	Correlation co-efficient (r)
Sedentary				
Stage 1	31	94 – 161	0.5 – 4	0.29
Stage 2	30	112 – 182	2 – 6	0.56**
Stage 3	27	149 – 188	4 – 9	0.29
Training				
Stage 1	75	86 – 144	0.5 – 2	-0.06
Stage 2	75	116 – 156	1 – 4	0.09
Stage 3	74	150 – 183	3 – 8	0.19
Stage 4	71	161 – 217	4 – 10	0.49**
Stage 5	24	173 – 202	7 – 10	0.52**

3.2.3.1 Sedentary group

The correlation coefficients between HR and RPE were in general low, with only the relationship during Stage 2 being greater than 0.5 and reaching significance (Table 3.8).

3.2.3.2 Training group

The correlation coefficients observed between HR and RPE were generally low, but increased progressively with each stage up to a maximum of 0.52 (Table 3.8).

3.3. Performance

3.3.1 Changes in VO_2 over the six-week study

For the sedentary participants, two submaximal treadmill fitness tests were scheduled, one in the first week and one in the sixth week of the study to monitor changes in VO_2 at 85% of maximum heart rate (HR_{max}). The malfunction of the mass spectrometer (mentioned earlier) prevented the analyses of data to determine if the sedentary participants had any change in VO_2 at 85% HR_{max}, therefore only mean results from Week 1 are presented here (Table 3.9).

The maximal treadmill fitness tests were designed for each training participant to achieve their VO_{2max}, but doubt exists as to whether each participant achieved this level. The doubt arose because some of the participants did not display a characteristic plateau in VO_2 at the end of the fitness test. Consequentially, the phrase VO_2 at maximum effort (VO_2 at max) will be used to indicate the VO_2 result at the maximum effort actually produced during the treadmill fitness test, rather than the true VO_{2max} result that the participant may have been capable of achieving.

Table 3.9: Range and mean ± SEM for VO_2 over the six-week study in sedentary and training participants. The VO_2 for the sedentary participants was at 85% of HR_{max} and the VO_2 for the training participants was at maximum effort.

Group	Week	VO_2 range (mL/min/kg)	Mean ± SEM VO_2 (mL/min/kg)
Sedentary	1	22.2 – 41.0	31.4 ± 1.4
Training	1	33.7 – 55.7	43.1 ± 1.5
Training	2	31.7 – 54.2	44.0 ± 1.7
Training	3	39.8 – 47.0	42.5 ± 0.9
Training	4	37.5 – 60.7	46.7 ± 1.8
Training	5	37.8 – 62.1	47.5 ± 3.3

VO_2 results can also be calculated using the equation (Mahler, *et al.*, 1995):

$$VO_2 = R + H + V$$

When: R (resting component) = 3.5mL/min/kg

H (horizontal component of the stage) = speed in metres/min * 0.2

V (vertical/resistive component of the stage) = grade * speed * 0.9

3.3.1.1. Sedentary group

Before each fitness test, the HR_{max} for each sedentary participant was estimated, using the equation given in Section 2.5.1.3 (Materials and Methods). When the participant's HR reached 85% of the HR_{max}, the fitness test was terminated and the VO_2 at that point was recorded. In this study this VO_2 result is referred to as VO_2 at 85% of HR_{max}, and it was assumed that the VO_2 recording approximated to that of the participants true 85% VO_{2max}.

The mean measured VO_2 at 85% of HR_{max} were compared to the calculated VO_2 results (Table 3.10). At Week 1, the mean measured VO_2 at 85% HR_{max} of 31.4 ± 1.4mL/min/kg was not significantly different from the calculated VO_2 of 31.8 ± 0.95mL/min/kg ($p>0.05$; paired t-test), using the equation of Mahler, *et al.*, (1995). The VO_2 results that might have been observed in Week 6 could be predicted using this equation.

For each sedentary participant, their HR at termination of the fitness test in the first week was compared to that in the sixth week. Overall there was no significant difference between HR recordings in Week 1 and Week 6 ($p = 0.296$; paired t-test). This supports the view that there were no significant increases or decreases in VO_2 at 85% of HR_{max} in sedentary participants during the study.

Table 3.10: Range, mean ± SEM and p-value for measured VO_2 compared to calculated VO_2 at the highest completed stage during a treadmill fitness test in both sedentary and training participants.

Group	Stage	Measured VO_2 range (mL/min/kg)	Measured VO_2 mean (mL/min/kg)	Calculated VO_2 (mL/min/kg)	p (t-test)
Sedentary	2	22.2 – 25.0	23.64 ± 1.4	24.0	0.84
	3	27.5 – 39.5	31.99 ± 1.1	32.3	0.78
	4	40.98	40.98	41.3	NA
Training	4	31.7 – 51.43	41.45 ± 0.7	41.3	0.88
	5	41.18 – 62.12	50.06 ± 1.2	51.2	0.35

3.3.1.2. Training group

The mean VO_2 at max values for each week was determined. All of the data from the training participants were analysed to determine if there was a statistically significant

change in VO_2 at max over the five weeks of reliable measured data. The mean overall increase in VO_2 at max was 3.3 ± 1.3mL/kg/min, from 36.2 ± 1.9 mL/kg/min in the first week to 39.6 ± 2.7 mL/kg/min during the fifth week of the study. The decrease seen in the third week (Figure 3.3) occurred following the return of the training participants from a New Zealand University Games tournament.

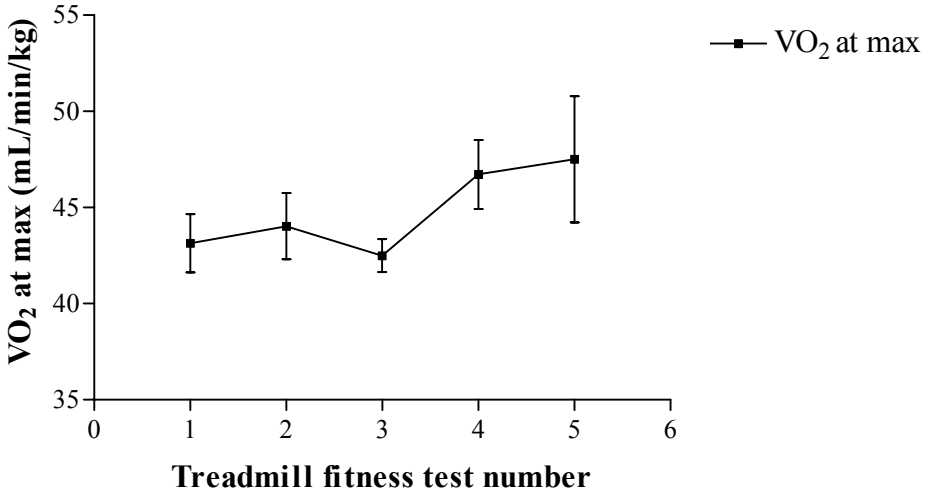


Figure 3.3: Mean VO_2 at max (mL/kg/min) over five weeks of training in training participants.

There was a significant overall increase in VO_2 at max values over the five weeks of the study for the training participants. Despite the decrease in mean VO_2 at max values seen in Week 3, the trend of increasing VO_2 during the study followed a linear pattern (84.6%; covariate analysis). The alcohol consumption during this tournament is discussed in section 3.2 below.

The mean measured VO_2 data available from Weeks 1 to 5 were not significantly different from the mean calculated VO_2 ($p>0.05$; t-test). Therefore, the VO_2 results were estimated for Week 6 using the data manually recorded during the fitness tests, as described above. The calculated VO_{2max} values for Week 6 range between 41.3 and 51.2mL/min/kg, with a mean of 45.3 ± 1.6, which is lower than but not significantly different to the measured mean VO_2 at max observed during Week 5 (47.5 ± 3.3mL/min/kg, $p=0.578$; paired t-test).

Individual analysis showed significant, positive increases in VO_2 at max between the first and fifth weeks in all participants, except two, who had lower VO_2 at max values in Week 5 than Week 1, although one of them reported feeling unwell in the fifth week, just before the test commenced. Some test results were not available as some participants could not undergo some fitness tests due to illness or injury.

3.3.1.3 Comparison of VO_2 between the sedentary and training groups

Because VO_2 results were limited to VO_2 at 85% of HR_{max} for the sedentary participants, 85%VO_2 results were calculated for the training participants and the resultant values were used to compare the sedentary and training groups. During the first week, the mean 85%VO_{2max} results for the sedentary group were significantly lower than for the training group, at 31.4 and 36.6mL/kg/min, respectively ($p=0.007$; t-test). Mass spectrometer malfunctions prevented a comparison of measured VO_2 in the sixth week.

3.3.2. Alcohol Consumption

The unexpectedly high level of alcohol consumption observed during this study led to an investigation into rates of drinking over the study. A total of 1,889 standard drinks were reported to have been consumed amongst the 31 participants during the six-week study. The definition of 'heavy drinking' in women is 4 or more standard drinks in a session (Alcohol Advisory Council of New Zealand, 2002) and, by this definition, of the 219 drinking episodes, participants consumed 4 standard alcoholic drinks or more on a total of 170 occasions. Figure 3.4 illustrates the range in the number of standard drinks consumed during the study. In the instance of a participant reporting "drank to excess" or "drank till vomited/passed out" the figure of 25 standard alcoholic drinks was used. No legal or illegal drug consumption was recorded in any sleep logs.

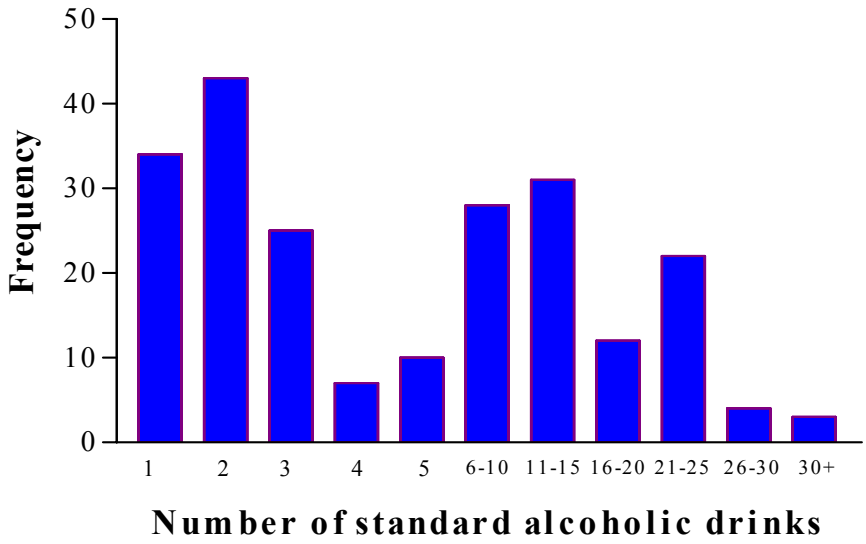


Figure 3.4: Frequency of the number of standard alcoholic drinks consumed in one session amongst all participants over the six-week study.

3.3.2.1 Sedentary group

There were five participants who abstained from alcohol within the sedentary group. The total number of recorded standard alcoholic drinks consumed was 360.5 over the six weeks. The mean weekly level of alcohol consumption per week ranged from 0.6 to 1.2 standard drinks. Most of the drinking was concentrated into the weekends, with 33 heavy drinking episodes (defined above) accounting for 89% of the consumed drinks. One sedentary participant attended the New Zealand University Games tournament but her mean alcohol intake during the duration of the tournament was not significantly different from means for the other five weeks of the study.

3.3.2.2 Training group

There were no training participants who abstained from alcohol consumption for the duration of the study. The training participants consumed 2,410 standard alcoholic drinks. The mean weekly intake was 1.6 to 9.9 standard drinks. The number of heavy drinking episodes was also higher in the training group totalling 137. These accounted for 98% of the total number of drinks consumed.

The highest mean weekly consumption amongst training participants occurred during the second week of the study. This increase coincided with the training group attending

a New Zealand University Games tournament. This tournament was noted in the sleep logs as a week typified by late nights and excessive alcohol consumption, in comparison to the preceding and following weeks (Figure 3.5).

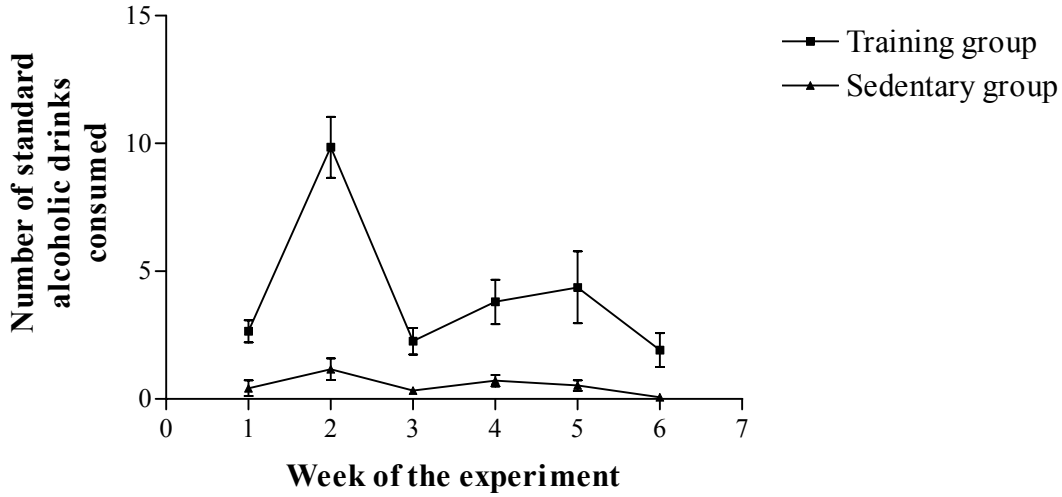


Figure 3.5: Mean ± SEM of standard alcoholic drinks consumed per week in sedentary and training participants during a six-week study.

When the level of alcohol consumption the night prior to a fitness test was examined, there were few nights when alcohol was consumed. As such, no further statistical analysis was conducted due to the lack of data which would preclude the detection of a relationship.

