Master of Science Thesis

Investigation of the Relationships that Exist between Athletic Training, Hormones and Sleep in Young Healthy Male Athletes

Lara Blackmore

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Abstract

Background. Many people engage in exercise for recreation, to promote personal health and as a profession. Accordingly there is wide ranging interest in the factors that affect a person's performance during exercise and in how that performance can both be assessed and enhanced. The physiological basis of exercise performance and its enhancement have been investigated for many years. Such investigations in people are impeded by the understandable reluctance of participants to provide significant numbers of blood samples by venepuncture. The recent development of an ultrasound method for non-invasive sampling of extracellular fluid, called transdermal electrosonophoresis (ESP), offers tremendous opportunities for benign monitoring of physiological responses involving changes in blood/extracellular fluid composition associated with exercise and indeed in clinical settings. Sleep quality/quantity is considered to have significant impact on training effectiveness and performance, with poor sleep correlated with poor athletic outcome. The link here is considered to involve growth hormone, as poor sleep quality/quantity diminishes growth hormone concentrations and reduced growth hormone concentrations impede training induced muscle development. Training effectiveness and recovery have been monitored in past research through measurement of blood hormone profiles, in particular the testosterone: cortisol ratio. The overall objective of this study was to validate the use of ESP as a non-invasive blood sampling technique through the study of the relationships that exist between exercise, fatigability, fitness, and hormone levels in blood, saliva and extracellular fluid and the investigation of the impact of spontaneous sleep disturbances on these relationships in young healthy male athletes. ***Methods.*** Plasma, ESP and saliva samples were taken regularly from 14 male rugby players during a four-week study. The plasma and ESP samples were analysed for testosterone, cortisol and growth hormone concentration. The saliva samples were analysed only for testosterone and cortisol levels. Fitness was assessed each week using a maximal treadmill test and fatigability was also investigated. Sleep quantity/quality was investigated using personal sleep logs which the participants filled out daily. In addition the participants' alcohol consumption was reported in the sleep log. ***Results.*** Correlations between hormone concentrations measured in plasma and ESP were higher than the correlations for plasma and saliva. The results here were highly significant. An equation was derived to estimate plasma concentrations of

testosterone, cortisol and growth hormone using the concentrations of the hormones measured in ESP samples. Few statistically significant relationships between hormone profiles, sleep quality/quantity and athletic training were revealed in the analysis of the results of this study. A negative correlation was found between the mean plasma cortisol concentration measured in the morning and the estimated VO_{2max}, and also between the cortisol concentration measured in the ESP sample and estimated VO_{2max}, but this did not quite reach significance. A negative correlation between estimated VO_{2max} and the mean total time asleep for the previous three or four nights was revealed. ***Conclusions.*** ESP sample analysis provides a more accurate estimation of testosterone, cortisol and growth hormone concentrations in plasma than saliva sample analysis does.

Contents

Chapter One: Literature Review

Tables

1.1 INTRODUCTION

New Zealand is a country of generally quite active people. The Hillary Commission (now Sport and Recreation New Zealand) defines the minimum level of physical activity required to improve a person's health and well-being as 2.5 hours per week and such person is called 'active' (Hillary Commission, 1999). In New Zealand, two out of three people over the age of five are active. In this study being 'active' will be referred to as partaking in some form of exercise. We can define exercise as the contraction of skeletal muscle for the purposeful movement of the body. These movements are controlled by the electrical conduction system of the central nervous system and include both reflex and voluntary contractions. The acute adaptations to exercise are mediated through the integration of the nervous and the endocrine systems. The above definition allows many activities to be considered as a form of exercise, from walking to sprinting, soccer to gymnastics and also other activities of daily life including work related physical activity. It is not only of interest to investigate the processes that occur during exercise but also to examine how other processes in the body essential for life, such as sleep, may impact on adaptations to exercise.

There were three objectives in this study:

1. With particular reference to testosterone, cortisol and growth hormone, to compare their concentrations in plasma and saliva with those derived by transdermal electrosonophoresis (ESP), a novel methodology. The aim here is to further validate the use of ESP as a non-invasive sampling methodology.
2. To explore relationships between exercise, fatigability (as a measure of fitness), fitness increases as assessed by maximum oxygen uptake (VO_{2max}) and the ventilatory anaerobic threshold, and the hormone levels in plasma, ESP and saliva samples.
3. Using spontaneous disturbances in sleep quality/quantity, to explore their impact on hormone profiles and athletic performance.

The physiological basis of human exercise performance and its enhancement have been investigated for many years. Such investigations are sometimes impeded by the reluctance of participants to provide significant numbers of blood samples by

venepuncture. The recent development of an ultrasound method for non-invasive sampling of extracellular fluid (transdermal electrosonophoresis) offers tremendous opportunities for benign monitoring of physiological responses involving changes associated with exercise (Cook, 2002). The opportunities in clinical settings are even wider. As this method is still in it infancy the extracellular fluid composition determined by electrosonophoresis must be correlated with the composition of plasma and/or saliva samples, in order to validate its development for widespread use. Cook (2002) investigated the best method of collecting transdermal exudate in sheep and humans. The result of this study was that high correlations between plasma and ESP sample concentrations were seen both at rest and during exercise and recovery in both species. This was unlike salivary concentrations where post exercise correlations with plasma decrease; this may be due to a time lag in partitioning of constituents between plasma and saliva which is complex and poorly understood (Quissell, 1993).

Sleep quality and quantity are considered to have significant impact on training effectiveness and performance, with poor sleep correlated with poor athletic outcome (Mougin et al, 1991), however, there is apparently no agreement in the literature about the effect of sleep deprivation on physiological responses to exercise. This will be discussed further below. Poor sleep is likely to have a negative impact on the effectiveness of athletic training and the link here is considered to involve growth hormone, as poor sleep quality/quantity diminishes nocturnal growth hormone secretion (Van Cauter et al, 1992). Reduced growth hormone concentrations impede training-induced muscle development (reviewed in Jenkins, 1999). Training effectiveness and recovery have been monitored in past research through measurement of plasma hormone profiles, in particular the testosterone:cortisol ratio (Hakkinen et al, 1987).

What was proposed for the present thesis research was a pilot study to investigate these phenomena. Before providing details of the present study, however, literature relevant to different aspects of the topic will be considered.

1.2 PHYSIOLOGICAL RESPONSES TO EXERCISE

1.2.1 Metabolic Demands of Exercise

1.2.1.1 Bioenergetic systems to support muscle contraction

The following details are well established and are reviewed extensively by Poortmans (1993). Energy in the form of ATP is synthesised in the body from energy-rich nutrients in food. When these nutrients (carbohydrates, fats, proteins) are metabolised they release ATP to fuel muscular contraction and other biological processes. Three bioenergetic systems exist to produce ATP, two of which are anaerobic and the other is aerobic. The phosphagen system comprises the stored ATP and phosphocreatine within cells; enough to maintain vigorous muscular activity for between three and 15 seconds. This anaerobic system is utilised during short duration, high intensity activities such as the 100m sprint or lifting a box. The other anaerobic system, the glycogen-lactic acid system, involves glucose mobilisation and anaerobic glycolysis producing pyruvate which in the absence of oxygen, is converted into lactic acid. It is able to produce ATP for longer than the phosphagen system but as the lactic acid accumulates in skeletal muscle, the decline in pH impedes the further breakdown of glucose. This system is primarily used in high intensity, short duration events such as a 400m sprint.

In the presence of oxygen, the pyruvate produced during glycolysis is converted into acetyl CoA. This then enters the tricarboxylic acid (TCA) cycle, producing 38 ATP molecules for every one acetyl CoA molecule compared with only 2 ATP molecules from anaerobic glycolysis. This third system, termed the oxidative system, is predominantly used during endurance type activities and yields far more ATP than either of the anaerobic systems. Fats can also be used to produce energy via the oxidative system, one 18-carbon chain fatty acid producing 147 ATP molecules. Most exercise activities require a blend of both anaerobic and aerobic metabolism. For example, the energy required for the beginning sprint and end sprint in a 1500m race will be derived from the anaerobic systems while the middle of the race will rely predominantly on the aerobic system. Table 1.1 compares the capacities of each of the bioenergetic systems in a 70kg male.

Table 1.1. Maximum rates of active phosphate production from different substrates and amounts available in a 70 kg man (muscle mass estimated to 28 kg) (Greenhaff et al, 1993).

System	Conversion	Rate (mol/min)	Amount Available (mol)
Phosphogen	ATP, PCr → ADP, Cr	4.4	0.67
Glycogen* – lactic acid	Glycogen* → lactate	2.35	1.6
Oxidative	Glycogen → CO_2	0.85 - 1.14	84
	Liver glycogen → CO_2	0.37	19
	Fatty acids → CO_2	0.40	4000

* Glycogen stored in muscle

1.2.1.2 Influence of intensity, duration and type of exercise

During rest the dominant bioenergetic system being used is the oxidative system. About two-thirds of the fuel being metabolised is fat, the remainder is glucose, as the contribution of protein is negligible (Greenhaff et al, 1993). During exercise both the anaerobic and aerobic systems contribute ATP, with their relative roles depending on the intensity of exercise, the state of training, and the diet of the athlete.

At exercise intensities below maximal oxygen consumption (VO_{2max}) the aerobic system dominates the supply of ATP for muscular contraction. As the exercise intensity increases there is a higher demand for ATP, but the aerobic system is limited in supplying adequate ATP for two reasons. Firstly, each person has an upper limit to the rate at which they can consume oxygen and secondly, it takes two to three minutes for ATP production to increase to a new higher level using this system. At low rates of oxygen consumption, ATP is predominately supplied by the oxidation of free fatty acids. However, during incremental exercise, as exercise intensity increases, a shift in substrate utilisation occurs with an increase in carbohydrate metabolism and a decrease in lipid metabolism – this is known as the "crossover concept" (Brooks and Mercier, 1994). Once the maximal rate of oxygen consumption by the aerobic system is reached, anaerobic systems contribute to ATP production at an increasing rate while the aerobic system continues to produce ATP.

Training, depending on the type, has several biochemical effects that improve the capacity of the bioenergetic pathways. Anaerobic training results in an increase in the

muscular stores of ATP and phosphocreatine (Eriksson et al, 1973), an increase in the activities and concentrations of enzymes involved in the phosphogen system (Thorstenson et al, 1975) and an increase in the activities and concentration of enzymes involved in the glycogen-lactic acid system (Costill et al, 1976; Eriksson et al, 1973; Gollnick et al, 1973).

Endurance (aerobic) training results in an increase in the myoglobin content of muscle (Mole et al, 1971), which is important in the delivery of oxygen from the cell membrane to the mitochondria where it is consumed. There is also an increase in the size and number of mitochondria present in skeletal muscle cells (Costill et al, 1971), the amount of glycogen stored (Gollnick et al, 1972), and an increase in the level of activity and concentration of enzymes involved in the tricarboxyclic acid (TCA) cycle and the electron transport chain (Gollnick et al, 1973). Additionally there is an increase in intramuscular stores of triacylglycerides (TAG), an increase in the mobilisation of free fatty acids from adipose tissue and an increase in the concentration of enzymes involved in the oxidation of fatty acids in the skeletal muscle cell (Gollnick, 1977). There is also a decrease the sympathetic nervous system responses to given submaximal exercise levels with aerobic training (Brooks and Mercier, 1994). These biochemical adaptations enhance lipid oxidation during mild- to moderate-training intensity so that trained individuals oxidise a greater proportion of fat at similar workloads than untrained individuals (Gollnick, 1977). Aerobic training therefore results in adaptations that conserve glycogen stores by utilising the body's stores of free fatty acids in adipose tissue. This prolongs the time to exhaustion as glycogen depletion results in exhaustion (Brooks and Mercier, 1994).

Likewise, diet can have an effect on athletic performance. In particular a high carbohydrate diet prior to competition (carbohydrate loading) increases the concentration of stored glycogen in muscle and prolongs time to exhaustion in endurance activities (Bergstrom et al, 1967). Consumption of a high fat meal prior to exercise can also improve performance by increasing the amount of fat available for use and therefore may prolong the time to exhaustion by glycogen sparing (Costill et al, 1977).

1.2.1.3 Specifics of rugby training and the game

The energy demands during a rugby union football match can be estimated by a combination of notational analysis and metabolic measurement (Nicholas, 1997). Notational analysis involves the observation and recording of game play. At this point it should be noted that, due to different player position requirements in a game, the physiological demands of particular positions may not be reflected correctly by studies that present mean values.

While a match is made up of two 40-minute halves plus the time that the referee adds at the end of a game, it has been estimated that the ball is in play for an average of 29 minutes (McLean, 1992). Morton (1978) estimated that the distance covered by players during a game is approximately 5.8km and that this is made up of 37% walking, 29% jogging and 34% striding or sprinting. In a study on Canadian players it was concluded that 85% of the time is spent in low-intensity activity, likely due to the need to resume formations in order for play to continue following a quick switch from one end of the field to the other as a result of tactical kicking and running with the ball (Docherty et al, 1988). The remaining 15% of the time is spent on intense exercise, 6% being related to running and 9% to tackling, pushing and competing for the ball.

Treadwell (1988) splits the analysis of time spent in movement modes into the two distinct playing units in a rugby union team, forwards[1] and backs[2]; the results are presented in Table 1.2 and Table 1.3 (In: Nicholas, 1997). Non-purposive running refers to all types of movement and stationary periods when a player is engaged in game play. Purposive running includes jogging and running up to three-quarters of maximal pace.

[1] Forwards – eight players located in three rows in the scrum; their role is to win and retain possession in set pieces (scrums and line-outs) and in open play (rucks and mauls).

[2] Backs – seven players including a half back (coordinates play between forwards and backs), a first five (coordinates the back line), two centres and two wings and a full back (the last defensive player); the role of the backs is to run into spaces in order to score tries.

Table 1.2. Combined time and motion analysis for rugby union forwards (Nicholas, 1997).

	Movement Category [min.sec (no.)]			
	Nonpurposeful Running	**Purposeful Running**	**Scrums**	**Rucks/ mauls**
Playing position				
Front five	47.18	17.12	9.14 (52)	6.32 (53)
Back row	55.10	18.00	6.28 (48)	4.02 (56)
Mean time	51.14	17.36	7.51 (50)	5.17 (54.5)
Proportion of total time (%)	63.80	21.50	9.19	6.26

Table 1.3. Combined time motion analysis for rugby union backs (Nicholas, 1997).

	Movement category (min.sec)			
	Nonpurposeful Running	**Purposeful Running**	**Sprinting**	**Time With Ball**
Playing position				
Half-back	51.04	21.28	2.16	1.52
Centre	55.16	14.42	2.14	1.20
Wing/full back	69.24	9.54	0.56	2.16
Mean time	58.35	15.21	1.49	1.29
Proportion of total time (%)	73.20	19.18	2.25	1.85

McLean (1992) investigated the density of work during a rugby union football match by timing work:rest ratios (W:RRs) and concluded that most work periods were 19-seconds in length with the most frequent W:RRs being in the range of 1:1 to 1:19. He also concluded that a scrum, line-out, ruck or maul occurred on average every 33-seconds. Notational analysis of a rugby union game may underestimate total match demands due to any additional energy expenditure required for accelerations, decelerations, running with the ball, running sideways, jumping in the lineouts, pushing in the scrums, rucks and mauls, tackling and other game related activities. For these activities the energy would be provided primarily by the phosphagen system.

Metabolic measurements, such as blood lactate levels, may provide additional information about what energy supplying systems are in use. Most studies report a blood lactate concentration of between 2.8-9.8mmol^{-1} (Reilly, 1997). Measurements of

blood lactate are taken during stoppages, at half time or at the end of the match. This may grossly underestimate the importance of the glycolytic pathway in the production of energy during a rugby match as lactate concentration at any one time is the balance between the rates of production and removal and is a measure of only the preceding few minutes of physical activity and metabolism (Brooks, 1985).

This information supports the classification of rugby union as an intermittent high intensity sport. Thus emphasis in training should be placed not only on the power of the anaerobic energy system in order to perform brief periods of maximal exercise, but also on improving the capacity of the aerobic system to enhance recovery between bursts of maximal exercise (Maud, 1984).

1.2.2 Physiological Support System Changes and Their Control

1.2.2.1 Cardiorespiratory adaptations to exercise

Exercise imposes stress upon many physiological systems throughout the body. In order to maintain optimal performance it is essential that a complex series of responses is coordinated and controlled. There are three regulatory roles of the cardiovascular system during exercise in the human, (1) the proper control of arterial blood pressure, (2) augmentation of blood flow to active muscle, and (3) the maintenance of internal body temperature (Seals et al, 1994). Turner (1991) describes the 'exercise reflex', which is initiated within the active muscle mass by a build up of metabolites due to a mismatch between perfusion and muscle metabolism. Chemoreceptors within the muscle register an imbalance and increase their firing rate in chemosensitive afferent nerves linked to the central nervous system (CNS). The efferent arm of the reflex arc then activates increases in ventilation, central and peripheral components of blood flow and blood pressure. Two neural mechanisms, central command and a reflex originating in contracting muscles, are known to play a large role in exercise-associated adjustments in cardiovascular and respiratory activity (Kramer and Waldrop, 1998). Relevant features of the nervous system's involvement in the changes that occur during exercise will be outlined here. Nervous control of the cardiorespiratory system is paramount to the overall functional efficiency of this system. The task of receiving information relative to pulmonary ventilation and blood flow, integrating it and then initiating a response to match mechanical and metabolic demands occurs within the

cardiovascular and respiratory control areas of the brain. These control areas are located specifically within the medulla oblongata of the brain stem (Kramer and Waldrop, 1998). The cardiovascular and respiratory areas are neurally interconnected, so that stimulation of one area will, via its connection with the other, generally affect both ventilation and blood flow (Harper, 1996).

Turner (1991) described three phases of moderate exercise. In phase 1 (0-15s) the cardiorespiratory responses are rapid. In fact they are so rapid that purely neural control mechanisms are thought to be responsible for the initial actions of the various physiological systems. The two most important neural control systems responding during phase one are (a) mechanical feedback reflexes originating from the active muscle mass and (b) a centrally generated feedforward motor pattern. The primary mechanism causing the increase in heart rate at this point is considered to be the decrease in efferent cardiac parasympathetic nerve activity to the sinoatrial node (Seals et al, 1994). This allows cardiac output and therefore blood pressure to rise. Additionally, it is likely that there is some sympathetic vasoconstrictor activity to the viscera so that blood can begin to be diverted to the metabolically more active skeletal muscle (Turner, 1991). At the same time, the operating point for the carotid artery and aortic baroreceptors is quickly reset to a higher level, which is likely to contribute to a further decrease in parasympathetic tone (DiCarlo and Bishop, 1992). As exercise proceeds to phase two (15s to two or three min), slower increases in cardiorespiratory variables occur until a new steady state is reached (phase three, three min onwards) during which neural and humoral mechanisms combine to bring about an appropriate response (Turner, 1991). During phase two, the cardiovascular and respiratory systems begin to attain a steady-state response profile and each physiological system comes increasingly under the influence of neural afferent inputs originating in the lungs, the heart, the carotid bodies, muscle chemoreceptors, the arterial baroreceptors and thermoreceptors (Turner, 1991). In addition, feedback control mechanisms, act directly on the central nervous system or indirectly via peripheral receptor systems (Turner, 1991). As the intensity of work effort increases, stimulation by efferent cardiac sympathetic neurons of the sinoatrial node and ventricular muscle causes release of noradrenaline which in turn binds to β-adrenergic receptors resulting in further increases in heart rate and myocardial contractility (Seals et al, 1994). The increase in sympathetic tone is caused by activation of the muscle metaboreceptor, by further

involvement of the baroreceptors, and possibly by activation of the muscle mechanoreceptors (O'Leary, 1993). During the steady state (phase three), prolonged exercise may be compromised by thermoregulatory and fluid homeostatic imbalances as well as limiting changes in substrate utilisation and delivery (Turner, 1991). At near maximal effort, parasympathetic activity is very low, sympathetic activity exists at greatly increased levels, and the release of adrenaline into the circulation will lead to further activation of the cardiac β-adrenergic system and greater stimulation of heart rate and ventricular contractility (Seals et al, 1994). As a result, heart rate, stroke volume and cardiac output all operate maximally. Following maximal exercise, heart rate decreases sharply due to a decrease in the enhanced sympathetic nerve activity; activation of parasympathetic activity plays a greater role once heart rate has partially recovered (Savin et al, 1982).

Foss and Keteyian (1998) classified the incoming stimuli into four functional groups: central command, humoral, physical and peripheral neural. These are summarised in Table 1.4.

Central motor command

Input into the cardiorespiratory centre occurs mostly via neurons that originate in the motor cortex and pass through the cardiorespiratory centre on their way to initiate a skeletal muscle action. The central command involves a parallel, simultaneous excitation of neuronal circuits controlling both the locomotor and cardiorespiratory systems. Actions initiated by the cardiorespiratory centres influence neural activity to the heart and blood vessels as well as neural outflow to the motor neurons innervating respiratory muscles. In addition central command can be initiated by mental conditions integrated by the hypothalamic region of the lymbic system.

Humoral stimuli

Humoral stimuli originate from changes in the chemical properties of blood or cerebro-spinal fluid, which ultimately influence receptors located elsewhere in the body. Once activated, these receptors provide afferent neural input to the cardiorespiratory area, which then evokes an appropriate response. The receptors are primarily chemoreceptors that are sensitive to changes in fluid chemistry, such as PO_2, PCO_2, K^+ concentration and/or pH. These chemoreceptors are located in the medulla (central chemoreceptors) or

elsewhere in the body, such as the aortic bodies or in the carotid bodies found at the bifurcation of the carotid arteries.

Physical stimuli

Changes in the physical characteristics of blood (e.g. pressure and volume) are classified as physical stimuli. There are pressure sensitive mechanoreceptors (the baroreceptors) located in the aortic arch and carotid arteries. Low pressure baroreceptors located in the atria, ventricles, pulmonary artery and pulmonary vein are also involved.

Peripheral neural stimuli

These stimuli originate from changes that take place in the lungs, muscles, joints, tendons and skin, and result in an afferent neural response being generated toward the cardiorespiratory areas in the brain. The feedback information they provide is concerned with (1) changes in the local chemistry (and possibly temperature) in and around the skeletal muscle; (2) muscle contraction and limb movement or tension development; and (3) intense pain, general discomfort and/or the presence of respiratory irritants.

During exercise, the respiratory rate increases to maintain optimal concentrations of O_2, CO_2 and H^+ in the tissues. There are similarities between control of the ventilatory system and cardiovascular regulation. Both the respiratory and the cardiovascular areas of the medulla receive impulses from the descending higher motor regions of the cerebrum and the ascending afferent impulses of the periphery (Weissman et al, 1979). The simultaneous increases in cardiac output and ventilation involves the direct activation of ventilation by a signal from the heart itself or from within the blood flowing from it – this is described as "cardiodynamic coupling' (Turner, 1991).

Table 1.4. Action of various stimuli on the cardiorespiratory areas of the brain stem and their effects on respiration and circulation (Foss and Keteyian, 1998).

	Action on Cardiorespiratory Areas				Effects		
			Receptor			Circulation	
Stimuli	**Feed-forward**	**Feed-back**	**Type**	**Location**	**Respiration Rate**	**Heart Rate**	**Arteriolar Tone**
Central command	✔				↑↑↑	↑↑↑	↑↑↑
Humoral							
Increased PCO_2, H^+, K^+	✔ ?	✔	Chemo	Brainstem, carotid arteries or aortic arch	↑↑	↑	↑
Decreased PO_2		✔	Chemo		↑	↑	↑
Increased (nor)adrenaline		✔	Chemo		↑	↑	↑
Physical							
Increased pressure		✔	Baro	Aortic arch, carotid arteries	↓	↓	↓
Increased volume		✔	Pressure or stretch	Right atrium/ pulmonary artery	↓	↓	↓
Peripheral neural							
Respiratory muscles		✔	Stretch or mechano	Muscle, joints, tendons	↑	↑?	?
Other skeletal muscle							
Mechanical		✔	Mechano	Muscle	↑?	↑	↑
Metabolic		✔	Metabo		↑?	↑↑	↑↑

1.2.2.2 Thermoregulation

Normally body temperature is regulated around a set point, and, generally, heat production is equal to heat loss (Gleeson, 1998). As heat production increases with exercise, heat is stored, increasing body temperature (Gleeson, 1998). Such a deviation from a set point is detected by sensory input from the body triggering reflexes that help to eliminate or in the case of core temperature being below the set point, to conserve heat. The thermoregulatory system of the body uses a thermal regulatory centre, thermal receptors and thermal effectors to carry out its function.

1.2.2.2.1 Thermal regulatory centre

The raphe nuclei located in the midbrain are key sites involved in integration of peripheral and central thermal stimuli and in processing of information ascending to the preoptic/anterior hypothalamic area (Bruck and Hinckle, 1980). Studies have shown that thermoafferent inputs from the skin converge in the hypothalamus (Cabanac, 1975; Ivanov et al, 1981) and it is at this site that incoming sensory information is coordinated with outgoing regulatory action (Gordon, 1986). The internal temperature is measured by receptors and compared with a set point and if the measured temperature deviates from the set point, the information is relayed to thermal effectors (see below), which correct the body temperature (McEwen and Heath, 1974).

1.2.2.2.2 Thermal receptors

Thermal receptors or sensors are sensitive to heat and cold stimuli and provide neural input to the co-ordinating centre. Central receptors are located in the anterior hypothalamus, peripheral receptors are located in the skin, thermoreceptors also exist in the spinal cord, abdominal viscera and deep body veins (Hensel, 1974). Peripheral receptors sense changes in ambient temperature while the internal receptors monitor changes in core temperature (Hensel, 1973). The heat receptors increase their rate of firing as the temperature of the blood perfusing the area increases, while the cold receptors increase their rate of firing when the temperature of the blood perfusing the area decreases (Hensel, 1974). There are three times as many heat receptors as cold receptors in the hypothalamus; these are sensitive to small temperature fluctuations within the arterial blood perfusing the area (Hensel, 1973). The heat, cold and pain receptors located just below the skin consist mainly of free nerve endings; the pain

receptors are sensitive to extremes of heat and cold (Hensel, 1973). In contrast to the hypothalamic receptors, there are three to ten times more cold than warm receptors in the peripheral and core body areas (Hensel, 1973).

1.2.2.2.3 Thermal effectors

Thermal effectors or organs are directed by the hypothalamic co-ordinating centre to produce regulatory or corrective changes these include skeletal muscle, smooth muscle encircling the arterioles that supply blood to the skin, the sweat glands, and certain endocrine glands (Benzinger, 1969). In warm conditions arterioles supplying blood to the skin dilate, facilitating heat dissipation and sweating occurs (Brengelmann, 1983). In the cold, cutaneous vasoconstriction occurs, decreasing the amount of heat lost to the environment through the skin, the metabolic rate is also increased by shivering and there may be an increase in the secretion of adrenaline from the adrenal medulla (Lossec et al, 1998). In addition to non-voluntary reflexes that help to eliminate or conserve body heat, cortical connections provide a means for voluntary (behavioural) regulation, for example seeking or avoiding shade, removing or adding clothing (Benzinger, 1969).

1.3 THE SLEEP-WAKE CYCLE

As noted in the introduction to this chapter, sleep is relevant to the present study. Peak secretion of many of the hormones in the human body relies on circadian rhythms as will be covered in a later section. We would therefore assume that for optimal physical training and performance good quality sleep is important. However it has been shown that physical and well learned tasks show little degradation due to sleep deprivation (Copes and Rosentswieg, 1972; Myles, 1985). A more comprehensive review of the physiology and the function of sleep may reveal more about the importance of sleep for optimal exercise performance.

1.3.1 Sleep Stages

Normal sleep consists of two types: non-rapid eye movement sleep (non-REM) and rapid eye movement (REM) sleep. Non-REM sleep consists of four stages, each of these are characterised below along with REM sleep (Savis, 1994). During a sleep episode the electrical activity of the brain cycles through stage one to stage four sleep then into REM sleep, the activity is then repeated starting from stage three sleep (Borbely, 1998). The features of non-REM and REM sleep are summarised in Table 1.5 and will be revisited in the discussion on the functions of sleep.

Stage 1:

This is the transition stage between waking and sleep it involves deepening respiration, decreased responsiveness and drifting thoughts. This stage is short, however anxiety and depression can lengthen it, delaying more restorative sleep.

Stage 2:

While this stage of sleep is not classified as restorative it makes up about 50% of total time asleep in adults. Decreasing time asleep over a period of weeks is at the expense of time spent in stage two sleep.

Stage 3 and Stage 4 (collectively know as delta sleep)

These are periods of relatively deep sleep and make up 15 to 20% of total time asleep. Delta sleep is considered as restorative sleep and during this time growth hormone secretion and cell division are at their circadian peak while metabolic rate, respiration, core temperature and heart rate are at their circadian low (Shapiro, 1982). When sleep is restricted, delta sleep is maintained.

REM Sleep

REM sleep which makes up 20 to 25% of the total sleep episode first occurs after about 90 minutes after sleep onset, it lasts only a few minutes but the duration of each episode lengthens throughout the sleep period. During this period cerebral blood flow, brain temperature, brain protein synthesis and heart rate are increased.

Table 1.5. Features of non-REM and REM sleep (Rechtschaffen, 1998).

Descriptive Features of Non-REM Sleep	Descriptive Features of REM Sleep
Systemic Features • Reductions in motor activity, postural tonus, behavioural responsiveness, metabolic rate, respiration rate, ventilatory response to CO_2, vasomotor tone, arterial blood pressure, brain and body temperatures, thermoregulatory setpoint, renal function, decreased intestinal motility • General parasympathetic dominance *Endocrine Features* • Reductions in release of cortisol and thyrotrophin • Increased secretion of growth hormone, aldosterone, testosterone, prolactin, insulin • Increased glucose levels *Cerebral Features* • Drifting, unfocused thought; occasional dreams; occasional reports of no mental activity • Decreased activation of forebrain by reticular system • Hyperpolarisation of thalamo-cortical neurons • Decreased neuronal firing in some areas; increases in others • Burst-pause firing pattern of neurons in several major brain areas • "Sleep-active" neurons in anterior hypothalamus, basal forebrain, amygdala, and nucleus of the solitary tract • Reduced cerebral metabolism during slow wave sleep • Cerebral blood flow varies regionally • Cerebral temperature decreases	*Motor and Vegetative Features* • Rapid eye movements • Diminished baroreflexes • Pupil constriction and phasic dilation • Reduced behavioural responsiveness • Irregular heart rate • Irregular respiration • Irregular blood pressure • Penile erections • Actively induced motor inhibition, interrupted by phasic twitches • Decreased temperature regulation • Further reductions of ventilatory response to CO_2 during phasic REM • Vasodilation in tonic REM (except in red muscle); constriction in phasic REM • Whole body metabolic rate increased *Cerebral Features* • Abundant dreaming • Suspension of reflective thought • Increased cerebral metabolism • Increased cerebral blood flow • Increased intracranial pressure • Increased brain temperature • Hypersynchronous hippocampal theta EEG • Increased neuronal activity in pyramidal tract; visual cortex; brainstem reticular formation; laterodorsal tegmental nucleus; pedunculopontine nucleus • Decreased neuronal activity in dorsal raphe and locus coeruleus • PGO spikes and associated phenomena

1.3.2 The Functions of Sleep

The specific functions of sleep have not been clearly defined, the roles of sleep are continually debated in the literature and it is likely that sleep plays a part in maintaining many of the processes that occur in the human body. There are five indicators that sleep is functionally important (Rechtschaffen, 1998):

1. Sleep is ever-present among mammals, birds and reptiles,
2. Sleep has persisted in evolution even though it is apparently maladaptive with respect to other functions,
3. Functional accommodations are made to permit sleep in different environments and life styles,
4. Sleep is homeostatically regulated, and
5. Serious physiological changes may result from prolonged sleep deprivation of animals.

Hirshkowitz et al (1997) outlined three theories of sleep function. The first is *the adaptive theory*, which states that sleeping increases an animal's survival probability by avoiding danger. For example, an animal that is visually oriented without highly adapted night vision becomes relatively immobile when the sun goes down; otherwise they become food. The second theory, *the energy conservation theory*, emphasizes overall decreased metabolism during sleep. Animals with a high metabolic rate sleep longer than those with lower metabolic rates. The metabolic rate is reduced during sleep by between 5 and 25% (Shapiro and Flanigan, 1993). In periods of high-energy consumption such as during adolescence, exercise, pregnancy and hyperthyroidism, more sleep is required by the individual, although a 90kg individual only saves 500 calories by sleeping 8hrs rather than remaining in a state of relaxed wakefulness (Shapiro and Flanigan, 1993). The last is *the restorative theory,* which states that the neural mechanisms suggested to underlie sleep-related restoration include neutralization of accumulated neurotoxins, responses to increased wakefulness-related, sleep-inducing substances, neurochemical synthesis and redistribution of brain chemicals (Drucker-Colin, 1979, In: Hirshkowitz et al, 1997).

In order to establish a further function of sleep perhaps we should consider the stages of sleep (outlined in section 1.3.1) independently, as it is evident from the features of the

sleep stages that they have different restorative purposes. Shapiro and Flanigan (1993) state that during non-REM sleep there is a high amount of macromolecular synthesis occurring as the rate of anabolism is thought to peak during non-REM sleep, while during REM sleep the synthetic products of delta sleep are removed to maintain synaptic connections. In support of the hypothesis that tissue restitution occurs during slow wave sleep, Adam and Oswald (1977) stated that there is an increase in tissue synthesis, cell division and growth hormone release during delta sleep. They also state that total delta sleep increases in situations when increased tissue restitution is required such as in athletes and children. The programming-reprogramming hypothesis states that REM sleep clears unimportant information from memory and consolidates more important experience (Dewan, 1968). Infants, whose brains are engaged in intense programming, sleep up to twice as long as adults, a large proportion of this is REM sleep (Hirshkowitz et al, 1997). Periods of intense learning in adults are accompanied by an increase in REM sleep (Hirshkowitz et al, 1997; Smith and Lapp, 1991).

1.3.3 The Physiology of Sleep

Sleep is regulated by homeostatic, circadian and ultradian processes (Borbely, 1998). Humans sleep and awaken in a fairly constant 24-hour cycle called a circadian rhythm that is established by an area of the hypothalamus called the suprachiasmatic nucleus. Borbely (1998) described a two-process model of sleep regulation in which a homeostatic process (Process S) rises during waking and declines during sleep, and interacts with a circadian process (Process C) that is independent of sleep and waking. Slow and delta wave activity, the marker of Process S, is shown to be determined mainly by homeostatic (i.e. sleep-waking dependent) factors, whereas REM sleep (or rather the REM sleep: non-REM sleep ratio) apparently depends on both homeostatic and circadian factors (Borbely, 1998).

The difference in electrical activity of the brain during waking and sleep appears to be due to the inherent activity of the reticular formation or more specifically the reticular activating system (RAS) (Garcia-Rill, 1997). There are two types of neurons in the RAS, cholinergic and noradrenergic, both send ascending projections to the substantia nigra and other basal ganglia structures, to the basal forebrain, hypothalamus, cerebellum and most areas of the thalamus (Garcia-Rill, 1997). In addition,

noradrenergic neurons also send ascending projections to the cerebral cortex and descending projections to the spinal cord (Garcia-Rill, 1997). Both the neuronal cell groups appear to be active during certain sleep-wake states but reciprocally active during others. Cholinergic neurons appear to be active during waking and REM sleep but not during non-REM sleep, whereas noradrenergic neurons appear to be more active during waking and non-REM sleep but inactive during REM sleep (Garcia-Rill, 1997). In other words during waking both the cortex and the spinal cord turned on, during non-REM sleep both are turned down or during REM sleep the cortex is turned on while the spinal cord is turned off.

Thalamocortical loops are formed through neuronal connections between the thalamus and the cortex (Hobson, 1995). The activity in the thalamocortical loops during the stages of the sleep-wake cycle is controlled by different mechanisms. During waking, the midbrain reticular formation is responsible for keeping the minimum level of activity high enough so that external data can be processed (Hobson, 1995). The circuit changes during non-REM sleep and it is believed that the mechanism for this change originates in neurons in the hypothalamus and lower brainstem and can be described as follows (Hobson, 1995). At the beginning of sleep, the brainstem excitatory drive upon the thalamocortical system falls, decreasing the level of activity in the thalamocortical loops. When the activity drops below a certain level the loops begin to oscillate. This causes both the thalamic and cortical neurons to fire; once oscillation is great enough this part of the brain is no longer able to process external information. The successive waves recorded by the EEG augment in amplitude, reach a peak, and then decrease as the inhibitory interneurons gradually impose more restraint. Hyperpolarisation of thalamocortical neurons during sleep induces fluctuations in membrane potential in the frequency range of the sleep EEG.

At the beginning of REM sleep there is a reactivation of the reticular formation, so that with its gradual rise in firing level the tendency for the thalamocortical loops to oscillate is suppressed (Hobson, 1995). During REM sleep, the central motor neurons are active causing eye movements, while the motor neurons in the spinal cord that control movement in other parts of the body are inhibited (Hobson, 1995). There is clustered firing of the motor neurons that send movement commands to the spinal cord, however no actual movement occurs as there is an inhibition to the spinal cord from the reticular

formation (Hobson, 1995). Many of the sensory systems are activated during REM sleep including those that relay information to the cortex regarding audition, vision and touch (Hobson, 1995).

1.3.4 Sleep and Athletic Performance

It has been shown that sleep quality and the sleep patterns of athletes are significantly different from those of non-athletes (Porter and Horne, 1981). Many factors can have an impact on a competitive athlete's sleep quality and quantity which could possibly result on compromised athletic performance. These factors during competition are often a result of travel and could include changes such as diet, ambient temperature, altitude, time zones and anxiety. Eastbound travel places greater adaptation demands than westbound, it is easier to delay the sleep episode in westbound travel than it is to contract it in eastbound (Davis, 1988). It is thought that anxiety resulting from travel and competition can result in frequent awakenings and less total sleep time (Adam et at, 1986). The positive effects of training such as increases in muscle mass and aerobic capacity, and the impact of poor sleep quality on these have not been studied as extensively as the effect of poor sleep on athletic performance during competition. Most studies show an increase in the amount and intensity of delta sleep with both aerobic and anaerobic training (Driver et al, 1988; Shapiro, 1975). This could indicate the importance of this sleep stage in training recovery.

Several studies have investigated the effect of sleep deprivation on physical performance by examining subjective and cardiorespiratory parameters. The results of these studies are conflicting and will be reviewed here. The differences in the outcome of these studies could be due to discrepancies in experimental design. It has been shown that 60 hours of sleep deprivation increases ratings of perceived exertion (RPE) on a Borg scale for exercise bouts of greater than eight minutes (Myles, 1985). In another study, involving a sleep deprivation regimen of only 30 hours, when participants were asked to self-select an intensity level on the treadmill to correspond to a set RPE there were no differences between the intensities selected by those in the sleep-deprived group and those in the control group (Martin and Haney, 1982). They also found that there was no difference in VO_2 or VCO_2 between the sleep deprived and control groups but did find that heart rate during exercise was lower in the sleep deprived group.

Martin and Gaddis (1981) found that after 30 hours of sleep deprivation the RPE was not significantly increased from initial values after light exercise (25% VO_{2max}) lasting eight minutes but was increased after moderate (50% VO_{2max}) and heavy exercise (75% VO_{2max}) of the same duration. This study found no physiological differences in heart rate, VO_2, minute ventilation, VCO_2 and mean blood pressure. A similar study, using a sleep deprivation period of 72 hours and exercise at 40%, 60% and 80% of VO_{2max}, found no differences between the sleep deprived group and the control group for heart rate, VO_2, VCO_2 and the respiratory quotient (Horne and Pettitt, 1984). In another study where participants experienced only three hours of sleep deprivation, heart rate and ventilation increased while final VO_2 reached during the test to exhaustion decreased (Mougin et al, 1991). Plyley et al (1987) investigated the effect of one hour of mild exercise (28% VO_{2max}) out of every three hours during a total period of 64 hours of sleep deprivation. They found that there was a significant decrease of 3.8ml/kg/min in VO_{2max} measured immediately after the sleep deprivation period. It has been shown that 36 hours of sleep deprivation causes 11% decreases in the time to exhaustion during treadmill exercise at 80% VO_{2max} (Martin, 1981). Examination of the studies together reveals that to date there is no agreement about the effect of sleep deprivation on athletic performance. They loosely indicate that sleep deprivation may result in an increased RPE at higher exercise intensities and a decrease in time to exhaustion, while the cardiorespiratory changes during exercise are not affected.

The effect of sleep deprivation on body temperature has also been investigated. It has been shown that core temperature, measured orally, maintains its circadian variation until at least 130 hours of sleep loss, however there was a lowering of the mean daily oral temperature (Horne and Pettitt, 1984). A sleep deprivation period of 50 hours results in a decrease in resting rectal temperature (Kolka et al, 1984). However, Martin et al (1986), showed no change in rectal temperature after only 36 hours sleep deprivation. It is not clear whether the effect of a lower core body temperature, as a result sleep deprivation, is beneficial or detrimental to physical performance. On the one hand, a lower core body temperature at the onset of exercise may delay heat stress that accompanies prolonged exercise or it may reflect a decreased metabolism which may adversely affect performance (VanHelder and Radomski, 1989). The effects of sleep deprivation on hormone secretion will be discussed later.

1.4 ALCOHOL

In New Zealand many young athletes engage in social drinking, particularly after competition. Table 1.6 shows the percentages of players who drink alcohol for three commonly played sports in NZ, rugby union, rugby league and netball. Table 1.7 shows the percentages of people who consume alcohol before and after training and before and after a game for the same three sports. The information communicated in these tables only refers to players under the age of 21 years for rugby union and rugby league, while a range of ages were surveyed for netball (Dowden, 1998). If all age groups had been studied in this case, the results may have differed.

As alcohol is consumed by a large proportion of people who participate in sport it is important that we are aware of the potential cumulative effects, if any, on fitness levels as well as on performance if consumption occurs the night before a training session or competition. While reviewing this we should remember that responses to alcohol differ widely between individuals and within an individual during different drinking episodes.

Table 1.6. Types of drinker (%) for three commonly played sports in NZ (Dowden, 1998).

Sport	Non-Drinker	Light-Drinker*	Heavy-Drinker**
Rugby Union	4	43	53
Rugby League	16	58	26
Netball	16	64	20

*A light drinker is defined as 6 or less drinks in one drinking session for a male and 4 or less for a female.

**A heavy drinker is defined as 7 or more drinks in one drinking session for a male and 5 or more for a female.

Table 1.7. The percentage of players participating in different sports who consume alcohol, of those who consume before and after training and before and after a game (Dowden, 1998).

Sport	% players over 20 years that Drink	Level of Drinking	Training		Game	
			24hrs Before	After	24hrs Before	After
Rugby Union	98	Heavy**	17	50	21	100
		Light*	14	26	8	62
Rugby League	77	Heavy	16	48	18	100
		Light	2	16	8	76
Netball†	82	Heavy	6	29	12	100
		Light	4	13	13	51

† small sample size, indicative only

*A light drinker is defined as 6 or less drinks in one drinking session for a male and 4 or less for a female.

**A heavy drinker is defined as 7 or more drinks in one drinking session for a male and 5 or more for a female.

1.4.1 Effect of Alcohol on Organ Systems

The consumption of alcohol can affect virtually every organ system in the body: nervous, cardiovascular, digestive, reproductive, endocrine, musculoskeletal, immune and respiratory.

1.4.1.1 Acute effects of alcohol consumption

Ethanol easily permeates the blood-brain barrier (Davson, 1955), after which it causes suppression of the increase in sodium conductance which normally accompanies stimulation, depressing membrane excitability and affecting the central nervous system (Armstrong and Binstock, 1964). Vision is impaired; visual acuity is diminished, tunnel vision develops and the field of vision narrows (Santamaria, 1989). Cognitive function is affected; thinking becomes difficult, concentration, learning, memory and judgement are impaired, self awareness decreases and the consumer has an inappropriate increase in self-confidence (Santamaria, 1989). Motor control is affected, reaction times are

slowed and there is an increasing degree of incoordination, as blood alcohol concentration increases these effects become exaggerated and additional effects including nystagmus and ataxia (Santamaria, 1989). The effects on behaviour vary between individuals and include vivacity, maudlin conduct, increasing apathy, moroseness, violent actions and deepening somnolence (Santamaria, 1989). At very high blood alcohol levels drowsiness gives way to deepening coma, respiratory paralysis and death (Santamaria, 1989).

1.4.1.2 Chronic effects of alcohol consumption

Several syndromes may occur in chronic alcoholics. The literature reviewed below does not define 'chronic ingestion', however the Alcohol Advisory Council of New Zealand defines chronic ingestion of alcohol as 21 or more standard drinks per week for men and 14 or more standard drinks per week for women, and states that at this level alcohol consumption is detrimental to health (Alcohol Advisory Council Pamphlet). Korsakoff's syndrome is a form of amnesia where the person has great difficultly in acquiring new information as memory is lost after a delay of only a few seconds and is associated with the destruction of structures close to the central axis of the brain (Santamaria, 1989). Wernicke's encephalopathy involves disturbed consciousness and confusion, abnormalities of ocular movements, ataxia, nystagmus and the inability to concentrate (Santamaria, 1989). Other permanent damage to the central nervous system can include cerebellar ataxia and demylination of nerve fibres in the pons and corpus callosum (Santamaria, 1989).

Alcohol has a number of adverse effects on the cardiovascular system including hypertension, cardiomyopathy and arrythmias (Regan, 1990). Chronic alcohol ingestion is likely to have a damaging effect on heart tissue leading to abnormal heart function, irreversible cell death and scarring (Reiken, 1991). The effect of chronic consumption of alcohol resulting in an increased chance of suffering from hypertension is extensively reviewed in Keil et al (1993). There is also an increased risk of suffering from stroke (van Gijn et al, 1993) and coronary heart disease (Renaud et al, 1993).

Alcohol can cause acute gastritis due to an increase in mucus production coupled with a decrease in acid production (Doll et al, 1993). Chronic alcohol abuse is likely to cause hypoglycaemia due to damage of the pancreas, alcoholic hepatitis, fatty liver and

cirrhosis (Rodes et al, 1993). Fertility in both males and females is decreased due to damage caused to the reproductive systems and sexual desire and function may also be affected indirectly via the endocrine system (Galaver and Van Thiel, 1989).

1.4.2 Effect of Alcohol on Sleep Patterns

Ingestion of alcohol prior to sleep has been shown to decrease sleep latency, meaning that the time taken to fall asleep decreases (Slade, 1989 IN: Savis, 1994), and significantly increase time spent in stage 4 sleep, primarily at the expense of time in stage 2 (Lands, 1999).

1.4.3 Effect of Alcohol and Alcohol Hangover on Athletic Performance

Participation in sport or exercise is more likely to occur when the participant is suffering from an alcohol hangover rather than while intoxicated. Upon reviewing the literature it is clear that there is a lack of research into the effect of an alcohol hangover on athletic performance. There is however a significant amount of research on both the acute and chronic effects of alcohol on both the physiological systems of the body and on athletic performance. Findings suggest that acute ingestion of alcohol before exercise should be actively discouraged as it adversely affects athletic performance (Stainback, 1997).

The medical hazards of alcohol intoxication during sport include; weakened left ventricle contraction, myocardial irritability resulting in arrhythmias which are potentially fatal, exercise induced anaphylaxis and asthma, and vestibular system dysfunction (O'Brien and Lyons, 2000). These then lead to the following changes to athletic performance; reduction in the heart's pumping ability and an increase in heart rate, an increase in blood pressure, impaired temperature regulation, excess loss of body heat caused by vasodilation of blood vessels, slowed reaction time, reductions in strength, balance, steadiness, and fine and complex motor coordination, and information processing (O'Brien and Lyons, 2000).

Alcohol hangover is characterised by headache, tremulousness, nausea, diarrhoea and fatigue, combined with impaired occupational, cognitive or visual-spatial skill performance (Wiese et al, 2000). Symptoms are caused by dehydration, hormonal

alterations, dysregulated cytokine pathways and toxic effects of alcohol (Wiese et al, 2000), shown in Table 1.8. The physiological characteristics include increased cardiac work with normal peripheral resistance, diffuse slowing shown on electroencephalography and increased levels of antidiuretic hormone (Wiese et al, 2000).

Table 1.8. Effects of alcohol intoxication and subsequent hangover on serum hormone concentration (adapted from: Wiese et al, 2000)

Hormones and Electrolytes Affected	Effects of Acute Alcohol Intoxication	Effects of Hangover	
		Hormone Level	Proportional to Severity
Antidiuretic hormone	Decreased	Increased	Yes
Aldosterone	Decreased	Increased	No
Renin	Decreased	Increased	No
Insulin response to glucose	Increased	Unknown	No
Glucagon	Increased	Unknown	Unknown
Cortisol	Unchanged or decreased	Increased	No
Thyroid stimulating hormone	Unchanged	Unchanged	No
Growth hormone	Unchanged	Decreased	No
Acid-base states	Metabolic acidosis	Metabolic acidosis	Yes
Electrolytes	Unchanged	Unchanged	No
Testosterone	Unchanged	Unchanged	No
Follicle-stimulating hormone and lutenising hormone	Unchanged	Unchanged	No
Prolactin secretion in response to thyroid stimulating hormone	Increased	Inhibited	Unknown

Sport performance is likely to be impaired in an athlete suffering from alcohol hangover due to a number of physiological changes that occur during a hangover; the severity of these changes may depend on the quantities of alcohol consumed. The haemodynamic changes include an increase in heart rate, left ventricular performance and blood pressure (Weise et al, 2000). Sports performance in chronic abusers of alcohol will be negatively affected by decreased cardiovascular efficiency, muscular strength and endurance, less than optimal body composition and poor nutrition (O'Brien and Lyons,

2000). Table 1.9 shows that those athletes who drink are also more likely to suffer from injury.

Table 1.9. The pattern of alcohol use and injury in 13 selected sports (O'Brien and Lyons, 2000).

Sport	% Who Drink	Mean Alcohol/Week (units*)	% Who Drink and Play/Train Next Day	Drinkers Injured (%)	Non-Drinkers Injured (%)
Gaelic football	77	15.3	75	71	18
Soccer	74	15	65	59	17
Hurling	84	22.1	71	71	14
Rugby	88	19.3	75	76	66
Basketball	68	10.3	68	69	16
US football	70	17.3	70	54	14
Track and field	61	10	56	64	22
Cycling	25	7.1	18	20	16
Rowing	48	10.7	36	44	14
Cricket	89	24.8	84	63	0
Tennis	40	13.3	40	83	33
Golf	76	15.8	70	38	0
Horse racing	20	10	20	0	75
Mean	63.08	14.69	57.54	54.77	23.46
SD	23.01	5.21	21.82	23.72	22.57

* 1 Unit = 1 standard drink = 10g alcohol

1.5 ENDOCRINOLOGY

Changes in plasma hormone concentrations during exercise and training are summarised in Table 1.10.

Table 1.10 Summary of the changes in plasma hormone levels during exercise and training (Foss and Keteyian, 1998).

Hormone	Exercise Changes	Training Changes	Significance
Hypothalamic releasing hormones	Some likely increase with increased intensity	Unknown but by association a dampened effect is likely	Close neural tie-in to movement/stress
Growth hormone	Increases with increasing exercise intensities	Lesser response in trained subjects	Increase in fatty acid mobilisation and gluconeogenesis
Thyroid stimulating hormone	Increases with increasing exercise intensities	Unknown	Unknown
Adrenocorticotropin	Increases with increasing exercise intensities	Unknown	Increases secretion of glucocorticoids
Thyroxine	Little or no change	Increase of thyroxin turn-over without toxic effects	Unknown
Calcitonin and parathyroid hormone	Unclear	Unknown	Important for normal bone development and bone health
Glucocorticoids (cortisol)	Increases with increasing exercise intensities	Increases less for the same work rate, may increase more with exhaustion	Increased glycogen deposition, liver gluconeogenesis, lipolysis and anti-inflammatory effect
Aldosterone	Increases with increasing rates of performing work	May increase less for the same absolute work rate	Maintenance of plasma volume

Hormone	Exercise Changes	Training Changes	Significance
Adrenaline	Little change with short periods of light work, increases with intensity and duration	Increases less for the same absolute work rate	Increases blood glucose, muscle blood flow, heart rate and contractility
Noradrenaline	Relative marked increases with increases in work rate; unsure of adrenal vs. sympathetic source	Increases less for the same absolute work rate	Blood pressure control, heart rate and contractility
Insulin	Decreases with increasing rates of performing work	Decreases less after training	Reduces the stimulus to utilise blood glucose
Glucagon	Increases with exercise duration but decreases from an elevated level with high intensity/short duration activity	Increases less following training	Increases blood glucose by glycogenolysis and gluconeogenesis
Erythropoietin	Unknown, but postulated to increase with exercise	Unknown	Increased production of red blood cells
Oestrogens and progesterone	Increase with higher rates of performing work	Unknown	Unknown
Testosterone	Increases with higher rates of performing work	Unknown	Unknown

1.5.1 Catecholamines

1.5.1.1 Non-exercise secretion of catecholamines

1.5.1.1.1 Control of catecholamine secretion

Principal among the systems that move the body to a new level of homeostasis during exercise are the central nervous system and the endocrine system. The central nervous

system is capable of making very rapid adjustments to large segments of the body, while the endocrine system can have a more global and far reaching effect but requires more time to respond (Mazzeo, 1991). Secretion of the catecholamines is initiated by sympathetic activity controlled by the brain as mentioned previously. The catecholamines both as the neurotransmitter, noradrenaline, released from sympathetic nerve terminals and the hormone, adrenaline, secreted by the adrenal medulla, have very powerful regulatory properties that exert control over a number of critical physiological and metabolic functions central to the ability to sustain physical exercise. Included among these responses are; their capacities to affect cardiac function and metabolism, to redirect blood flow to working muscle, and to promote substrate mobilisation and utilisation (Mazzeo, 1991). Noradrenaline spills over from sympathetic nerve terminals into the blood stream and during rest is 3-4 times greater in blood than adrenaline as its release from the adrenal medulla is low (Mazzeo, 1991).

1.5.1.1.2 Factors affecting catecholamine secretion

Several factors may modulate sympathetic activity. Sleep deprivation has been shown to increase the concentration of catecholamines in plasma (Opstad et al, 1980) while in another study this effect was not seen (Fiorica et al, 1968). During exercise even small decreases in plasma glucose concentration enhance adrenaline secretion, while plasma noradrenaline is less influenced by glucose concentrations (Kjaer et al, 1987). Insulin availability in the time preceding exercise is also inversely related to the catecholamine response, and secretion of the catecholamines is exaggerated in states of insulin deficiency such as fasting, during periods when a high fat diet is consumed or during poorly controlled diabetes mellitus (Kjaer et al, 1987).

1.5.1.2 Secretion of catecholamines during exercise

The extent of the catecholamine response to exercise is dependent on exercise intensity, duration and the training status of the individual. During progression to VO_{2max}, plasma catecholamines show a positive exponential relationship to workload (Mazzeo, 1991). Galbo et al (1975) stated that there is a six-fold increase in adrenaline and noradrenaline concentrations during graded exercise. The ratio of noradrenaline to adrenaline in plasma remains similar to that at rest. This suggests that the response is predominantly mediated by the sympathetic nervous system as opposed to an adrenal response alone

(Mazzeo, 1991). During submaximal prolonged exercise to exhaustion, the adrenal response is elicited in response to reductions in blood glucose concentration (Mazzeo, 1991). Catecholamine recovery to resting levels takes longer after prolonged exercise to exhaustion than after short duration, more intense exercise; plasma levels returned to normal after 30-minutes following graded exercise (Galbo et al, 1975).

1.5.1.3 Training effects

It is well documented that for a given submaximal workload both plasma noradrenaline and adrenaline levels are reduced in the endurance-trained individuals compared with sedentary counterparts (Mazzeo, 1991). This most likely represents reduced sympathetic nervous system stimulation as a result of the exercise being less of a relative stress as well as the individual having a better ability to maintain blood glucose, thereby reducing the need for secretion of catecholamines (Mazzeo, 1991). The ability to synthesise catecholamines in the adrenal medulla is enhanced with endurance training, and at maximal exercise their secretion in trained individuals is greater than for untrained persons (Mazzeo, 1991).

1.5.2 Testosterone

1.5.2.1 Non-exercise secretion of testosterone

1.5.2.1.1 Control of testosterone secretion

Testosterone is the primary hormone in males responsible for reproductive function and the development of secondary sex characteristics (Hadley, 1996a). The secretory pathway of testosterone begins at the hypothalamus where gonadotropin-releasing hormone (GnRH) is secreted into the hypothalamic-pituitary portal blood flow and stimulates the secretion of two hormones, lutenizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary gland (Hadley, 1996b). Lutenizing hormone then stimulates the secretion of testosterone from the Leydig cells within the testes (Hadley, 1996a).

1.5.2.1.2 Patterns of testosterone secretion

In young adult men, the nocturnal rise of testosterone begins shortly after falling asleep, peaks at about the first REM sleep period and remains constant until awakening

(Luboshitzky et al, 1999). This nocturnal rise in testosterone is probably controlled by hormones other than LH as the increase in LH levels is of modest magnitude and inconsistent (Luboshitzky et al, 1999). In older men, decreases in sleep efficiency, the number of REM sleep episodes and an increase in REM sleep latency are associated with lower circulating testosterone levels (Schiavi et al, 1993). In young subjects with sleep disorders, an inverse correlation between the severity of sleep apnoea and testosterone levels has been demonstrated (Grunstein et al, 1989).

1.5.2.2 Secretion of testosterone during exercise

There is an increase in plasma testosterone concentration relative to the intensity of exercise, and the size of the muscle mass involved (Cadoux-Hudson et al, 1985). However endurance exercise of a long duration (three hours) produces a decrease in blood testosterone concentration which can last from hours to days (Urhausen, and Kindermann, 1987).

1.5.2.3 Training effects

A decrease in basal levels of testosterone has been reported in endurance trained athletes (Hackney, 1996). This can have negative effects on both sperm count and motility and is likely to be caused by disruptions to the hypothalamic-pituitary-gonadal axis as a result of such changes as increased plasma cortisol levels, suppressing LH receptors (Hackney, 1996).

1.5.3 Cortisol

1.5.3.1 Non-exercise secretion of cortisol

1.5.3.1.1 Control of cortisol secretion

Cortisol is a glucocorticoid hormone which is the end product of the hypothalamic-pituitary-adrenocortical system. Corticotropin releasing factor (CRF) is secreted by the hypothalamus which stimulates the anterior pituitary gland to release adrenocorticotropic hormone (ACTH), which then stimulates the adrenal cortex to release cortisol (MacLaren et al, 1999). Cortisol exerts a negative feedback on the hypothalamus and anterior pituitary gland inhibiting the secretion of CRF and ACTH,

thereby inhibiting further cortisol secretion by the adrenal cortex (MacLaren et al, 1999).

1.5.3.1.2 Patterns of cortisol secretion

The cortisol secretory pattern is dominated by a circadian rhythm which closely reflects the circadian pattern of adrenocorticotropic activity (Weitzman et al, 1971). In normal young adults levels of cortisol are highest in the early morning, decline throughout the day, are lowest around midnight and rise rapidly during the second half of the sleep episode when REM sleep predominates, reaching a peak just before waking (Weitzman et al, 1971). Sleep onset has an inhibitory effect on cortisol secretion that persists through the first few hours of sleep, whereas remaining awake during the usual sleep time results in elevated cortisol levels (Weitzman et al, 1971). If sleep occurs during the day the changes in cortisol levels are less marked than at night (Pietrowsky et al, 1994). During the day changes in cortisol concentration are due to changes in pulse amplitude rather than pulse frequency (Follenius et al, 1992).

Weitzman et al (1971) analysed the data obtained from twenty-minute samples of seven twenty-four hour studies and from the results divided adrenocortical activity over the twenty-four hour sleep-wake cycle of normal men into four phases, as described below. Phase 1 and phase 3 shown in this study are supported in a study by Quabbe et al (1982) who demonstrated that plasma cortisol showed a circadian rhythm with a late evening minimum and an early morning maximum.

Phase 1: (Minimal secretory activity)

This episode begins approximately four hours before and continues for two hours after sleep onset. During this time cortisol secretion is minimal.

Phase 2: (Preliminary nocturnal secretory episode)

A single isolated secretory episode occurs during the third and fifth hours of sleep.

Phase 3: (Main secretory episode)

This occurs during the sixth and eighth hours of sleep and continues through the first hour of wakefulness, involves a series of three to five secretory episodes and is due to the close clustering of secretory episodes. It represents the most intense time of adrenal cortical activity and accounts for nearly half of the total hormone secreted over twenty-four hours.

Phase 4: (Intermittent waking secretory activity)

Between four and nine secretory episodes are found in this eleven-hour compartment spanning the second to twelfth hours of the waking period; there is considerable variability in the secretory output.

During sleep deprivation it has been shown that cortisol maintains its circadian secretory rhythmicity (Hellman et al, 1970). It has also been shown that sleep deprivation of up to 80 hours does not change plasma cortisol concentration (Martin et al, 1986). The twenty-four hour protocol of cortisol concentration in participants on a regular sleep wake schedule is largely comparable to that of participants remaining awake throughout the night on a constant routine protocol (Czeisler and Klerman, 1999). Steiger et al, (1987) raised the question as to whether the secretory patterns of cortisol and growth hormone opposed each other, however from their research they failed to establish such a relationship.

1.5.3.1.3 Factors affecting cortisol secretion

Several factors independent of exercise can affect plasma cortisol concentration such as hypoglycaemia (Ellis et al, 1990), pain (Mellor et al, 2002), restraint (Geverink et al, 2002), trauma (Offner et al, 2002), burns (Murton et al, 1998) and shock (Moran et al, 1994).

1.5.3.2 Secretion of cortisol during exercise

Early studies reported that blood cortisol concentration only increased at heavy workloads (Hartley et al, 1972). Since then further studies have shown that the concentration of cortisol increases linearly, in proportion to the exercise intensity (Chicharro et al, 1998). Elevated cortisol levels return to basal levels within hours of completing exercise (Urhausen et al, 1995). Like GH it has been shown that the cortisol response to a 30 minute cycling test at 70% VO_{2max} is greater after three and five days of experiencing no organised sleep than on the first day (Opstad et al, 1980).

1.5.3.3 Training effects

The effect of training on the cortisol response to exercise is not well understood. However it is thought that as cortisol is secreted in humans in response to physical

stress, secretion of cortisol in untrained individuals is greater compared to trained individuals at similar workloads (Chicharro et al, 1998).

1.5.4 Growth Hormone

1.5.4.1 Non-exercise secretion of growth hormone

1.5.4.1.1 Control of growth hormone secretion

Growth hormone is secreted from the anterior pituitary gland (Muller et al, 1999) and is regulated by two hypothalamic peptides. Growth hormone releasing factor (GRF) is secreted by the hypothalamus into the hypothalamic-pituitary portal blood where it binds to receptors on the membrane of somatotrophs to stimulate growth hormone secretion and transcription of new growth hormone mRNA (Muller et al, 1999). Somatostatin is also secreted by the hypothalamus and suppresses growth hormone release in two ways; (1) neurons in the preoptic area release somatostatin targeted at GRF-producing hypothalamic cells, thus suppressing GRF release, and (2) somatostatin is secreted into the hypothalamic-pituitary portal circulation and suppresses growth hormone release from the pituitary gland following binding to receptors on somatotrophs in the anterior pituitary (Muller et al, 1999).

Growth hormone is released from the anterior pituitary gland in response to GRF stimulation when somatostatin levels are low (Plotsky and Vale, 1985). This response to GRF is blunted when somatostatin levels are elevated (Plotsky and Vale, 1985). Pulsatile secretion of growth hormone in humans is correlated with fluctuations in plasma somatostatin levels, even during continuously elevated GRF levels (Vance et al, 1985). Somatostatin and growth hormone down-regulate pituitary somatostatin receptor levels, reducing the inhibitory effects of somatostatin at the level of the pituitary, in turn enhancing the responsiveness of the pituitary to GRF (Strobl and Thomas, 1994).

Growth hormone infusion in humans rapidly and completely inhibits GRF-stimulated release of growth hormone from the pituitary and it is hypothesised that growth hormone negative feedback is due to a stimulation of somatostatin release (Kelijman and Frohman, 1991). Growth hormone release is also inhibited by Insulin-Like Growth Factor-I, and several mechanisms have been implicated in rodents: (1) inhibition of

growth hormone gene transcription within the pituitary, (2) inhibition of growth hormone secretion from the pituitary, and (3) hypothalamic effects suppressing pulsatile growth hormone secretion from the pituitary (Strobl and Thomas, 1994). The neurons that control the changes in somatostatin secretion from the hypothalamus are an important determinant of plasma growth hormone levels in humans. Cholinergic pathways are involved in the increased secretion of growth hormone stimulated by sleep and exercise. Central cholinergic tone modulates the activity of central α- and β-adrenergic nerves, which in turn dictate somatostatin and possibly also GRF secretion (Ghigo et al, 1992). Central adrenergic receptors affect plasma growth hormone levels in two ways; (1) α-adrenergic agonists stimulate the release of growth hormone, and (2) β-adrenergic agonists inhibit growth hormone secretion (Ghigo et al, 1992).