3.4. Sleep Quality Ratings and Sleep Duration

The sleep logs completed daily by each participant provided a large data set from which investigations into sleeping patterns and factors impacting on sleep could be conducted. Analysis of the data revealed group- and time-specific changes over the course of the study. Sleep quality ratings (SQR) ranging from 0 to 10 were determined each morning and were a reflection of a number of factors such as the duration of sleep and the number of sleep disturbances that night. In addition, factors such as the frequency and duration of naps taken the previous day and the effect of any medication or alcohol consumed the previous day or night were considered. Sleep duration (SD) was determined from the sleep logs.

There were no differences between the sedentary and training group for mean SQR values, although there were differences in mean SD, which are discussed below ($p=0.983$ and 0.012, respectively; ANOVA). The means of both SQR and SD differed from week to week, as seen in Figure 3.6 below ($p= 0.001$ and 0.000, respectively; repeated measures ANOVA). Mean SQR and SD values among the participants taking oral contraceptives (OC) were lower than those who did not ($p=0.001$ and $p=0.000$; ANOVA). However, the difference was not significant when the different weeks of the study were included as factors ($p= 0.128$ and $p=0.152$; ANOVA).

3.4.1. Sedentary group

The mean SQR and SD for each variable during the study are reported in Table 3.11. There were no significant differences in mean SQR during the six weeks of the study ($p= 0.570$; repeat measures ANOVA), as illustrated in Figure 3.6. However the mean SD over the six weeks within the sedentary group was significantly different from the means reported by the training group (described below). When the participants were distinguished by group (sedentary or training) and OC use, SQR ratings were significantly different from each other ($p= 0.006$; ANOVA). However, the magnitude of the difference was of little biological relevance. There were no significant differences in mean SD amongst the subgroups ($p= 0.153$; ANOVA).

Table 3.11: Range and mean ± SEM for sleep quality rating (SQR) and sleep duration (SD) from 0 to 10 for sedentary and training participants over the course of the six-week study.

Group	Variable	Range	Mean ± SEM
Sedentary	SQR	2 – 10	6.7 ± 0.1
	SD	1.5 – 13.4	7.9 ± 0.1
Training	SQR	0 – 10	6.7 ± 0.1
	SD	2 – 14	7.7 ± 0.1

3.4.2 Training group

The mean SQR and SD for each variable during the study have been reported in Table 3.11. Mean SD exhibited a significant reduction during the second week of the study (Figure 3.6) when the training participants were competing in a New Zealand University Games tournament (p=0.000, repeat measures ANOVA). The mean SD for Week 2 was significantly lower compared to mean values in both Week 1 and Week 3.

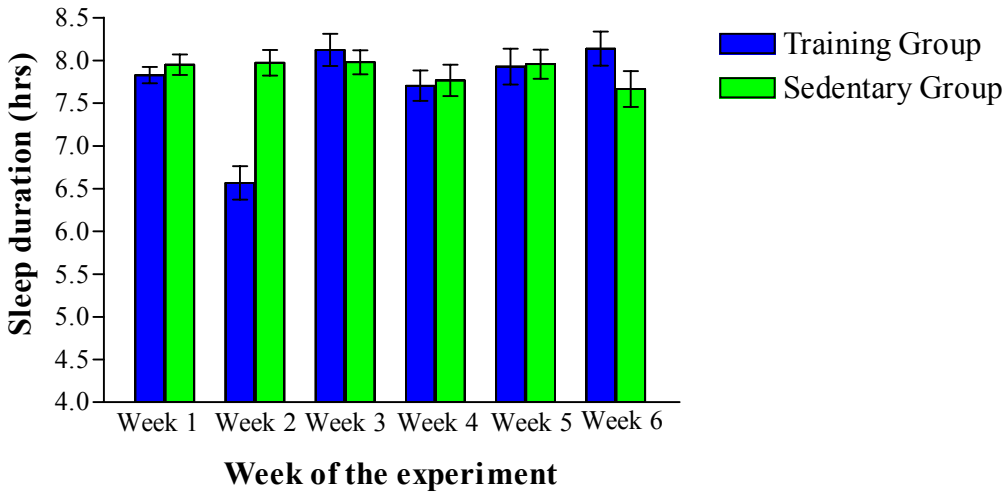


Figure 3.6: Changes in mean ± SEM sleep duration (hrs) over a six-week study in both sedentary and training groups.

3.5. Cross-Correlation analysis

3.5.1 VO_2 and Sleep Quality Ratings or Sleep Duration

The results from the sedentary group are from Week 1 only as the VO_2 at 85% of HR_{max} results were unavailable due to the malfunction of the mass spectrometer. In both the sedentary and training group neither sleep quality rating (SQR) nor sleep duration (SD) were related to VO_2 at 85% HR_{max} (Table 3.12).

Table 3.12: Relationships between VO_2 at 85% maximum heart rate (HR_{max}) or test duration or VO_2 at max and sleep quality rating (SQR) or sleep duration (SD) for sedentary and training groups over a six-week study. NB.: n_{tests} = Number of tests.

Group	Relationship	Correlation co-efficient (r)	n_{tests}
Sedentary	VO_2 at 85% HR_{max} and SQR	0.48	14
	VO_2 at 85% HR_{max} and SD	-0.10	14
Training	VO_2 at max and SQR	0.20	57
	VO_2 at max and SD	-0.14	57

3.5.2 Other relationships with sleep quality ratings or sleep duration

SQR was not correlated with SD (hours) (r = 0.207; p=0.000; correlation analysis). However, this analysis did not take into account whether any naps had been taken the previous day or the frequency of disturbances during the night.

There were no significant differences in SQR between the sedentary and training groups, and neither oral contraceptive (OC) use nor the week of the study had any effect (Table 3.13). Sleep duration was related to the week of the study as mentioned earlier but was unrelated to OC use. There was no relationship between alcohol consumption and either SQR or SD within the participants who drank heavily (r = -0.125 and -0.270; correlation analysis).

Table 3.13: Relationships between sleep quality rating (SQR) and oral contraceptive (OC) use, week and OC use, and alcohol consumption for all participants as a whole.

Group	Relationship	p (ANOVA)
Whole	SQR and OC use	0.98
	SQR and Week*OC	0.13
	SQR and alcohol consumption	0.14

3.5.3 Menstrual cycle effects

One of the objectives of this research was to investigate possible effects of the menstrual cycle phase on the variables measured in this study. An extensive literature review was conducted and information was collected from the participants regarding the timing of their cycles and negative symptoms they experienced during different phases of the menstrual cycle. The information gathered was analysed to ascertain if relationships existed between menstrual cycle phase and performance (as defined by VO_2 results), sleep quality ratings or sleep duration. No significant relationships were observed for any of the relationships (Table 3.14), however this could be related to lack of data confirming the phase of the menstrual cycle. The length of the literature review in general and in particular the length of the section regarding the menstrual cycle and oral contraceptive use, combined with the paucity of results, led to the removal of the background literature review and results pertaining to the menstrual cycle. No progesterone assays were conducted or basal body temperature monitored during the study to confirm the menstrual cycle phase.

Table 3.14: Correlation coefficients (r) of relationships between the day of the menstrual cycle (MC) and sleep quality rating (SQR), VO_2 at 85% of maximum heart rate (VO_2 at 85% HR_{max}), test duration and VO_2 at maximum effort (VO_2 at max) for all participants and sedentary and training groups.

Group	Relationship	Correlation co-efficient (r)
Whole	MC and SQR	0.01
	MC and SQR around Day 1	0.01
Sedentary	MC and VO_2 at 85% HR_{max}	0.02
	MC and test duration	-0.28
Training	MC and VO_2 at max	0.07

3.6. Endocrine Data

3.6.1 Hormone Profiles

Concentrations of cortisol, testosterone and growth hormone in plasma were first evaluated in the study population as a whole, then in different groups and subgroups of participants, and finally according to level of alcohol consumption (abstainers, drinkers and heavy drinkers). If a significant difference in hormone concentration between groups or subgroups was observed, an investigation into whether the same difference was observed in interstitial fluid was conducted. Group trends and differences in cortisol and testosterone concentration in saliva were not examined because the primary purpose of saliva sampling was to assist in the evaluation of the electrosonophoretic method.

3.6.1.1 Hormone concentrations from the group as a whole

A. Cortisol

Although two of the 31 participants showed a decreasing trend in plasma cortisol concentrations as the study progressed, in general plasma cortisol levels (Table 3.15) displayed no consistent patterns within participants across the entire group. The mean plasma concentration for the sedentary group was not significantly different from that of the training group ($p=0.353$; ANOVA). Despite the apparent trend towards decreasing plasma cortisol levels in the sedentary group as the study progressed (Figure 3.7, top panel) the trend was not significant ($p=0.845$; ANOVA). No notable changes in plasma cortisol levels were detected immediately following fitness tests.

Table 3.15: Range and mean ± SEM concentrations of cortisol (ng/mL) in plasma, interstitial fluid (IF) and saliva in all participants and according to sedentary group (SG) or training group (TG). NB. n_p= Number of participants, n_s= Number of samples.

Group	Fluid	Range	Mean ± SEM	n_p	n_s
Whole	Plasma	4.9 – 111	30 ± 1.3	31	217
	IF	0.1 – 26	3.3 ± 0.1	31	398
	Saliva	0.8 – 58	5.5 ± 0.2	31	492
SG	Plasma	4.9 – 94	31 ± 1.7	16	119
	IF	0.1 – 26	3.5 ± 0.2	16	189
	Saliva	0.8 – 58	6.0 ± 0.3	16	236
TG	Plasma	6.6 – 111	28 ± 1.9	15	98
	IF	0.7 – 17	3.0 ± 0.2	15	198
	Saliva	1.1 – 22	4.9 ± 0.2	15	245

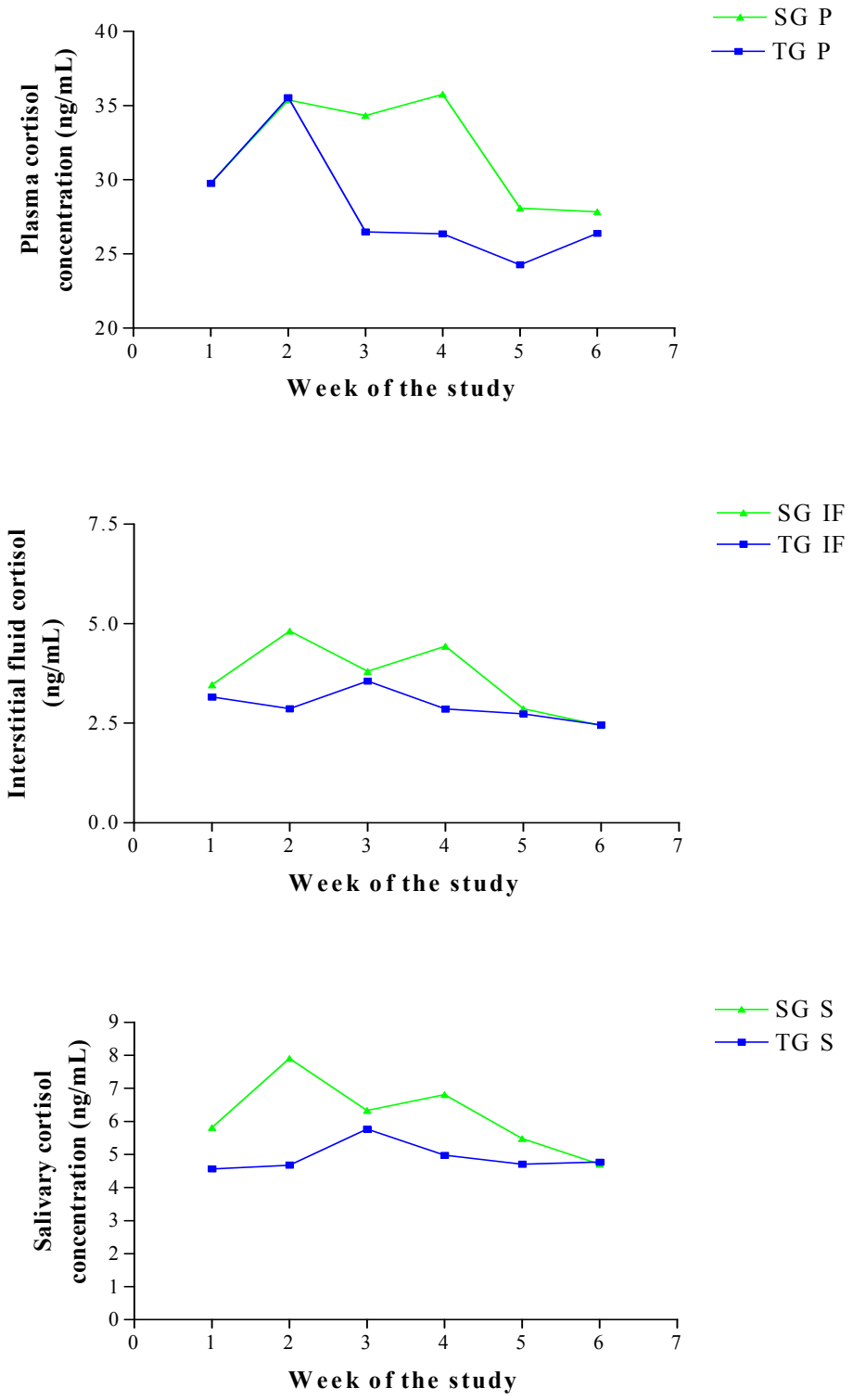


Figure 3.7: Mean weekly cortisol concentration (ng/mL) in plasma (P), interstitial fluid (IF) and saliva (S) in both the sedentary group (SG) and training group (TG) during a six-week study.

B. Testosterone

There were no significant changes or patterns in plasma testosterone levels for any participants during the study (Figure 3.8). There was no significant difference in plasma testosterone levels between the sedentary and training participants (Table 3.16) (p= 0.874; ANOVA), and no change in plasma testosterone levels were detected immediately following treadmill fitness tests.

In 5 out of the 31 participants, plasma testosterone concentrations on the first day of the study were considerably higher than the mean for the remainder of the study. The range of plasma testosterone levels on the first day was from about 400 to 601pg/mL, while the means for the remainder of the study were from about 190 to 245pg/mL. The mean plasma testosterone (488 ± 44.8pg/mL) on the first day of the study for the 5 participants was significantly higher than the mean for the rest of the participants (221 ± 19.0pg/mL) (p=0.002; independent-samples t-test).

Table 3.16: Range and mean ± SEM concentrations of testosterone (pg/mL) in plasma, interstitial fluid (IF) and saliva in all participants and according to sedentary group (SG) and training group (TG). NB. n_p= Number of participants, n_s= Number of samples.

Group	Fluid	Range	Mean ± SEM	n_p	n_s
Whole	Plasma	93 – 601	210 ± 5.8	31	199
	IF	9.1 – 94	23 ± 0.6	31	370
	Saliva	28 – 597	130 ± 3.9	31	477
SG	Plasma	93 – 601	218 ± 8.9	16	109
	IF	9.1 – 93.8	23 ± 0.9	16	178
	Saliva	36 – 457	123 ± 5.2	16	234
TG	Plasma	105 – 592	208 ± 8.9	15	92
	IF	11 – 78.3	24 ± 0.9	15	194
	Saliva	28 – 597.0	137 ± 5.6	15	245

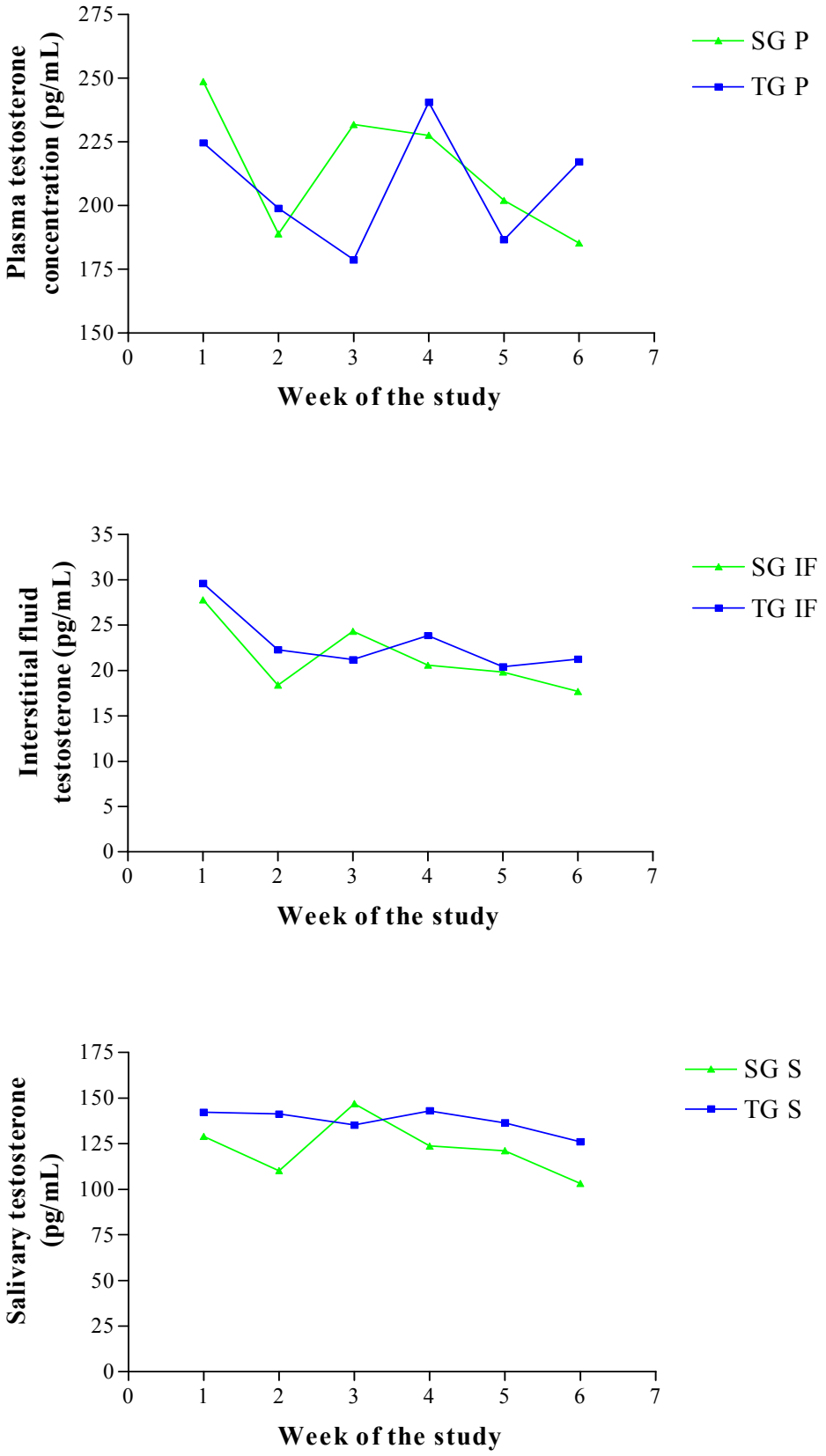


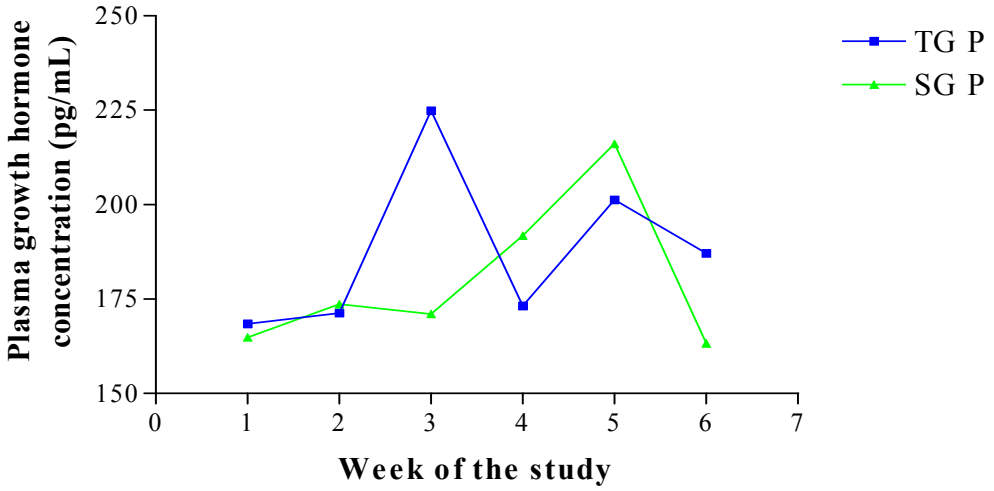
Figure 3.8: Mean weekly testosterone concentration (pg/mL) in plasma (P), interstitial fluid (IF), and saliva (S) in both the sedentary group (SG) and training group (TG) during a six-week study.

C. Growth Hormone

A range in plasma growth hormone (GH) concentrations among groups was observed over the study (Table 3.17). Growth hormone concentrations in the training group were numerically but not significantly higher than those in the sedentary group (p= 0.294; ANOVA). No patterns in plasma or interstitial fluid GH during the study were detected (Figure 3.9). No increases or decreases in interstitial fluid GH levels taken immediately following cessation of the fitness test or during the study were observed.

Table 3.17: Range and mean ± SEM concentrations of growth hormone (pg/mL) in plasma (P) and interstitial fluid (IF) in all participants and according to sedentary group (SG) and training group (TG). NB. n_p= Number of participants, n_s= Number of samples.

Group	Fluid	Range	Mean ± SEM	n_p	n_s
Whole	Plasma	48 – 452	184 ± 6.8	31	149
	IF	3.9 – 41	18 ± 0.7	31	153
SG	Plasma	48 – 416	178 ± 9.1	16	81
	IF	3.9 – 41	17 ± 0.9	16	80
TG	Plasma	50 – 452	192 ± 10.3	15	68
	IF	4.3 – 41	19 ± 1.0	15	73



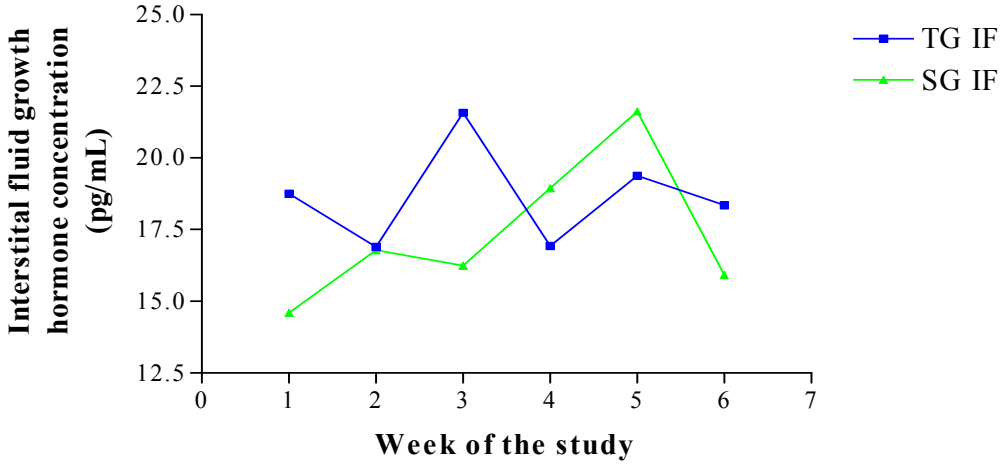


Figure 3.9: Mean weekly growth hormone concentration (ng/mL) in plasma (P), and interstitial fluid (IF) in both the sedentary group (SG) and training group (TG) during a six-week study.

3.6.1.2 Hormone concentrations according to subgroups

The following hormone results were considered according to oral contraceptive (OC) use, then a combination of sedentary or training group with OC use, and finally by the level of alcohol consumption. Further division of the participants by main group and by alcohol consumption was not warranted as none of the training participants abstained from alcohol during the six-week study. Analyses of variance were performed to test the significance of any possible effect that groups or subgroups had on hormone concentration, and the results are reported here.

Subgroup 1: Oral contraceptive use

1A. Cortisol

Plasma cortisol concentrations displayed a large range (Table 3.18). The plasma cortisol levels were lower in the participants taking OCs compared to those who did not, with the difference approaching significance (p= 0.057; ANOVA). However this difference was not significant in interstitial fluid cortisol levels (p= 0.944; ANOVA). Further division of the participants into subgroups showed with respect to plasma cortisol concentrations that there was no significant difference between the OC and non-OC users within the sedentary group, but within the training group the OC users (22.9 ± 1.7ng/mL) had a significantly lower mean cortisol concentration than non-OC users (35.2 ± 4.0ng/mL) (p=0.002; ANOVA). Similar results were seen in interstitial fluid, with the mean cortisol levels in the sedentary group being numerically but not significantly lower for the OC group than the non-OC group, and significantly lower levels in the training group for individuals taking OCs than those not taking OCs (p= 0.000; ANOVA).

Table 3.18: Range and mean ± SEM of cortisol concentrations (ng/mL) in plasma, interstitial fluid (IF) and saliva in participants on oral contraceptives (OC), not on oral contraceptives (NOC) and according to sedentary group (SG) or training group (TG) and oral contraceptive use. NB. n_p= Number of participants, n_s= Number of samples.

Subgroup	Range	Mean ± SEM	n_p	n_s
On OC				
Plasma	5.8 – 80	27 ± 1.6	16	108
IF	0.6 – 26	3.2 ± 0.2	16	210
Saliva	1.1 – 58	5.4 ± 0.3	16	257
Not on OC				
Plasma	4.9 – 111	32 ± 2	15	109
IF	0.1 – 17	3.3 ± 0.2	15	177
Saliva	0.8 – 22	5.6 ± 0.2	15	224
SG and OC				
Plasma	5.8 – 80	33 ± 2.8	7	47
IF	0.6 – 26	4.0 ± 0.4	7	86
Saliva	1.3 – 58	6.7 ± 0.6	7	106
SG and NOC				
Plasma	4.9 – 94	30 ± 2.2	9	72
IF	0.1 – 11	3.1 ± 0.2	9	103
Saliva	0.8 – 16	5.5 ± 0.3	9	130
TG and OC				
Plasma	6.6 – 68	23 ± 1.7	9	61
IF	0.7 – 6.7	2.6 ± 0.1	9	124
Saliva	1.1 – 10	4.4 ± 0.2	9	151

TG and NOC				
Plasma	8.9 – 111	35 ± 4	6	37
IF	0.8 – 17	3.6 ± 0.3	6	74
Saliva	1.3 – 22	6.7 ± 0.4	6	94

1B. Testosterone

The participants taking OCs had a lower plasma testosterone concentration compared with the participants not taking OCs (Table 3.19), although this difference only approached significance (p=0.092; ANOVA). The same relationship was not observed in interstitial fluid (p= 0.407; ANOVA). Further subdivision of participants revealed a greater range of plasma testosterone concentrations (Table 3.19), although no significant effect of group, OC use or a combination of both was observed (p=0.874, p=0.194 and p=0.501, respectively; ANOVA).

Table 3.19: Range and mean ± SEM of testosterone concentrations (pg/mL) in plasma, interstitial fluid (IF) and saliva in participants on oral contraceptives (OC), not on oral contraceptives (NOC) and according to sedentary group (SG) or training group (TG) and oral contraceptive use. NB. n_p= Number of participants, n_s= Number of samples.