1.5.4.1.2 Insulin-like growth factors

The release of growth hormone stimulates the release of insulin-like growth factors (IGFs) –I and –II from the liver and these are responsible for the growth promoting actions of growth hormone (Daughaday and Rotwein, 1989).

1.5.4.1.3 Patterns of growth hormone secretion

In normal young adults the twenty four-hour profile of growth hormone secretion consists of stable low levels abruptly interrupted by secretory pulses (Van Cauter et al, 1992). Quabbe et al (1966) were the first to show that in young adult males, a primary secretory episode of growth hormone occurs within the first four hours of sleep and is associated with slow wave sleep. They also showed that this increase in growth hormone secretion is not due to changes in concentrations of glucose or insulin in the plasma. Since this initial finding others have also demonstrated that maximum growth hormone release consistently occurs within minutes of the onset of slow wave sleep and the amount secreted is closely related to the duration of the slow wave sleep episode (Holl et al, 1991; Plotnick et al, 1975; Sassin et al, 1969; Takahashi et al, 1968). In contrast to this, in young adult females growth hormone pulses occur throughout the day and night (Quabbe et al, 1966). In a major sleep episode there may be more than one pulse of growth hormone and this is associated with the recurrence of slow wave sleep (Takahashi et al, 1968).

Growth hormone levels in fact rise in the late evening before the secretory episode begins (Plotnick et al, 1975). In the absence of sleep during a constant routine there is typically a secretory episode of growth hormone near habitual bedtime, however this episode is markedly reduced in amplitude and is no longer a prominent component of the daily growth hormone profile in young men (Czeisler and Klerman, 1999). A pulse of growth hormone is more likely to occur at the usual time of sleep onset, whether or not the individual sleeps at that time (Takahashi et al, 1968), however, the secretory episode is smaller than if sleep had occurred (Van Cauter and Refetoff, 1985).

It has been proposed that cortical centres in the brain that regulate the onset of slow wave sleep, simultaneously direct the release of growth hormone from the anterior pituitary gland (Holl et al, 1991). Interruptions of slow wave sleep by awakenings or rapid eye movement stages are found to coincide with marked reductions in pituitary growth hormone secretion (Van Cauter et al, 1992). It has also been shown that injection of growth hormone in rats increases the amount of REM sleep experienced, suggesting that the growth hormone released during non-REM sleep allows the onset of REM sleep (Drucker-Colin et al, 1975). Upon resumption of sleep there is often a second secretory pulse of growth hormone (Beck et al, 1975). For delayed sleep, the maximum growth hormone level, the amount secreted and the maximum secretory rate are the same as if the sleep episode began at the habitual bedtime (Van Cauter et al, 1992). In a study of military cadets, growth hormone levels increased during a five day rangers course where the participants experienced no organised sleep (Aakvaag et al, 1978). It is thought that this increase in growth hormone concentration can be attributed to the stress caused by sleep deprivation, although confounding factors such as constant physical activity and energy deficits may also influence plasma growth hormone concentrations.

When sleep occurs during the day growth hormone secretion is more dependent on the occurrence of slow wave sleep than on the fact that sleep had occurred (Karacan et al, 1974). The increase in pulse frequency seen during nocturnal sleep is not seen when sleep occurs during the day (Weibel et al, 1997). Sleep after a time shift associated with jet lag is reportedly associated with increased total growth hormone secretion due to increased pulse amplitude rather than pulse frequency (Golstein et al, 1983).

1.5.4.1.4 Factors affecting growth hormone secretion

Circulating growth hormone concentrations arise from a complex pattern of pulsatile hormone release by the anterior pituitary gland which can be influenced by gonadal steroids, nutrition, body composition and sleep (Muller et al, 1999). In humans, the daytime pulsatile pattern of growth hormone secretion is influenced by the effect of dietary metabolites in a way that preserves lean body mass during periods of caloric restriction (Muller et al, 1999). Oral glucose and free fatty acid administration provokes an acute and marked suppression of growth hormone secretion that is followed by a delayed increase in plasma growth hormone three to five hours later (Valcavi et al, 1992). The effects of glucose on growth hormone secretion are thought to be mediated by a 'glucoceptor' within the hypothalamus that stimulates somatostatin release (Strobl and Thomas, 1994). Hypoglycaemia results in elevated plasma growth hormone levels (Strobl and Thomas, 1994). In addition, ingestion of a high protein meal or infusion of arginine or other amino acids stimulates growth hormone secretion (Strobl and Thomas, 1994).

Changes in growth hormone secretion can occur in several physiological states, including the following (Strobl and Thomas, 1994):

1. Obesity: plasma growth hormone is decreased due to a 3-fold decrease in pulse frequency and an increase in clearance from blood.
2. Diabetes/starvation: growth hormone secretion is increased 2-3-fold.
3. Acromegally: high levels of growth hormone due to a high frequency of release of GRF that is unresponsive to negative feedback by IGF-I.
4. Hypercortisolism: chronic cortisol elevations lead to a decrease in plasma growth hormone levels.
5. Hypothyroidism: there is a blunted growth hormone response to hypoglycemia and arginine and a low level of circulating IGFs.
6. Pregnancy: growth hormone is increased in both the maternal and fetal circulation.

1.5.4.2 Secretion of growth hormone during exercise

Growth hormone levels rise in response to acute exercise with a threshold level of approximately 30% VO_{2max} (Felsing et al, 1992). Exercise increases the secretion of growth hormone and the increase is proportional to the intensity of the work (Sutton and Lazarus, 1976). The rise begins within ten minutes of the onset of exercise (Sutton and

Lazarus, 1976), and is dependent not only on the intensity of the exercise but also on the type, with levels increasing by up to 100-fold, particularly in response to anaerobic exercise and hypoxia (Felsing et al, 1992). Pritzlaff et al (2000) stated that during exercise recovery there is an increase in fat oxidation and that it increases as a function of exercise intensity and is directly related to growth hormone release.

It has been shown that the growth hormone response to exercise is greater while experiencing sleep deprivation. For a group of military cadets on a five-day rangers course with no organised sleep, growth hormone secretion during a 30-minute cycling test at 50% VO_{2max} was greater on days three and five than on the initial day (Opstad et al, 1980). This finding is supported in a study of 50 hours sleep deprivation, where the growth hormone response to exercise at 70% VO_{2max} was greater in the sleep deprived group compared with the normal controls (VanHelder and Radomski, 1989).

1.5.4.3 Training Effects

After endurance training, basal levels of growth hormone increase and the growth hormone response to exercise is increased (Plotnick et al, 1975). This has the effect of increasing and maintaining lean body mass and increasing the use of fatty acids for energy production in adults.

1.5.5 Aspects of the Hormonal Control of Blood Glucose Concentration

As the hormonal control of blood glucose concentration was not investigated in the present study, only a brief description of the involvement of insulin and glucagon will be given here. Since both insulin and glucagon are secreted by the Islets of Langerhans within the pancreas and are involved in the maintenance of blood glucose concentration they will be considered together. While insulin has many roles in metabolism, its main role is to lower plasma glucose concentration by facilitating glucose uptake in muscle and adipose cells and by inhibiting hepatic glucose output (Newsholme et al, 1992). On the other hand, glucagons role is to increase plasma glucose by increasing hepatic glycogenolysis and stimulating gluconeogenesis (Kimball and Murlin, 1924).

1.5.5.1 Control of secretion of Insulin and Glucagon by nutrients

The primary determinant of the rate of both insulin release and glucagon release is the concentration of blood glucose. Consumption of a meal containing carbohydrates causes an increase in blood glucose concentration. This results in an increase in the secretion of insulin from the β-cells of the pancreas while causing a decrease in the secretion of glucagon from the α-cells (Muller, et al, 1970). This resulting rise in the ratio of insulin: glucagon produces a decline in glycogenolysis and gluconeogenesis and an increase in glycogenesis (Jennings et al, 1977). The fall in blood glucose concentration between meals has an immediate inhibitory effect on insulin secretion thereby limiting a further fall in blood glucose concentration (Hedeskov, 1980). As the primary role of glucagon is to increase blood glucose, its secretion rises (Hans-Jurgen and Ziegler, 1977). Glucagon acts on the liver to increase glycogenolysis and gluconeogenesis and decrease glycogenesis (Jennings, 1997).

An increase in blood amino acid concentration following a high protein meal causes a parallel increase in both insulin and glucagon (Muller et al, 1971). The increase in glucagon secretion when amino acid concentration is high is thought to occur to prevent hypoglycemia occurring from the increased insulin concentration (Unger et al, 1969).

1.5.5.2 Secretion of Insulin and Glucagon during exercise

As the glucose available in the blood is used up at the onset of exercise, there will be obvious affects on the plasma concentration of both insulin and glucagon. The concentration of insulin in plasma decreases in proportion to the duration and intensity of the exercise while the concentration of glucagon increases (Galbo et al, 1975; MacLaren et al, 1999). In addition to the stimulus of the falling blood glucose concentration, the stimulation of the sympathetic nervous system resulting in the release of adrenaline and noradrenaline, inhibits insulin secretion (Dunning and Taborsky, 1988) and increases glucagon secretion, therefore maintaining plasma glucose levels during exercise (Woods and Porte, 1974).

1.6 MONITORING PHYSIOLOGICAL CHANGES DURING EXERCISE

1.6.1 Measuring Hormone Concentration in Body Fluids

1.6.1.1 Plasma

As testosterone, cortisol and growth hormone are released from glands into the blood stream, secretion of these hormones can be measured by extracting blood to analyse the plasma for the concentration of the hormone (Beck et al, 1975; Bonifazi and Lupo, 1996; Crowley and Matt, 1996; Follenius et al, 1992, Weitzman et al, 1971; Wideman et al, 1999). Once collected, the blood sample is centrifuged to separate the plasma from the red blood cells. It is important to note that anyone collecting and handling the blood sample is at risk of being exposed to infectious disease such as HIV and hepatitis so sterile techniques and hygiene are very important during collection and handling of the sample. Blood collection in research requires skin pricking, venepuncture or catheterisation. Deciding which method to use during a study depends on how often collection is required and the size of the sample. These three methods are invasive and can be painful so studies can be limited by participants' willingness to tolerate the sampling.

Skin pricking usually uses the ear or fingers to collect small blood samples, the benefit being that it is relatively easy and does not require instruction to perform. Sample collection here is limited by blood flow to the area. Venepuncture can remove larger samples of blood than skin pricking but is not appropriate for repeated blood collection. In this case catheterisation might be more appropriate. Both venepuncture and catheterisation require the researcher to be trained in the technique. These three methods of blood collection are also used in diagnostic medicine.

Regular removal of blood, using any method, involving significant volumes can have an influence on the concentration of the hormone due to a decrease in blood volume. This is particularly so for research participants with small blood volumes such as neonates.

1.6.1.2 Saliva

Saliva can be used as a means of monitoring the concentration of steroid hormones in the body as it shows a good correlation with plasma concentration (Cook and Jacobsen, 1995). Diffusion of the steroid hormone through the salivary gland epithelium into the primary secretory fluid within the acinar-intercalated duct complex is driven by the concentration of unbound hormone (Quissell, 1993). Because of the slow dissociation rate of the steroid hormone from high-affinity, specific hormone binding protein in plasma, it is free or weakly bound steroid hormone that dissociates and passes through these membrane structures as the blood passes through the salivary gland during saliva formation (Quissell, 1993). Therefore saliva levels of steroids reflect the concentration of the free hormone in plasma. If the steroid does not have a high-affinity, high-capacity binding protein in the plasma, the saliva levels will correlate with the total plasma hormone concentration (Quissell, 1993). While growth hormone is not commonly measured in saliva, it has been reported in the literature to be measurable at a concentration 1000^{th} of that found in plasma (Rantonen et al, 2000).

The advantages of using saliva to monitor changes in steroid hormone concentration are; (1) it is non-invasive and therefore allows for multiple sampling and easy collection, (2) blood sampling raises ethical issues, such as informed consent to take blood, blood ownership and safety issues, particularly for groups such as neonates, the elderly and the mentally and physically handicapped - such issues are less pressing with saliva sampling (Quissell, 1993). Problems associated with using saliva collection include; (1) insufficient sample sizes after exercise, (2) the time delay of a hormone concentration change in the blood and being detected in the saliva, (3) the metabolism of the steroid hormone by salivary gland epithelial cells during transcellular movement, (4) metabolism of the hormone by oral bacteria in saliva (Quissell, 1993), and (5) contamination of samples through tissue trauma or from crevicular fluid due to poor oral hygiene (Quissell, 1993). In addition, some hormones that have high-affinity, high-capacity binding protein will have a very high plasma ratio of total to free hormone; such samples are hard to assay as plasma contamination in saliva can give falsely high values as the assay cannot distinguish between free and bound hormone (Quissell, 1993).

1.6.1.3 Transdermal electrosonophoresis by ultrasound

The outer layer (stratum corneum) of the skin provides a barrier against the environment (Suhonen et al, 1999). Low frequency ultrasound (sonophoresis) applied to the skin has been shown to increase the permeability of skin up to 4100 fold (Tezel et al, 2003) by increasing the number of imperfections in the lipid bilayer of the stratum corneum creating 'pores' through which solutes can diffuse (Malghani et al, 1998). This technology has been used in previous studies for non-invasive measurement of solutes (Cook, 2002; Kost et al, 2000). The addition an electric field to the skin during sonophoresis (electrosonophoresis) the movement of the plasma constituents towards the skin surface is improved (Cook, 2002). These phenomena have been the basis for constructing a device that allows collection of blood constituents without actual blood removal or invasion through the skin into the bloodstream, as blood is not actually removed and the constituent reflects only an osmotic equilibration across tissue.

A hand held device has been constructed that delivers ultrasound and a small electric current to the skin's surface for 10-30 seconds per sample. This has proven to be painless and innocuous (Dr C.J. Cook, personal communication). An extensive description of the measurement head attached to a hand held ultrasound device has been summarised by Cook (2002). Briefly, the plastic head fitted to the ultrasound gun has a small chamber covered with a semipermeable membrane at the end and connects with the skin. The chamber is constantly fed ethanol through an entry and exit port allowing for the collection of the sample. On either side of the head electrodes supply a 9V current to the skin when connected.

In practice, the device is simply placed against the skin anywhere on the body, the ultrasound and current are applied for 10-30 seconds, the flow of ethanol through the head of the device is started and the sample is collected and removed for analysis by ELISA methods. Alternatively, a small biosensor can analyse the sample immediately. No side effects or problems have been detected in healthy or diseased patients with repeated sampling (Dr C.J. Cook, personal communication). The ultrasound dose used in both Cook (2002) and Kost et al (2000) was at a frequency of 20kHz and an intensity of $10Wcm^2$. Kost et al (2000) states that a two minute dose at this intensity is below the maximum energy dose used in therapeutic ultrasound (Antich, 1982).

Using this method, glucose, insulin, 17-β estradiol, testosterone and cortisol has been successfully measured in sheep and humans both before and after exercise (Cook, 2002). Kost et al (2000) have also used transdermal electrosonophoresis to measure glucose levels in humans and in rats. All measurements had a linear relationship with concentrations in the blood and thus could be arithmetically related to actual blood concentrations.

1.6.2 Assessment of Sleep Quality

1.6.2.1 EEG Activity

Electroencephalogram (EEG) is considered to be the best means of quantifying sleep (Roehrs et al, 1990; Shapiro, 1982) and was first achieved on humans by Hans Berger in 1928 (Berger, 1929). It involves recording the electrical activity of the brain during waking and sleep by placing two electrodes, connected to an amplifier-recorder, on the scalp and measuring the voltage between the two (Cooper et al, 1974). Although the exact source of the EEG in the brain is still not known, each deviation in the record has some yet to be precisely defined relationship to the activity of the many neurons in the vicinity of the two electrodes (Cooper et al, 1974). As the amplitude and frequencies of the various electrical patterns of each sleep stage varies, they can be differentiated on the EEG recording and these are characterised in Table 1.11 (Rechtschaffen and Kales, 1968). The electrical activity of the brain during REM sleep is very similar to that of stage one non-REM sleep (Rechtschaffen and Kales, 1968). The use of EEG to measure sleep patterns and therefore sleep quality is not always possible and other methods have been developed.

Table 1.11. Characteristics of EEG non-REM sleep stages.

Stage	Frequency (cylces per second)	Amplitude (microvolts)	Wave Form
I	4-8	50-100	Theta waves
II	8-15	50-150	Spindle waves
III	2-4	100-150	Slow waves and spindles
IV	0.5-2	100-200	Delta waves

1.6.2.2 The Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) is a subjective measure of the quality and patterns of sleep in older adults (Smyth, 1999). It differentiates "poor" from "good" sleep by measuring seven areas: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping medication and daytime dysfunction over the last month. The user self-rates each of these seven areas of sleep.

1.6.2.3 Daily Sleep Log

It has been shown that self-reported estimates of sleep are highly correlated with polygraphic measures of sleep quality and quantity (Jacobs et al, 1988). Filled out every morning, sleep logs consist of several questions ranging from what was ingested prior to sleep to how much sleep was had, and include a personal rating of the quality of sleep.

1.6.3 Monitoring Respiratory and Related Changes during Exercise

The following is summarised from the American College of Sports Medicine's Guidelines for exercise testing and prescription.

1.6.3.1 Oxygen uptake (VO_2)

Measuring the rate of oxygen uptake (VO_2) can provide useful information:

1. Under certain conditions, it provides a measure of the energy cost of exercise.
2. The rate of oxygen uptake during maximal exercise (VO_{2max}) indicates the capacity for oxygen transport and utilisation during exercise.
3. VO_{2max} also serves as the criterion measure of aerobic fitness for inter- and intra-individual comparisons.
4. In combination with the measured rate of carbon dioxide output (VCO_2), VO_2 provides general information about the fuels being used for exercise.

1.6.3.1.1 Measuring VO_{2max}

Maximal oxygen uptake (VO_{2max}) is a measure of the maximal rate at which oxygen can be consumed and reflects the capacity of the aerobic system. It involves analysis of expired air samples collected while the participant performs exercise of progressing intensity and is typically expressed relative to bodyweight. Procedures for estimating

VO_{2max} have been developed and validated by measuring: (a) the correlation between directly measured VO_{2max} and the VO_{2max} estimated from physiological response to submaximal exercise (e.g. heart rate at a specific power output), (b) the correlation between directly measured VO_{2max} and test performance (e.g. time to run 1 mile or time to volitional fatigue using a standard test protocol) (American College of Sports Medicine, 1995).

1.6.3.1.2 Estimating VO_{2max} using submaximal VO_2

Since maximal exercise testing is not often feasible, submaximal exercise tests have been developed that can be used to estimate VO_{2max}. During submaximal tests the relationship between VO_2 and heart rate is defined by measuring VO_2 and heart rate at two or more submaximal exercise intensities. VO_{2max} may then be estimated by extrapolating the relationship out to theoretical maximum heart rate (220-age, (American College of Sports Medicine, 1995)).

1.6.3.1.3 Discontinuous vs continuous protocols

Discontinuous protocols alternate rest and work intervals resulting in a plateau in VO_2 with increasing intensity. This occurs more often than in a continuous protocol due to the participant not experiencing the degree of local muscular fatigue experienced with a continuous protocol. The discontinuous protocol takes longer to administer than the continuous and is therefore less often used due to time constraints.

1.6.3.2 Respiratory exchange ratio (RER) and the respiratory quotient (RQ)

Both are measured as VCO_2/VO_2 and are unitless. The difference between the two is that the respiratory exchange ratio (RER) can be measured over a very short period of time (one minute) while the respiratory quotient (RQ) measures cellular respiration, which implies that the measurement has been made over a longer period (15 minutes or more). RQ provides information about substrate utilisation at the cellular level. 1.0 is pure carbohydrate oxidation, 0.7 is mixed fat oxidation and approximately 0.8 is mixed protein oxidation. RER, on the other hand, can exceed 1.0 during heavy non-steady state or maximal exercise due to hyperventilation, which increases VCO_2 disproportionately or due to buffering of lactic acid in the blood.

1.6.3.3 Subjective rating scales

Borg's rating of percieved exertion scale was developed to allow the exerciser to subjectively rate his/her feelings during exercise, taking into account personal fitness level, environmental conditions and general fatigue levels. The results correlate highly with measured exercise heart rates and calculated O_2 consumption levels. The revised scale accounts for both the linear increase in VO_2 and heart rate during exercise and non-linear responses of variables such as blood lactic acid accumulation and ventilation.

1.6.4 Measuring Body Temperature Changes during Exercise

As measurement of the temperature of the human brain is virtually impossible for the purpose of medicine and research, alternate sites must be used to assess body temperature. No one single site is used to measure or approximate core body temperature though oral, rectal, tympanic and axillary are most commonly used (Sund-Levander et al, 2002). Brain temperature can be approximated by the non-invasive measurement of tympanic membrane temperature within the auditory meatus of the ear and is generally considered as a good index of the central drive for physiological temperature regulation (Stolwijk and Hardy, 1966). The temperature of the blood leaving the heart on its way to perfuse the body core is an ideal measure of internal body temperature, however while it is possible to obtain aortic blood temperature it is too invasive to be acceptable so as a compromise oesophageal temperature within the thoracic cavity at the level of the heart can be used to obtain measurements very similar to that of aortic blood (Gerbrandy et al, 1954). The older, traditional site of the rectum is easier to access than oesophageal temperature but also has limitations including a low level of systemic perfusion, leading to thermal inertia and a preferential venous drainage of exercise-heated blood from the legs returning via hemorrhoidal veins (Mead and Bomarito, 1949).

1.7 PLAN OF THIS THESIS

There were three aims in this study. The first aim was to validate the use of transdermal electrosonophoresis as a non-invasive sampling method by comparing concentrations of testosterone, cortisol and growth hormone in the transdermal electrosonophoresis samples to the concentrations measured in plasma and saliva. Secondly, to explore the relationships between fitness increases and fatigability with hormone levels in the three fluids. Lastly, to explore the impact of sleep quality and quantity on hormone profiles and athletic performance.

The remainder of this thesis consists of three chapters. Chapter two outlines the materials and research methods employed during this study. This is followed by Chapter three, the Results, where the data obtained during the research period is presented and analysed using statistical methods. The absence of a control group during this research dictates that relationships within the group were sought in the analysis rather than a comparison made between two or more groups of participants. Chapter four discusses these results and attempts to provide reason for the data obtained. Due to the nature of this pilot study a large portion of the discussion revolves around a critique of methods used and alternate methods not employed here, and the implications for further studies.

Chapter Two: Materials and Methods

2.1 INTRODUCTION

In order to investigate the three objectives of this research a four-week study of young, male, rugby players was designed. During the study plasma, transdermal electrosonophoresis (ESP) and saliva samples were taken regularly from participants to allow for analysis of testosterone, cortisol and growth hormone levels. Fitness was assessed each week using a maximal treadmill test and fatiguability was also investigated. The details of the study are outlined below and a list of all of the equipment and consumables used are included in Appendix 1. Participants were allocated with a random number to ensure anonymity during the study and in publication of the results.

2.2 PARTICIPANT SELECTION

After obtaining ethical approval from the Massey University Human Ethics Committee (ethics application see Appendix 2), potential participants for the study were approached at a local rugby football club. The coach of a competitive team comprising male players between the ages of 19 and 22 was consulted on his willingness to have his players involved in a study of this nature. The team was then approached as a group on two occasions. At these meetings, they were presented with a verbal outline of what would be expected of them during the study, given an information sheet (Appendix 3) and had the opportunity to ask questions about the study.

To be accepted into the study participants had to satisfy certain inclusion criteria. The participants had to be male, between the age of 18 and 30 years, participate in regular sports training, non-injured and medically healthy. Participants were not accepted into the study if they had health conditions incompatible with regular training and exercise, they used recreational or performance enhancing drugs or were uneasy with any of the aspects of the study.

2.3 PRE STUDY MEASUREMENTS

Those interested in participating underwent a thorough medical assessment by a general practitioner before being admitted into the study (medical assessment see Appendix 4). Only those passed fit were included. Informed consent was obtained in writing (consent form see Appendix 5). The participants were weighed and their heights measured before the study began. Weight was measured with calibrated scales (Jadever, JPS-2030, Jadever Scale Co Ltd, Taiwan) and height using a stadiometer (Seca 222, Vogel and Halke GmbH Co, Hamburg, Germany).

2.4 MEASUREMENTS DURING THE STUDY

2.4.1 Sleep Log

Upon waking each morning the participants filled out a sleep log consisting of seven simple questions modeled from (Hawkins and Shaw, 1992). The questions covered time that the participant went to bed, sleep latency, time awake in morning, whether sleep disturbances were experienced, if dreams were remembered, whether the person had taken any naps during the day and a rating of perceived sleep quality. A rating of 0 denoted the participant had no sleep at all, while a rating of 10 was the best sleep that the participant ever remembers having. An additional question was added to the sleep which required the participant to recall the number of standard drinks of alcohol they had consumed in the twenty-four hours prior to waking. The participants had a table available which stated the number of standard drinks present in popular alcoholic beverages in measured amounts.

2.4.2 Morning Sample Collection

Each participant came into the laboratory at a consistent time three days a week (Monday, Wednesday and Friday). They provided blood, ESP and saliva samples. The sampling period for all participants was between 0900 and 1200 hours.

2.4.3 Blood Collection and Treatment

Blood was taken only on Mondays and Fridays using sterile techniques. The participants immersed their hands in hot water for three to five minutes before blood collection to promote vasodilation of the blood vessels in the fingers and improve blood flow. The participant's fingers were sterilized with 70% isopropyl alcohol (Sterets pre injection swabs, Seton Prebbles Ltd, England). Once the alcohol had dried, the two fingers were lanced using an automatic lancet (Accu-Chek Softclix Pro, Roche Diagnostics NZ Ltd) and blood was collected into 100μL heparinised capillary tubes (Chase Scientific Glass Inc, Tennessee, USA). Each finger was massaged in order to

promote blood flow, but gently enough to avoid haemolysis. Approximately four to six capillary tubes were taken at each time, depending on the flow of blood.

The capillary tubes were centrifuged (IEC Micro Capillary Centrifuge, International Equipment Company, Massachusetts, USA) for five minutes to separate the red blood cells from the plasma. The plasma was removed by filing the capillary tube above the line dividing the two halves and snapping the tube. The plasma was then transferred into a 1.5mL-microtest tube (3810X, Eppendorf, Germany). As the samples were collected they were stored on ice until all samples from the morning were obtained and then they were transferred to a deep freeze at -20°C where they were kept until assay.

2.4.4 Transdermal Electrosonophoresis Samples

ESP samples were collected in a manner similar to that described by (Cook, 2002). The ultrasound transmission gel (Aquasonic, Parker Laboratories Inc, New Jersey, USA) was applied to the area on the anterior surface of the arm where the modified ultrasound head was in contact with the skin. This head generates and seal to the skin and allowed collection from the skin of any exudates caused by the ultrasound transdermal flux across a semi-permeable size exclusion membrane into a fluid chamber. A 9V generated current was applied across the arm simultaneously with the ultrasound as this increases hormonal flux (Cook, 2002). The size of the current varied between participants due to differing skin resistances. A continuous ultrasound pulse of 20kHz, 10Wcm^2, (ITO Physiotherapy and rehabilitation unit, Tokyo, Japan) was applied for 15 seconds, and then the arm was removed from the ultrasound. Any metabolites, hormones or other plasma constituents that were drawn out of the arm were collected from the head chamber into an associated removable collection vial in 70% ethanol. To ensure that all of the substances were collected, flow through the chamber continued for an additional 60 seconds after the arm was removed. This created a total fluid sample of approximately 0.7-1.0ml. The sample was collected in 1.5ml microtest tubes (3810X, Eppendorf, Germany), which were stored at -20°C until they were assayed.

2.4.5 Saliva Samples

Saliva samples were provided by the participants on all three mornings. Participants were advised at the beginning of the study to avoid eating apples or other coarse textured food, brushing their teeth, or drinking coffee during the hour before supplying saliva samples. Approximately 5mL of saliva were required and samples were supplied by spitting into 25mL sterile containers (LBS3534, Labserve, Auckland, New Zealand). The samples were stored at -20°C until the time of assay.

2.4.6 Post Training Sprint Testing

Every Monday night at 1730 hours, the 14 participants trained for between 30 and 45 minutes. Once training was completed the participants ran three 40-meter sprints with a five-meter rolling start, each sprint was followed with a three minute rest period. The participants performed the sprints on an all weather track and were wearing sneakers. The sprints were performed at 3-minute intervals. The sprints were timed using a Speedlight Sports Timing System (Swift Performance Equipment, Australia). Each participant provided saliva samples once the three sprints were completed. These were then stored at -20°C until assay. It was intended that an ESP sample would also be collected at this time but an absence of a power source on site prevented this.

2.4.7 Fitness Testing

Each participant completed a fitness test once a week on either a Wednesday or Thursday afternoon between 1300 and 1700 hours. The test was designed to increase oxygen uptake until the participants reached their maximal oxygen uptake (VO_{2max}). The criteria that were used to determine whether they had reached their VO_{2max} was failure of heart rate to increase with further increases in work (American College of Sports Medicine, 1995). The test was performed in a temperature-controlled laboratory at approximately 21°C with humidity below 60%. The maximal fitness test was performed on a Payne wide-bodied treadmill (TM4-NZ, Stanton Engineering Pty Ltd, New South Wales, Australia) controlled by Payne software using a ramp protocol designed by myself (see Appendix 7). Heart rate was monitored using a polar heart rate monitor (Polar Vantage NV™, Polar Electro Oy, Finland). After the test the data were

downloaded from the monitor onto a computer using an interface (Polar Advance Interface System™, Polar Electro Oy, Finland).

The treadmill protocol involved a two-minute warm up at a speed of 108.3m/min (6.5kph) which was either a brisk walk or a light jog, the treadmill then increased its speed at a constant acceleration 0.42m/min (of 0.025kph/s) with a grade of 8%, the participants continued to run until they could not keep up with the increasing speed. At this point, the treadmill switched to a warm down protocol comprising a very slow walk. The peak speed that the participant reached was used in the following equation to calculate VO_{2max}:

General equation:

VO_2 Mode = Resting Component (R) + Horizontal Component (V) + Vertical or Resistive Component (V)

Equation specific to running:

VO_2 (ml/kg/min) = 3.5ml/kg/min + walking speed (m/min) X 0.2 + Grade (fraction) X peak speed (m/min) X 0.9

After the fitness test, the participant gave a saliva and an ESP sample. These samples were stored at -20°C until assay.

2.4.7.1 Measurement of VO_2, VCO_2 and RER

It was initially planned that maximal oxygen uptake (VO_{2max}) would be measured by continual monitoring of oxygen use using open circuit spirometry. In addition to monitoring oxygen use, carbon dioxide production (VCO_2) was going to be measured and used to determine the respiratory exchange ratio and anaerobic threshold. In the event, equipment malfunction led to the abandonment of this plan.

2.5 SAMPLE ANALYSIS

Plasma samples were analysed for the concentration of testosterone, cortisol and growth hormone, as were the ESP samples. Saliva samples were only analysed for testosterone and cortisol concentrations as growth hormone is not present in saliva. Testosterone and cortisol concentration in ESP and samples were analysed by enzyme-linked immunosorbent assay (ELISA) methods using manufacturer's instructions (Salimetric, Pennsylvania, USA). Plasma testosterone and cortisol concentrations in plasma were analysed using DRG Diagnostics plasma kits (DRG Diagnostics, Marburg, Germany). Growth hormone was assayed by a direct radioimmunoassay kit for both plasma and ESP samples, as per manufacturer's instructions (DRG Diagnostics, Marburg, Germany).

2.6 STATISTICAL ANALYSIS

2.6.1 Relationships between hormone levels in the three fluids

Comparisons between hormone concentrations in plasma, ESP and saliva samples were made using Pearson correlations. Correlations were made for the group and for each individual participant. The results for the group were compared for the sample collected both in the morning and after physical activity, including both the fitness test and the sprints. Significance was accepted at $P \leq 0.05$. Analysis of variance was used to determine whether there were any significant differences between the data for each individual.

Regression analysis was used to establish a calibration constant to convert ESP sample concentrations into corresponding plasma concentrations. This was done for all three hormones.

2.6.2 Relationships between fitness measures and hormone profiles

The relationships between predicted VO_{2max} and hormone profiles of the participants were examined twice. After viewing the scattergraphs produced by plotting the predicted VO_{2max} calculated during a test and the participant's mean morning hormone concentrations, the relationships were examined by calculating Pearson correlations coefficients. These were calculated for all three hormones in all three sample types. Pearson correlations were also calculated to establish whether there were any significant relationships between the VO_{2max} of the participants and their testosterone and cortisol concentrations in ESP and saliva after the test.

2.6.3 Alcohol consumption and hormone concentration

To test whether alcohol consumption had any effect on hormone profiles Pearson correlations between alcohol consumption in a 24-hour period and the hormone concentrations in the three fluids the following morning were examined. This was done for both the group and the individual participant's data.

2.6.4 Sleep and hormone concentrations

Scattergraphs for sleep rating, sleep latency and total time asleep were plotted against hormone concentrations measured the following morning. Pearson Correlations were then used to examine whether there were relationships between the quality and quantity of sleep for the participants and their hormone concentrations the following morning. Correlations were also calculated using mean values for sleep measures for the previous four, three and two nights.

Effects of sleep disturbances on the concentration of the hormones in plasma, ESP and saliva the following morning were investigated using an independent-samples t-test. Levene's test was used to assess for equality of variances. This method was also used to assess the effect of dreaming on the concentrations of the hormones in plasma, ESP and saliva.

2.6.5 Sleep and fitness measures

Plots of sleep quality and sleep quantity measures, sleep rating, sleep latency and total time asleep, against predicted VO_{2max} were viewed. Pearson correlations were used to examine whether there existed any relationships between on the predicted VO_{2max} for the participants and these measures. Correlations were also calculated using mean values for sleep measures for the previous four, three and two nights.

2.6.6 Analysis of sprint times

Mean sprint times were calculated for the following:

- Each week (i.e. week one, week two, week three)
- Each sprint (i.e. first, second the third attempt)

One-way analysis of variance (ANOVA) was used to investigate whether the differences seen from week to week and from sprint to sprint were significant.

Chapter Three: Results

Tables

Figures

3.1 PARTICIPANTS

The participants in the present study were all males within a narrow age range of 19 to 22 years, and they played in the same rugby union football team. Most had a similar ethnic background, as 13 of the 14 classed themselves as New Zealand European, although one of those 13 also indicated that he was Maori, the 14th indicated that he was Tongan. All of the participants were tertiary students, living in flats.

Key features of the 14 male are summarised in Table 3.1. Their mean age, height and weight were 20 years, 181cm and 86kg respectively, and their mean body mass index (BMI) was 26.2kg/m^2. Each participant's VO_{2max} was estimated using final running speed reached by the participant and gradient of the treadmill (described in section 2.2.3 and 3.4). The mean VO_{2max} was 42.3mg/kg/min. The concentrations of testosterone, cortisol and growth hormone for each participant are listed in Table 1 are the mean values for all plasma samples taken during each morning of the study. The group means for testosterone, cortisol and growth hormone concentrations in plasma were 5.4ng/ml, 132ng/ml and 12.0ng/ml respectively.

The concentration ranges for hormones in samples taken during the morning for each participant in each fluid are shown in Table 3.2. The concentration range for testosterone in plasma samples was 2.1 to 10.5ng/ml, in ESP samples was 0.11 to 1.03ng/ml and in saliva was 0.07 to 0.59ng/ml. The concentration range for cortisol in plasma samples was 39 to 217ng/ml, in ESP samples was 3.5 to 25.8ng/ml and in saliva was 0.73 to 15.84ng/ml. The concentration range for growth hormone in plasma samples was 4.5 to 20.4ng/ml and in ESP samples was 0.49 to 2.09ng/ml.

Table 3.1. Anthropometric values, VO_{2max} and mean plasma hormone concentrations (ng/ml) from each participant .

						Mean morning plasma hormone concentrations (ng/ml)		
Participant Code	Age	Height (cm)	Weight (kg)	BMI (kg/m^2)	Estimated VO_{2max} (ml/kg/min)	Testosterone	Cortisol	Growth Hormone
10	21	192.0	89.7	24.3	43	3.9	131	7.6
16	22	173.4	85.5	28.4	41	6.7	183	15.4
18	19	177.4	79.8	25.4	43	4.0	80	8.5
23	20	170.8	69.4	23.8	43	6.3	130	14.4
25	20	184.5	95.6	28.1	44	4.6	95	11.0
28	19	175.5	90.3	29.3	42	6.7	119	14.3
34	20	184.2	97.1	28.6	41	5.1	149	11.5
38	20	177.8	88.5	28.0	39	5.6	153	11.7
40	22	173.7	84.4	28.0	42	6.8	122	15.1
42	21	184.8	85.6	25.1	43	6.1	117	13.0
48	20	188.4	88.7	25.0	43	4.8	148	11.4
51	20	185.8	73.0	21.1	44	5.6	171	13.0
55	19	179.5	76.8	23.8	43	4.5	126	9.8
57	20	188.3	100.3	28.3	42	5.3	132	11.5
Mean	20.2	181.2	86.1	26.2	42.3	5.4	133	12.0
SD	0.98	6.56	8.92	2.46	1.3	0.99	27	2.37

BMI, body mass index; SD, standard deviation

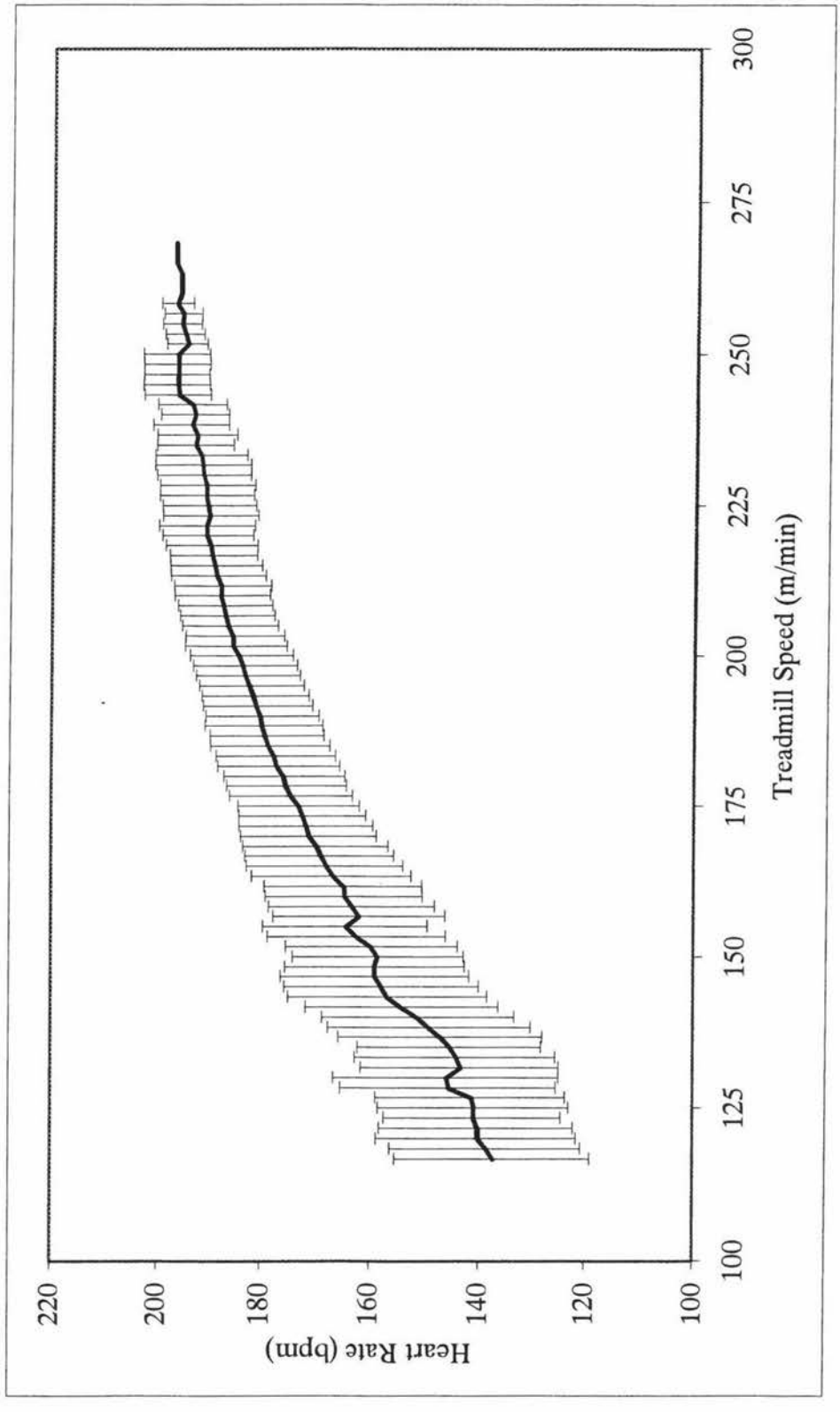


Figure 3.6. Mean heart rate with increasing treadmill speed.

Table 3.3 shows the summary details of the hormone concentrations in plasma, ESP samples and saliva. For plasma, the hormone concentrations in the calculation of these figures include only those samples taken in the morning as no plasma was sampled following a fitness test or a sprint test. For ESP sample concentrations, concentrations in samples taken in the morning and in samples taken after the fitness tests are included. For saliva sample concentrations, those in morning samples, the samples taken after the fitness tests and the samples after the sprint tests are included.

Table 3.3. Ranges of hormone concentrations in all participants in samples taken using the three methods.

Hormone	Sampling Method	N	Minimum	Maximum	Mean	SD
Testosterone	Plasma	99	2.1	10.5	5.5	1.8
	ESP	166	0.11	1.03	0.52	0.18
	Saliva	245	0.00	0.59	0.24	0.10
Cortisol	Plasma	99	39	217	132	37
	ESP	166	3.5	25.8	13.5	4.0
	Saliva	249	0.04	15.8	3.57	2.28
Growth	Plasma	99	4.5	20.4	12.1	4.2
Hormone	ESP	101	0.49	2.09	1.26	0.42

N, number of samples; SD, standard deviation

3.2 CORRELATIONS OF PLASMA HORMONE CONCENTRATIONS WITH THOSE IN ESP AND SALIVA SAMPLES TAKEN AT THE SAME TIME

3.2.1 Group Analysis

Table 3.4 shows the correlation coefficients for the concentration of each hormone in the three fluids. Samples taken at all times (morning, after the fitness test, after the sprints) for all participants were included in these calculations. Correlation coefficients were calculated for plasma and ESP, plasma and saliva, and ESP and saliva for each of the three hormones measured, except for growth hormone in saliva. The positive correlations between concentrations of testosterone and cortisol in plasma and ESP samples were much higher than those between plasma and saliva samples. Growth hormone also had very strong positive correlations between plasma and ESP, but cannot be measured in saliva samples. The correlations are represented graphically in Figure 3.1, Figure 3.2 and Figure 3.3.

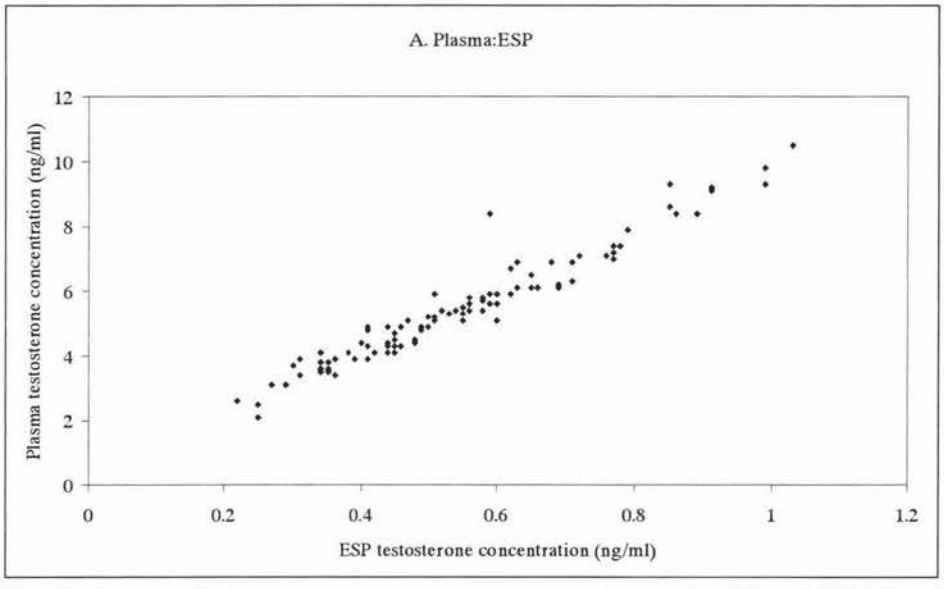
Table 3.4. Correlations between plasma, ESP and saliva hormone concentrations.

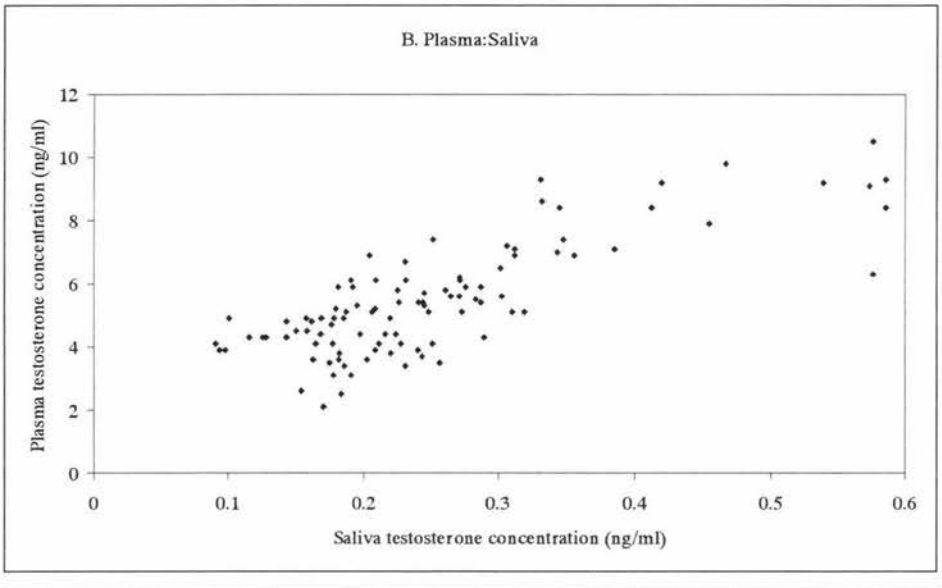
Comparison	Statistic	Testosterone	Cortisol	Growth Hormone
Plasma – ESP	r	0.970**	0.965**	0.977**
	p	<0.001	<0.001	<0.001
	N	99	99	99
Plasma – Saliva	r	0.791**	0.612**	-
	p	<0.001	<0.001	-
	N	99	99	-
ESP – Saliva	r	0.698**	0.522**	-
	p	<0.001	<0.001	-
	N	166	166	-

N, number of samples; r, correlation coefficient; p, significance level

**Correlation significant at the 0.01 level

Figure 3.1. Relationship between testosterone concentrations measured in the three fluids.





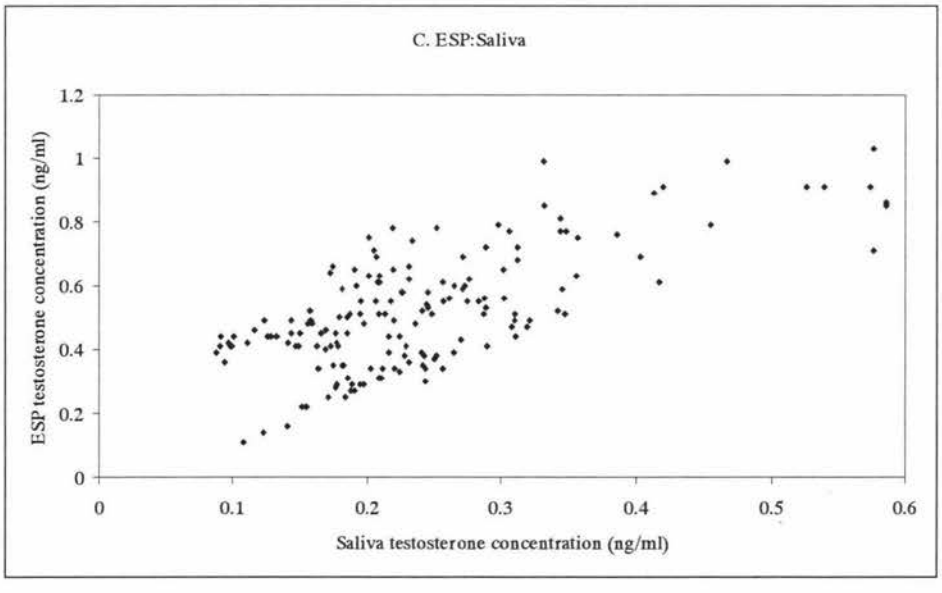


Figure 3.2. Relationships between cortisol concentrations measured in the three fluids.

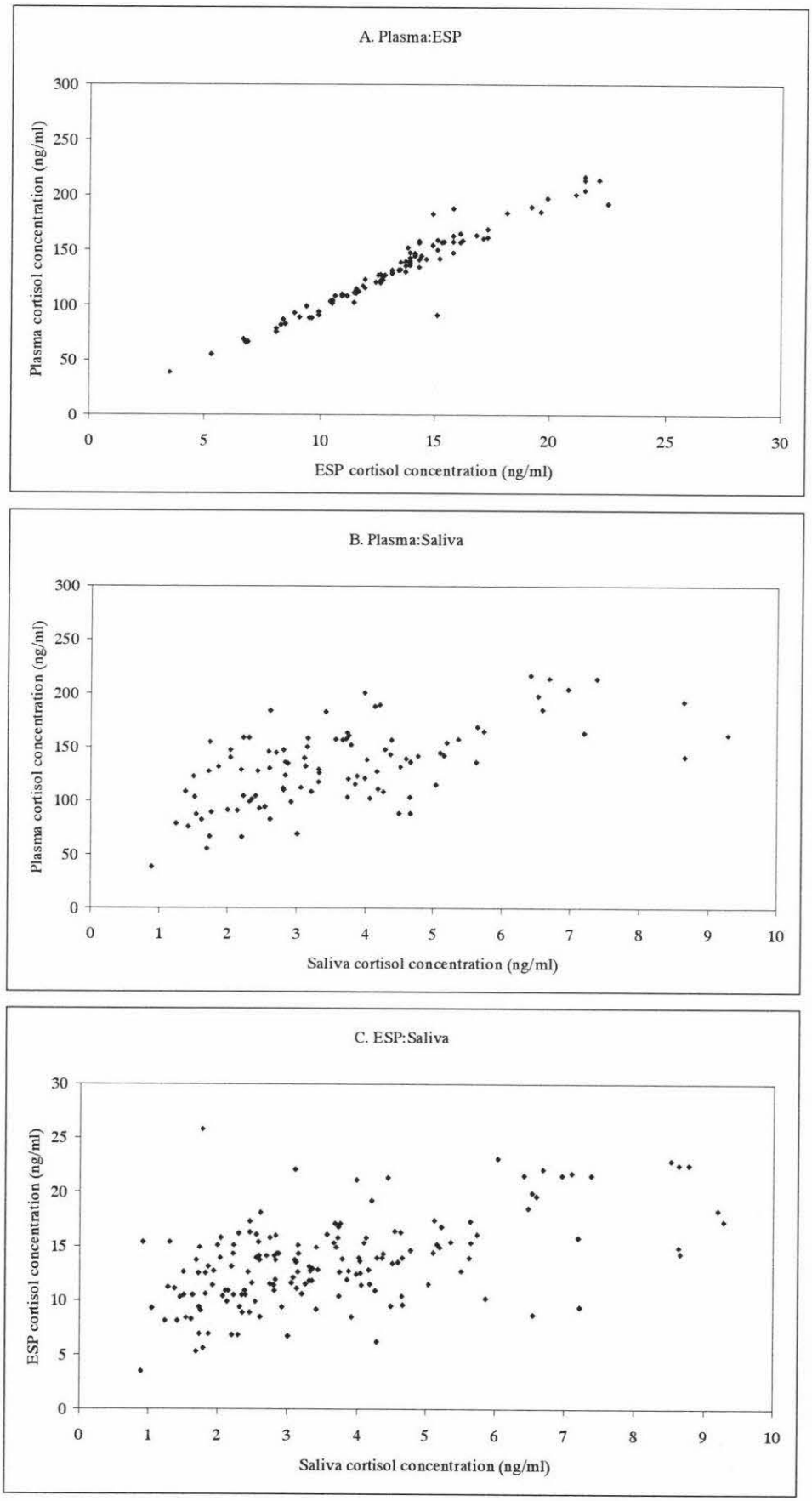
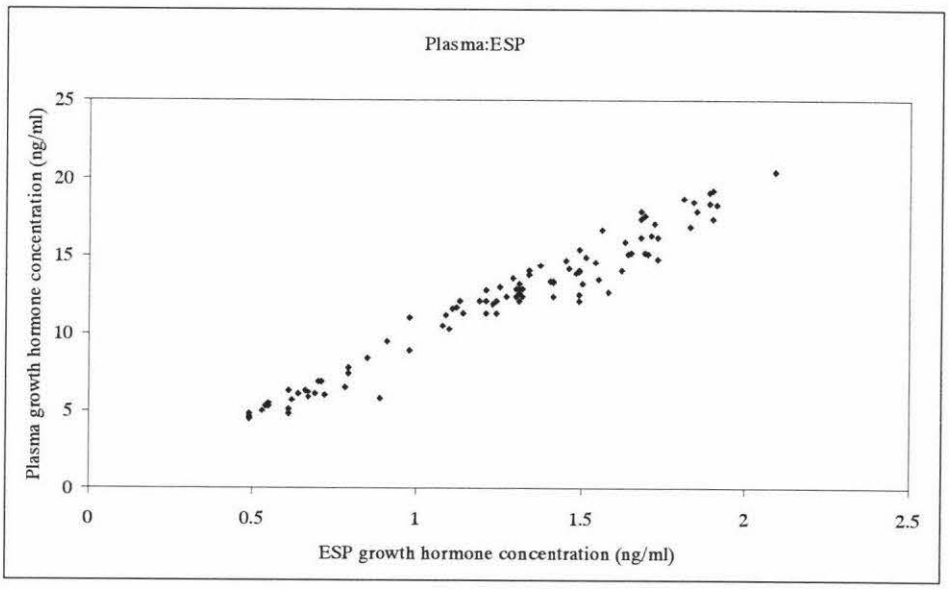


Figure 3.3. Relationships between growth hormone concentrations measured in plasma and ESP samples.



3.2.2 Individual Analysis

We can subdivide the analysis according to each participant to establish whether differences occur between individuals. Pairwise correlation coefficients between the selected pairs of three fluids for each individual participant are shown in Table 3.5, Table 3.6 and Table 3.7. We can see from these results that there were very high, significant positive correlations between the hormone concentrations in plasma and the ESP samples for each of the hormones for all participants, except for participant 28's plasma:ESP correlation for cortisol.

The plasma: ESP testosterone concentration correlations (Table 3.5) were significant for each participant. However only half (seven) of the plasma: saliva testosterone concentration correlations and only eight of the ESP: saliva testosterone correlations were significant.

Table 3.5. Selected pairwise testosterone correlations for each participant.

Participant	Plasma: ESP		Plasma: Saliva		ESP: Saliva	
Code	N	r	N	r	N	r
10	4	0.996**	4	0.166	6	0.318
16	6	0.941**	6	0.813*	8	0.866**
18	7	0.887**	7	-0.429	10	-0.412
23	7	0.985**	7	0.710	10	0.686*
25	8	0.979**	8	0.153	10	0.318
28	7	0.988**	7	0.892**	9	0.810**
34	7	0.925**	7	0.243	9	0.161
38	8	0.802*	8	0.796*	10	0.808**
40	8	0.994**	8	0.899**	10	0.857**
42	8	0.951**	8	0.892**	10	0.851**
48	8	0.995**	8	0.719*	10	0.577
51	7	0.983**	7	0.651	9	0.681*
55	7	0.994**	7	0.749	9	0.694
57	7	0.985**	7	0.813*	10	0.601*

N, number of samples; r, correlation coefficient

*Correlation significant at the 0.05 level

**Correlation significant at the 0.01 level

As with testosterone, each participant's plasma: ESP cortisol concentration correlation was significant (Table 3.6). However only three of the individual pairswise correlations were significant for plasma: saliva and only five were significant for ESP: saliva. It is evident for plasma and saliva, there are more significant pairwise correlations for testosterone than for cortisol concentrations. Seven significant pairwise correlations for testosterone compared with three for cortisol, suggests that testosterone secretion is more stable than cortisol so that the concentration of cortisol measured in saliva represents the concentration of cortisol in plasma some time before.

Table 3.6. Selected pairwise cortisol correlations for each participant.

Participant	Plasma: ESP		Plasma: Saliva		ESP: Saliva	
Code	N	r	N	r	N	r
10	4	0.980*	4	0.666	6	0.828*
16	6	0.941**	6	0.576	8	0.708*
18	7	0.998**	7	0.274	10	0.212
23	7	0.948**	7	-0.002	10	-0.181
25	8	0.975**	8	0.226	10	0.742*
28	7	0.734	7	0.851*	10	0.691*
34	7	0.992**	7	0.850*	9	0.895**
38	7	0.897**	7	0.455	9	0.527
40	8	0.995**	8	0.371	10	0.369
42	8	0.977**	8	0.331	10	0.456
48	8	0.987**	8	0.649	10	0.591
51	7	0.918**	7	0.751	9	0.651
55	7	0.990**	7	0.573	9	0.295
57	7	0.967**	7	0.894**	10	0.360

N, number of samples; r, correlation coefficient

*Correlation significant at the 0.05 level

**Correlation significant at the 0.01 level

Table 3.7. Correlation of plasma and ESP growth hormone levels in individual subjects.

Participant	Plasma: ESP	
Code	N	r
10	4	0.986*
16	6	0.920**
18	7	0.947**
23	7	0.981**
25	8	0.975**
28	7	0.991**
34	7	0.978**
38	8	0.975**
40	8	0.994**
42	8	0.992**
48	8	0.960**
51	7	0.928**
55	7	0.996**
57	7	0.980**

N, number of samples; r, correlation coefficient

*Correlation significant at the 0.05 level

**Correlation significant at the 0.01 level

3.2.3 Exercise and Electrosonophoresis

Although plasma was not sampled after exercise in this study, it has been shown in other studies that high correlations between plasma and ESP samples remain during exercise and recovery (Cook, 2002). We can therefore examine the effect of exercise on the correlations between hormone concentrations in ESP and saliva samples for testosterone and cortisol. The data for testosterone and cortisol were separated into measurements made in the morning and those made after the fitness test was completed. From Table 3.8 we can see that after exercise the correlation between ESP and saliva was still strong for testosterone, however the correlation was no longer significant for cortisol. This is depicted graphically in Figure 3.4 and Figure 3.5. We can see that while the highly significant, positive correlation for testosterone concentration in ESP and saliva samples remains after exercise the same correlation for cortisol does not. No effect of exercise on the correlation between growth hormone concentration in ESP and saliva samples could not be investigated as growth hormone is not present in saliva samples.

Table 3.8. Correlations between the hormone concentrations in ESP and saliva samples obtained in the morning and after exercise.

	Testosterone		Cortisol	
Statistic	Morning	After exercise	Morning	After exercise
r	0.748	0.822	0.559	-0.002
p	<0.001	<0.001	<0.001	0.994
N	130	22	130	22

N, number of samples; r, correlation coefficient; p, significance level

Figure 3.4. Relationships between ESP and saliva sample testosterone concentrations in the morning and after exercise.

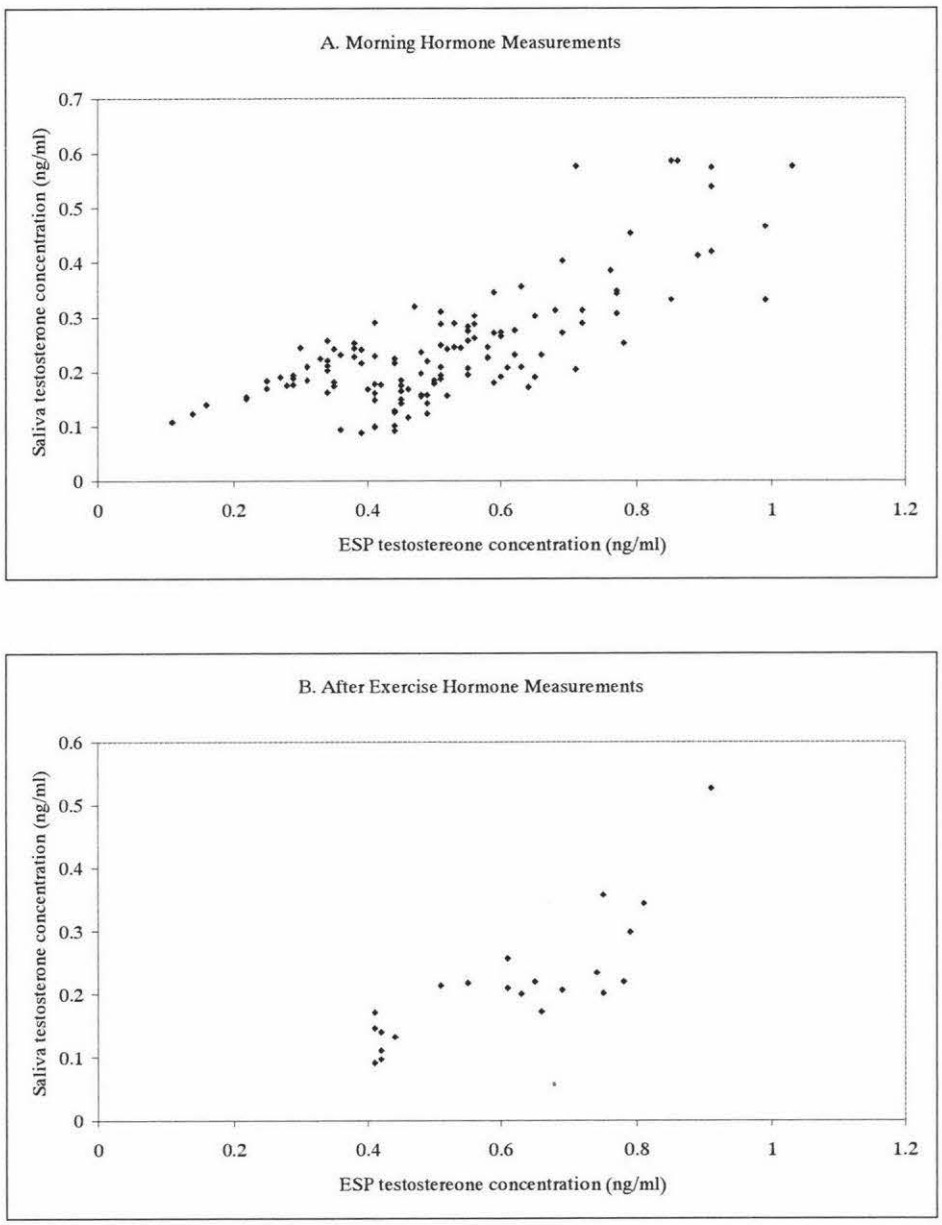
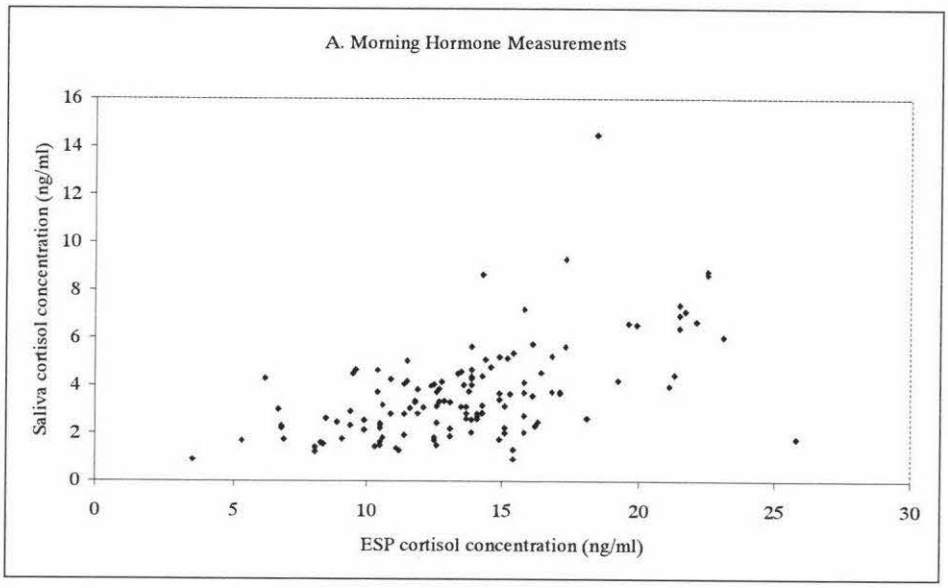
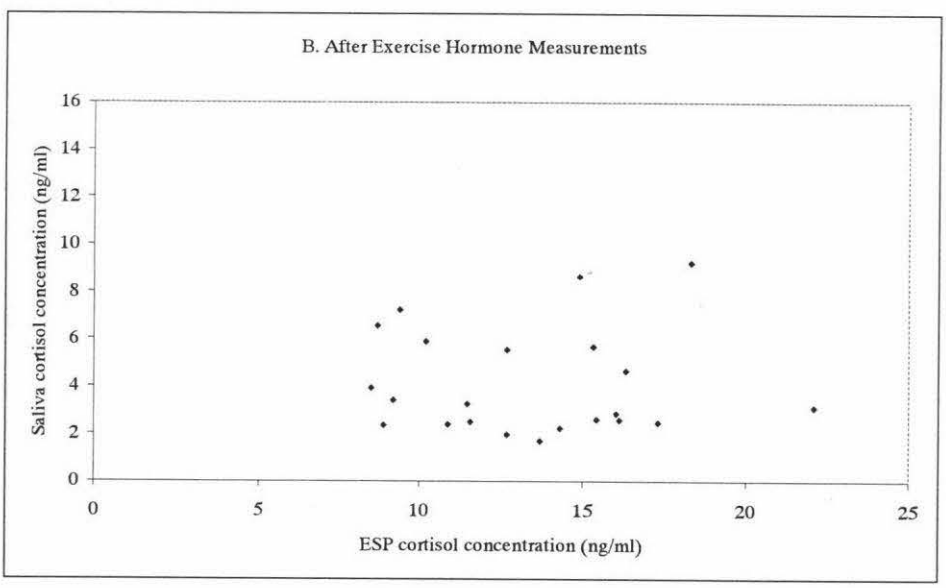


Figure 3.5. Relationships between ESP and saliva sample cortisol concentrations in the morning and after exercise.