Subgroup	Range	Mean ± SEM	n_p	n_s
On OC				
Plasma	93 – 601	202 ± 8.7	16	101
IF	9.1 – 78	23 ± 0.9	16	195
Saliva	28 – 466	116 ± 4.5	16	254
Not on OC				
Plasma	103 – 490	225 ± 9.1	15	100
IF	10.5 – 94	23 ± 0.8	15	177
Saliva	32 – 597	146 ± 6.3	15	225
SG and OC				
Plasma	93 – 601	210 ± 14.5	7	44
IF	9.1 – 67	22 ± 1.2	7	76
Saliva	36 – 412	113 ± 7.3	7	102
SG and NOC				
Plasma	103 - 490	224 ± 11.4	9	65
IF	11 – 94	23 ± 1.2	9	102
Saliva	37 - 457	130 ± 7.2	9	132
TG and OC				
Plasma	111 – 592	196 ± 10.8	9	57
IF	11 – 78	24 ± 1.2	9	119
Saliva	28- 466	118 ± 5.7	9	152
TG and NOC				
Plasma	105 – 411	227 ± 15.2	6	35
IF	11 – 41	23 ± 1.0	6	75
Saliva	32 – 597	169 ± 10.8	6	93

1C. Growth Hormone

No significant relationships were observed between OC use and either plasma or interstitial fluid GH levels in this study (p= 0.666, p= 0.857, respectively; ANOVA). The wide ranges in plasma GH concentrations seen in the subgroups (Table 3.20), may have precluded the possibility of detecting significant differences between the means for the subgroups (p=0.675; ANOVA).

Table 3.20: Range and mean ± SEM of growth hormone concentrations (pg/mL) in plasma and interstitial fluid (IF) in participants on oral contraceptives (OC), not on oral contraceptives (NOC) and according to sedentary group (SG) or training group (TG) and oral contraceptive use. NB. n_p= Number of participants, n_s= Number of samples.

Subgroup	Range	Mean ± SEM	n_p	n_s
On OC				
Plasma	51 - 452	182 ± 9.5	16	76
IF	5.9 – 41	18 ± 1.0	16	77
Not on OC				
Plasma	48 – 382	186 ± 9.9	15	73
IF	3.9 – 37	18 ± 0.9	15	76
SG and OC				
Plasma	51 – 416	173 ± 13.5	7	35
IF	5.9 – 41	16 ± 1.3	7	32
SG and NOC				
Plasma	48 – 376	181 ± 12.4	9	46
IF	3.9 – 37	18 ± 1.2	9	48
TG and OC				
Plasma	86 – 452	190 ± 13.4	9	41
IF	8.7 – 41	20 ± 1.3	9	45
TG and NOC				
Plasma	50 – 382	194 ± 16.5	6	27
IF	4.3 - 32	18 ± 1.6	6	28

Subgroup 2: Alcohol Consumption

Hormone results were considered in relation to the participants' alcohol consumption. Heavy drinkers were defined as participants who consumed 4 standard alcoholic drinks or more in one session on more than one occasion (Alcohol Advisory Council of New Zealand, 2002). Moderate drinkers were participants who drank less than 4 standard alcoholic drinks, and abstainers were participants who did not drink any standard alcoholic drinks during the study.

2A. Cortisol

The three levels of alcohol consumption (abstainers, moderate drinkers and heavy drinkers) had no significant influence on plasma cortisol levels (Table 3.21). Neither time nor the interaction between time and alcohol consumption in either moderate drinkers or heavy drinkers had any effect on plasma cortisol levels (p=0.784 and p=0.984, respectively; ANOVA).

Table 3.21: Range and mean ± SEM of cortisol concentrations (ng/mL) in plasma, interstitial fluid (IF) and saliva in all participants according to the level of alcohol consumption. Moderate= less than 4 standard drinks in one session during the six-week study; heavy drinkers= at least one heavy drinking session of 4 or more standard alcoholic drinks during the six-week study. NB. n_p= Number of participants, n_s= Number of samples.

Subgroup	Range	Mean ± SEM	n_p	n_s
Abstainers				
Plasma	6.3 – 58	30 ± 2.6	5	37
IF	0.6 – 6.9	3.0 ± 0.2	5	51
Saliva	1.1 – 12	5.3 ± 0.4	5	60
Moderate drinkers				
Plasma	4.9 – 111	30 ± 1.5	8	180
IF	0.1 – 26	3.3 ± 0.1	8	336
Saliva	0.8 – 58	5.5 ± 0.2	8	421
Heavy drinkers				
Plasma	5.8 – 111	29 ± 1.7	18	140
IF	0.7 – 17	3.1 ± 0.1	18	258
Saliva	1.1 – 22	5.2 ± 0.2	18	323

2B. Testosterone

Plasma testosterone concentrations in non-drinkers were significantly higher than in both moderate drinkers and heavy drinkers (Table 3.22) (p=0.014; ANOVA). The same relationship was not observed in interstitial fluid. There was no significant effect of the combination of time and alcohol consumption in either moderate or heavy drinkers on plasma testosterone levels (p=0.151 and p=0.711, respectively; ANOVA).

Table 3.22: Range and mean ± SEM of testosterone concentrations (pg/mL) in plasma, interstitial fluid (IF) and saliva in all participants according to the level of alcohol consumption. Moderate= less than 4 standard drinks in one session during the six-week study, Heavy drinkers= at least one heavy drinking session of 4 or more standard alcoholic drinks during the six-week study. NB. n_p= Number of participants, n_s= Number of samples.

Subgroup	Range	Mean ± SEM	n_p	n_s
Abstainers				
Plasma	111 – 490	244 ± 18	5	32
IF	11 – 49	23 ± 1.4	5	49
Saliva	47 – 385	144 ± 11	5	59
Moderate drinkers				
Plasma	93 – 601	208 ± 10	8	77
IF	9.1 – 94	23 ± 1.1	8	129
Saliva	36 – 457	116 ± 5.9	8	175
Heavy drinkers				
Plasma	96 – 601	213 ± 8.1	18	129
IF	9.1 – 94	24 ± 0.8	18	252
Saliva	28 – 597	137 ± 5.0	18	322

2C. Growth Hormone

There was no significant difference in mean plasma GH concentration (Table 3.23) in the groups differentiated on the basis of alcohol consumption (p=0.742; ANOVA). There was also no significant impact of time or the interaction of time and alcohol consumption in either moderate or heavy drinkers on plasma GH levels in this study (p= 0.668, p= 0.261 and p= 0.789, respectively; ANOVA). There was no consistency in the nature of the response of plasma GH concentration the morning following alcohol consumption, in either heavy or moderate drinkers, and as such no comment is made here on the distinction between moderate and heavy alcohol consumption on plasma GH concentration.

Table 3.23: Range and mean ± SEM of growth hormone concentrations (pg/mL) in plasma and interstitial fluid (IF) in all participants according to the level of alcohol consumption. Moderate= less than 4 standard drinks in one session during the six-week study, Heavy drinkers= at least one heavy drinking session of 4 or more standard alcoholic drinks during the six-week study. NB. n_p= Number of participants, n_s= Number of samples.

Subgroup	Range	Mean ± SEM	n_p	n_s
Abstainers				
Plasma	51 – 305	163 ± 14	5	24
IF	4.8 – 30	16 ± 1.4	5	24
Moderate drinkers				
Plasma	48 – 416	184 ± 12	8	57
IF	3.9 – 41	18 ± 1.1	8	56
Heavy drinkers				
Plasma	48 – 452	193 ± 8.8	18	97
IF	3.9 – 41	19 ± 0.9	18	101

3.7. The electrosonophoretic method as a technique for estimating plasma hormone levels

An assessment of the value of electrosonophoresis as a sampling methodology to estimate plasma concentrations was conducted. Hormone concentrations in interstitial fluid (obtained using electrosonophoresis), were correlated with those in plasma. Similarly, correlation analysis was conducted on hormone concentrations in plasma and saliva and also between interstitial fluid and saliva. Regression analysis was then performed to characterise the relationship between hormone levels in plasma and those in interstitial fluid.

3.7.1 Correlation analysis

The correlation coefficients for the above noted relationships (Table 3.24) were highest for the relationship between the concentrations in plasma and interstitial fluid, ranging from 0.988 to 0.989 for the three hormones. Lower ranges were obtained for both the relationships between plasma and saliva (0.752 to 0.958) and saliva and interstitial fluid (0.396 to 0.939) concentrations. No salivary GH levels have been reported as it cannot be assayed for in saliva.

Table 3.24: Correlation coefficients (r) and the r^2 value for relationships between the concentrations of cortisol, testosterone and growth hormone in plasma, interstitial fluid (IF) and saliva for all participants. NB. n= number of paired samples; ** denotes significance at the 0.01 level (2-tailed).

Relationship	n	Correlation coefficient (r)	r^2
Cortisol			
Plasma and IF	191	0.99**	0.98
Plasma and Saliva	211	0.96**	0.92
Saliva and IF	386	0.94**	0.88
Testosterone			
Plasma and IF	182	0.99**	0.98
Plasma and Saliva	200	0.75**	0.57
Saliva and IF	374	0.40**	0.16
Growth Hormone			
Plasma and IF	144	0.99**	0.98

Figures 3.10 to 3.12 depict the relationships between the concentrations of the three hormones in plasma, interstitial fluid and saliva. In the graphs below, outlying cortisol and testosterone concentrations in saliva and interstitial fluid samples can be observed.

These samples may have been taken on Wednesdays (when no plasma samples were taken).

1. Cortisol

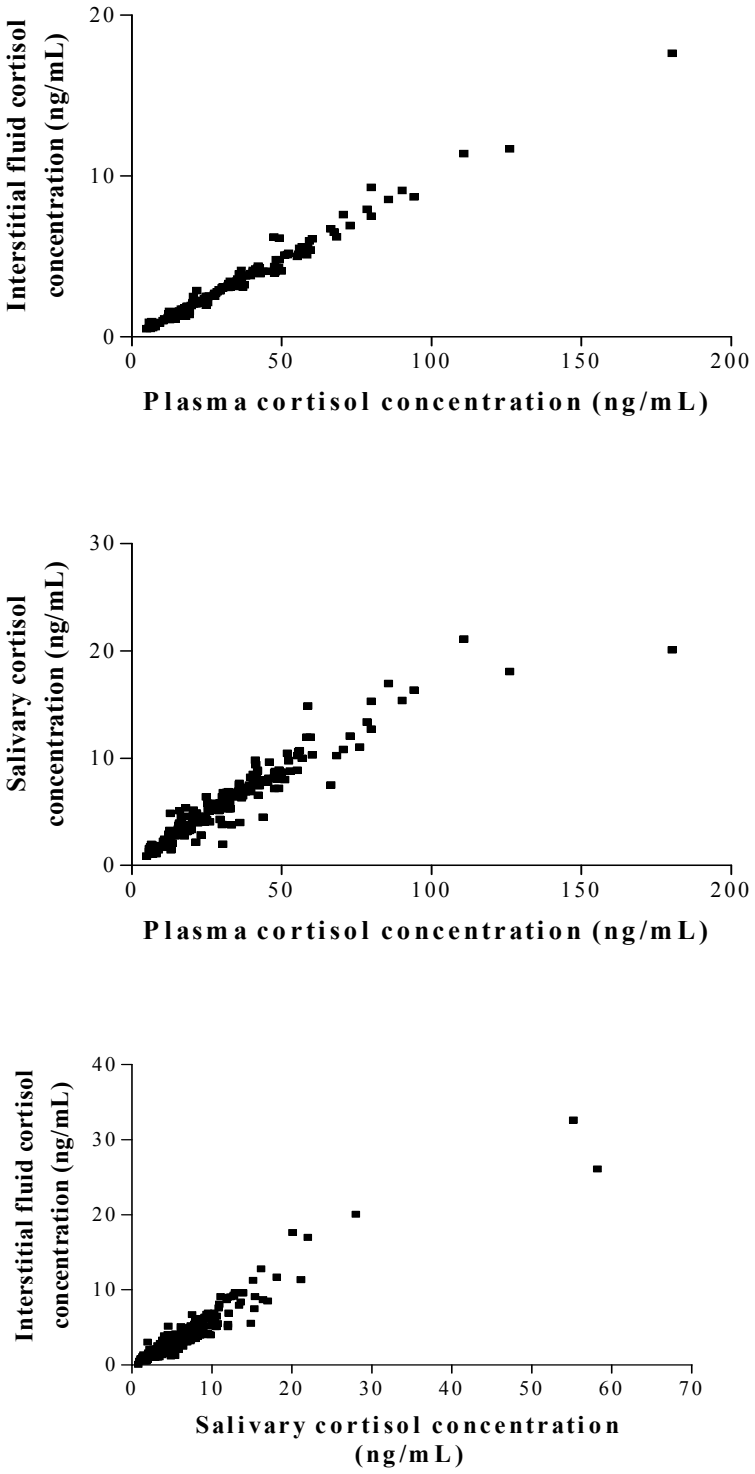


Figure 3.10: Cortisol concentrations (ng/mL) in Plasma vs. Interstitial fluid (n=193), Plasma vs. Saliva (n= 213) and Saliva vs. Interstitial fluid (n=389).