3.3 ANALYSIS OF PLASMA AND ESP DERIVED DATA TO ESTABLISH A CONVERSION EQUATION TO CALCULATE PLASMA HORMONE CONCENTRATION FROM ESP SAMPLE HORMONE CONCENTRATION

Measurement of the concentration of a hormone in an ESP sample may allow its concentration in plasma to be estimated. In order to do this, linear regression analysis of the hormone concentrations measured in both plasma and ESP samples was done to calculate a conversion equation. An ESP sample hormone concentration could then be inserted into this equation to estimate the concentration of that hormone in plasma.

3.3.1 Individual Analysis

In order to determine whether a conversion equation was consistent between each individual and between each hormone a regression equation for each participant for each hormone was calculated (shown in Table 3.9). The *individual participant's* slopes for testosterone varied from 7.739 to 10.441 and the intercepts varied from -0.167 to 0.901. For cortisol the slopes varied from 7.491 to 11.882 and the intercepts from -18.337 to 36.551. For growth hormone the slopes varied from 6.8496 to 10.935 and the intercepts from -2.028 to 4.428. The *mean slopes* for the three hormones varied from 9.020 to 9.504 and the *mean intercepts* for the three hormones varied from 0.164 to 7.705.

The individual participant's slopes for the regression graph and intercepts were analysed for variance. There were no significant differences between the slopes of the equations for the 14 participants for any of the hormones (Table 3.10). There were no significant differences between the intercepts of the equations for the 14 participants for any of the hormones (Table 3.11). Moreover the intercepts of the participant's regression equations were not significantly different from zero (Table 3.12).

Table 3.9. Slope and intercept coefficients of the variation of ESP and plasma levels of various hormones individual participants obtained by linear regression.

Participant	Testosterone		Cortisol		Growth Hormone	
Code	Slope	Intercept	Slope	Intercept	Slope	Intercept
10	7.739	0.901	11.353	-18.337	10.648	-0.942
16	9.693	0.076	8.072	31.183	6.850	4.428
18	10.441	0.018	9.218	6.492	9.063	-0.020
23	9.666	0.085	11.882	-17.266	10.411	-0.695
25	8.582	0.625	9.908	-1.892	8.651	0.866
28	9.435	0.592	7.491	20.769	10.935	-2.028
34	8.719	0.777	9.424	6.548	9.392	0.500
38	9.135	0.824	8.337	26.946	9.084	0.399
40	9.097	0.719	9.967	-0.740	10.183	-0.581
42	8.681	0.766	9.728	0.447	9.815	0.143
48	10.085	-0.167	8.881	17.074	9.6780	0.027
51	8.535	0.660	8.048	36.551	9.398	-0.079
55	7.911	0.838	10.560	-6.799	9.746	-0.197
57	8.568	0.655	9.330	6.891	9.206	0.480
Mean	9.020	0.526	9.443	7.705	9.504	0.164
SD	0.781	0.358	1.261	16.970	1.003	1.428
Min	7.739	-0.167	7.491	-18.337	6.8496	-2.028
Max	10.441	0.901	11.882	36.551	10.935	4.428

Table 3.10. Analysis of variance for slope coefficients for the variation of ESP and plasma levels of three hormones of individuals obtained on linear regression.

Source	DF	SS	MS	F	p
Participant	13	13.554	1.043	0.96	0.508
Hormone	2	1.942	0.971	0.90	0.420
Error	26	28.108	1.081		
Total	41	43.604			

DF, degrees of freedom; SS, sum of squares; MS, mean square; F, f-statistic; p, significance level

Table 3.11. Analysis of variance for intercept coefficients for the variation of ESP and plasma levels of individuals obtained on linear regression.

Source	DF	SS	MS	F	p
Participant	13	1328.30	102.18	1.09	0.410
Hormone	2	506.42	253.21	2.69	0.086
Error	26	2443.53	93.98		
Total	41	4278.25			

DF, degrees of freedom; SS, sum of squares; MS, mean square; F, f-statistic; P, significance level

Table 3.12. Test of significance of departure from zero of slope and intercept parameters obtained by linear regression of the variation of ESP and plasma levels of three hormones individual participants.

Variable	N	Mean	SD	SE Mean	95%CI	T	p
Intercept	42	2.8	10.22	1.58	(-0.38, 5.98)	1.78	0.083

N, number of samples; SD, standard deviation; SE Mean, standard error of the mean; 95%CI, ; T, students T; p, significance level

3.3.2 Group Analysis

On the basis of the individual analysis it became apparent that the slopes of the regression equations describing the relationships between the hormones in plasma and ESP samples were similar, exhibiting mean values of 9.020 to 9.504. The first question addressed in this group analysis was to assess whether the relationships between plasma and ESP sample concentrations was similar for all three hormones. This can be examined by fitting a general equation in the form:

$$[Pl] = K + \alpha_0[ESP] + \alpha_1 X_1[ESP] + \alpha_2 X_2[ESP] \qquad \text{(equation a)}$$

Where; [Pl] is the concentration of the hormone in plasma,
K is a constant,
α_0, α_1, α_2 are parameters,
[ESP] is the concentration in the ESP sample, and
X_1 and X_2 are indicator variables

While any of the three hormones could have been used as the reference hormone in the following calculations in this case testosterone was used, thus X_1 and X_2 are indicator variables used multiplicatively for cortisol and growth hormone respectively. Accordingly the equations for each of the hormones are as follows:

Testosterone:$[Pl]_T = K + \alpha_0[ESP]_T + \alpha_1 X_1[ESP]_T + \alpha_2 X_2[ESP]_T$

However, X_1 and X_2 are both equal to 0 as they relate to cortisol and growth hormone, therefore the equation becomes:

$[Pl]_T = K + \alpha_0[ESP]_T$ (equation b)

Cortisol:

$[Pl]_C = K + \alpha_0[ESP]_C + \alpha_1 X_1[ESP]_C + \alpha_2 X_2[ESP]_C$

However, X_2 is equal to 0, therefore the equation becomes:

$[Pl]_C = K + \alpha_0[ESP]_C + \alpha_1 X_1[ESP]_C$

Which is equivalent to:

$[Pl]_C = K + (\alpha_0 + \alpha_1)[ESP]_C$ (equation c)

Growth Hormone:

$[Pl]_{GH} = K + \alpha_0[ESP]_{GH} + \alpha_1 X_1[ESP]_{GH} + \alpha_2 X_2[ESP]_{GH}$

However, X_1 is equal to 0, therefore the equation becomes:

$[Pl]_{GH} = K + \alpha_0[ESP]_{GH} + \alpha_2 X2[ESP]_{GH}$

Which is equivalent to:

$[Pl]_{GH} = K + (\alpha_0 + \alpha_2)[ESP]_{GH}$ (equation d)

The resulting fitted equation is

$[Pl] = 1.251 + 7.885[ESP] + 1.921X_1[ESP] + 0.840X_2[ESP]$ (equation 1a)

with parameters as detailed in Table 3.13. Or as separate hormone equations

Testosterone:	$[Pl] = 1.251 + 7.885[ESP]$	(equation 1b)
Cortisol:	$[Pl] = 1.251 + 9.806[ESP]$	(equation 1c)
Growth Hormone:	$[Pl] = 1.251 + 8.689[ESP]$	(equation 1d)

Table 3.13. Parameters for a regression equation describing the relation between the levels of three hormones in plasma and in ESP fluid as detailed in equation 1a.

Predictor	Coefficient	SE of coefficient	T	p
Constant	1.251	1.084	1.154	0.249
ESP	7.885	2.046	3.855	0.000
X_1ESP	1.921	1.980	0.970	0.333
X_2ESP	0.840	1.479	0.568	0.570

SE, standard error of estimation; T, student's t; p, significance level

The constant (K) in equation 1a was not significantly different from 0 and may therefore be omitted giving the resulting fitted equation

$$[Pl] = 9.954[ESP] + 0.06054X_1[ESP] - 0.334X_2[ESP] \quad \text{(equation 2a)}$$

with parameters as detailed in Table 3.14. Or as separate hormone equations

Testosterone: $[Pl] = 9.954[ESP]$ (equation 2b)

Cortisol: $[Pl] = 10.01454[ESP]$ (equation 2c)

Growth Hormone: $[Pl] = 9.620[ESP]$ (equation 2d)

Table 3.14. Parameters for a regression equation describing the relation between the levels of three hormones in plasma and in ESP fluid, excluding a constant term as detailed in equation 2a.

Predictor	Coefficient	SE of Coefficient	T	p
ESP	9.954	0.985	10.106	0.000
X_1ESP	0.06054	0.986	-0.061	0.951
X_2ESP	-0.334	1.073	-0.312	0.755

SE, standard error of estimation; T, student's t; p, significance level

Noting that the coefficients of X_1ESP and X_2ESP do not approach statistical significance, nor are they large numerically, in relation to ESP, which was itself very highly significant, the data were refitted without these indicator variables, resulting in and equation of the form

$$[Pl] = 9.891[ESP] \quad \text{(equation 3a)}$$

with parameters as detailed in Table 3.15.

Table 3.15. Parameters for a regression equation describing the relation between the levels of three hormones in plasma and in ESP fluid, excluding a constant term or indicator variable as detailed in equation 3a.

Predictor	Coefficient	SE of Coefficient	T	p
ESP	9.891	0.041	243.575	0.000

SE, standard error of estimation; T, student's t; p, significance level

These results provide strong evidence that a single multiplier, estimated to be 9.891 with a standard error of estimation of 0.041, can be applied to ESP sample concentrations of these three hormones in order to estimate plasma concentrations, with reasonable accuracy. Note that in effect, 9.891 is the calibration constant to convert the concentration of the hormone in the ESP sample to the concentration in plasma for this equipment and the protocol described.

3.4 RELATIONSHIPS BETWEEN FITNESS LEVELS AND HORMONE PROFILES

As was described in the Chapter 2 the participant's completed a fitness test once a week on a treadmill. The test involved a three minute warm up walk at 108.3m/min (6.5kph) followed by a test period of increasing treadmill speed at an acceleration of 0.42m/min/s (0.025kph/s). The test was terminated when the participant chose to stop. Table 3.16 shows the results of each completed fitness test. Only those tests for which heart rate was recorded successfully have been included in the table. In most cases there were two fitness tests for each participant. Where two tests did occur, the difference between the maximum heart rates reached on each occasion was no greater than four beats per minute and in most cases the rates were greater than or very near the theoretical maximum heart rate for the participant's age using the equation: HR_{max} = 220 – age (years). The only participants who did not appear to have reached their maximum heart rate were 18, 38 and 40. Hence, for their tests it is likely that VO_{2max} was not achieved. These three participants only completed one test each.

VO_{2max} was estimated using the equation given below {American College of Sports Medicine 1995 #6800}. The walking speed that was used for all participants was 108.3m/min (6.5K/h). This was the warm up speed for each test, which was equivalent to walking speed for most participants. The grade of the treadmill was 8% and was constant throughout the test.

VO_2 (ml/kg/min) = 3.5ml/kg/min + walking speed (m/min) X 0.2 + Grade (fraction) X peak speed (m/min) X 0.9

Figure 3.6 graphs the mean heart rate recorded for all participants for all of the fitness tests against the speed of the treadmill. It shows that heart rate increased as the speed of the treadmill increased during the fitness test. Recording of heart rate did not begin until the start of the test, at which time the participants had been walking briskly on the treadmill for two minutes. Figure 3.7 and Figure 3.8 depict the individual heart rate recorded plotted against the treadmill speed for two fitness tests, one for participant 25 (Figure 3.7) and one for participant 51 (Figure 3.8).

Table 3.16. Maximal fitness tests: speed reached, maximum heart rate and estimated VO_{2max}.

Participant Code	Week of Study	Speed Reached (K/h)	Speed Reached (m/min)	Theoretical Maximum Heart Rate (beats/min)	Maximum Heart Rate Reached (beats/min)	Estimated VO_{2max} (ml/kg/min)
16	3	13.0	216.7	198	192	40.8
18	4	14.0	233.3	201	173	42.0
23	3	15.5	258.3	200	201	43.8
	4	14.0	233.3	200	198	42.0
25	3	16.1	268.3	200	197	44.5
	4	15.5	258.3	200	198	43.8
28	3	14.3	238.3	201	206	42.4
	4	14.0	233.3	201	204	42.0
34	3	13.3	221.7	200	203	41.2
	4	13.4	223.3	200	199	41.3
38	4	12.0	200.0	200	176	39.6
40	3	14.2	236.7	198	183	42.2
42	3	15.0	250.0	199	207	43.2
	4	15.0	250.0	199	206	43.2
48	3	14.5	241.7	200	187	42.6
	4	14.5	241.7	200	185	42.6
51	3	15.5	258.3	200	195	43.8
	4	15.4	256.7	200	192	43.7
55	3	15.2	253.3	199	193	43.4
	4	14.5	241.7	199	191	42.6
57	4	13.2	220.0	200	194	41.0

NB: only those tests for which heart rate was recorded are included in this table.

Table 3.2. Individual ranges of hormone concentrations for samples taken in the morning.

Participant Code	Testosterone Concentration (ng/ml)			Cortisol Concentration (ng/ml)			Growth Hormone Concentration (ng/ml)	
	Plasma	ESP	Saliva	Plasma	ESP	Saliva	Plasma	ESP
10	2.6-4.4	0.11-0.45	0.10-0.22	112-143	10.3-13.9	1.27-4.37	4.8-12.9	0.61-1.30
16	5.1-8.6	0.31-0.89	0.21-0.58	139-217	13.5-23.1	4.21-15.84	11.0-17.6	0.98-1.90
18	2.5-4.9	0.14-0.44	0.07-0.18	39-109	3.5-16.3	0.73-2.61	5.0-13.5	0.53-1.55
23	5.1-9.2	0.31-0.91	0.19-0.42	69-183	6.2-17.1	1.63-4.28	7.8-19.2	0.79-1.90
25	3.5-6.9	0.34-0.72	0.16-0.29	66-140	6.8-18.4	1.73-14.49	5.5-16.2	0.55-1.68
28	3.7-9.8	0.30-0.99	0.21-0.59	76-161	8.1-22.5	1.30-9.29	6.5-18.7	0.78-1.91
34	3.6-5.9	0.28-0.59	0.12-0.29	108-197	6.4-19.9	2.30-6.60	7.4-14.0	0.79-1.49
38	3.8-8.4	0.22-0.77	0.14-0.37	127-184	10.6-18.1	1.72-4.13	5.3-15.4	0.54-1.69
40	3.4-10.5	0.31-1.03	0.17-0.58	101-158	10.4-15.8	2.35-5.04	5.1-20.4	0.61-2.09
42	4.1-9.1	0.34-0.91	0.17-0.57	79-145	8.1-15.2	1.23-5.16	4.6-18.5	0.49-1.84
48	2.1-7.4	0.25-0.77	0.16-0.34	99-204	9.4-21.5	1.50-7.20	4.5-17.4	0.49-1.68
51	3.1-7.9	0.27-0.79	0.15-0.58	129-214	12.5-22.1	2.22-6.69	6.0-16.7	0.72-1.70
55	3.1-7.0	0.16-0.77	0.12-0.34	89-158	8.9-21.3	2.46-8.65	4.8-16.3	0.49-1.71
57	4.4-7.1	0.29-0.76	0.14-0.39	89-169	9.1-25.8	1.75-7.11	6.1-15.9	0.64-1.63

Figure 3.7. Participant 25: heart rate with increasing treadmill speed.

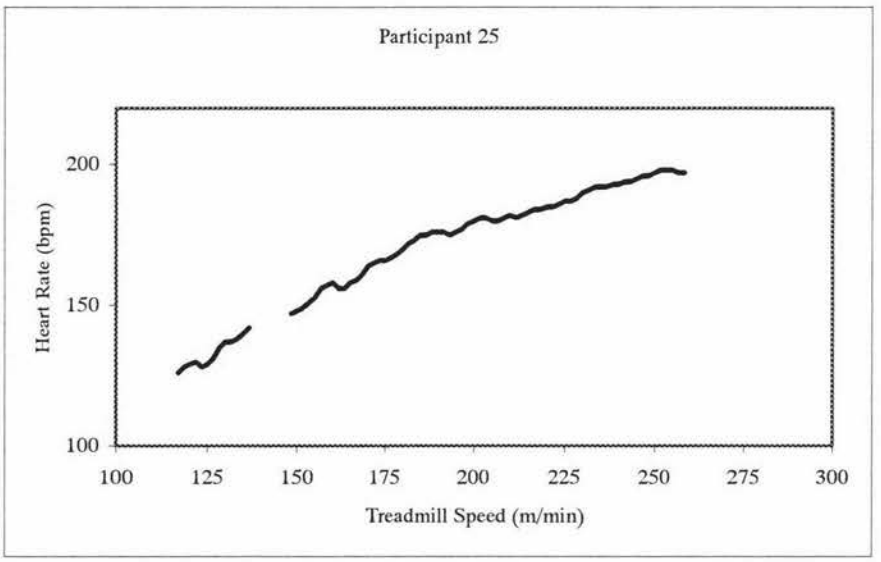
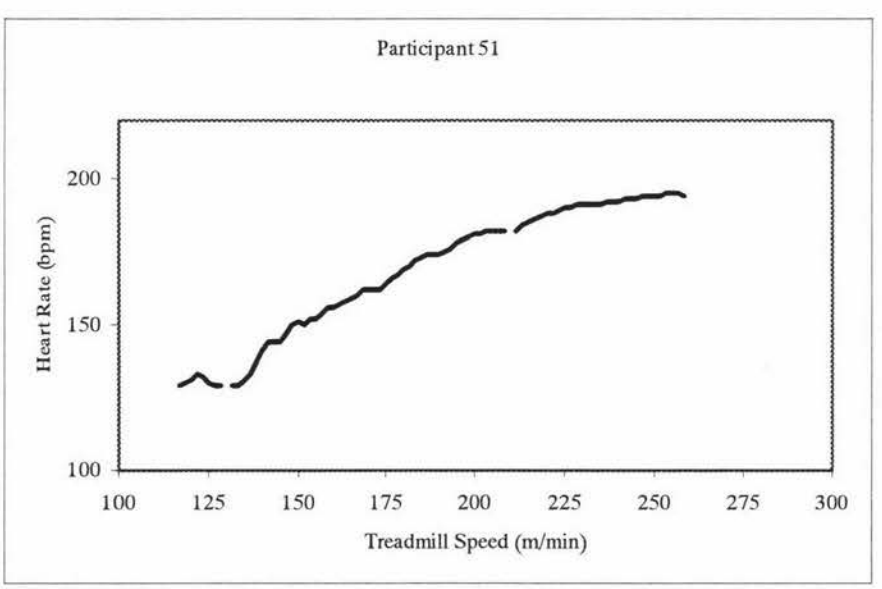


Figure 3.8. Participant 51: heart rate with increasing treadmill speed.



In order to establish whether any relationships existed between estimated VO_{2max} and the hormone profiles of each participant, correlation coefficients were calculated using each participant's mean morning hormone concentrations in plasma, ESP and saliva samples, and their VO_{2max} estimated from the equation. Mean morning hormone concentrations were used instead of morning concentrations on the same day, as fitness tests were not always performed on days where sampling occurred in the morning. The results are shown in Table 3.17. There was a weak, but significant, negative correlation between plasma cortisol concentration in the morning and the estimated VO_{2max} and a

similar negative correlation, which did not quite reach significance, between ESP cortisol concentration and the estimated VO_{2max}.

Table 3.17. Relationships between the estimated VO_{2max} and the mean morning concentration of each hormone.

Sample	Statistic	Testosterone	Cortisol	Growth Hormone
Plasma	r	-0.228	-0.346	-0.122
	p	0.174	0.036*	0.473
ESP	r	-0.034	-0.316	-0.134
	p	0.844	0.057	0.428
Saliva	r	-0.186	-0.081	
	p	0.271	0.632	

r, correlation coefficient; p, significance level

*Correlation significant at the 0.05 level

Table 3.18 and Table 3.19 show the mean ESP and saliva sample concentrations of testosterone and cortisol for the morning, after the fitness test and after the sprints for each participant.

Table 3.18. Comparison of mean ESP and saliva sample testosterone concentrations for each participant during the morning, after the fitness test and after the sprints.

Participant Code	Saliva Concentrations						ESP Sample Concentrations					
	Morning		After Fitness Test		After Sprints		Morning		After Fitness Test		After Sprints	
	[C]	N	[C]	N	[C]	N	[C]	N	[C]	N	[C]	N
10	0.14	8	0.12	1	0.18	4	0.35	6	-	-	0.37	1
16	0.32	10	0.25	2	0.28	3	0.62	8	0.69	1	0.47	1
18	0.12	12	0.11	3	0.23	4	0.34	10	0.43	2	0.34	1
23	0.26	12	0.29	3	0.31	4	0.57	10	0.75	2	0.31	1
25	0.22	12	0.29	4	0.14	3	0.47	10	0.60	2	0.61	1
28	0.34	11	0.34	3	0.39	4	0.64	9	0.78	2	0.51	1
34	0.22	10	0.25	3	0.28	4	0.47	9	0.52	2	0.52	1
38	0.25	12	0.32	3	0.24	4	0.48	10	0.76	2	0.27	1
40	0.33	11	0.19	2	0.27	3	0.62	10	0.41	1	0.43	1
42	0.27	12	0.27	3	0.26	4	0.59	10	0.73	2	0.49	1
48	0.21	12	0.24	3	0.18	4	0.50	10	0.51	2	0.29	1
51	0.27	11	0.21	3	0.30	4	0.55	9	0.60	2	0.44	1
55	0.18	10	0.17	3	0.26	4	0.42	9	0.47	2	0.39	1
57	0.20	12	0.32	2	0.20	3	0.48	10	-	-	0.49	1

[C], concentration in ng/ml; N, number of samples

Table 3.19. Comparison of mean ESP and saliva sample cortisol concentrations for each participant during the morning, after the fitness test and after the sprints.

Participant Code	Saliva Concentrations						ESP Sample Concentrations					
	Morning		After Fitness Test		After Sprints		Morning		After Fitness Test		After Sprints	
	[C]	N	[C]	N	[C]	N	[C]	N	[C]	N	[C]	N
10	2.5	8	2.6	2	1.6	4	12.3	6	-	-	11.5	1
16	6.8	10	2.0	3	2.8	3	19.0	8	8.9	1	11.1	1
18	1.5	12	2.0	3	4.9	4	9.8	10	12.6	2	18.5	1
23	2.8	12	6.5	4	2.6	4	12.0	10	16.6	2	12.8	1
25	3.6	12	1.9	3	4.2	4	11.1	10	13.3	2	10.9	1
28	4.0	11	7.0	3	7.8	4	14.5	9	12.9	2	22.9	1
34	4.2	10	3.6	3	2.3	4	14.0	9	10.9	2	10.4	1
38	2.5	12	3.7	3	2.7	4	14.4	10	3.7	3	5.6	1
40	3.7	11	3.5	3	6.9	3	12.4	10	13.7	1	17.4	1
42	3.1	12	4.5	3	2.2	4	11.9	10	10.7	2	10.9	1
48	3.6	12	2.4	3	1.0	4	14.6	10	14.4	2	9.3	1
51	4.2	11	5.7	3	4.6	4	16.2	9	11.9	2	15.3	1
55	4.5	10	2.4	3	2.2	4	13.7	9	13.9	2	6.9	1
57	3.5	12	3.8	2	1.6	3	15.6	10	-	-	9.4	1

[C], concentration in ng/ml; N, number of samples

Correlation coefficients were also calculated using the ESP and saliva sample concentrations of testosterone and cortisol after the fitness test was completed (Table 3.20). No significant correlations were found.

Table 3.20. Correlating predicted VO_{2max} with ESP and saliva hormone levels after the fitness test.

Sample	Hormone	N	r	p
ESP	Testosterone	22	-0.154	0.492
	Cortisol	22	-0.202	0.368
Saliva	Testosterone	37	-0.248	0.139
	Cortisol	37	0.032	0.849

N, number of samples; r, correlation coefficient; p, significance level

3.5 RELATIONSHIPS BETWEEN SLEEP QUALITY/QUANTITY AND HORMONE PROFILES

3.5.1 Relationships between hormone concentrations and sleep rating, length of the sleep period and time to fall asleep.

The third objective of this research was to investigate the possible effects of sleep quality and quantity on hormone profiles and vice versa. The results from the sleep logs are summarised in Table 3.21. The mean score for the sleep quality rating was 6.4, however the quality of sleep that the participants felt they had, ranged from a value of zero, indicating no sleep, to ten, indicating the best sleep they felt possible. The time taken to fall asleep ranged from one minute to three and a half hours, with a mean time to fall asleep of 24 minutes. The total amount of sleep in one night ranged from five minutes to 17 hours with a mean total time asleep of approximately eight hours.

Table 3.22 shows the correlations of the hormone concentrations in morning ESP samples with four of the sleep quality/quantity variables taken from the sleep logs on the night prior to when the sample was taken. No significant correlations were found.

Table 3.21. Sleep quality and sleep quantity of the participants.

Participant Code	Sleep Rating			Sleep Latency (min)			Total Time Asleep (min)		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
10	0-9	6.5	2.0	5-120	23	26	5-565	415	129
16	5-9	7.1	1.0	1-60	31	17	290-660	533	90
18	4-9	6.9	1.1	5-150	24	32	330-615	511	65
23	3-7	5.4	1.0	1-30	9	7	253-689	462	114
25	3-8	5.9	1.2	5-60	27	13	325-655	476	69
28	1-8	6.2	1.2	2.60	15	13	285-650	504	102
34	6-10	8.2	1.3	1-30	11	9	359-1020	580	135
38	5-9	6.9	1.0	5-60	24	13	355-630	528	76
40	2-9	6.6	1.6	5-75	30	16	210-690	469	81
42	3-8	6.0	1.4	1-30	18	10	320-625	466	81
48	4-8	6.7	1.0	5-120	46	28	250-600	478	80
51	3-8	6.4	1.2	1-60	21	20	215-630	475	101
55	1-8	5.1	1.9	20-120	34	22	180-675	469	112
57	2-8	6.3	1.6	5-90	16	18	225-630	485	107
Group	0-10	6.4	1.5	1-150	24	21	5-1020	489	104

Table 3.22. Correlations between hormone levels and sleep quality and sleep quantity measures.

Hormone Measured in ESP Sample	Statistic	Sleep Rating	Sleep Latency	Total Time Asleep
Testosterone	r	-0.022	-0.090	0.046
	p	0.809	0.316	0.612
	N	125	125	125
Cortisol	r	0.020	0.025	0.022
	p	0.826	0.784	0.804
	N	125	125	125
Growth Hormone	r	0.055	-0.080	0.075
	p	0.594	0.440	0.470
	N	96	96	96

N, number of samples; r, correlation coefficient; p, significance level

*Correlation significant at the 0.05 level

We can investigate whether a relationship existed between the accumulation of sleep quality and quantity over several days and the hormone concentration in plasma by correlating the mean total time asleep during those days with the concentrations of testosterone, cortisol and growth hormone. Table 3.23 details the correlations between the mean sleep quality rating for the previous four, three and two nights and hormone concentrations in ESP samples. No significant correlations were found.

Table 3.23. Correlations between hormone levels in ESP samples and sleep quality rating.

		Mean previous nights sleep rating		
Hormone	**Statistic**	**4**	**3**	**2**
Testosterone	r	0.034	0.030	0.079
	p	0.708	0.740	0.379
	N	125	125	125
Cortisol	r	0.062	0.030	0.079
	p	0.546	0.740	0.379
	N	96	125	125
Growth Hormone	r	0.062	0.033	0.049
	p	0.546	0.752	0.635
	N	96	96	96

N, number of samples; r, correlation coefficient; p, significance level

Table 3.24 details the correlations between the mean total time asleep for the previous four, three and two nights and hormone concentrations in ESP samples. No significant correlations were found.

Table 3.24. Correlations between hormone levels in ESP samples and total time asleep.

		Mean previous total time asleep		
Hormone	**Statistic**	**4**	**3**	**2**
Testosterone	r	0.031	-0.072	-0.022
	p	0.729	0.428	0.805
	N	125	125	125
Cortisol	r	0.153	0.119	0.082
	p	0.089	0.188	0.363
	N	125	125	125
Growth Hormone	r	0.111	0.040	0.130
	p	0.280	0.696	0.208
	N	96	96	96

N, number of samples; r, correlation coefficient; p, significance level

3.5.2 Sleep disturbances and hormone concentrations

We examined whether experiencing sleep disturbances was associated with different hormone concentrations the following morning. Table 3.25 shows the mean hormone concentrations the morning after nights with no sleep disturbances and after nights during which sleep disturbances occurred.

Levene's test was performed to test whether the variation of the concentrations for the two groups (no sleep disturbances and sleep disturbances) is the same. Equal variance was assumed when the test significance was greater than 0.05. Where the significance was less than 0.05, equal variance is not assumed. The only sample set where the significance was less than 0.05 was for ESP growth hormone concentration; in this case equal variance was not assumed. For all others equal variance was assumed as the test significance's were greater than 0.05.

The concentration of cortisol in plasma and ESP samples during the morning after participants had experienced sleep disturbances in the previous sleep episode were significantly lower than when sleep disturbances were absent. Sleep disturbances had no

significant effect on testosterone and growth hormone concentrations in the same samples.

Table 3.25. Mean hormone concentrations on days following nights of undisturbed and disturbed sleep in the same group of subjects.

Hormone	Sample	Disturbances	N	Mean (ng/ml)	SD	SE	t-test sig.
Testosterone	Plasma	No	55	5.4	1.59	0.21	0.892
		Yes	39	5.4	1.91	0.31	
	ESP	No	66	0.51	0.18	0.02	0.976
		Yes	59	0.51	0.21	0.03	
	Saliva	No	83	0.23	0.10	0.01	0.404
		Yes	71	0.24	0.11	0.01	
Cortisol	Plasma	No	55	144	32.7	4.41	<0.001
		Yes	39	114	36.8	5.89	
	ESP	No	66	14.5	3.55	0.44	0.026
		Yes	59	12.9	4.49	0.58	
	Saliva	No	83	3.8	2.24	0.25	0.301
		Yes	71	3.4	2.09	0.25	
Growth Hormone	Plasma	No	55	12.0	3.92	0.51	0.927
		Yes	39	12.1	4.63	0.74	
	ESP	No	55	1.3	0.38	0.05	0.994
		Yes	41	1.3	0.48	0.07	

N, number of samples; SD, standard deviation; SE, standard error of the mean

3.5.3 Relationship between dream remembering and hormone concentrations

We can perform a similar analysis for sleep episodes when the participant remembers dreams and for sleep episodes where no dreams were remembered. The results from this are shown in Table 3.26.

Using Levene's test, equal variance was assumed for all hormone samples except salivary cortisol concentration where equal variance was not assumed. None of the differences in mean concentrations of hormones were significant, although the lower mean cortisol concentration in saliva when dreams were remembered closely approached significance (p=0.051).

Table 3.26. Mean hormone concentrations on days following nights of sleep with remembered dreams and nights with no remembered dreams in the same group of subjects.