2. Testosterone

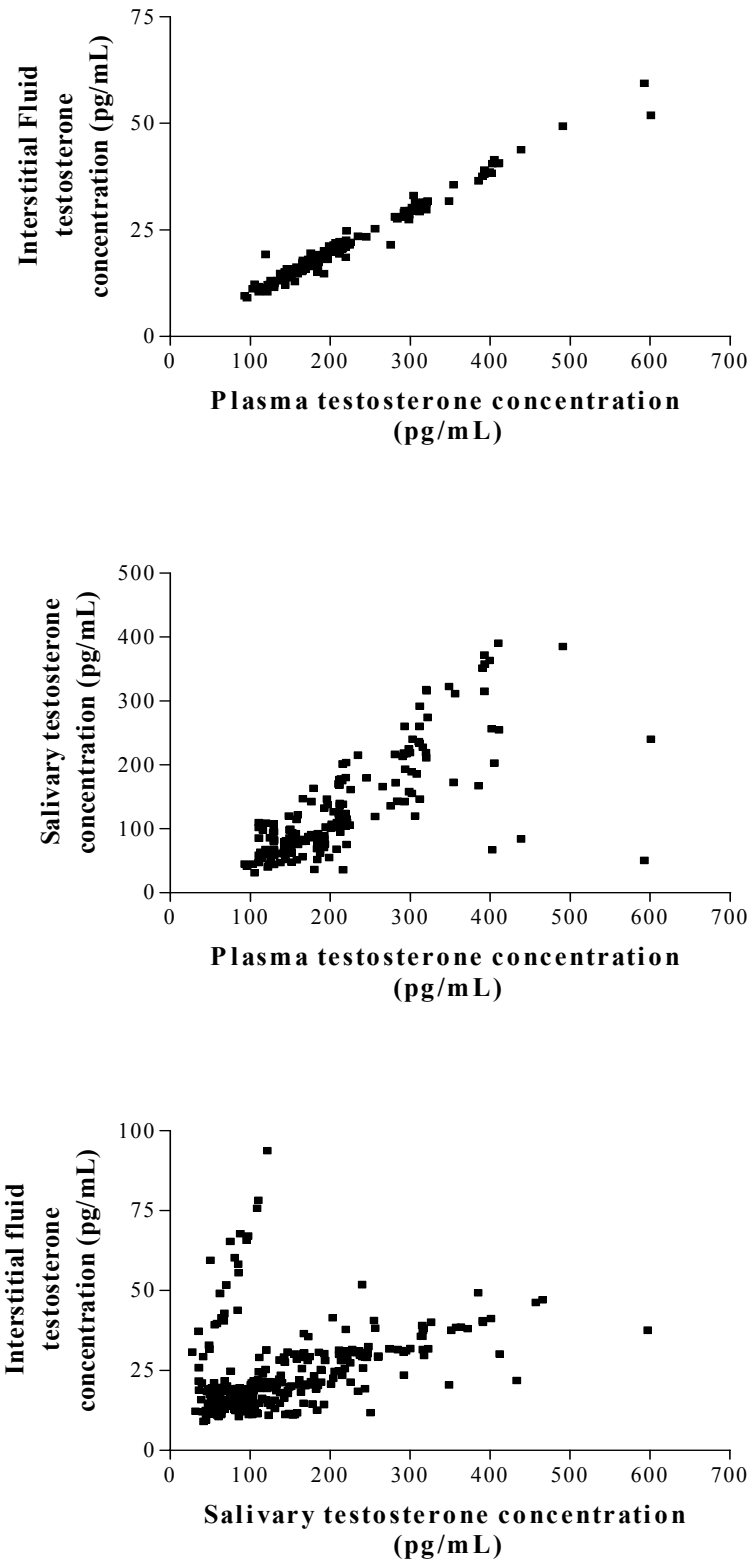


Figure 3.11: Testosterone concentrations (pg/mL) in Plasma vs. Interstitial fluid (n=178), Plasma vs. Saliva (n=197) and Saliva vs. Interstitial fluid (n= 364).

The mean testosterone concentration ratio between plasma and interstitial fluid was 9.4, whereas the mean ratio for plasma and salivary testosterone concentration was 1.8.

3. Growth Hormone

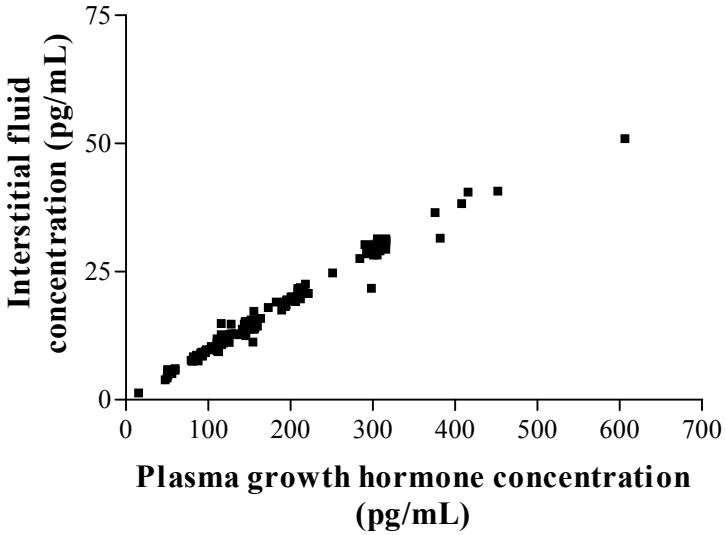


Figure 3.12: Growth hormone concentrations (pg/mL) in Plasma vs. Interstitial fluid (n= 150).

3.7.2 Regression analysis

The data set for each hormone was combined, and multiple regression analysis was performed to investigate if the linear relationship between the partitioning of hormones between plasma and interstitial fluid was the same or similar for each of the three hormones. If similar relationships were found for all three hormones, a simple conversion equation could then be created to predict plasma hormone levels from the concentrations in the interstitial fluid samples obtained by electrosonophoresis.

The initial regression equation below used cortisol as the reference hormone, with testosterone and growth hormone represented by the indicator variables Z_1 and Z_2 respectively. Cortisol was an arbitrary choice for the reference hormone, and the analysis was conducted to compare if the relationships for each hormone were similar.

$$[P] = \beta_0 + \beta_1 Z_1 + \beta_2 Z_2 + \alpha_0 X + \alpha_1 Z_1 X + \alpha_2 Z_2 X$$

Where: [P] = the plasma concentration of cortisol, testosterone or GH

α = the slope

β = the y-intercept

X = the concentration of hormone in interstitial fluid.

When each hormone is reviewed separately the regression equations became:

Cortisol: $[P] = \beta_0 + \alpha_0 X$

Testosterone: $[P] = \beta_0 + \beta_2 + (\alpha_0 + \alpha_2) X$

Growth hormone: $[P] = \beta_0 + \beta_1 + (\alpha_0 + \alpha_1) X$

The regression results suggested that the slopes for cortisol and testosterone were not significantly different. As the y-intercepts were not significantly different from zero, an equation using only a conversion factor could be generated which could be used to estimate the plasma concentration of either cortisol or testosterone from their respective concentrations in interstitial fluid.

$$\text{Cortisol or testosterone: } [P] = 10.2X$$

The regression analysis of the relationship between plasma and interstitial fluid for growth hormone concentrations resulted in a similar conversion factor to that observed for cortisol and testosterone, but it had a y-intercept that was significantly different from zero. The resultant linear model was produced:

$$\text{Growth Hormone: } [P] = -4.3 + 10.6X$$

The significant y-axis intercept could be due to cross-reactivity of another protein or similar sized molecule with the growth hormone antibody, when the assay was performed. The gradient of the line, at 10.6, was not significantly different from that determined for cortisol and testosterone, so that GH evidently displayed similar partitioning from plasma into interstitial fluid.

3.7.3 Accuracy of the electrosonophoretic method

The sensitivity of the electrosonophoretic (ESOP) method is related to the sensitivity of the methods used to measure the concentrations of hormones or constituents of plasma, interstitial fluid and saliva, and to any variability inherent in the ESOP method itself. The sensitivity of the enzyme linked immunosorbent assays (ELISA) assays used to determine cortisol, testosterone or growth hormone concentrations were ± 3 ng/mL, ± 2 pg/mL and ± 1 pg/mL, at the highest end of the measured ranges.

The plasma concentrations of the three hormones were close to 10-times those in interstitial fluid in each case, indicating a partitioning ratio of approximately 10:1. Nevertheless, the sensitivity of the hormone assays in each case allowed changes in the plasma concentrations over wide ranges to be detected via interstitial fluid. An example of this can be seen in Figures 3.10 to 3.12 above. Increases in plasma GH concentrations observed in the training group during the third and fifth week of the study, were also observable in interstitial fluid samples.

Chapter Four

Discussion

Research Objective One

Did aerobic fitness, as assessed by VO_2 results, increase among training participants over the six-week study when compared to sedentary participants?

Regular aerobic training can lead to an increase in cardiovascular fitness (Mahler *et al.*, 1995). In the present study, a significant increase in aerobic fitness was observed in the training group between the first and the fifth week, when compared to a sedentary control group (Section 3.3.1.2). Mean values for the maximum oxygen consumption (VO_{2max}) within the training group increased by 3.3 ± 1.3mL/kg/min between the first and fifth week of the study. In studies of similar duration, increases of VO_{2max} in females have ranged from 1mL/kg/min (Brock and Legg, 1997) to 4.3mL/kg/min (Carter, *et al.*, 1999).

An increase in cardiovascular fitness is achieved through improving associated physiological variables. Repeated bouts of aerobic training can lead to improved efficiency and perfusion of the lungs and an increased stroke volume, through an enlarged heart size. Such adaptations to training improve the transport of oxygenated blood to working muscles and the removal of waste products from the increased metabolic activity within the muscles. This therefore increases the ability of the individual to perform aerobic work before the anaerobic energy system is utilised (ASCM, 1990; Powers and Howley, 1997; Xu and Rhodes, 1999).

The study by Carter, *et al.* (1999), referred to above, is similar to the present study as the training participants were active in recreational sport, but not highly trained, prior to the study commencing. The fitness level of the female participants in the study by Brock and Legg (1997) prior to the study was not reported. Both of these studies had

more frequent training intervals, ranging from 3 to 10 sessions per week, than the present study. The training sessions were also of shorter duration (20 to 40 minutes) than in the present study (90 minutes; see Table 2.3). Neither Brock and Legg (1997) nor Carter *et al.*, (1999) reported the intensity of training sessions or attendance at training sessions. Nor were these recorded in the present study, to minimise study demands on the participants. Quantification of training intensity through monitoring of heart rates at each stages of the training session over several weeks to ensure consistency of intensity would have required a marked increase in time commitment.

Assessment of VO_{2max} is one of the most definitive measures of an individual's aerobic power (Macsween, 2001) and repeated VO_{2max} fitness tests are commonly used in both medical and sport science fields to assess cardiovascular fitness. Once baseline levels of cardiovascular fitness have been determined, changes in VO_{2max} as a result of training programme can be detected. The Bruce treadmill exercise test protocol used in the present study was chosen because of its suitability for both trained and healthy untrained young individuals (Table 2.2). In addition, it was chosen because the recommended test duration (8 to 12 minutes) minimises both the discomfort and the perception of difficulty, while obtaining the highest VO_{2max} compared to other protocols (Fielding, *et al.*, 1997; Lear *et al.*, 1999; Senaratne *et al.*, 2000; Kang, *et al.*, 2001). Maximal tests are thought to be inappropriate for sedentary or patient populations therefore recognised submaximal versions of common maximal protocols (such as the Bruce protocol) are used to estimate VO_{2max} by linear extrapolation from submaximal VO_2 data (Mahler, *et al.*, 1995).

A comparison of the changes in VO_2 at 85% of maximum heart rate between the first and sixth week among the sedentary group did not occur. This was because in the final week of the study all of the data were lost, due to mass spectrometer malfunction (as mentioned previously). This prevented an assessment of possible changes in VO_2 for each sedentary participant, a comparison of mean VO_2 between the sedentary and training groups, and a comparison of changes in VO_2 over the six weeks within each group.

Measured VO_2 results from the fitness tests from both sedentary and training participants were compared to predicted VO_2 results using the equation described by

Mahler *et al.*, (1995) (Table 3.10). There was no significant difference between the measured VO_2 and predicted VO_{2max} for the sedentary group. However, the submaximal fitness tests were designed to take each sedentary participant to 85% of VO_{2max}. When the calculated VO_{2max} results were converted to 85% VO_{2max}, the measured VO_2 results were significantly higher than the calculated 85% VO_{2max}. This discrepancy may be due to the fact that the calculation for VO_{2max} does not take into account either the length of time spent in the final stage of the treadmill fitness test or the gender, age or weight of the participant. Another explanation could be that the sedentary participants reached 85% of their VO_{2max} before the treadmill fitness test was terminated. The latter explanation then implies that VO_{2max} might occur at heart rates higher than the predicted maximum heart rate (HR_{max}) which is determined by the equation:

$$HR_{max} = 220 - \text{age of participant}$$

Although this equation is commonly used to determine the end-point of an exercise test, its reliability is questionable due to the variability in heart rate (Lear *et al.*, 1999). This explanation also suggests that during a fitness test, the VO_2 at 85% of HR_{max} is not equivalent to 85% VO_{2max}.

During the study, it was thought that some of the training participants did not achieve VO_{2max} during their fitness tests for several reasons. In some participants, there was an absence of the characteristic 'plateau' in the graph of VO_2 over time as described by Mahler, *et al.*, (1995). In addition, mean values for the rating of perceived exertion (RPE) in the final stages of the fitness test were lower than expected (Table 3.7). The ratings of perceived exertion at the end of a maximal test would be expected to be very high, at 9 or 10 on the 0 to 10 scale (Borg, 1982). Observed means for Stages 4 and 5 (7.1 and 8.8, respectively) were lower than expected. Possible reasons for this observation could have been that the participants did not fully understand the nature of the RPE system, or that they did not achieve VO_{2max}. A lack of motivation may also have impacted on the performance of some of the training participants.

A decrease in resting heart rate is a common indication of increased cardiovascular fitness (Powers and Howley, 1997). This variable was not measured in the present study, however heart rates at low intensities of exercise were recorded. It has been

shown that a lower heart rate at a matched submaximal work rate is an indication of the cardiovascular system response to exercise training (McInnis and Balady, 1994; Bjarnason-Wehrens, 1999). Within the training group, the mean heart rate at low exercise intensities tended to be lower in Week 6 than in Week 1, with the difference approaching significance ($p=0.077$; paired t-test) (Section 3.2.1). In comparison, there was no decrease in mean heart rate at low exercise intensity for the sedentary group between the first and final week.

It must be noted here that the failure to record resting HR prior to the fitness tests commencing was a significant flaw in the methodology of the study. Resting HR may have decreased during the course of this study.

Assessing changes in resting HR would have been an appropriate alternative method of monitoring changes in cardiovascular fitness given that VO_2 data were not available. Kim, *et al.,* (1999) have reported mean resting heart rates of 72.4 ± 11bpm in Caucasian females of a similar age (25.4 ± 3.4 years) to the participants in the present study. Although some of the participants in the present study may have had similar resting heart rates, the variability inherent in heart rate and the wide range of body mass index observed here mean that it cannot be assumed that the mean resting heart rate was similar to that observed by Kim, *et al.*, (1999), which unfortunately prevents an estimate-based analysis.

To summarise: the specific, significant changes observed in both the oxygen uptake (VO_2) and heart rate (HR) data during repeated maximal testing suggest the aerobic fitness of the training participants increased across the study. If so, the rugby training undertaken elicited changes in variables associated with cardiovascular fitness and increased maximal oxygen consumption.

Research Objective Two

(I) Is VO_2 affected by sleep quality or sleep duration or by the consumption of alcohol?

One of the original objectives of this study was to investigate effects of the day or the phase of the menstrual cycle on VO_2 results. As described in the introduction, the lack of findings resulted in all information pertaining to the menstrual cycle being removed. The stage of the menstrual cycle was not confirmed in participants using hormone assays or monitoring of temperature. In addition, fitness tests were not scheduled to coincide with particular days of the menstrual cycle. Therefore it is possible that an affect may have been present and could not be detected with the study protocol.

Maximal and submaximal oxygen consumption is known to be influenced by such factors as age, gender, fat-free mass, health, heredity and training status (Robergs and Roberts, 1997; Lear, *et al.*, 1999). Sleep quality, sleep duration and the occurrence of spontaneous sleep disturbances were monitored each night during the study. Particular attention was paid to variables of sleep quality during the night prior to each fitness test, to assess whether there were associations with the VO_2 measured during the fitness test. No relationships were found between sleep quality variables and VO_2 for either sedentary or training participants (Table 3.1.2).

Relationships may have existed between VO_2 and sleep quality variables. However, within the sedentary group there were insufficient data to detect relationships, as only two fitness tests were completed by each participant, and because all of the VO_2 data for the final week were lost. Although the majority of the training participants completed all six fitness tests, it is not likely that this increase in the volume of data would have been sufficient to detect significant relationships between VO_{2max} and sleep quality or duration, because the magnitude of the sleep disturbances that occurred here was probably too low.

Experimental perturbations in sleep quality and quantity, as seen in studies by Bond, *et al.*, (1986) and Martin, (1988), can influence fitness test variables such as peak VO_2,

motivation and lactate variables. However, it is important to note that the duration of sleep deprivation or the type of disturbances employed in these previous studies were not mirrored by any of the spontaneous sleep disturbances encountered during the present study. While no significant associations between sleep quality variables and VO_2 were detected here, such associations might have risen if the sleep disturbances or deprivation had been more marked.

An increase in alcohol consumption was noted in Week 2 of the experiment (Figure 3.5). However, most participants abstained from alcohol the night prior to a fitness test. As described in Section 3.3.2.2, the paucity of data prevented statistical analysis. Therefore a relationship may exist between alcohol consumption and VO_2 achieved during a fitness test, but the design of this study and the absence of management of alcohol consumption prevented the detection of a relationship.

In summary: the lack of detected relationships between VO_2 and sleep quality variables in either sedentary or training participants is likely to be due to the small amount of VO_2 data (due to mass spectrometer malfunction) and the low magnitude of sleep disturbances. The absence of a detected relationship between alcohol consumption and VO_2 is due to the abstinence of alcohol consumption in the majority of participants the night prior to a fitness test.

Research Objective Three

Were plasma or interstitial fluid concentrations of cortisol, testosterone and growth hormone affected either acutely by treadmill fitness tests in sedentary or training participants or chronically by six weeks of rugby training among training participants?

The ranges of plasma concentrations of cortisol, testosterone and growth hormone observed in the present study and how they compare with those reported in the literature are presented below (Section 3.6). Following this, an examination of the effects of treadmill fitness tests, the training period, oral contraceptive use and alcohol consumption on plasma and interstitial fluid hormone levels is conducted.

Plasma cortisol concentrations in the present study ranged from about 5 to 111ng/mL (Table 3.15). Reported plasma cortisol concentrations in females at different times of the day, and under differing experimental conditions ranged between 50 and 350ng/mL (Stupnicki, *et al.*, 1995; Galliven, *et al.*, 1997; Sarkola, *et al.*, 1999; Consitt, *et al.*, 2001; Copeland, *et al.*, 2002). The participants in the aforementioned studies were all women, aged between 16 and 33 years. Some reports refer to cortisol levels at rest (Consitt, *et al.*, 2001; Copeland, *et al.*, 2002) or at different times of the day (Galliven, *et al.*, 1997) while others deal with the effects of exercise (Stupnicki, *et al.*, 1995) or alcohol consumption (Sarkola, *et al.*, 1999). The ranges of plasma cortisol concentrations reported in these particular studies were similar to those detected in the present study.

Two of the 31 participants displayed decreasing cortisol levels as the study progressed (Section 3.6.1.1). Stress is known to elicit an increase in the activity of the hypothalamic-pituitary-adrenal axis (Prinz, *et al.*, 1980; Minton, 1994) and stress perceived by the participant as result of the study may have decreased during the study due to familiarisation with the procedures and researchers. In general, however, there was no significant change in mean plasma cortisol levels during the study in either the sedentary or training group.

Plasma testosterone concentrations ranged from about 93 to 490pg/mL (Table 3.16), which in general corresponded to the ranges in females published by others (Shangold,

et al., 1981; Oka, *et al.*, 1988; Martin, *et al.*, 1999). The ages of the participants in these studies were similar to those in the present study, at 15 to 47 years, and as with the cortisol studies (noted above) involved experimental conditions which were similar to aspects of the present study.

Despite an interesting observation that 5 of the 31 participants displayed markedly higher plasma testosterone levels on the first day of the study, no significant changes in mean plasma testosterone levels occurred across the study (Figure 3.8). The higher plasma testosterone concentrations observed on the first day could have been due to a number of mechanisms. An anticipatory increase in testosterone levels prior to a competition has been reported (Cumming, *et al.*, 1987) and the results observed on the first day of the present study may in some ways have mimicked this. The higher plasma testosterone concentrations may have been due to stress, caused by an unfamiliar situation, blood sampling and the presence of new technology.

Plasma growth hormone (GH) concentrations ranged from 47 to 452pg/mL (Table 3.17). In several other studies, plasma GH ranges of 200 to 10,300pg/mL have been reported (Välimäki, *et al.*, 1983; Cano, *et al.*, 1999; Consitt, *et al.*, 2001). Although the range in ages of the participants in these studies (20 to 46 years) was greater than that of the present participants (18 to 25 years), all were premenopausal women (Table 3.1). The ethnicity of the participants was not reported in any of the studies, so that differences between ethnic groups could not be commented on. Although ethnicity was reported in the present study (Section 3.1) all participants were Caucasian bar two, preventing an ethnicity-based comparison. These studies all controlled for diet, training status, phase of the menstrual cycle and the time of day that the experiment was conducted. However none of the studies reported the season, environment or climate in which the study was conducted.

The wide range in GH concentrations detected both in the present study (Table 3.17) and in the studies above is primarily due to biological variation. This variation results from the pulsatile nature of GH release and the range of both endogenous and exogenous factors that can affect GH release. The types of assays used to detect GH in the studies mentioned above can also affect results due to the varying detection limits (10 to 50pg/mL) and degrees of inter- and intra- assay coefficients of variation (1.7 to

7%). However this variation is small in comparison to the large biological variation in GH levels.

Acute changes in plasma hormone concentrations

Plasma cortisol concentrations apparently increase linearly during endurance exercise in proportion to intensity[6] and duration, and can remained elevated for several hours following cessation of exercise (Chicarro, *et al.*, 1998; Scheen *et al.*, 1998). Long duration endurance exercise, lasting six hours, has initiated a rise in plasma cortisol (Lac and Berthon, 2000). In contrast, a decrease in plasma cortisol levels after 40 minutes of endurance exercise has been reported by Copeland, *et al.*, (2002). During the present study, no acute increases in plasma or interstitial fluid concentrations of cortisol were observed in response to the treadmill fitness tests which lasted 6 to 15 minutes (Section 3.6.1.1, A). As endurance exercise apparently needs to be at least 20 minutes in duration at an intensity of at least 60% of VO_{2max} to elicit significant changes in plasma cortisol levels (Urhausen, 1995), the absence of significant changes in plasma cortisol concentration in the present study was not surprising. Had blood samples been taken from the training participants following the rugby training sessions, an increase in cortisol concentration may have been evident because of the 90-minute duration of these training sessions (Table 2.3). This would support the idea that the intensity of training can affect blood cortisol concentrations, however this is merely speculation.

Testosterone concentration is thought to increase in females following exercise of short duration (approximately two hours) (Webb *et al.,* 1984; Bonen and Keizer, 1987). Both the intensity and volume of exercise influence the magnitude of the change, and increases remain elevated for at least 30 minutes (Urhausen, 1995; Consitt, *et al.*, 2002). In the present study, no significant changes in plasma or interstitial fluid testosterone concentration following the fitness tests were detected (Section 3.6.1.1, B). It is likely that the short duration of the fitness tests was insufficient to elicit a response from the hypothalamic-pituitary-gonadal axis.

[6] As noted in Section 1.1.1.3 of the Introduction, low exercise intensity is ≤45% $VO_{2max,}$ moderate intensity is 50-65% VO_{2max} and heavy intensity exercise is ≥65 VO_{2max} (Brooks and Mercier, 1994).

No acute response of plasma GH levels to the fitness tests was detected (Section 3.6.1.1, C). As with cortisol and testosterone concentration responses (see above), exercise apparently must last for at least 20 minutes in order to elicit a response (Felsing, *et al.*, 1992; Kanaley, *et al.*, 1997). An acute increase in plasma GH levels following 40 minutes of aerobic exercise has been observed (Consitt, *et al.*, 2001; Pritzlaff-Roy, *et al.*, 2002). Presumably changes in plasma GH concentrations were reflected in interstitial fluid, although in these studies, such samples were not taken. In the present study, therefore, the lack of a significant increase in interstitial fluid GH concentration in response to the fitness test is also likely to have been due to the short duration of the fitness tests.

Chronic changes in plasma hormone concentrations

No significant changes in mean plasma cortisol concentrations were detected within the present training group as result of the six-week training period (Figure 3.7). This accords with the observation of Filiare *et al.*, (1998) that there were no difference in resting salivary cortisol levels across an even longer training period (sixteen weeks) and higher frequency of training (five or six sessions weekly). Although Filiare, *et al.*, (1998) monitored salivary cortisol and not plasma cortisol, it is assumed that neither plasma nor salivary cortisol changed significantly.

Endurance training programmes are thought to decrease plasma cortisol levels over time (Consitt, *et al.*, 2002). However, debate remains over the duration of training required to cause a significant change in plasma cortisol levels, and the exact nature of chronic changes.

The apparent absence of significant changes in plasma or interstitial fluid testosterone levels during the present six-week training period (Figure 3.8) accords with the absence of significant changes in plasma testosterone levels after six or twelve weeks in groups undergoing strength training, endurance training or a combination of both (Bell, *et al.*, 2000). Literature regarding the chronic changes of plasma testosterone concentrations in women following prolonged endurance training is equivocal. Although no change in resting plasma testosterone levels have been reported (Prior, 1987), other researchers reported that resting concentrations are lower in endurance trained female athletes

(Urhausen, *et al.*, 1995; Consitt, *et al.,* 2002). In contrast, resistance exercise programmes can apparently elicit a significant increase in plasma testosterone levels in females after a 12-week training period (Marx *et al.,* 2001). It is possible that if the present study had been of longer duration, decreases in resting free testosterone levels among the training group compared to the sedentary group may have been observed.

Increases in both circulating GH concentrations and one if its effectors (IGF-1) have been detected following two weeks of regular endurance or resistance training (Jenkins, 1999). A non-significant rise in GH concentration was detected in the present training group in comparison to the sedentary group following the six-week training period (Table 3.17). This might suggest that the training was beginning to affect the pathway leading to production and secretion of GH. However, the strength of this conclusion is limited by the fact that GH secretion is pulsatile and affected by many factors, including stress, temperature and prandial state (Spiegel, *et al.*, 2000).

Oral contraceptive use

Oral contraceptive (OC) use had varying effects on plasma concentrations of cortisol, testosterone and GH (Section 3.6.1.2, Subgroup 1). Significantly lower mean free plasma cortisol levels were observed in participants taking OCs compared to participants who did not (Table 3.18). This finding was also seen within the training group, where OC users displayed significantly lower mean free plasma cortisol levels than those not taking OCs.

There is evidence supporting the theory that OCs decrease resting plasma free cortisol levels and inhibit the response of the hypothalamic-pituitary-adrenal axis to stressors. Free plasma cortisol levels in women taking OCs was significantly lower than in women who did not take OCs (Pruessner *et al.,* 1997). Also, women taking OC have displayed decreased free salivary cortisol response to both *psychosocial* stress (Kirschbaum *et al.,* 1995) and *psychological* stress (Komesaroff *et al.,* 1999). In addition, an attenuated cortisol response to HPA axis stimulation following administration of oestrogen was observed in postmenopausal women (Puder *et al.,* 2001).

One mechanism underlying these effects may be that synthetic oestrogens increase plasma levels of corticosteroid-binding globulin, which increases total cortisol levels and decreases the amount of bioavailable free cortisol (Durber *et al.,* 1976; Carr *et al.,* 1979; Ruokonen *et al.,* 1982; Pruessner *et al.*, 1997; Kirschbaum *et al.*, 1999). Also, oestrogens may inhibit cortisol production and action via several mechanisms described by Komesaroff, *et al.*, (1999). Inhibition of production is apparently achieved through attenuation of ACTH secretion at the level of the anterior pituitary or hypothalamus and through actions on CRH gene expression, and cortisol action may be modulated by oestrogen altering glucocorticoid receptor function.

Another possible mechanism for this effect is that the persistently elevated levels of progestagens in the circulation from OCs act to inhibit cortisol release, possibly through negative feed-back mechanisms. This is because progesterone and cortisol receptors are structurally homologous (Deroo and Archer, 2002) and progesterone receptor antagonists bind to both progesterone and glucocorticoid receptors (Zhang *et al.*, 2002).