Hormone	Sample	Dreams	N	Mean (ng/ml)	SD	SE	t-test Sig.
Testosterone	Plasma	No	39	5.3	1.90	0.30	0.831
		Yes	55	5.4	1.60	0.22	
	ESP	No	49	0.51	0.21	0.03	0.860
		Yes	76	0.51	0.18	0.02	
	Saliva	No	63	0.24	0.11	0.14	0.441
		Yes	91	0.23	0.10	0.01	
Cortisol	Plasma	No	39	135	40.80	6.53	0.520
		Yes	55	130	34.90	4.70	
	ESP	No	49	13.5	4.10	0.59	0.548
		Yes	76	14.0	4.09	0.47	
	Saliva	No	63	4.1	2.68	0.34	0.051
		Yes	91	3.3	1.68	0.18	
Growth Hormone	Plasma	No	39	11.8	4.54	0.73	0.616
		Yes	55	12.2	3.98	0.54	
	ESP	No	39	1.2	0.45	0.07	0.670
		Yes	57	1.3	0.41	0.54	

N, number of samples; SD, standard deviation; SE, standard error of the mean

3.6 FITNESS MEASURES AND SLEEP QUALITY/QUANTITY

Table 3.27 shows the results of correlations between estimated VO_{2max} in the fitness test and the sleep quality parameters of the previous night's sleep. The results from 37 fitness tests were used in these calculations. No significant correlations were found.

Table 3.27. Correlation of estimated VO_{2max} with quality of previous nights sleep.

Sleep Measure	r	p
Sleep Rating	-0.201	0.234
Sleep Latency	-0.067	0.694
Total Time Asleep	-0.275	0.100

r, correlation coefficient; p, significance level

We can investigate whether accumulating sleep quality and sleep quantity has an effect on the estimated VO_{2max} by the participants. Table 3.28 shows the correlations of total sleep time and sleep rating with estimated VO_{2max} when a mean of the previous four, three and two nights scores are used in the calculation. There was a weak, negative but significant correlation between total time asleep and estimated VO_{2max} for the mean of the previous four and three nights and a negative correlation with total time asleep during the previous two nights that closely approached significance.

Table 3.28. Correlations between estimated VO_{2max} and the mean sleep rating and total time asleep during the four, three and two nights before exercise testing.

Sleep Measure	Mean of previous number of nights	r	p
Sleep Rating	4	-0.280	0.093
	3	-0.289	0.082
	2	-0.276	0.099
Total Time Asleep	4	-0.388*	0.018
	3	-0.360*	0.029
	2	-0.323	0.051

R, correlation coefficient; p, significance level

*Correlation significant at the 0.05 level

3.7 ALCOHOL CONSUMPTION AND HORMONE CONCENTRATIONS

Alcohol consumption of the participants was measured by reporting the number of standard drinks consumed during every twenty-four hour period prior to rising in the morning. The relationships alcohol consumption and subsequent hormone concentrations were investigated both collectively and individually. Table 3.29 shows the number of times during the study each participant consumed alcoholic beverages, how many times the drinking session was light (6 or less standard drinks) or heavy (7 or more standard drinks) and the amount consumed in standard drinks.

Table 3.29. Number of times alcohol was consumed and amount consumed by each participant over the four week period of the study.

Participant Code	Number of Times Alcohol Consumed			Number of Standard Drinks Consumed		
	Total	Light[a]	Heavy[b]	Range	Mean	SD
10	11	5	6	1-50	16	17
16	6	1	5	4-52	31	16
18	0	-	-	-	-	-
23	12	3	9	2-45	19	12
25	5	2	3	2-20	10	9
28	10	5	5	1-45	20	19
34	7	1	6	2-50	31	16
38	5	1	4	5-20	16	6
40	4	2	2	1-30	10	14
42	10	3	7	1-23	13	7
48	9	1	8	3-40	26	11
51	10	1	9	2-40	23	11
55	8	2	6	2-28	16	9
57	0	-	-	-	-	-
Group	97	27	70	1-52	19	12

[a]Light drinking is defined as 6 or less standard drinks in one drinking session

[b]Heavy drinking is defined as 7 or more standard drinks in one drinking session

Twelve of the fourteen participants engaged in regular consumption of alcoholic beverages. The number of standard drinks consumed during any one drinking session

ranged from one to 52; however 70 of the 97 drinking sessions that were recorded can be defined as heavy with 19 being the mean number of standard drinks consumed.

The amount of alcohol consumed, measured in standard drinks, in the twenty-four hours prior to sample collection was compared with hormone concentrations within the three samples to investigate whether a relationship could be established, i.e. between the amount of alcohol consumed and the concentrations of hormones in plasma, ESP or saliva samples. Table 3.30 displays the resulting correlations between alcohol consumption during the previous twenty-four hours measured in standard drinks, and the plasma, ESP sample and salivary concentrations of the three hormones the following morning. The concentration of testosterone and cortisol in saliva exhibited weak, but significant, positive correlations with alcohol consumption in the previous twenty-four hours.

Table 3.30. Relationships between alcohol consumption and plasma, ESP and saliva sample hormone concentrations the following morning.

Sample	Hormone	r	p	N
Plasma	Testosterone	0.053	0.613	94
	Cortisol	0.152	0.145	94
	Growth Hormone	0.051	0.623	94
ESP	Testosterone	0.115	0.202	125
	Cortisol	0.033	0.718	125
	Growth Hormone	0.051	0.624	96
Saliva	Testosterone	0.173*	0.026	153
	Cortisol	0.180*	0.026	153

N, number of samples; r, correlation coefficient; p, significance level

*Correlation significant at the 0.05 level

Due to the limited amount of data available where alcohol had been consumed and plasma, ESP and saliva samples taken the following morning the data were grouped together in terms of hormone concentrations when alcohol had been consumed in the previous twenty-four hours and hormone concentrations when alcohol had not been consumed. Levene's test was performed to test whether the variation of the concentrations for the two groups (alcohol consumed and no alcohol consumed) was the same. Equal variance was assumed when the test significance was greater than 0.05.

Where the significance was less than 0.05, equal variance was not assumed. Equal variance was not assumed for all samples. The absence of significant differences (Table 3.31) suggests that the consumption of alcohol did not effect the hormone concentrations of samples taken the following morning.

Table 3.31. Mean hormone concentrations in the morning when alcohol had and had not been consumed in the previous twenty four hours.

Hormone	Sample	Alcohol Consumed	N	Mean (ng/ml)	SD	SE	t-test Sig.
Testosterone	Plasma	No	72	5.3	1.781	0.210	0.665
		Yes	22	5.5	1.544	0.329	
	ESP	No	101	0.50	0.194	0.019	0.091
		Yes	24	0.56	0.163	0.033	
	Saliva	No	118	0.23	0.104	0.010	0.105
		Yes	36	0.26	0.115	0.019	
Cortisol	Plasma	No	72	129	37.1	4.37	0.124
		Yes	22	142	36.9	7.87	
	ESP	No	101	13.7	4.23	0.421	0.702
		Yes	24	14.0	3.46	0.706	
	Saliva	No	118	3.5	2.04	0.187	0.233
		Yes	36	4.0	2.55	0.425	
Growth Hormone	Plasma	No	72	11.9	4.23	0.500	0.636
		Yes	22	12.4	4.16	0.888	
	ESP	No	74	1.2	0.432	0.050	0.529
		Yes	22	1.3	0.405	0.086	

N, number of samples; SD, standard deviation; SE, standard error of the mean

The relationships between alcohol consumption in the previous twenty-four hours and hormone concentrations in the three samples the following morning were investigated

and the significant correlations that were found are listed in Table 3.32. These correlations were generally strong and highly significant. However there were very low numbers of samples for each participant where alcohol consumption had been followed by a morning of sample collection (between one and five) and therefore the results are not very convincing.

Table 3.32. Significant correlations between an individual's alcohol consumption and their hormone concentration the following morning.

Sample	Hormone	Participant Code	r	p	N	N[1]
Plasma	Growth Hormone	40	-0.927*	0.023	5	1
ESP	Cortisol	25	0.718*	0.019	10	1
ESP	Growth Hormone	40	-0.886*	0.045	5	1
Saliva	Testosterone	16	0.809**	0.005	10	2
Saliva	Testosterone	23	0.730**	0.007	12	5
Saliva	Testosterone	51	0.650*	0.030	11	4
Saliva	Cortisol	16	0.891**	0.001	10	2
Saliva	Cortisol	38	0.787**	0.002	12	2

N, number of samples; r, correlation coefficient; p, significance level

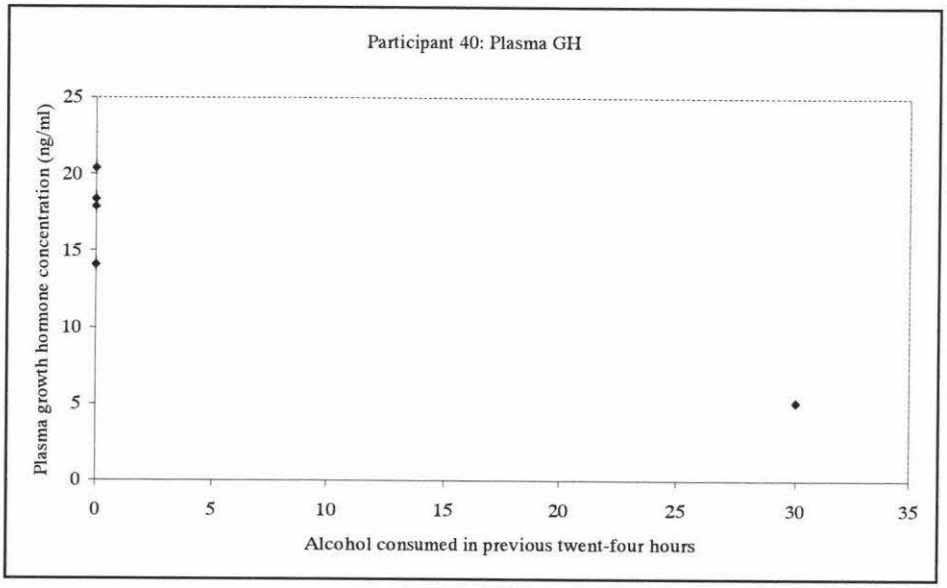
[1] Number of samples included in correlation when alcohol consumed in previous 24 hours

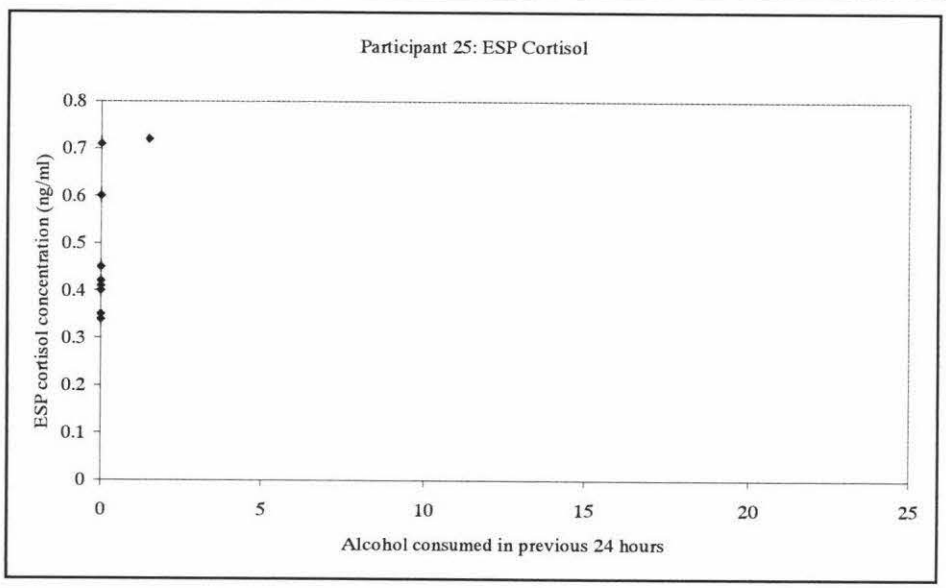
**Correlation significant at the 0.01 level

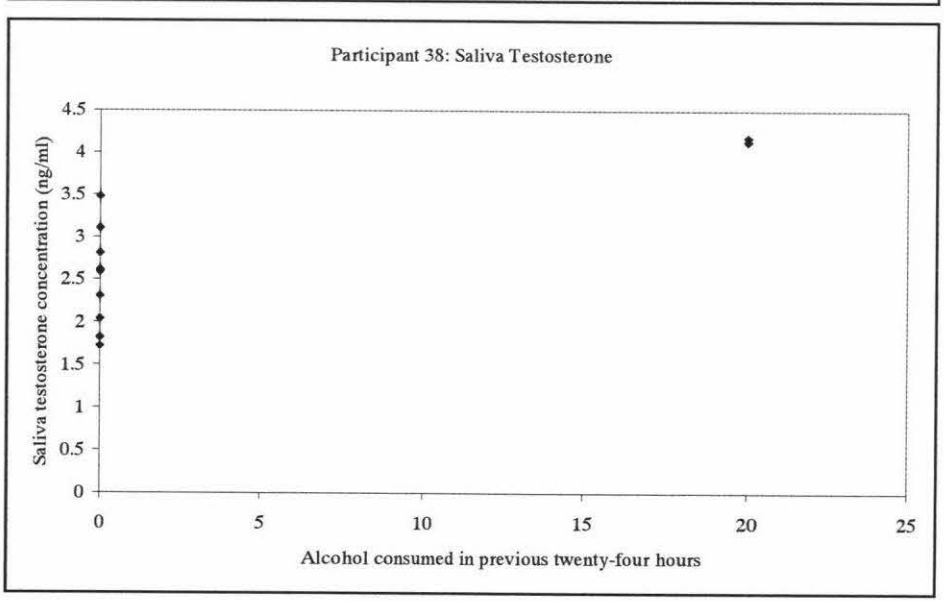
*Correlation significant at the 0.05 level

Three of these relationships are graphed in Figure 3.9. While the results are significant we can see that we cannot draw any confident conclusions, as there are very few points on the graphs were alcohol has been consumed.

Figure 3.9. Relationships between alcohol consumption by participants in the previous twenty-four hours and hormone concentrations.







3.8 SPRINT DATA ANALYSIS

Each week, three 40m sprints were completed by each participant. The mean time taken for a sprint during each week is detailed in Table 3.33.

Table 3.33. Mean sprint times for each week.

Week	Mean sprint time (s)	SD	SE
1	5.435	0.251	0.041
2	5.114	0.231	0.037
3	5.202	0.288	0.049

SD, standard deviation; SE, standard error of the mean

The analysis of the differences between sprint times for each week is detailed in Table 3.34. The third column in the table lists the differences of the mean sprint times between each week. So for example the difference in mean sprint time between week one and two was 0.322 seconds. There was a significant difference between sprint time for week one and week two (p=<0.001) and for week one and week three (p=0.001). No significant difference existed between the mean sprint time for week two and week three. The mean time taken to sprint 40m decreased from week one to week two, whereas from week two to week three the mean time taken to sprint 40m effectively remained constant.

Table 3.34. Analysis of differences in sprint times between weeks.

Week (A)	Week (B)	Mean Difference (A-B) (s)	SE	p
1	2	0.322*	0.059	<0.001
1	3	0.233*	0.060	0.001
2	1	-0.322*	0.059	<0.001
2	3	-0.089	0.059	0.299
3	1	-0.233*	0.060	0.001
3	2	0.089	0.059	0.299

SE, standard error of the mean; p, significance level

*mean difference significant at 0.01 level

The mean time taken for a sprint for each attempt (i.e. first, second and third sprint) is detailed in Table 3.35. The differences in mean time of each of the sprints were analysed. The third column in Table 3.36 lists the difference of the mean time between each sprint. So for example the difference in mean sprint time between first and second sprint was 0.043 seconds. None of the differences were significant.

Table 3.35. Mean sprint times for each sprint attempt.

Sprint Attempt	Mean sprint time (s)	SD	SE
1st	5.279	0.294	0.047
2nd	5.237	0.297	0.049
3rd	5.225	0.280	0.047

SD, standard deviation; SE, standard error of the mean

Table 3.36. Analysis of differences in sprint times for the first, second and third sprint.

		Mean Difference		
Sprint (A)	Sprint (B)	(A-B)	SE	p
1	2	0.043	0.067	0.800
1	3	0.054	0.067	0.704
2	1	-0.043	0.067	0.800
2	3	0.011	0.068	0.985
3	1	-0.054	0.067	0.704
3	2	-0.011	0.068	0.985

SE, standard error of the mean; p, significance level

Chapter Four: Discussion

Table of Figures

4.1 INTRODUCTION

This study produced some interesting results in terms of validating the use of transdermal electrosonophoresis (ESP) as a method of measuring a person's testosterone, cortisol and growth hormone level. These will be discussed in further detail within this chapter.

For establishing the relationships between hormone profiles, sleep and athletic training the results are not as revealing. Due to the unforeseen problems and the limited experimental design of this pilot study, this thesis has revolved around a critique of the methods used and alternate methods not employed here, and the implications for further studies.

4.2 FINDINGS OF THIS STUDY

The major findings of this pilot study are outlined here and will be reviewed in more detail in the discussion below:

1. When the participants' samples were analysed collectively, the correlation coefficients for the hormone concentrations in the samples were highest for the plasma: ESP comparison, ranging from 0.965 to 0.977 ($p<0.001$). The correlation coefficients for the plasma: saliva comparison ranged from 0.612 to 0.791 ($p<0.001$) and for the ESP: saliva comparison from 0.522 to 0.698 ($p<0.001$). As can be seen from the p values these correlations are highly significant.

2. When the participant's samples were analysed individually, the plasma: ESP correlation coefficients for testosterone ranged from 0.802 to 0.996 (13 significant at $p<0.01$, one significant at $p<0.05$). For cortisol, the correlations ranged from 0.734 to 0.998 (12 significant at $p<0.01$, one significant at $p<0.05$) and for growth hormone the correlations ranged from 0.920 to 0.996 (13 significant at $p<0.01$, one significant at $p<0.05$).

3. When the effect of exercise on the ESP: saliva testosterone and cortisol concentration correlations were analysed, the strong, positive, significant correlation for testosterone with no exercise remained with sample analysis after exercise ($r=0.822$, $p<0.01$). This was not the case with cortisol, as the sample analysis after exercise did not reveal a strongly correlated, significant relationship ($r=0.539$, $p=0.994$).

4. Regression equations of the hormone concentrations in plasma and ESP samples were derived for each hormone in each participant and were found to be similar. The individual participants' slopes for the three hormones ranged from 6.85 to 11.88 while the intercepts ranged from -18.34 to 36.55. There were no significant differences in the slopes and in the intercepts of the equations for each hormone. The intercepts were not significantly different from zero. This

finding allowed the data to be grouped together for collective analysis and calculation of a single set of equations for converting an ESP sample concentration into an estimated plasma sample concentration.

5. When the individual participant's hormone data were combined it was found that the intercept was not significantly different to zero and that the regression equation for the three hormones were not significantly different from each other. Therefore a single equation could be derived to estimate the plasma concentrations of testosterone, cortisol or growth hormone from their respective ESP concentrations. The resulting equation was:

   $$[Pl] = 9.891[ESP]$$

   Where; [Pl] is the concentration of the hormone in plasma, and
   [ESP] is the concentration of the hormone in the ESP sample.

6. There existed a weak, negative but significant correlation ($r=-0.346$, $p=0.036$) between the mean plasma cortisol concentration measured in the morning and the estimated VO_{2max}. A very similar correlation coefficient existed for the relationship between the cortisol concentration measured in the ESP sample and estimated VO_{2max} but this did not quite reach significance ($r=-0.316$, $p=0.057$).

7. No relationship could be established between estimated VO_{2max} and the testosterone or cortisol concentrations in the ESP or saliva samples after the treadmill test. This same relationship was not investigated for growth hormone as the samples were not analysed for growth hormone because its concentration in saliva is below the detection limit of the assay.

8. Sleep quality rating, sleep latency and total time asleep did not exhibit any significant relationship with the hormone concentrations measured in the samples taken the following morning.

9. No relationship appeared to exist between hormone levels in the samples when comparing them with mean sleep rating and total time asleep over the previous two, three and four nights.

10. The presence of sleep disturbances was associated with a decrease in the following morning's cortisol concentrations in both plasma and ESP samples. In plasma the decrease was from a mean cortisol concentration of 145ng/ml with no sleep disturbances to 115ng/ml with sleep disturbances. This decrease is significant (t-test: $p<0.001$). In the ESP samples the mean cortisol concentration decreased from 14.5ng/ml to 13.0ng/ml ($p=0.026$).

11. No relationship was established between estimated VO_{2max} and the sleep quality and sleep quantity measures.

12. There existed a weak, negative but significant correlation between estimated VO_{2max} and the mean total time asleep for the previous three or four nights ($r= -0.360$, $p=0.029$ and $r=-0.388$, $p=0.018$, respectively).

13. A weak, positive but significant correlation was established between alcohol consumption and the concentration of testosterone and cortisol measured in saliva the following morning ($r=0.173$, $p=0.026$ and $r=0.180$, $p=0.026$, respectively) when all of the individual participant's data were analysed collectively.

14. When the participant's data were analysed separately to establish relationships between alcohol consumption and hormone concentrations the following morning some highly significantly correlations were found (r from –0.927 to 0.891, p from 0.001 to 0.045).

15. There was a decrease in the mean time taken to sprint 40m by the participants from week one (5.435 seconds) to week two (5.114 seconds), with a significance of $p<0.001$. There was no significant change in the mean time to sprint 40m from week two to week three.

16. There was no change in the mean time taken to sprint 40m on the participants first, second and third attempts within each week, i.e. they did not appear to be getting faster or slower.

4.3 VALIDATING THE USE OF ESP TO ESTIMATE PLASMA HORMONE CONCENTRATIONS

The present work contributes to the research that shows that ESP may be used to monitor constituents of plasma quickly and safely in a non-invasive manner. The testosterone, cortisol and growth hormone concentrations in plasma and ESP samples demonstrated strong correlations (Table 3.4). The results suggest that ESP sampling to estimate plasma hormone concentrations of testosterone, cortisol and growth hormone may offer a more accurate alternative to saliva sampling, this will be discussed shortly. These results support and extend previous studies for these and other plasma constituents. Kost et al (2000) used this technology to demonstrate that glucose can be extracted from the blood stream through the skin in both rats and humans. Cook (2002) measured cortisol, testosterone, 17- estradiol, insulin and glucose using ESP sampling technology in sheep and humans.

4.3.1 Ease of use and safety of ESP

In addition to the statistical evidence presented in this thesis, observations made during the study lend further support to the use of this device in preference to saliva sampling. No adverse physical sensations were reported by the participants relating to the use of the device during this study, as reported by Cook (2002) and Kost et al (2000) who both state that there was no reported pain or discomfort during their studies. Often, when the device had been left continuously running for some time during sampling, it felt warm although the temperature was not hot enough to cause any discomfort or pain. The ultrasound pulse applied to the arm to collect the ESP sample had a frequency of 20kHz and an intensity of $10Wcm^2$. These settings were also used in studies on humans by Cook (2002) and Kost et al (2000). Kost et al (2000) state that a two minute dose at this intensity is below the maximum energy dose used in therapeutic ultrasound (Antich, 1982) so that the dose used in the present study is well within the guidelines as this same intensity was only applied for 15 seconds. The 9V applied to the skin for the 15 second duration of ultrasound was not felt by 13 of the 14 participants. The one who could feel it reported it as a mild tingling sensation.

ESP samples were collected five times a week, representing a total of 20 collections from each participant during the four-week study. Visual observation of the participant's skin revealed no obvious reactions to the ultrasound waves, voltage or ultrasound gel applied to the area. Moreover no visible effects were seen by Kost et al (2000).

4.3.2 Is ESP sample analysis preferable to plasma sample analysis?

Accessing extracellular fluid composition using ESP technology allows plasma composition to be estimated due to exchange of substances across capillary walls. Capillaries, which form large branching networks, are made from a single layer of epithelial cells which allow rapid exchange of materials both from plasma to extracellular fluid and vice versa (Taylor and Moore, 1999). There are three methods of capillary exchange; diffusion, vesicular transport and bulk flow (Taylor and Moore, 1999). Most plasma constituents diffuse through the capillary wall down their concentration gradients either directly through the lipid bilayer or through fenestrations or intracellular clefts (Taylor and Moore, 1999).

Hormone concentrations in plasma are used as the primary reference measurement during endocrine studies and in clinical diagnosis. In fact, relationships have been established between plasma composition and the functioning of virtually every tissue, organ and organ system in the body. In the present study the plasma: ESP sample correlations for testosterone, cortisol and growth hormone ranged from 0.965 to 0.977 and all were highly significant. However, although the ESP values correlate very well with plasma values, they do not give us the same results as plasma, which is further indicated by the fact that the correlation coefficients are not 1.000. In fact, ESP estimates for bound hormones may only represent the free plasma concentrations and not the total. Accordingly, ESP estimates are an approximation to plasma concentrations, albeit in these cases a close approximation.

Despite this limitation, the correlations are good making ESP sampling an excellent substitute for plasma sampling. The main reason for seeking this substitute is to avoid the discomfort and pain caused by penetrating the skin during blood collection. The use of needles for skin pricking, venepuncture and catheterisation may result in an

unwillingness of volunteers to participate in studies involving blood collection. An additional benefit of this sampling methodology is that once refined it is a simple and safe method that non-specialists can use.

4.3.3 Is ESP sample analysis preferable to saliva sample analysis?

As a time delay exists for steroid hormones to cross the partition between blood and saliva, changes in the concentration of steroid hormones in plasma are not immediately reflected in saliva (Quissell, 1993). The results of the present study demonstrate this by the poor plasma: saliva hormone concentration correlations (r=0.612-0.791) compared with plasma: ESP hormone concentration correlations (r=0.965-0.970). Cook (2002) measured testosterone, cortisol, 17-β estradiol, insulin and glucose in humans using ESP technology and showed that plasma concentration correlated better with the ESP sample concentration (r=0.91) than with the saliva sample concentration (r=0.83). In Cook's study the concentration in the ESP sample was between 8% and 12% of that measured in blood at the same time.

For relatively labile hormones such as cortisol the time delay results in even poorer correlations between plasma and saliva values as the concentration in plasma often changes. This effect can be seen as the correlation between plasma and saliva is higher for the hormone testosterone (r=0.791) than for cortisol (r=0.612). When the testosterone and cortisol concentrations are divided into samples taken in the morning and samples taken after the fitness test the effect of rapidly changing hormone concentrations on the plasma or ESP sample and saliva correlations is further demonstrated. As cortisol is secreted by the adrenal cortex in response to physical stress the cortisol concentration in plasma is likely to be increased after the fitness tests (Scheen et al, 1998). The ESP sample concentrations were correlated with the saliva concentrations because plasma samples were not taken following the fitness tests and ESP sample hormone concentrations were very highly correlated with plasma sample hormone concentrations. The results show that the significant correlation of testosterone in ESP samples with testosterone in saliva samples remains fairly constant with exercise ($r=0.748$, $p<0.01$ no exercise; $r=0.822$, $p<0.01$ after exercise), however the significant correlation of cortisol in ESP samples with cortisol in saliva samples is lost after exercise ($r=0.559$, $p<0.01$ no exercise, $r=-0.002$, $p=0.994$ after exercise).

Another study has compared correlations between plasma and saliva and between plasma and ESP samples both before and after exercise for testosterone, cortisol, 17-estradiol and glucose (Cook, 2002). In that study, the high plasma: ESP sample concentration correlation before exercise (r=0.91) remained for the same correlation after exercise. However the plasma: saliva sample concentration correlation was reduced after exercise (r=0.83 before exercise; r=0.64 after exercise). From the present results and those from Cook (2002) we can conclude that ESP sampling is preferable to saliva sampling for at least testosterone and cortisol, particularly when sampling occurs following exercise.

Growth hormone is only found in minute concentrations in saliva (Rantonen, 2000), therefore saliva sampling is not commonly used as a non-invasive method of estimating plasma growth hormone concentration. As the concentration of growth hormone can be measured in ESP samples, as seen during this study, and the correlation between its plasma and ESP sample concentrations is very high, ESP sampling introduces another non-invasive method of estimating plasma growth hormone concentration which is obviously more useful in this respect than saliva sampling.

Several problems associated with saliva collection were outlined in Chapter One of this thesis. These problems do not exist with ESP sampling. In contrast to saliva collection, there was never a problem with collecting a large enough quantity of ESP sample for analysis of all three hormones. The volume of the sample collected depends on the time for which the device is operated while in contact with the skin. Therefore should a researcher wish to analyse more plasma hormones or other substances, thereby requiring a greater sample volume, then the operation protocol can be lengthened to provide a larger sample. Some of the problems associated with saliva sampling result from the time delay between changes in plasma concentrations being reflected in salivary concentrations (Quissell, 1993). This is in contrast to the rapid exchange of plasma constituents between capillaries and extracellular fluid as explained above. Moreover, metabolism of steroid hormones by salivary gland epithelial cells during transcelluar movement and by oral bacteria in the saliva may alter the concentration measured in saliva which would then not truely reflect the plasma concentration (Quissell, 1993). Finally, saliva samples can often be contaminated through tissue trauma and poor oral hygiene; these appear not to be problems with ESP sampling.

4.3.4 Immunosensors for immediate sample analysis

Alternative to sample collection, removal and analysis by ELISA methods in a laboratory, immunosensors can be fitted to the ultrasound device at the exit point of the sampling head chamber. This technique was used to measure the concentration of testosterone, cortisol, 17- estradiol and insulin is described in Cook (2000). This allows for immediate measurement of hormone concentrations and increases the range of applications of the ESP technology and will be discussed below.

4.3.5 Opportunities for application of ESP

The potential opportunities for use with humans will be discussed here. It is important to note however that many of these opportunities will also be relevant to animal research and veterinary medicine. This technology provides a range of opportunities for research in sport and exercise where a low invasive method would maximise full participation in such trials. A positive effect of this new device is an increase in the number of suitable participants available for research. Due to the reduced need for blood sampling by venepuncture it is likely that management staff of elite athletes and the athletes themselves would be more likely to agree to be involved in studies that would otherwise have required blood sampling by skin or venepuncture. This technology also increases the number of samples that could be taken from human participants in a set time period, as the method is non-invasive, painless and the small volume of exudates required will not measurably alter extracellular composition.

Monitoring training effectiveness may also be possible with ESP. For example, blood composition can be examined for potential hormonal markers of overtraining such as low testosterone: cortisol ratios (Urhausen et al, 1995). ESP may also provide a method of sampling during competition, due to its speed in sample collection and low invasiveness. This could be applied at all levels of competition and has applications in many areas. For example, analysis of bioenergetic metabolites in plasma can provide trainers with a clear picture of which energy systems are being used and in what capacity during competition (Poortmans, 1993). They can then apply a training programme that would improve these systems in relation to their use. Other potential

applications of this technology are drug testing, nutritional studies and patient monitoring.

Study design in endocrine and other research involving neonates, critical care patients and the elderly are often limited as blood removal can pose problems in these groups. Problems such as location of veins for blood removal, pain of the procedure and the small amount of blood that can be removed from these participants without adverse effects can limit study design. The use of ESP technology in these studies would increase the number of samples that could be collected therefore improving study design and the interpretive value of the results. For the same reasons this technology has benefits in the field of medicine. Because the method is painless and does not remove large amounts of blood it would be particularly good for monitoring hormone concentrations in neonates, critical care patients and the elderly. Addition of immunosensors to the device for immediate measurement of blood hormone concentration would enhance its uses particularly for monitoring the status of a patient. Kost et al (2000) discussed the development of technology in the maintenance of blood glucose levels in people with type I diabetes. A wristwatch/patch type device that senses and displays blood glucose concentration could potentially be combined with delivery of insulin in response to physiological requirements. This type of technology could also be developed for administration of many other hormones or drugs.

4.3.6 Areas of further investigation

As this technology is relatively new there are still a number of areas that require extensive investigation to allow the wide application of this technology. Listed below are some of the factors that may have an effect on the accuracy on ESP sample measurement and still require investigation.

1. Individual differences in body composition, age, gender and skin properties.
2. Differing physiological states including extreme hormonal ranges and core and skin temperature.
3. The effect of perspiration.
4. Effect of site of ultrasound application.
5. Environmental changes such as ambient temperature.

Safety concerns, including the effect of repeated use on skin and underlying tissues also require consideration.

4.4 CRITIQUING METHODS

A review of the research methods used and of others not used in the present study is included here.

4.4.1 Estimation of body composition

Body composition was not extensively monitored during this study. Body mass index (BMI) was calculated using the participants' weights and heights measured at the beginning of the four-week study. Ten of the fourteen participants had BMIs within the range of 25 to 29.9kg/m^2, a range that is considered to be grade one obesity (first grade above the desired range) (Jaequier, 1987). For most of the participants included in this range, the high BMI is likely to be due to a large observable muscle mass. While BMI is commonly used when determining a persons risk of developing obesity-related diseases (Wagner and Heyward, 1999), for this study it has not given an accurate measure of the body composition of the participants. When selecting the method to use for assessing body composition there are several considerations; (1) cost, (2) ease of operation, (3) technician training and skill, (4) subject cooperation and comfort, (5) number of participants and time available for assessment, (6) body composition variables to be quantified and purpose of assessment, and (7) whether or not the assessment will be conducted on multiple occasions to assess changes in body composition (Wagner and Heyward, 1999). Several other methods exist that can be used to determine body composition; they have varying degrees of accuracy, complexity and cost.