In the present study, plasma testosterone levels were numerically but not significantly lower in OC users compared to non-OC users (Table 3.19). Similar observations have been made in premenopausal females by others (Gaspard *et al.,* 1983; Hammond *et al.,* 1984; Luthold *et al.,* 1993; Sarkola, *et al.*, 2000). The participants in the study by Sarkola *et al.*, (2000) were of the same ethnicity (Caucasian) and of a similar age (26 ± 4yrs) to those in the present study. The possible mechanism is that OCs act to suppress ovarian function; in addition to preventing ovulation, the ovarian production of both testosterone and testosterone precursors is apparently inhibited (Greenspan and Gardner, 2001).

GH concentrations have been reported to be higher in OC users than non-users, through oestrogen stimulating both GH production and secretion (Bemben, 1992). In the present study, no significant effect of OC use on plasma GH levels was detected (Table 3.20). As the duration of OC use in each participant was not recorded, linkages between that and possible effects on GH synthesis and secretion cannot be assessed. Bemben (1992) also noted that lower dose OC formulations (35μg oestrogen and ≤1mg progestin) did not have a significant impact on plasma GH levels when compared to older formulations. Original OCs contained significantly higher levels of synthetic oestrogen

(150μg) than the 20-35mg seen in the low-dose formulations prescribed today (Cerel-Suhl and Yeager, 1999). All of the present participants, except one, who were taking OCs, were on low-dose formulations (Table 3.3). Thus, it may be that the lower dose OC formulations were insufficient to elicit any significant changes in GH synthesis and secretion.

Alcohol consumption

The present study was not designed to monitor any changes in plasma cortisol, testosterone or growth hormone levels following alcohol consumption. In addition, the majority of the drinking sessions occurred during the weekends, when no sampling of blood, interstitial fluid or saliva occurred. Moreover, there was no regulation of the amount of alcohol consumed or the timing of sampling following consumption. This is in contrast to most published studies. Nevertheless, some comment is merited.

In the present study, no relationships were detected between plasma cortisol levels and alcohol consumption, either acutely or across the study (Table 3.21). This finding is contrary to reports that alcohol consumption can initiate a stress response in individuals, both during its consumption (Frias, *et al.*, 2000) and during a hangover (Linkola, *et al.*, 1979). As already noted, this study was not originally designed to monitor endocrine changes associated with alcohol consumption, so that the protocol was inadequate to detect effects which might have been there.

Alcohol consumption in females can apparently acutely increase plasma testosterone levels. In the present study however, plasma testosterone levels were not related to the alcohol consumption the previous night (Section 3.6.1.2, 2B). This is in contrast to studies in females showing that plasma testosterone levels peak about 12 hours after consuming alcohol (Välimäki, *et al.*, 1983). A proposed mechanism is that plasma testosterone levels are apparently increased through the combined effects of the stimulation of the adrenal glands by prolactin, ACTH and CRH and the inhibition of hepatic catabolism (Frias, *et al.*, 2000; Sarkola, *et al.*, 2001). It may be that plasma testosterone concentration increased immediately following alcohol consumption, but that its levels had returned to resting values by the time sampling occurred the next morning. The lack of regulation of alcohol consumption or the timing of drinking

sessions is likely to account for the absence of detectable associations in the present study.

In the present study the participants who abstained from alcohol had significantly higher mean plasma levels of testosterone than either the moderate drinkers or the heavy drinkers (Table 3.22). This contrasts with a report of increased resting plasma testosterone levels in female alcoholics (Pettersson *et al.,* 1990). A possible reason for this discrepancy is that alcohol consumption during the present study may not have been sufficiently regular to induce a significant change in resting plasma testosterone levels. Testosterone concentrations change depending on the phase of the menstrual cycle (Oka, *et al.*, 1998), and as such the increased testosterone levels may have been related more to the phase of the menstrual cycle than to alcohol consumption. As no hormone assays were conducted here to confirm the phase of the menstrual cycle this idea cannot be evaluated.

No significant acute response of plasma GH levels on the morning following alcohol consumption was detected (Section 3.6.1.2, 2C). This contrasts with a reported acute decrease in plasma GH levels as a result of alcohol consumption (Prinz, *et al.*, 1980). The mechanism behind this decrease in plasma GH concentration apparently involves glucose. Alcohol consumption is known to increase blood glucose levels (Masuda, *et al.*, 1985). Hyperglycaemia in turn inhibits the release of GH (Välimäki, *et al.*, 1983; Frias, *et al.*, 2000). A reason for the present observation may be that transient decreases in plasma GH concentrations may have been complete, with resting levels restored by the time sampling occurred the following morning. Also, as mentioned above, most alcohol consumption occurred during the weekends so that no follow-up sampling occurred. Therefore changes in plasma GH concentration may have been elicited but were not observed due to the sampling schedule.

In the present study, no acute effect of alcohol consumption on plasma GH levels was detected. In contrast, a chronic effect of alcohol consumption on plasma GH concentration was detected. Plasma GH levels in the group of heavy drinking participants were significantly higher than those in the moderate drinkers and in the abstainers (Table 3.23). However, this effect may not be alcohol related and needs to be examined more rigorously, as numerous factors can affect both production and secretion

of plasma GH concentration (Lamberts *et al.*, 1997; Cano, *et al.*, 1999; Speigel *et al.*, 2000).

In summary: the short duration of the fitness tests was probably not sufficient to elicit significant changes in the secretion of cortisol, testosterone or growth hormone of sufficient magnitude to alter their plasma concentrations. In addition, the present participants were exposed to a relatively short, six-week, training period of low frequency (two sessions weekly) of what is presumed to have been of a moderate intensity. The basis of this presumption is the reporting by the training participants of the activities during the training sessions (Table 2.3). Evidently these factors collectively were insufficient to elicit any significant changes in plasma concentration of cortisol and testosterone, although a non-significant trend towards a small effect on plasma GH secretion may have occurred. Significantly lower resting plasma cortisol levels were detected in oral contraceptive users, possibly resulting from competition with synthetic progestagens at the cellular receptor level. No effect of oral contraceptive use on either plasma testosterone or growth hormone concentration was detected. This may be related to the lower levels of both synthetic oestrogens and progestagens in oral contraceptives prescribed today. Alcohol consumption had no acute detectable effects on the plasma concentrations of cortisol, testosterone or growth hormone. Mean resting levels appeared to be affected by alcohol consumption, with higher plasma testosterone levels observed in participants who abstained from alcohol, and higher plasma growth hormone levels detected in heavy drinkers. These results are in contrast to reports in the literature. However, alcohol consumption was not controlled for in the present study which may be related to the unusual observations.

Research Objective Four

(I) How accurate was the electrosonophoretic method in predicting plasma concentrations of cortisol, testosterone and growth hormone and can equations be derived to predict plasma concentrations of these hormones from interstitial fluid concentrations?

The exchange of cortisol, testosterone and GH between plasma and interstitial fluid apparently occurs rapidly, because the concentrations of the respective hormones in interstitial fluid and plasma exhibited very strong, significant positive relationships (r = 0.988 to 0.989) (Table 3.24).

The concentrations of GH in plasma and interstitial fluid over the ranges observed appear to exhibit approximately the same ratio as those observed for testosterone and cortisol (10.6 as opposed to 10.2 and 10.2, respectively) (Figures 3.10 to 3.12). A notable difference was that for the GH graph, in contrast to those for cortisol and testosterone, the y-intercept was significantly different from zero. The y-intercept could result from cross-reactivity during the assay from peptides of similar molecular weight to GH (such as insulin-like growth factors) or from other molecules with similar binding sites. The nature of the relationship between plasma and interstitial fluid for cortisol and testosterone led to the development of an equation applicable to both hormones (Section 3.7.2). Equations 1 and 2 (below) can be used to predict plasma concentrations, however it must be noted that each equation is only applicable when the technique described in the Materials and Methods section is adhered to, and is only applicable to plasma cortisol and testosterone (Equation 1) and growth hormone (Equation 2).

1. Cortisol or testosterone: $[Pl] = 10.2X$

2. Growth Hormone: $[Pl] = -4.3 + 10.6X$

Where Pl is the plasma concentration of the hormone in question and X is the interstitial fluid concentration derived using electrosonophoresis.

Lower correlation coefficients for each hormone were observed for relationships between saliva and plasma (cortisol, testosterone and GH) or saliva and interstitial fluid (cortisol and testosterone) (Table 3.24). The lower correlations seen between hormone levels in saliva and either plasma or interstitial fluid are likely to due to the variability of saliva secretion rate which is determined by autonomic nervous system activity, which can in turn be stimulated by exercise (Davenport, 1982). The exchange of a hormone from plasma into saliva is complex and not well understood, and debate exists as to whether saliva represents the unbound fraction (Hofman, 2001) or total hormone concentration (Cook, 2002).

An unusual spread of the data was observed for testosterone concentrations in saliva compared to interstitial fluid (Figure 3.11, bottom panel). Two distinct groups of data were detected. The first was a small group of data points that displayed a ratio of testosterone concentrations in salivary and interstitial fluid that was smaller and inconsistent when compared to the second group. The second group included the majority of the data points and displayed a more consistent concentration ratio for testosterone in saliva and interstitial fluid. The majority of the data points in the first group were taken in the first three days of the study and when closely examined displayed no other distinguishing characteristics. It is therefore suggested that this cluster of data points was a result of experimental variation which probably arose from a combination factors. Technical problems that were encountered using the electrosonophoretic machine (described below) may also have contributed to the unusual values.

The concentration of solutes in the sample of interstitial fluid is influenced by several factors. Factors include the flow-time characteristics of the ethanol moving through the collection head unit and the volume of ethanol in the reservoir beneath the semi-permeable membrane (Figure 2.1). Problems either with the pump that moves the ethanol through the collection head unit or with the tubing containing the ethanol can result in inconsistent sample concentrations. As numerous problems were experienced with the tubing during the first week of the study, it is possible that this contributed to the unusual values that were observed.

Disruptions to the integrity of the semi-permeable membrane may have resulted in molecules of large molecular weight entering the harvested interstitial fluid. Cross-reaction involving some of these larger molecules during the testosterone assay (Section 2.8) may have resulted in the unusual testosterone levels described above.

The temperature of the skin and the amount of sweat on the surface of the skin at the time of application may have affected the conductance of the electric current and ultrasound waves. This in turn may affect the movement of fluid and therefore concentration of blood constituents in samples. Some of the participants may have experienced anxiety and stress in the initial days of the study, which can stimulate the activity of the sympathetic nervous system, which can increase sweat production. This may have contributed to the unusual testosterone results seen. As the study progressed, familiarisation with the experimental procedures may have occurred, which may have reduced nervousness or anxiety levels. This in turn may have increased the consistency of skin conditions. It is also possible that the unusual testosterone levels described above were the result of contamination of sample collection containers by the hormones present on the surface of the skin (Zouboulis, 2000). However this explanation is unlikely due the small diameter of the sample containers.

There are many components that comprise the electrosonophoretic device and each component provides a potential source of problems (Figure 2.1). In the discussion above, several such problems are presented which may have led to unusual testosterone concentrations. Other factors that could impact on constituent concentrations are examined in the general discussion below.

In summary, the electrosonophoretic method apparently provides an accurate, painless, low-invasive method for prediction of the plasma levels of these three hormones. This technology has far-reaching implications for research into both human and animal endocrinology, as it provides a replacement method for continuous sampling, without the need for in-dwelling catheters and the possible associated infection problems. Continuous sampling using electrosonophoresis may lead to better understanding of acute and chronic hormone responses to engineered or natural challenges. Other fields may also benefit from the ability to predict blood constituents through electrosonophoresis, such as medical, sport science and animal welfare fields.

Chapter Five

General Discussion

The present study looked at a wide variety of variables in a relatively small population. The large number of variables examined and the fact that the participants were human and not animals, limited how invasive each measurement technique could be to minimise the level of intrusion on the participants. Other limitations imposed on the study were financial and ethical, so that several procedures which could have added to the precision of the research were not included. In the following section, alternative research designs are presented.

The following research projects have been designed with regard to and incorporate the lessons learnt from conducting the present study. The hypothetical research projects below have been developed without regard to monetary or some ethical constraints and complete compliance from the participants is assumed. It is recognised and acknowledged that this situation would not occur in reality, but the account below is provided in order to communicate understanding of the scientific rigour required. Instead of a single study, several studies are proposed here with different research designs and more tightly defined experimental objectives, in order to demonstrate how biologically significant results might be achieved more rigorously.

The effect of training on VO_2 in sedentary and training participants

A study to investigate changes in VO_{2max} as a result of a training programme should be at least fifteen weeks in duration. The increased length of the study is in line with the American College of Sport Medicine Position Stand which recommended 15-20 weeks as a minimum length for a training experiment (ASCM, 1990). Two groups (sedentary and training) should be established, with participants who are of the same gender, and are matched for age and weight.

Baseline fitness levels of each participant need to be established, using a series of aerobic and anaerobic fitness tests before the start of the training programme. When dealing with a sports team the tests used should be sport-specific, such as the 'speed-endurance test' as described by Deutsch and Slievert, (2000). Other sport-specific tests are described by Gore, (2000). The 'speed-endurance test' determines both speed endurance and speed decrement, and the distance over which the test occurs can be adjusted to be sport-specific.

The testing programme would provide a series of indices, such as VO_{2max}, lactate threshold values, short-distance sprint times or long-distance endurance times, which can then be reassessed at completion of the training period in both groups to allow for comparative analysis. This prior testing would also serve to familiarise the participants with the nature of the fitness tests and other monitoring systems, such as RPE recordings, that they would encounter during the study. The timing of the study needs to coincide with the pre-season training phase for a sports team, during which the proficiency of the energy systems are expected to increase (Powers and Howley, 1997).