In addition to a person's weight and height, as used for measuring BMI, anthropometry includes the measurement of girths of various body segments and skeletal breadths using bony landmarks. It uses two principles, firstly that circumferences reflect the free fat mass (FFM) and fat mass of that area and secondly that skeletal size is directly related to lean body mass (Wagner and Heyward, 1999). Several prediction equations have been developed that utilise the circumference measurements in combination with skin fold measurements to estimate body fat. Skin fold measurement involves the use of callipers to measure the thickness of a fold of skin and its underlying subcutaneous fat. When this is repeated at several sites over the body the measurements can be inserted

into an equation (derived from regression models) to calculate body fat percentage (Jackson and Pollock, 1978). This method employs two assumptions, that skin fold is a good measure of subcutaneous body fat and there is a good relationship between subcutaneous fat and total body fat. Skin fold measurement has three potential errors; firstly due to the nature of the measurement there is a large potential technical error; secondly it is likely that variations exist in the relationship between subcutaneous and total fat between individuals; and lastly it is also likely that variations in the ratio of skin fold thickness to subcutaneous fat exist. During regression modelling to establish equations for calculating body fat percentage using this method Jackson and Pollack (1978) made two important observations. The first is that the relationship between the skin fold thickness and body density was quadratic; therefore the prediction errors are larger at the extremes of body fat percentage if a linear regression line is used to fit the data. The other is that age accounted for a significant proportion of variation in body density. In light of these observations, while skin folds can be used confidently to measure body fat percentage in lean, athletic individuals, it is perhaps not as accurate for measuring older or obese populations.

Bioelectrical impedance analysis (BIA) is a simple procedure that measures the resistance encountered when a small electrical current, which cannot be felt, is passed through the body. Fat free tissue is a good conductor of electrical current whereas fat is not, therefore the resistance measured is inversely proportional to FFM (Wagner and Heyward, 1999). Resistance or opposition to electrical flow is related to the length of the conductor and its cross-sectional area. The use of BIA to estimate body fat percentage assumes that the body is a cylindrical shape with uniform length and area, however the body is more like a series of cylinders. The cylinders of the extremities of the body have the smallest area and therefore greatest influence on whole body resistance while the trunk contains 50% of body weight but contributes only a small percentage of total body resistance (Wagner and Heyward, 1999). The method also assumes that the conducting material within the cylinder is homogenous and that resistance is constant, whereas in fact it is influenced by hydration status, microstructure and electrolyte concentration (Wagner and Heyward, 1999). Estimation of body fat percentage in obese individuals using this method is underestimated while in very lean individuals it is overestimated (American College of Sports Medicine, 1995). To summarise (Houtkooper et al, 1996) state that "with proper standardisation of methods,

instrumentation, and subject preparation, this non-invasive body composition approach can quickly, easily and relatively inexpensively provide accurate and reliable estimates of free fat mass and total body water in healthy populations" (pp.446S-447S). However, interpretive care is required at the extremes.

Hydrostatic weighing (hydrodensitometry) is based on Archimedes' Principle which states that a body immersed in water is buoyed by a force equal to the weight of the displaced fluid (Wagner and Heyward, 1999). As bone and muscle are more dense than water, while fat is less dense, a person with more FFM for the same total body weight, weighs more in water, has a higher body density and a lower percentage of body fat, than a person with less FFM (American College of Sports Medicine, 1995). As air present in the lungs affects buoyancy, the underwater measurement must be taken once the individual has exhaled completely and residual volume measured. The measurements can then be inserted into a formula to calculate body density, which can then be converted into body fat percentage. The body density calculation assumes that densities of FFM and fat mass are constant for all individuals, however, as FFM varies with age, gender, ethnicity, level of body fatness and activity level, there are several population specific formulas, which nevertheless do not account for individual variations in bone density (Wagner and Heyward, 1999). The disadvantages of this method are that the special equipment that is required is expensive, the process is relatively complicated and time consuming and the process of weighing a person while fully submerged in water is not practical for many populations and can cause anxiety in some individuals (American College of Sports Medicine, 1995).

The final method to assessing body composition to be considered here is air displacement plethysmography using a Bod Pod (Life Measurements Inc. Concord, CA, USA). The Bod Pod is a large, egg-shaped, fibreglass container that uses the relationship between pressure and volume (Poisson's Law) to derive a body volume for a person seated inside the chamber (Wagner and Heyward, 1999). While wearing minimal clothing the person seated inside breathes normally for 20 seconds while body volume is measured. Lung volume is also measured and subtracted from body volume. Respiratory movements, gas and water vapour exchange, and the heat generated by the person inside affect the measurement (Wagner and Heyward, 1999). While this method

of air displacement plethysmography uses the same assumptions as hydrodensometry, it is more accurate, rapid, safe and comfortable (Wagner and Heyward, 1999).

4.4.2 Estimating maximal oxygen consumption

VO_{2max} was estimated from an equation using final speed reached on the treadmill during the fitness test and ranged from 39.6 to 44.5ml/kg/min with a mean of 42.3ml/kg/min. A total of 21 maximal fitness tests were performed during this study where heart rate was measured and recorded throughout the test. Maximum heart rate reached during the test was very close to the theoretical maximum heart rate calculated from the equation 'maximum heart rate = 220 – age' of participant during 18 of the 21 tests. While heart rate reached at the completion of the test was not used in the calculation of VO_{2max}, it was useful to record as if maximum heart rate was close to theoretical maximum heart rate, it supported the view that the participant had, in fact, reached their actual VO_{2max} at the completion of the fitness test.

The intended purpose of the RAMP tests of increasing speed on a treadmill was to directly measure the participant's VO_{2max} using open-circuit spirometry. The following criteria were to be used to determine whether the participant had reached his VO_{2max}: (1) failure of heart rate to increase with further increases in work intensity, (2) a plateau in oxygen uptake with increased workload and (3) a respiratory exchange ratio of greater than 1.15 (American College of Sports Medicine, 1995). However, the respiratory mass spectrometer that was to be used for gas analysis during the fitness test unexpectedly malfunctioned throughout the study. Therefore the fitness tests were performed without gas analysis and VO_{2max} had to be estimated according to the equation noted above (section 3.4). Without the use of the respiratory mass spectrometer, the only criterion that was monitored was heart rate, as VO_2 and the respiratory exchange ratio could not be monitored.

As actual VO_{2max} could not be measured directly, it was estimated from the final speed that the participant reached at the end of the fitness test. It was assumed for the purpose of calculating VO_{2max} that when the participant stopped the test they had reached their VO_{2max}. Some of the participants had heart rates that reached a plateau near to or above their theoretical maximum and it is more likely that they had reached their VO_{2max} than those whose heart rates did not plateau. When a participant felt that he could no longer

continue the test, there was no way to determine whether it was due to reaching his maximal aerobic capacity or whether other factors were influencing the way he felt. For this fitness test aerobic capacity is not likely to be the only factor influencing the final speed reached on the treadmill. Health and well-being on the day was likely to have an effect. Ten of the fourteen participants were suffering from colds at some point during the research and often some would perform the test while suffering from an alcohol hangover as they did not inform the researchers of their condition until after the test.

Use of the equation to convert the final speed on the treadmill reached by the participant into a VO_{2max} should be done with the following cautionary notes in mind (American College of Sports Medicine, 1995):

1. The measured VO_2 at a given work rate is highly reproducible for a given individual; however, the intersubject variability in measured VO_2 may have a standard error of estimate as high as 7%.
2. These equations are appropriate only for steady state submaximal aerobic exercise. Failure to achieve steady state will result in an overestimation of VO_2.
3. Anything that changes the mechanical efficiency such as gait abnormalities, wind, snow or sand will result in loss of accuracy.

To establish whether any relationships existed between aerobic fitness and hormone concentrations and aerobic fitness and sleep by way of correlations this method was deemed suitable as the overestimation of VO_{2max} using this method during analysis is consistent between all tests.

Submaximal tests have been developed as it is not always feasible to exercise a person to the point of volitional fatigue. While submaximal tests provide an estimate of VO_{2max} without the risks associated with maximal testing they make several assumptions which are not usually met leading to errors and are therefore not as precise as maximal exercise testing (American College of Sports Medicine, 1995). The assumptions are:

1. That a steady state heart rate is obtained for each exercise work rate,
2. That a linear relationship exists between heart rate and oxygen uptake (or work rate if VO_2 is not measured),
3. That the maximal heart rate for a given age is uniform,
4. That mechanical efficiency (i.e. VO_2 at a given work rate) is the same for everyone.

The method of submaximal testing involves determining the relationship between the participant's heart rate and VO_2 during an increasing intensity fitness test using the Bruce or the Balke protocol as describe in American College of Sports Medicine (1995). VO_{2max} can then be estimated using the theoretical maximum heart rate. Using this method it is possible to also monitor changes in cardiorespiratory fitness if the heart rate response to a given workload changes over time irrespective of VO_{2max}.

4.4.3 Measuring sleep quality and sleep quantity

In this study only one significant relationship was seen between sleep quality and hormone profiles the following morning. This was a decrease in cortisol concentration when sleep disturbances were experienced (from 145ng/ml to 115ng/ml, $p<0.001$, in plasma). Peak cortisol secretion occurs during REM sleep which is also the stage of sleep most difficult to wake up from (Weitzman et al, 1971). Disturbances during sleep may affect cortisol secretion as, once woken the sleep cycle begins again (Borbely, 1998), therefore time in REM stage when cortisol secretion is greatest is less than if the person was not woken at all during the sleep episode. Disturbances occurring during REM sleep would have to be relatively disruptive in order to wake the person up and therefore likely to keep them awake for longer.

The lack of relationships identifiable within this data may indicate a weakness in the ability of sleep logs to provide accurate information about sleep quality and quantity. However, self-reported estimates of sleep have been shown to correlate highly with polygraphic measures of sleep quality and quantity (Jacobs et al, 1988). Another possibility could be that the samples were taken at a time of day when the influence of sleep on hormone profiles was no longer obvious.

4.5 CRITIQUE OF EXPERIMENTAL DESIGN

4.5.1 Participant selection

In some respects the participants were fairly homogeneous, as they were all males within a narrow age range (19-22 years), and they played the same sport for the same team. All participants had a similar ethnic background, as 13 out of the 14 classed themselves as New Zealand European, although one of those 13 also indicated that he was Maori, the other indicated that he was Tongan. All of the participants were tertiary students, living in flatting situations.

In view of the relative homogeneity between the participants we expected to see similar results for hormone ranges. Each participant's hormonal ranges in plasma were mostly within what is considered the normal range (Wilson et al, 1998). The results from this study could have been more varied if a heterogeneous group was chosen. The aims of this study were to investigate the relationships between hormone concentrations, level of fitness and sleep in young male rugby players so the choice of participants is limited in age range and sport played, but they were all well suited for the purpose of this part of the study. For the validation of the use of ESP as a method of estimating plasma hormone levels, a heterogeneous group could have provided a wider range of hormone levels, particularly low levels of testosterone as occurring in females. A heterogeneous group would also provide data for comparison of age, gender, and skin properties but would have needed to be much larger in sample size.

4.5.2 Measurement of hormone concentrations in the morning

4.5.2.1 Establishing a basal hormone concentration

Samples were taken in the morning in an effort to establish a basal concentration for testosterone, cortisol and growth hormone. The mean plasma testosterone concentration was 5.4ng/ml with a range of between 2.1 to 10.5ng/ml. The mean plasma cortisol concentration was 133ng/ml with a range of 39 to 217ng/ml and the mean plasma growth hormone concentration was 12.0ng/ml with a range of 4.5 to 20.4ng/ml. It was difficult to establish a basal plasma hormone concentration as the data were limited by

poor compliance of the participants. Seven of the fourteen participants gave samples on all of the twelve required days, three people missed only one day, another three missed two days and one missed four. None of the participants gave their samples at the same time each day contrary to what had been organised. The later in the morning the samples were given, the greater the deviation from basal hormone concentration is likely to have been, as the hormone concentrations can be effected by factors including diet (Valcavi et al, 1992; Strobl and Thomas, 1994) and exercise (Cadoux-Hudson et al, 1985; Felsing et al, 1992; Chiccaro et al, 1998).

4.5.2.2 Relationships between morning hormone concentrations and estimated VO_{2max}

The only significant correlation that was evident in the relationships between hormone concentrations measured during the morning and VO_{2max} of the participants was a weak, negative correlation between mean morning cortisol concentration in plasma and VO_{2max} ($r=-0.346$, $p=0.036$). While significant, this correlation indicates that only 12% of the variation in VO_{2max} can be attributed to the mean morning cortisol concentration in plasma ($r^2=0.12$). When visually comparing the significant relationship between mean morning plasma cortisol concentration and estimated VO_{2max} with a non-significant similar correlation; (in this case mean morning plasma testosterone), (Figure 4.1 and Figure 4.2), the graphs were very similar. Therefore the weak relationship between mean morning plasma cortisol concentration and estimated VO_{2max} is not very convincing. A larger population size is needed to investigate this relationship further and examine whether the relationship disappears or becomes stronger.

Figure 4.1. Relationship between a participant's mean morning cortisol concentration and their VO_{2max}.

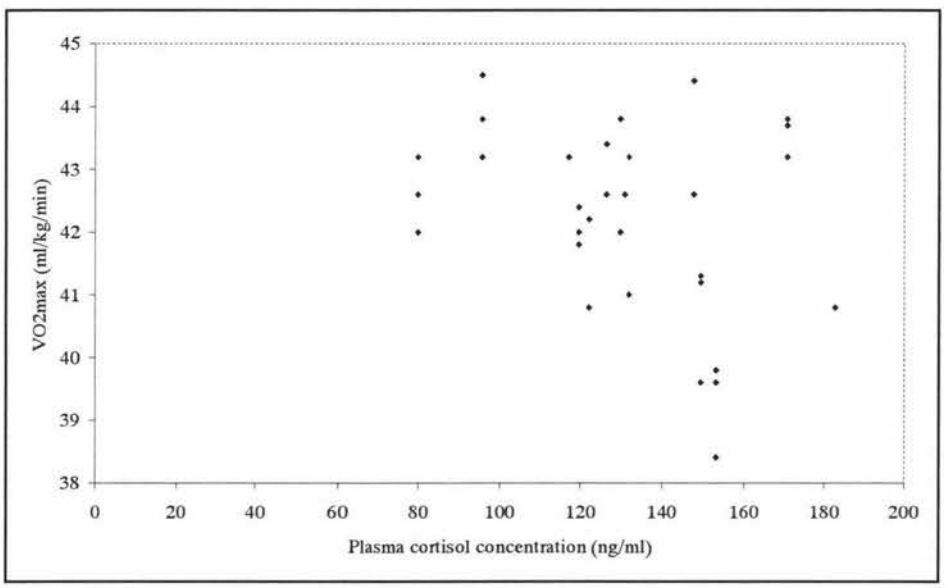
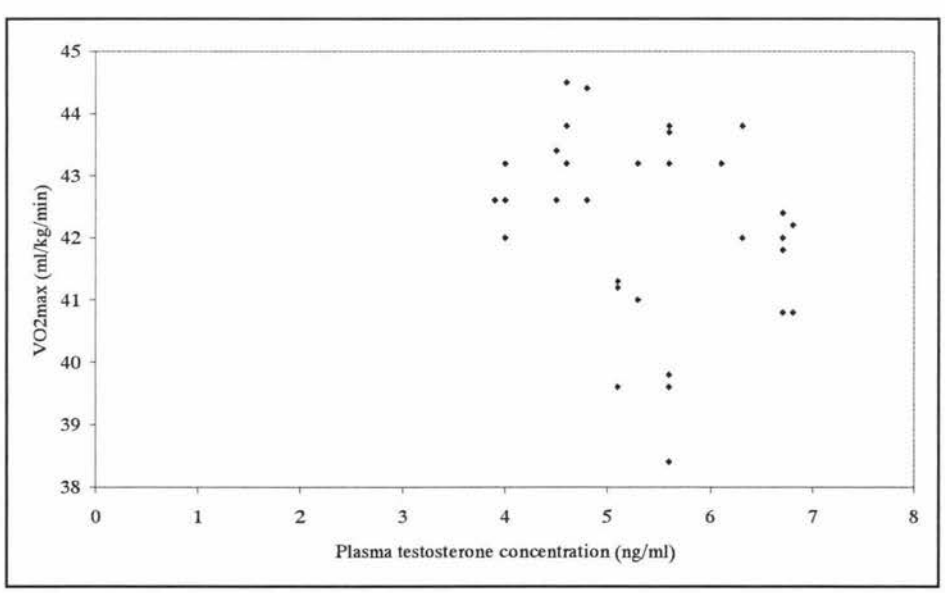


Figure 4.2. Relationship between a participant's mean morning testosterone concentration and their VO_{2max}.



4.5.3 Measurement of hormone concentrations after the fitness test

Hormone concentrations measured after the fitness tests did not reveal any relationships between a person's final VO_2 achieved and their hormonal response. This could have been due to the difficulties measuring VO_{2max} but could also have been due to sampling

methods. If a participant felt that they could not complete the warm down they could stop the test, this produced differences between participants in the time delay from reaching final VO_2 and giving the sample. This may have had an influence on hormone concentrations in the samples. Plasma concentrations of both testosterone and cortisol change over time during recovery (Jurimae et al, 2001). In the saliva samples the time delay would allow for more of the hormone to cross from the blood to the saliva.

It would also have been useful to have taken samples before the fitness test as many had engaged in activities such as exercise prior to completing the test. If this approach had been adopted, *changes* in hormone levels from start to finish of the test could have been used in analysis and may have been more informative.

4.5.4 Examining relationships between sleep quality or sleep quantity and VO_{2max}

There was no evidence that the previous night's sleep has an effect on final VO_2 achieved during the fitness test. The lack of a relationship here could be due to sleep requirements differing between individuals. In this study results for the fitness tests were compared between individuals whereas a relationship may be seen if more data existed for each individual, thereby allowing comparison within an individual.

4.5.5 Examining relationships between alcohol consumption and hormone concentrations

In total, 97 drinking episodes were recorded in the sleep logs. The number of standard drinks consumed in any twenty-four hour period ranged from zero to 52. Seventy of the drinking episodes could be classed as heavy drinking due to the number of drinks consumed at the time, as the mean number of standard drinks taken was 19. When all of the data was analysed collectively for relationships between standard drinks consumed during a drinking episode and the hormone concentrations measured in samples taken the following morning, a weak, positive but significant relationship was found with the concentration of testosterone and cortisol measured in saliva. These relationships are shown graphically in Figure 4.3 and Figure 4.4.

Figure 4.3. Relationship between saliva testosterone concentration in the morning and the amount of alcohol consumed in the previous twenty-four hours.

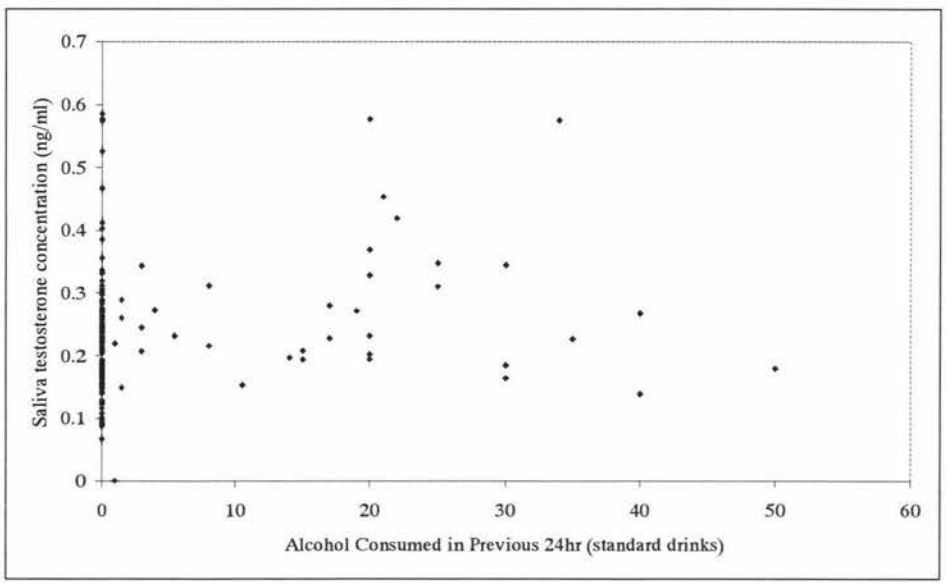
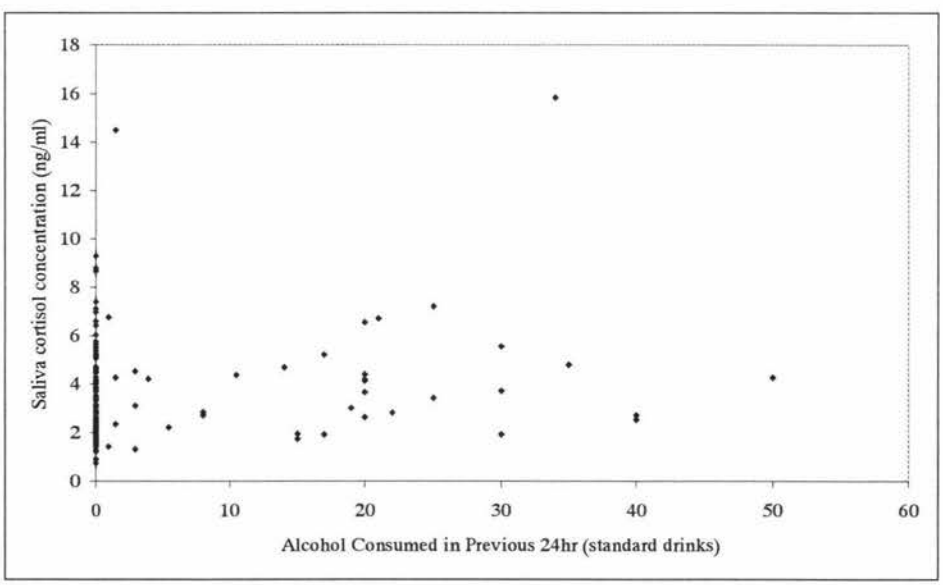


Figure 4.4. Relationship between saliva cortisol concentration in the morning and the amount of alcohol consumed in the previous twenty-four hours.



4.6 WEAKNESSES OF THE STUDY

To summarise, this study had the following weaknesses:

- No sedentary control group for comparison
- Short duration of the study
- Malfunction in equipment

4.7 IMPROVED EXPERIMENTAL DESIGN

In consideration of the above listed weaknesses and the problems encountered during this study, as previously discussed, we can improve the design of the study to produce more reliable and scientifically informative results. Accordingly, presented below is an account of how some features of the experimental design could be improved in hypothetical future studies.

4.7.1 Participant selection

Two groups of participants should be recruited for the study, so that, in addition to an experimental group of participants who play rugby for the same team, a group of sedentary males between the ages of 18 and 24 would be studied. Each group should consist of at least 20 participants. Group A, the sedentary group, will provide a comparison between athletes and non-athletes for every part of the study. They must participate in less than two hours of moderate exercise a week to be included. Group B, the athletes, compete in one of two sports teams (to allow for sufficient samples size) and are to be classified within a playing unit (forwards or backs), they could then be compared as a whole group or as positional units. All members of this group will complete the same training regimens. The study will span six weeks and both groups are to abstain from alcohol for the duration. The study would begin on the first week of the local club rugby union football competition.

The participation of two such groups would allow a more valid analysis of possible casual links between exercise/training and differences in hormone profiles than was possible here. The present pilot study, in observing only one group, could at best provide evidence of associations between different factors which, if significant enough would then need to be investigated with more precision using a carefully devised and more rigorous multi-group experimental design.

4.7.2 Sample collection

4.7.2.1 In the morning

The basal hormone level of each participant are to be established by providing samples upon waking and prior to rising each morning of the study. These measurements will be more accurate than those in this study as activities prior to this time can vary from day to day and are likely to influence hormone secretion. For example running from the car park to a building to hand in an assignment on time could increase cortisol levels.

4.7.2.2 Associated with the fitness test

Plasma, ESP and saliva samples will be taken immediately before fitness tests and sprints as well as upon finishing. This would allow for monitoring changes in testosterone, cortisol and growth hormone concentration associated with exercise and also for more precise comparison of how well plasma hormone concentrations are correlated with ESP sample hormone concentrations and with saliva hormone concentrations following exercise.

4.7.3 Fitness testing

The treadmill test protocol will be different from that used in this study. A test consisting of two-minute stages of the same speed with an increasing incline is proposed. Those participants in the sedentary group will have VO_{2max} estimated from a submaximal test where they will continue only until their heart rate reached 75% of their theoretical maximum (220-age). They will complete two submaximal tests, one in the first and one in the last week of the study. The members of the athletes group will stop the test when they feel they cannot continue, anticipated to be the point at which they have reached their VO_{2max}. They will complete four maximal tests separated by two weeks each.

Prior to the test a resting heart rate will be established for each participant and recovery will be monitored until heart rate has decreased to resting + 30%. VO_2 will be measured during rest and throughout the test; it will be measured in both ml/min/kg of lean body weight and total body weight.

4.7.4 Measuring fatigability

To investigate fatigability both group A (sedentary) and group B (athletes) will complete sprint tests after an initial warm up of a 200 meter light jog. Each participant will complete as many sprints as required to observe an increase of 25% in the time to sprint 40 meters. The first sprint will be used as a practice, while the time for the second sprint will be used as the reference. Plasma and ESP samples will be taken before the warm up and following each sprint. In addition to being analysed for testosterone, cortisol and growth hormone, the samples will also be analysed for lactic acid to investigate the relationship between fatigue and lactic acid build up in plasma. Excluding the warm up, the tests will be performed following a 24 hour period of no exercise.

4.7.5 Measuring sleep quality/quantity

EEG recording will be used instead of sleep logs to assess the time spent in each of the sleep stages. In order to investigate the relationships between sleep quality/quantity and hormone concentrations the following morning, and sleep quality/quantity and athletic performance, sleep will be manipulated. Group A and Group B will be further divided into four sub-groups with five participants in each group receiving different sleep treatments. Each participant is in bed with EEG connected. They will be encouraged to sleep from 2230 hours and woken up at 0700 hours the following morning. The sleep schedules for each group are outlined below. These schedules should allow for the effect of sleep quality/quantity on hormone concentrations the following morning to be investigated. It will also allow effects of the accumulation of poor sleep quantity to be investigated.

Group 1: No induced sleep disturbances, any naturally occurring disturbances are noted.

Group 2: Every night the participant is woken up every two hours for 10 minutes.

Group 3: Every night the participant is woken up every two hours for 30 minutes.

Group 4: Every night the participant is woken up every two hours for 60 minutes.

4.7.6 Alcohol consumption

The relationships between alcohol consumption and hormone concentrations will not be investigated in this theoretical study and therefore the participants would be asked to abstain for the six-week period. Alcohol consumption was recorded in the present study because not allowing consumption during the four weeks of it would have reduced the number of people volunteering as participants. To properly investigate the effect of alcohol consumption on hormone concentrations a separate study could be organised. This would be a multigroup study involving males of the same weight and body composition. Every evening between 1800 and 2200 hours the five groups would consume a set amount of alcohol. One group would not consume any alcohol for the duration of the study, the others would consume enough to maintain a blood alcohol level of one, two, three or four times the legal blood alcohol limit for driving a vehicle in New Zealand.

In an attempt to achieve this the groups would be instructed to drink a multiple of the Alcohol Advisory Council of New Zealand's safe driving recommendation for standard drinks per hour. For example, those participants in the group required to maintain a blood alcohol level of three times the legal driving limit during the four-hour period would be instructed to drink three times the safe driving recommendation. It is recognised that blood alcohol concentration as a results of consumption varies between individuals. All participants would have plasma, ESP and saliva samples taken the following morning upon waking. Testosterone and cortisol will be measured in each fluid and growth hormone measured in plasma and ESP samples. In addition alcohol content of plasma and ESP samples will also be measured. Sleep and physical activity would be standardised across the two groups.

4.7.7 Other measurements

- Weight, height and body fat will be measured once a week at the same time each week to enable changes in them to be monitored.
- Weight will also be measured immediately prior to fitness tests as it influences power on the treadmill.

- Body fat will be measured by bioelectric impedance analysis (BIA), which can also indicate a person's basal metabolism.
- Other anthropometric measurements taken for each participant would include somatotypes and leg length in order to establish whether size of an individual could affect performance on a treadmill due to biomechanical restrictions.

4.7.8 Ethical Approval and Participants' Compliance

All research involving human participants is subject to human ethics committee approval. It is obvious that some of the protocols outlined here, perhaps especially the one proposed as a theoretical exercise for alcohol, may not be acceptable. Of course, it is also recognised that the willingness or unwillingness of participants to complete each and every part of a proposed study limits what can be done. Regarding the above ideas for improved study design it is assumed that complete compliance would be obtained, although it is acknowledged that in reality such full cooperation may not be forthcoming.

4.8 CONCLUSIONS

When referring back to the three original objectives of this study we can draw several conclusions from the results obtained. The correlations between plasma sample hormone concentration and ESP sample hormone concentration ranged from 0.965 to 0.977 and were highly significant. The ESP sample concentrations analysed in the present study represented a figure of approximately 10% of the plasma sample concentrations measured at the same time. The correlations for plasma hormone concentration with saliva sample hormone concentration were not as high; they ranged from 0.612 to 0.791. These results suggest that ESP sample analysis provides a more accurate estimation of testosterone, cortisol and growth hormone concentrations in plasma than saliva sample analysis.

The ability to establish any relationships between basal concentrations of testosterone, cortisol and growth hormone and athletic performance was limited due to malfunction of equipment and limitations in sample collection and analysis. A negative correlation was found between the mean plasma cortisol concentration measured in the morning and the estimated VO_{2max}, and also between the cortisol concentration measured in the ESP sample and estimated VO_{2max}, but this did not quite reach significance. No relationships were seen between athletic performance and testosterone or cortisol concentrations in ESP or saliva samples following the fitness test.

The presence of sleep disturbances as noted in the sleep logs was associated with a decrease in the following morning's cortisol concentrations in both plasma and ESP samples. No relationship could be established between athletic performance and the previous night's sleep quality and sleep quantity measures. However there was a negative correlation between estimated VO_{2max} and the mean total time asleep for the previous three or four nights.

While establishing a relationship between alcohol consumption and testosterone, cortisol and growth hormone concentration the following morning was not one of the three initial objectives of the study, it was investigated as it can have an influence on athletic performance, sleep quality and hormone concentrations. A correlation was

established between alcohol consumption and the concentration of testosterone and cortisol in saliva the following morning. Some highly significant correlations were found between alcohol consumption and the hormone concentrations the following morning when the participant's data were analysed separately to establish relationships between alcohol consumption. However, the very low numbers of samples that were taken when alcohol had been consumed limit interpretation of these results, perhaps a larger sample would reveal whether the relationship remained or disappeared.

The limitations in the design of this study were revealed during analysis of the results obtained. Discussed within this chapter were improvements to the design to further investigate the objectives of the present study. In summary of these improvements; (1) the study would be longer, (2) a control group would be used, (3) sample collection would be more frequent and have stricter guidelines for when the samples are taken, (4) the fitness testing method would be modified to an increase in incline rather than speed and VO_2 would be measured throughout the test, (5) fatigability would be induced by continual sprints until a decrease in performance is seen, (6) sleep would be measured using EEG technology and sleep deprivation would be manipulated, and (7) the effect of alcohol on athletic performance and hormone concentrations would be studied separately.