Training sessions can be described using training impulses (TRIMPS). This unit takes into account the frequency, intensity and duration of the session as well as different heart rate variables, in order to create a 'dose' of training experienced by the participant. A TRIMP is comprised of a fitness response and a fatigue response which each have unique characteristics (Morton, 1991). A study investigating increases in fitness over a season can define the season using a profile of TRIMPS so that it can be easily reported, quantified and repeated by other researchers. Utilising this model may help to maximise benefit from a training programme and prevent overtraining or 'overreaching' syndrome (Urhausen, 1995).

For each individual, increases in VO_{2max} or in other indices of fitness resulting from a defined number of TRIMPS can then be determined. Then a general prediction can be made regarding the increase in fitness indices resulting from a given number of TRIMPS. However, such results are only representative of the population from which they are sampled, and therefore extrapolation to participants of another gender, age,

training history, ethnicity, sports code or to different training programmes should be done with caution.

Care needs to be taken to standardize the time of the fitness test for each participant, and the timing and composition of food intake prior to the test. In addition, environment, temperature and humidity and order of events should be regulated to minimise variation (Mahler, *et al.*, 1995). A careful choice of the on-line gas analysis systems or use of an alternative is recommended, to avoid the possible loss of data, as seen in the present study.

The effect of different phases of the menstrual cycle on VO_2

Due to the inherent variation in menstrual cycle parameters (Harlow and Matanoski, 1991), pre-screening of participants is recommended before beginning a study of this nature. Healthy, eumenorrhiec women without a history of menstrual irregularities, who display characteristic, observable changes surrounding ovulation should be selected for both the training and sedentary groups. Ovulation can be confirmed using hormone assays to detect a decrease in plasma oestrogen concentration and a surge in plasma LH levels, and an increase in temperature following ovulation due to increased plasma progesterone concentrations (Chabbert Buffet *et al.*, 1998; Stachenfeld *et al.*, 2002).

It should be noted here that the requirements regarding characteristic changes in progesterone will exclude women who do not display changes in progesterone, but are otherwise healthy.

It is recommended that an observation phase occurs, during which each participant is monitored for several menstrual cycles before the experiment begins. This is done to confirm 'normal' cycling and that endocrine and temperature changes are detectable around ovulation (Silberstein and Merriam, 2000).

During the observation phase, the researchers should ensure that familiarisation with both the nature of the fitness test and the equipment used during the fitness test occurs. Once the study has begun, the exercise tests should be scheduled to occur on days that

coincide with confirmed phases of the menstrual cycle. It is recommended that two exercise tests are conducted, one during the follicular and another during the luteal phase of the menstrual cycle, over several cycles. This will increase the precision of the results, although this extends the duration of the experiment. Careful reporting of negative menstrual symptoms (pain, water retention, nausea, etc.) should occur to detect trends or relationships between negative symptoms. A large cohort of women is recommended owing to the varied nature of responses, and repeated testing over a series of menstrual cycles would increase the precision of results obtained.

The effect of sleep quality, sleep duration and spontaneous or generated sleep disturbances on hormone profiles

Initially, resting values for each hormone being studied should be determined for each individual, taking into account pulsatility of hormone release (e.g. growth hormone) or circadian rhythms (e.g. cortisol) (Santiago, *et al.*, 2001). Increased precision of measurements will be achieved through keeping the sampling time and the timing of meals prior to sampling consistent. The most sensitive assays possible should be used and it is recommended that the samples are analysed in one continuous batch by the same technician to standardise techniques and minimise variation. When investigating the influence of factors such as sleep duration or spontaneous sleep disturbances, blood, saliva or interstitial fluid samples should be taken at several times during each day. This will allow monitoring of changes to baseline values and changes in circadian rhythms of hormone release, if hormones are influenced by circadian rhythms.

If sleep logs are the method used to monitor behaviours and occurrences that might affect sleep quality and duration, careful attention needs to be paid to the questions and design of the sleep log. Details regarding behaviours such as naps or alcohol consumption or spontaneous disturbances during sleep are required so that a more accurate indication of sleep quality and duration can occur. As sleep quality is a subjective measure, investigation into an alternative method for reporting sleep quality other than that used in the present study is suggested.

Other methods for increasing the accuracy of monitoring the duration and composition of sleep exist include actigraph monitors, such as the SleepWatch® (Ambulatory Monitoring Inc., New York), which is based on correlating movement to alertness. The monitor can display the duration of sleep as well as the duration of both REM and non-REM sleep. Sleep laboratories require an increased level of commitment from the participants, but provides the opportunity to investigate other indices of sleep, such as electroencephalogram (EEG) recordings and muscle tone, as well as obtaining video recordings of the sleep period to even more accurately assess sleep disturbances. This form of monitoring allows for a comparison between unbroken sleep and spontaneous disturbances during sleep.

The use of sleep logs is far simpler and easier for the participants to comply with compared to monitoring of sleep in sleep laboratories. However, sleep logs have limitations, for example there is not possible determine the effect of different variables on the structure of sleep and the duration of each type of sleep (REM and non-REM). In addition, the participants may not record all events that occur during each night which may have an effect on the analysis of sleep quality or sleep duration. Sleep quality itself is a subjective measure which makes it difficult to analyse and make comparisons with objective variables.

A study could be conducted to assess the value of sleep logs. A large group of participants (again matched for age and gender) would undergo several nights of sleep, which were either undisturbed, or with increasing levels of generated disturbances. The nature of the generated disturbances would vary. Examples include a delayed sleep onset (ranging in duration), awakening during the night at pre-defined intervals (again for a range of duration), or when a certain type of sleep occurred (i.e. non-REM or REM sleep). The last type of disturbance requires monitoring of EEG recordings to ensure accurate timing of disruptions. A week or more would separate each experimental procedure in order to allow each participant several nights to normalise sleeping habits. Following each experimental night of sleep, sleep logs would be completed and assessed, for example, to see if disturbances correlated with a decrease in sleep quality ratings. This would provide a measure of the usefulness of the sleep logs.

A study regarding the effects of sleep deprivation on resting hormone concentrations should include different lengths of sleep deprivation to clearly define the endocrine response. A large group of participants who are matched for age, ethnicity and gender is recommended, due to the wide range in resting hormone concentration. Before any participants undergo sleep deprivation, baseline levels of each hormone should be established, as described above. In addition, each participant should be assessed to determine their optimal sleep length.

In the first experiment regarding sleep deprivation, sleep deprivation will be achieved by delaying sleep onset. Each participant will experience all levels of sleep deprivation, in random order to eliminate an order-effect. The length of sleep deprivation will range from two to twenty-four hours, with each level increasing by two hours, resulting in twelve stages that each participant will experience. A week or more will separate each experimental procedure. The separation of experimental procedures will lengthen the duration of the experiment but will ensure that each participant has recovered from the previous weeks sleep deprivation. The recommendations regarding endocrine studies are discussed above and such will not be repeated here.

Following this experiment, similar studies should occur on the same participants, but with altered timing and nature of the sleep deprivation. For example, shorter but more frequent periods of sleeplessness that are imposed at different times throughout the night should be investigated to compare different endocrine responses. The use of either sleep laboratories or actigraph monitors would allow selective sleep deprivation, preventing either non-REM or REM sleep, at different parts of the night, which would extend the ability to assess the relationships between sleep structure and hormone secretion.

Another research design is proposed here, regarding the effects of alcohol consumption on the structure of sleep. In this design, increasing levels of alcohol consumption are consumed by participants prior to sleeping, and physiological parameters relating to sleep are monitored using EEG monitors. Due to the harmful physiological effects of alcohol consumption, especially in large amounts (Section 1.5), it is acknowledged that this level of intrusion raises ethical issues. It would therefore be very unlikely to gain

acceptance from ethical committees due to the possible range of negative physiological consequences.

A range of alcohol consumption would be provided to two groups of participants, one male group and one female group (both matched for age, health status and ethnicity). Blood alcohol concentrations would be monitored during the alcohol consumption period until the pre-determined level is reached, at which point consumption ceases. The experiment would be conducted over several weeks in order to allow rest period between drinking sessions and to allow sleeping habits to normalise. Each participant would experience each level of alcohol consumption but in varying order to eliminate any order-effects. Following the alcohol consumption, the participant would be monitored in a sleep laboratory for sleep onset and various neurological variables, monitored using an EEG. This experiment would provide data on the response of sleep structure to alcohol consumption at many different levels, and whether or not there is a gender difference in the response.

To repeat, due to the harmful physiological effects of alcohol consumption, especially in large amounts (Section 1.5), it is acknowledged that this level of intrusion raises ethical issues, and that such an experiment would, quite rightly, probably not be approved by an ethics committee.

The accuracy of the electrosonophoretic method in predicting plasma hormone levels

The sensitivity of the electrosonophoretic (ESOP) method is a function of both the sensitivity of the assays used to test for hormone levels, and the ability of the ESOP method to harvest interstitial fluid samples. The ability to monitor small changes in hormone levels accurately is affected by the type of assay chosen as inter- and intra-assay coefficients of variation can vary widely (Pringle *et al.,* 1989; Wilkin *et al.,* 1989; Shimada *et al.,* 1995; Boots *et al.,* 1998). Altering several variables related to ESOP method may affect the ratio of hormone in interstitial fluid to that measured in plasma and as such should be investigated. Variables include a longer application time, a longer ultrasound pulse and a smaller volume of ethanol within the head unit (Figure 2.1).

Further research could focus on the difference in the yield of interstitial fluid (and the concentrations of blood constituents) from one specific ESOP technique[7] in people of different age, ethnicity, fitness level and health status. These studies could be extended to include other ESOP techniques to find the most appropriate technique for each group.

As discussed in the section above regarding Research Objective Four, components of the electrosonophoretic method can affect the concentration of hormones in the interstitial fluid. As such, a series of experiments should occur in order to clarify the nature and magnitude of changes in each component.

Previous research has revealed no permanent change to the structure of the skin at the site of application, even after repeated applications (Tamada, *et al.*, 1995; Kost, *et al.*, 2000). Despite these findings, it is recommended that an experiment occurs into changes in the structure of the skin following different lengths of application and different ultrasound frequencies. Findings from these studies could provide support for the suggestion that this methodology is benign in nature. Also, studies investigating the effect of temperature of the skin or the amount of sweat on the skin on the application of the ultrasound or electric current would lead to ways to standardise skin conditions for optimal use of the ESOP equipment.

In addition to assessing possible changes in skin structure, the effect of different lengths of application or frequencies of ultrasound on constituent concentration could be investigated. The flow-time characteristics of the fluid passing through the collection head unit can also affect the concentration of hormones collected. An experiment utilising a range of the aforementioned variables would allow for the most efficient use of the ESOP method.

[7] The technique includes the length of application, the length of ultrasound waves and the speed of fluid movement through the collection head unit.

Conclusions

The present study provided a significant number of samples (over 600) from participants from similar cohorts. All the participants were female students of a similar age and the majority were Caucasian. Half of the participants were regularly participating in rugby training and half were sedentary.

A significant increase in aerobic fitness (as assessed by VO_2) among the training participants was observed across the six-week training programme. Although the intensity of training sessions was not quantified, it appears that rugby union training programmes of short duration can increase aerobic fitness in healthy young females over a period of six weeks. Due to the absence of information regarding training intensity and the small sample size of the training group (n=15), extrapolation of these findings to other populations is not recommended.

No significant impact of either sleep duration or sleep quality on VO_2 was detected during the study. This was likely to be due to several factors, including the subjective nature of sleep quality ratings and the variation among what individuals define as a 'good' night of sleep. Sleep patterns were not manipulated in this study which may also have contributed to the lack of observed relationships. In addition, no relationships were detected between alcohol consumption the night before a fitness test and the VO_2 obtained during that test. It was not intended that the effects of alcohol consumption on the variables measured in the present study be assessed. The lack of regulation of timing of consumption or the amount of consumption is likely to be the reason for the lack of an observed relationship.

The treadmill fitness tests elicited no observable acute response in plasma cortisol, testosterone or growth hormone concentrations. The duration of the treadmill fitness tests was apparently insufficient to cause acute changes in the operational dynamics of production and secretion of the three hormones. No significant chronic changes in resting plasma levels of these hormones occurred across the six-week study. This may have been related to the frequency and intensity of training sessions, although the

intensity of these sessions was not monitored. Also, the short duration of the study may have precluded the development of changes in resting hormone levels.

In both training and sedentary participants, oral contraceptive use was found to be linked to decreased plasma cortisol concentrations. This added support to published reports of similar responses. This effect is likely to be due to separate effects of the synthetic oestrogen and progestagen elements. The oestrogen component apparently increases corticosteroid binding globulin, which increases total cortisol levels and decreases free cortisol levels. The progestagen component apparently competes with cortisol at glucocorticoid receptors and may enhance negative feedback, thereby further decreasing cortisol levels.

No acute or chronic changes as a result of alcohol consumption were detected in either plasma cortisol or plasma testosterone concentrations. While no acute changes in plasma growth hormone levels were detected, chronic levels in heavy drinkers were higher when compared to levels in moderate drinkers or abstainers. This novel observation may be an artefact of the pulsatile nature of growth hormone release and the number of factors which can influence production and secretion, rather than an unusual effect of alcohol upon the hypothalamus or pituitary glands.

The electrosonophoretic (ESOP) method has many advantages over traditional methods of sample collection. For example, the machine itself is straightforward to use and causes no pain or discomfort to the participants, in contrast to blood sampling. In addition, the skin is not punctured during application, and no damage to the structure of the skin occurs, both of which reduce the risk of infection, again in contrast to blood sampling. Although saliva sampling in humans is both convenient and painless, the variability of salivary hormone concentrations decreases the usefulness of this method.

The extremely high correlations between measurements of each of the three hormones in interstitial fluid and plasma provided more precise estimation of plasma concentrations when compared to saliva. The relative concentrations of the hormones in the two fluids over the concentration ranges observed (determined by regression analysis) and the resulting equations provide a simple and straightforward technique for predicting plasma hormone levels.

The above factors, combined with the lack of discomfort or pain experienced by participants provides a more accurate alternative to results obtained from saliva samples and a painless and safer alternative to blood sampling. It is strongly recommended that validation of the ESOP technique be continued to discover the range of its benefits for use in research into sport science, medical and animal fields.

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