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MATERIALS

Table 1. Equipment Required

Equipment	Model Used	Company	Country
Weight Scales	Jadever JPS-2030	Jadever Scale Co. Ltd.	Taiwan
Stadiometer	Seca 222	Vogeland Halke GmbH Co.	Germany
Lancet	Accu-Chek Softclix Pro	Roche Diagnostics NZ Ltd.	New Zealand
Centrifuge	IEC Micro Capillary Centrifuge	International Equipment Co.	USA
Timing System	Speedlight Sports Timing System	Swift Performance Equipment	USA
Treadmill	Payne Widebodied Treadmill TM4-NZ	Stanton Engineering PTY Ltd.	Australia
Heart Rate Monitor	Polar Vantage NV™	Polar Electro Oy.	Finland
PC transfer Interface	Polar Advantage Interface System™	Polar Electro Oy.	Finland
Respiratory Mass Spectrometer	PulmoLab Ex 670	Morgan Medical Ltd.	England
Microwell	Organon Teknika Microwell System	Organon Teknika	Netherlands
Microwell Reader	Organon Teknika Reader 230S	Organon Teknika	Netherlands
Ultrasound	ITO Physiotherapy and Rehabilitation unit	ITO	Japan

Table 2. Consumables Required

Consumable	Details	Company	Country
Alcohol Swabs	Sterets Pre-injection swabs, 70% isopropyl alcohol	Seton Prebbles Ltd.	England
Lancet Refills		Roche Diagnostics NZ Ltd.	New Zealand
Capillary Tubes	100µL heparinised capillary tube	Chase Scientific Glass Inc.	USA
Microtest Tube	1.5mL, 3810X	Eppendorf	Germany
25mL Sterile Container	LBS3534	Labserve	New Zealand
Ultrasound Gel	Aquasonic gel	Parker Laboratories Inc.	USA
Cotton Swabs			
70% Ethanol			
Salivary Blood Contamination Kit	Salivary Blood Contamination Enzyme Immunoassay Kit	Salimetrics LLC	USA
Salivary Testosterone Kit	Salivary Testosterone Enzyme Immunoassay Kit	Salimetrics LLC	USA
Salivary Cortisol Kit	HS-Cortisol High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit	Salimetrics LLC	USA
Plasma Testosterone Kit	Testosterone ELISA EIA 1559	DRG Instruments GmbH	Germany
Plasma Cortisol Kit		DRG Instruments GmbH	Germany
Plasma Growth Hormone Kit	Dual RIA DRG Diagnostic assays	DRG Instruments GmgH	Germany

Appendix Two

NATIONAL APPLICATION FORM FOR ETHICAL APPROVAL OF A RESEARCH PROJECT

PART I : BASIC INFORMATION

Protocol number and date received (for office use only)

1. Full project title

Validation of electrosonophoresis as a non-invasive blood sampling technique through the study of the relationships that exist between exercise, fatigability, fitness, and hormone levels in blood, saliva and extracellular fluid and the investigation of the impact of spontaneous sleep disturbances on these relationships in young healthy male athletes.

2. Short project title (lay title)

Investigation of the relationships that exist between athletic training, hormones and sleep in young healthy male athletes.

3. Lead Principal Investigator's name and position

Lara Blackmore – Master of Science Student

4. Address of lead Investigator

Institute of Food Nutrition and Human Health	Work ph	06 350 7330
Massey University	Home ph	06 [redacted]
Private Bag 11222	Fax	06 350 5657
Palmerston North	E-mail	Lar[redacted]

5. Lead investigator's qualifications and experience in past 5 years (relevant to proposed research)

Lara Blackmore completed a BSc with a major in physiology in 1999. In 2000 she began an MSc majoring in
sport science. Her first year comprised of 4 full time, full year papers including Human Performance and Training, Energy Metabolism and Its Control, Respiratory Physiology and Food Composition and Nutrition.

6. Co-investigators' name(s) and position(s)

A	David J. Mellor – Professor
B	Christian Cook – Senior Research Scientist (Honorary Lecturer at Massey University)
C	Hugh Morton – Associate professor
D	Heather Purnell – Master of Science student

7. Address of co-investigator A

Institute of Food Nutrition and Human Health	Work ph	06 350 4807
Massey University	Home ph	
Private Bag 11222	Fax	06 350 5657
Palmerston North	E-mail	D.J.Mellor@massey.ac.nz

8. Address of co-investigator B

Technology Development Group	Work ph	07 858 4743
Hort Research	Home ph	
Private Bag 3123	Fax	07 858 4705
Hamilton	E-mail	c.c[redacted]

9. Address of co-investigator C

Institute of Food Nutrition and Human Health	Work ph	06 350 4265
Massey Universtity	Home ph	
Private Bag 11222	Fax	06 350 5657
Palmerston North	E-mail	H.Morton@massey.ac.nz

10. Address of co-investigator D

Institute of Food Nutrition and Human Health	Work ph	06 356 9099 Extn 7763
Massey Universtity	Home ph	
Private Bag 11222	Fax	06 350 5657
Palmerston North	E-mail	H.M.Purnell@massey.ac.nz

11. Where this is supervised work

11.1 Supervisor's name	Hugh Morton
Position	Associate Professor
Day time phone number	06[redacted]

11.2 Signature of supervisor (where relevant)
Declaration: I take responsibility for all ethical aspects of the project

12. List any other New Zealand Ethics Committees to which this project has been submitted and attach their letters of approval where available: Not Applicable

13. I wish the protocol to be heard in a closed meeting ☐ Yes ☑ No
(If yes the reason should be given in a covering letter)

14. I request a fast track procedure ☐ Yes ☑ No

15. Proposed starting date (dd/mm/yy)	1st June 2001
16. Proposed finishing date (dd/mm/yy)	1st December 2001
17. Duration of project (mm/yy)	6 months
18. Proposed final report date (mm/yy)	1st June 2002

PART II : PROJECT SUMMARY

1. Multicentre proposals

(Important: read the guidelines, Appendix 1)

	Yes	No
1.1 Is this a multicentre study? **(if no, go to question 2)**	√	
1.2 Is this committee the primary ethics committee?	√	

If **no**, name the primary ethics committee

1.3 Has the protocol been submitted to any other ethics committees in New Zealand? (If **yes**, attach copies of relevant correspondence) ☐ Yes ☑ No

1.4 Who is the lead investigator or institution in New Zealand?	Massey University
1.5 List the other New Zealand centres involved, and the Principal Investigator for each centre	Hort Research Institute – Dr Christian Cook Insurance cover for Dr Cook being negotiated between Massey University and the Hort Research Institute, participation is dependent upon a successful negotiation.
1.6 If the study is based overseas, what other countries are involved?	Not Applicable

2. Scientific Assessment

Has this project been scientifically assessed by independent review? ☑ Yes ☐ No

If **yes**, by whom? (name and position) A copy of the report should also be attached	As a multicentre proposal a range of scientists have been involved in devising this.
If **no**, is it intended to have the project scientifically assessed, and by whom?	

3. Data and Safety Monitoring Board (DSMB)

11.3 Is the trial being reviewed by a data and safety monitoring board?		Yes	√	No
If yes, who is the funder of the DSMB?		Sponsor		HRC

4. Summary

Give a brief summary of the study (not more than 200 words, in lay language)

A Master of Science student at Massey University will conduct this study. It involves the collection of blood, saliva and extracellular fluid samples by electrosonophoresis from young male athletes. The participants will be of the same sporting code that participate in routine training for their sport. The number of participants that will be included in the study is 16. The samples will be analysed for concentrations of growth hormone, testosterone, cortisol and glucose at Hort Research in Hamilton. Base line concentrations will be established through morning collections while the effect of training on hormone profiles will be measured post-training. The impact of training will be measured through increases in fitness, as measured on a treadmill and through decreases in fatigability, as measured with 40-meter sprint times. The effect of spontaneous sleep disturbances on effectiveness of training will also be investigated requiring the participant to regularly fill in a sleep log. The study has also been designed to investigate the use of the electrosonophoresis device as a non-invasive blood sampling methodology.

PART III: PROJECT DETAILS

SCIENTIFIC BASIS

1. Aims of Project

1.1 What is the hypothesis/research question(s)? (state briefly)

To assess whether electrosonophoresis can be used to monitor the concentrations of hormones, in blood and saliva, of relevance to training performance in young male athletes.

1.2 What are the specific aims of the project?

There are three objectives in this study of the training of healthy young adult males:

11.3.1.1.1 With particular reference to growth hormone, cortisol, testosterone and glucose, to compare their concentrations in blood and saliva with those derived by electrosonophoresis. The aim being to further validate that use of electrosonophoresis as a non-invasive sampling methodology.

11.3.1.1.2 To explore relationships between exercise, fatigability (as a measure of fitness), fitness increases as assessed by maximum oxygen uptake (VO_2max) and the ventilatory anaerobic threshold (VAT), and the hormone levels in these three fluids.

11.3.1.1.3 Using spontaneous disturbances in sleep quality/quantity to explore their impact on hormone profiles and athletic performances.

2. Scientific Background of the Research

Describe the scientific basis of the project (300 words maximum) Where this space is inadequate, continue on a separate sheet of paper. *Do not* delete page breaks or renumber pages.

Many people engage in exercise for recreation, to promote personal health and as a profession. Accordingly there is wide ranging interest in the factors that affect a person's performance during exercise and in how that performance can both be assessed and enhanced.

The physiological basis of exercise performance and its enhancement have been investigated for many years. Such investigations in people are impeded by the understandable reluctance of participants to provide significant numbers of blood samples by venepuncture. The recent development of an ultrasound method for non-invasive sampling of extracellular fluid (electrosonophoresis) offers tremendous opportunities for benign monitoring of physiological responses involving changes in blood/extracellular fluid composition associated with exercise and indeed in clinical settings. As this method is still in its infancy, to further develop its widespread use, the composition of the ultrasound samples must be correlated with the composition of blood and/or saliva samples.

Sleep quality/quantity is considered to have significant impact on training effectiveness and performance, with poor sleep correlated with poor athletic outcome. The link here is considered to involve growth hormone, as poor sleep quality/quantity diminishes growth hormone concentrations and reduced growth hormone concentrations impede training induced muscle development. Training effectiveness and recovery have been monitored in past research through measurement of blood hormone profiles, in particular the testosterone: cortisol ratio. What is proposed is a pilot study to investigate these phenomena.

3. Participants

3.1 How many participants is it intended to recruit?	Up to 16 (12 would be minimal) – allows for withdrawals.
3.2 How will potential participants be identified?	Those who belong to a winter sporting club.

3.3 How will participants be recruited? (e.g. advertisements, notices)	A sporting group will be approached and after explanation of project and provision of written details, volunteers will be sought.
3.3.1 Where will potential participants be approached? (e.g. outpatient clinic) If appropriate, describe by type (e.g. students)	Massey University, Palmerston North club team.
3.3.2 Who will make the initial approach to potential participants?	Miss Lara Blackmore and Dr Christian Cook
3.3.3 Is there any special relationship between the participants and the researchers? e.g. doctor/patient, student/teacher	Not Applicable

3.4 Briefly describe the inclusion/ exclusion criteria and include the relevant page number(s) of the protocol or investigator's brochure

Inclusion criteria are:

11 Passes physical/medical exam before participation,
12 Engaged in regular sports training,
13 Healthy, non-injured 18-30 year old male.

Exclusion criteria are:

11 Performance enhancing or recreational drug use,
12 Injury during trial,
13 Health conditions incompatible with regular training and exercise,
14 Unease with any aspect of the study.

3.5 If randomisation is used, explain how this will be done

Not Applicable

4. Study Design

4.1 Describe the study design. Where this space is inadequate, continue on a separate sheet of paper. *Do not* delete page breaks or renumber pages.

The following will be the optimum involvement of each participant:

11 Each participant will be given a thorough medical check by a general practitioner before being allowed to participate in the study. Only those who pass the check will be included. Self exclusion by those who participate in performance enhancing or recreational drug use will be requested.

12 The study will run for four weeks.

13 Upon waking every morning seven days a week, a sleep log consisting of eight simple questions will be filled out. In addition to these questions any alcohol consumption during the past 24 hours will be noted in the sleep log. Filling out the log will take approximately two minutes each day.

14 Each participant will come into the laboratory three days a week (Monday, Wednesday and Friday) for approximately 15 minutes. Each participant will be given a specific appointment, which will occur at the same time throughout the study. The following tests will be performed:

14.3 A fitness test will be given on each Monday and the last Friday. It will involve running on a treadmill at an increasing speed until the participant chooses to stop the machine. This test will give an indication of the participant's maximal oxygen consumption and their anaerobic threshold. If this number of fitness tests is not acceptable to participants an alternative of one fitness test on the first Monday and one on the last Friday of the trial will be offered.

The following samples will be taken:

14.4 Blood samples (approximately 200μL) will be taken if possible on Mondays and Fridays or on Mondays and on the last Friday dependant on what is acceptable to the participants. These will be taken by finger tip or thumb tip pricking with a sterile lancet, to cause bleeding and the blood collected in a heparinised capillary tube.

14.5 Saliva samples will be collected on all three days. Participants will spit directly into a container or blow into a straw. When the fitness test occurs the sample will be taken before and after the test.

14.6 Electrosonophoresis samples will be collected on all three days. When the fitness test occurs the sample will be required before and after the test.

15 Once a week after a routine training session each participant will be required to:

15.3 Immediately after training give saliva and electrosonophoresis samples.

15.4 Perform no less than three 40-meter sprints with one-minute recovery time between each sprint.

15.5 Give saliva and electrosonophoresis samples after they have completed the sprints.

All blood, saliva and electrosonophoresis samples will be analysed for concentrations of growth hormone, cortisol, testosterone and glucose.

4.2 How many visits/admissions of participants will this project involve? Give also an estimate of total time involved for participants.

The maximum time commitment over a four week period for each participant is 6 hours and 30 minutes.
Broken down the visits would involve:
Morning – 4 weeks, three times per week – 20 minutes per visit.
Post-training – 4 weeks, once a week – 20 minutes per visit.
Filling in the sleep log – 28 times – 2 minutes per log.

4.3 Describe any methods for obtaining information. Attach questionnaires and interview guidelines.

Question and answer information sheet (see appendix one). Sleep log filled out by participants (see appendix two). Quantitative data acquisition by observation and measurement.

4.4 Who will carry out the research procedures?

Miss Lara Blackmore and Heather Purnell under the supervision of Professor David J. Mellor and Dr Christian Cook.

4.5 Where will the research procedures take place?

Massey University Recreation Centre and/or Human Performance Laboratory.

4.6 If blood, tissue or body fluid samples are to be obtained, state type, use, access to, frequency, number of samples, total volume, means of storage and labelling, length of proposed storage and method of disposal.

Up to 8 finger tip/thumb tip 200μl blood samples (total 1600μl), up to 24 5ml saliva samples by spitting (total 120ml), and up to 24 transcutaneous harvests of extracellular fluid samples (see appendix three). All samples will be deep-frozen. See appendix four for treatment,

transportation, storage and disposal of samples.

4.7 Will data or other information be stored for later use in a future study? [√] Yes [ ] No

If yes, explain how: Maybe used for comparative similar study of female athletes. Will be stored in locked filing cabinet with no individuals names linked to data sheets. Any future use of the data would be subject of a new application to the Massey University Human Ethics Committee.

4.8 Will any samples go out of New Zealand? [ ] Yes [√] No

If so where, and for what purpose?

5. Research Methods and Procedures

5.1 Is the method of analysis quantitative or qualitative? Quantitative

(If the method of analysis is qualitative, go to question 5.2)

If the method of analysis is **wholly or partly quantitative**, complete the following:

5.1.1 Describe the statistical method that will be used

Correlation analysis, repeat measures analysis of variance. Parametric and non-parametric analytical techniques will be considered. Standard computer statistical packages will be used.

5.1.2 Has specialist statistical advice been obtained? Yes ~~No~~

If **yes**, from whom? Associate Professor Hugh Morton

(A brief statistical report should be included if appropriate)

5.1.3 Give a justification for the number of research participants proposed, using appropriate power calculations.

This is a pilot study to help determine variance, which should allow power calculations to be made.

5.1.4 What are the criteria for terminating the study?

See exclusion criteria section 3.4.

5.2 If the method of analysis is **wholly or partly qualitative**, briefly describe the analysis. If interviews are to be used include the general areas around which they will be based. Copies of any questionnaires that will be used should be appended.

Not Applicable

6. Risks and benefits

6.1 What are the benefits to research participants of taking part?
Regular fitness tests over four weeks, assessment of sleep quality, advice as a group concerning the effectivenss of training, and a team incentive.

6.2 How do the research procedures differ from standard treatment procedures?
During training the athletes will provide small finger prick blood samples, saliva samples and will undergo
electrosonophoresis assessment.

6.3 What are the physical or psychological risks, or side effects to participants or third parties? Describe what action will be taken to minimise any such risks or side effects.
The only anticipated risks are those associated with fitness testing and due cre will be taken to minimise those risks as much as possible. Participants will be advised to train and exercise within their normal capacity, during the treadmill fitness test adverse affects are avoided by an automatic off switch, which the participant can use if he is feeling uncomfortable. An automatic off switches exist on the treadmill that can be used by the operator, and the machine itself has an automatic stopping system if the participant is not keeping up with the pace. Sterile sampling techniques will be used in every case, electrosonophoresis employs ultrasound levels below those used for diagnostic imaging and will be applied for no more than 30 seconds as determined by an automatic shutdown timer (see appendix tree for more details).

6.4 What arrangements will be made for monitoring and detecting adverse outcomes?
Self reporting and visual inspection of finger tip lancet wounds and of skin areas where the electrosonophoresis device is applied. If accidents occur during fitness testing in the laboratory, a telephone is available for emergency calls. There is a first aid kit available in the laboratory and the researchers are trained in CPR. A mobile phone will be made available during all field-testing.

6.5 Will any potential toxins, mutagens or teratogens be used? ~~Yes~~ No
If **yes**, specify and outline the justification for their use

6.6 Will any radiation or radioactive substances be used? ~~Yes~~ No
Note: If any form of radiation is being used please answer the following. If no, go to question 6.8

6.6.1 Under whose license is the radiation being used?

6.6.2 Has the National Radiation Laboratory (NRL) risk assessment been completed? Yes No
If **yes**, please enclose a copy of the risk assessment, and the contact name and phone number
If **no**, please explain why

6.7 What facilities/procedures and personnel are there for dealing with emergencies?

6.8	Will any drugs be administered for the purposes of this study?	~~Yes~~	No
	If **yes** is SCOTT approval required?	Yes	No
	Has SCOTT approval been given? (please attach)	Yes	No

7. Expected outcomes or impacts of research

7.1 What is the potential significance of this project for improved health care for Maori and non Maori, and for the advancement of knowledge?

Improved monitoring of athletic performance in terms of safety and enhancement. Further assessment of the electrosonophoresis device for assessing blood composition non-invasively and painlessly. Advancing understanding of sleep quality and quantity on hormone responses of relevance to training effectiveness.

7.2 What steps will be taken to disseminate the research results?

The research will be used as a basis of a Master of Science thesis, depending on continuation of sufficient participants for statistical validity, will be prepared for publication in a peer reviewed scientific journal and at an appropriate professional society meeting.

PART IV: BUDGET AND USE OF RESOURCES

8. Budget

8.1 How will the project be funded?

Hort Research will meet all sampling and chemical analytical costs. The Massey University Institute of Food Nutrition and Human Health will meet all other costs including travel expenses and consumables.

8.2 Does the researcher, the host department or the host institution, have any financial interest in the outcome of this research? Please give details.

Massey University has no financial interest on the outcome of this research. Hort Research owns the patent for the electrosonophoresis device.

8.3 Will the researcher personally receive payment according to the number of participants recruited, or a lump sum payment, or any other benefit to conduct the study? If so, please specify:

No

8.4 What other research studies is the lead investigator currently involved with?

None

9. Resource Implications

9.1 Does the study involve the use of healthcare resources? ~~Yes~~ No

If **yes**, please specify:

9.2 What effect will this use of resources have on waiting list times for patients ie., for diagnostic tests or for standard treatments?

10. Financial Costs and Payments to Participants

10.1 Will there be any financial cost to the participant? Give examples including travel.

Travel costs to participants will be reimbursed. In addition, a contribution of $500 to the club travel fund will be made at the end of the study.

10.2 Will the study drug/treatment continue to be available to the participant after the study ends? ~~Yes~~ ~~No~~ N/a

If **yes**, will there be a cost, and how will this be met?

10.3 Will any payments be made to participants or will they gain materially in other ways from participating in this project? Yes ~~No~~

If **yes**, please supply details Participants will be reimbursed for travel expenses and a donation will be made to their sport team's travel fund.

11. Compensation for Harm Suffered by Participants

Is this a clinical trial under Accident Rehabilitation and Compensation Insurance Corporation Guidelines? (see form guidelines) ~~Yes~~ No

If **yes**, please answer the following:

11.1 Is the trial being carried out principally for the benefit of a manufacturer or distributor of the drug or item in respect of which the trial is taking place? Yes No

(a) If the answer to 11.1 is **yes**, please complete **Statutory Declaration Form B** and answer questions 11.2, 11.3 and 11.4

(b) If the answer to 11.1 is **no** please complete **Statutory Declaration Form A**

11.2 What type of injury/adverse consequence resulting from participation in the trial has the manufacturer or distributor undertaken to cover? (please tick the appropriate box/es) **Yes** **No**

a) any injury (mental or physical)
b) only serious or disabling injuries.
c) only physical injuries
d) only physical injuries resulting from the trial drug or item, but not from any other aspect of the trial
e) physical and mental injury resulting from the trial drug or item, but not from any other aspect of the trial.
f) any other qualification (explain)

11.3 What type of compensation has the manufacturer or distributor agreed to pay? **Yes** **No**

a) medical expenses
b) pain and suffering
c) loss of earnings
d) loss of earning capacity
e) loss of potential earnings
f) any other financial loss or expenses
g) funeral costs
h) dependants' allowances

11.4 Exclusion clauses:

a) Has the manufacturer or distributor limited or excluded liability if the injury is attributable to the negligence of someone other than the manufacturer or distributor? (such as negligence by the investigator, research staff, the hospital or institution, or the participant). **Yes** **No**

b) Has the manufacturer or distributor limited or excluded liability if the injury resulted from a deviation from the study protocol by someone other than the manufacturer or distributor?

c) Is company liability limited in any other way?

If yes, please specify

12. Information and Consent

Consent should be obtained in writing, unless there are good reasons to the contrary. If consent is not to be obtained in writing the justification should be given and the circumstances under which consent is obtained should be recorded. Attach a copy of the information sheet and consent form.

12.1By whom, and how, will the project be explained to potential participants?

Miss Lara Blackmore and Dr Christian Cook will explain project both orally and in written form.

12.2 When and where will the explanation be given?	At the sports club
12.3 Will a competent interpreter be available, if required?	Not required
12.4 How much time will be allowed for the potential participant to decide about taking part?	One – two weeks
12.5 Will the participants be capable of giving consent themselves? - if not, to whom will the project be explained and who will give consent?	Yes
12.6 In what form (written, or oral) will consent be obtained? If oral consent only, state reasons.	Written
12.7 Are participants in clinical trials to be provided with a card confirming their participation, medication and contact phone number of the principal investigator?	Not applicable Yes No

13. Confidentiality and Use of Results

13.1 How will data including audio and video tapes, be handled and stored to safeguard confidentiality (both during and after completion of the research project)?	A name-number list will be kept separate to all data sheets. Numbered data sheets will be kept in a separate locked filing cabinet.
13.2 What will be done with the raw data when the study is finished?	Raw data will be stored on CD, floppy disks or on a pass-worded computer hard disk. Disks will be kept in a locked filing cabinet.
13.3 How long will the data from the study be kept and who will be responsible for its safe keeping?	Data will be kept for 5 years under the responsibility of David Mellor and Christian Cook.
13.4 Who will have access to the raw data and/or clinical records during, or after, the study?	Lara Blackmore, David Mellor and Christian Cook.
13.5 Describe any arrangements to make results available to participants, including whether they will be offered their audio tapes or videos.	A written record of individual data will be given to each participant.
13.6 If recordings are made, will participants be offered the opportunity to edit the transcripts of the recordings?	Not applicable / Yes / No
13.7 Is it intended to inform the participant's GP of individual results of the investigations, and their participation, if the participant consents?	~~Yes~~ / No
If **no**, outline the reasons	In the event of any outlying results, participants will be advised to consult their GP.
13.8 Will any restriction be placed on publication of results?	Yes / ~~No~~
If **yes**, please supply details	The electrosonophoresis results may not be published for up to 18 months, otherwise there will be no restriction.

14. Treaty of Waitangi

14.1 Have you read the HRC booklet, "Guidelines for Researchers on Health Research involving Maori"? Yes ~~No~~

14.2 Does the proposed research project impact on Maori people in any way? ~~Yes~~ No

14.3 Explain how the intended research process is consistent with the provisions of the Treaty of Waitangi

Any Maori participant will be given full opportunity to make any special requirements known.

14.4 Identify the group(s) with whom consultation has taken place, and attach evidence of their support

Not applicable

14.5 Describe the consultation process that has been undertaken **prior** to the project's development

Not applicable

14.6 Describe any ongoing involvement the group consulted has in the project

Not applicable

14.7 Describe how information will be disseminated to participants and the group consulted at the end of the project

As stated in section 13.5.

15. Other Issues

15.1 Are there any aspects of the research which might raise specific cultural issues? Yes ~~No~~

If **yes**, please explain Possibly blood collection if there are any Maori participants. Steps will be taken to ensure that any potential participants cultural concerns can be met before they volunteer to take part. If any cultural concerns cannot be met by the researchers, we will ask the participant not to volunteer.

15.1.1 What ethnic or cultural group(s) does your research involve?
Describe what consultation has taken place with the group prior to the project's development

The research does not specifically involve certain ethnic groups

15.1.2 Identify the group(s) with whom consultation has taken place and attach evidence of their support

Not applicable

15.1.3 Describe any ongoing involvement the group consulted has in the project
Not applicable

15.1.4 Describe how you intend to disseminate information to participants and the group consulted at the end of the project
Individual results will be discussed with each participant confidentially once all of the data had been collected. Each participant has the right to receive a summary of the findings.

16. Ethical Issues

16.1 Describe and discuss any ethical issues arising from this project, other than those already dealt with in your answers?
None

Thank you for your assistance in helping us assess your project fully

Please now complete:

- **the declarations (Part V)**
- **a drug administration form (if applicable)**
- **an Accident Rehabilitation and Compensation Insurance Corporation form A or B**

PART V: DECLARATIONS

1. DECLARATION BY PRINCIPAL INVESTIGATOR

The information supplied in this application is, to the best of my knowledge and belief, accurate. I have considered the ethical issues involved in this research and believe that I have adequately addressed them in this application. I understand that if the protocol for this research changes in any way I must inform the Ethics Committee.

NAME OF PRINCIPAL INVESTIGATOR (PLEASE PRINT): Lara Blackmore

SIGNATURE OF PRINCIPAL INVESTIGATOR: LBlackmore

DATE: 10/5/2001

2. DECLARATION BY THE HEAD OF THE DEPARTMENT IN WHICH THE PRINCIPAL INVESTIGATOR IS LOCATED OR APPROPRIATE DEAN OR OTHER SENIOR MANAGER **

I have read the application and it is appropriate for this research to be conducted in this department I give my consent for the application to be forwarded to the Ethics Committee.

NAME AND DESIGNATION (PLEASE PRINT): R. H. MORTON

SIGNATURE: R Hugh Morton

DATE: 10/5/01 DESIGNATION: Assoc. PROFESSOR

** *(NOTE: WHERE THE HEAD OF DEPARTMENT IS ALSO ONE OF THE INVESTIGATORS, THE HEAD OF DEPARTMENT DECLARATION MUST BE SIGNED BY THE APPROPRIATE DEAN, OR OTHER SENIOR MANAGER.*

IF THE APPLICATION IS FOR A STUDENT PROJECT, THE SUPERVISOR SHOULD SIGN HERE).

3. DECLARATION BY THE GENERAL MANAGER OF THE HEALTH SERVICE IN WHICH THE RESEARCH IS BEING UNDERTAKEN (IF APPLICABLE)

I have reviewed the proposal for cost, resources, and administrative aspects and issues regarding patient participation and staff involvement. The proposal has my approval subject to the consent of the Ethics Committee.

NAME OF GENERAL MANAGER (PLEASE PRINT):

SIGNATURE:

Appendix Three

INFORMATION SHEET

For the study on:

Hormone Profiles, Training Performance and Sleep Quality

Researcher, Supervisors and Contacts

Hi, I am Lara Blackmore, a postgraduate student in the Institute of Food, Nutrition and Human Health and principal researcher in this study. Also involved in this study are Professor David Mellor, Associate Professor Hugh Morton, Dr Christian Cook and Heather Purnell. Heather Purnell is another postgraduate student in the same Institute and will be assisting me during the collection of samples.

Contact Phone Numbers:

Lara Blackmore
Professor David Mellor
Associate Professor Hugh Morton
Dr Christian Cook
Heather Purnell

Purpose and Objectives of the Study

Body function as it affects exercise performance and its enhancement has been studied for many years. Such studies in people are sometimes impeded by a reluctance to provide blood samples. The recent development of a safe ultrasound method for non-invasive sampling of skin fluids, which have a composition resembling blood, offers tremendous opportunities for benign monitoring of physiological responses involving blood composition associated with exercise. The new method has the clinical name of electrosonophoresis but we like to think of it as "Star Trek" technology.

We have a particular interest in training effectiveness and recovery, and in sleep quality/quantity. Training effectiveness and recovery have been monitored in the past by measuring blood hormone profiles, in particular the testosterone: cortisol ratio. Sleep quality/quantity is considered to have significant impact on training effectiveness and performance, the link here is considered to be via growth hormone. Blood glucose concentrations are also of interest because they reflect energy supply.

There are three objectives in this study:

1) With particular reference to testosterone, cortisol, growth hormone and glucose, to compare their concentrations in blood and saliva with those derived by electrosonophoresis. The aim being to further validate that use of electrosonophoresis as a non-invasive way of sampling.
2) To explore relationships between exercise, fatigability (as a measure of fitness), fitness increases (as assessed by maximum oxygen uptake (VO_2max) and the ventilatory anaerobic threshold (VAT)), and the hormone levels in blood, saliva and skin fluid.
3) To explore the impact of spontaneous disturbances in sleep quality/quantity on hormone profiles and athletic performance.

The experiment has been reviewed critically by scientists, a medical practitioner and a human ethics committee, and has been checked for safety and value of results. This project has been reviewed and approved by the Massey University Human Ethics Committee, PN Protocol 01/67.

I would like to invite you to participate in this study because of you winter sports club activities. What the study will involve, if you agree to participate, is outlined below.

Being a Participant would involve

The following is an outline of the full programme that we would like you to engage in:

- We would arrange a thorough physical/medical check up by a general practitioner before the study. This will not involve you giving blood or urine samples. Only those who passed fit will be included.
- Please do not volunteer to participate if you engage in recreational and/or performance enhancing drug use.
- The study will run for four weeks and will be conducted at Massey University.
- When you wake up each morning of the week, we ask you to fill out a sleep log consisting of eight simple questions. We also want you to note any alcohol consumption during the previous 24 hrs. Filling out the log will take you about 2 minute each day.
- We ask you to come to the laboratory three days a week (Monday, Wednesday and Friday) for a maximum of 20 minutes each time. We would like you to perform the following fitness test:
  - On each Monday and the last Friday of the 4-week trial we want you to do a fitness test. It will involve running on a treadmill at an increasing speed until you choose to stop the machine. This test will give an indication of your maximal oxygen consumption (VO2max) and ventilatory anaerobic threshold (VAT). If this number of tests is too many for you, we would like you to do a minumum of one test on the first Monday and another test on the last Friday of the study.

  We want to take the following samples:
  - Small blood samples (about 200μL) will be taken on each Monday and Friday or on each Monday and the last Friday depending on what you find acceptable. These samples will be taken by a sterile lancet to cause a small amount of bleeding and the blood droplet collected by a capillary tube.
  - We want you to provide a saliva sample on all three days each week. We will ask you to spit directly into a container or blow saliva down a straw. When you do each fitness test we would like you to provide a sample both before and after the test.
  - We would like to sample your skin fluid by the painless and non-invasive electrosonophoresis method on all three days each week. When you do each fitness test we would like you to provide a sample both before and after the test.
- Once a week after a routine training session we would like you to do the following:
  - Immediately after training give us a saliva sample and a electrosonophoresis sample.
  - Then perform three 40-meter sprints with one-minute recovery time between each sprint.

- Finally, give another saliva sample and another electrosonophoresis sample after you have completed the sprints.

Benefits to you as a participant:

- You will be able to confidentially discuss your own results with researcher.
- Your sports club will receive advice on the effectiveness of your training as a group.
- A donation will be given to your team travel fund; this is not dependent on how many people finish the trial.
- You will receive a personal fitness assessment over a four-week period.
- You will learn how to assess your sleep quality.

Risks you as a Participant

No risks or side affects are anticipated. You will be advised to train and exercise within your normal capacity. During the treadmill fitness test, adverse affects are avoided by an automatic off switch, which you can use if you are feeling uncomfortable. If accidents occur during fitness testing in the laboratory, a telephone is available for emergency calls. There is resuscitation equipment available during testing and technicians able to operate the equipment in the building. A mobile phone will be made available during all field-testing. Sterile sampling techniques will be used in every case, electrosonophoresis employs ultrasound levels below those used for diagnostic imaging (during pregnancy) and will be applied for no more than 30 seconds as determined by an automatic shutdown timer. Self reporting and visual inspection of the small lancet wounds and of skin areas where the electrosonophoresis device is applied will allow early medical intervention in the unlikely event that any problems are encountered.

The Inclusion/Exclusion Criteria

To be included you must:

- Pass physical/medical exam before participation,
- Be engaged in regular sports training,
- Be a healthy, non-injured 18-30 year old male.

We would ask you to withdraw from the study if you become injured during the period of the trial.

We do not want you to volunteer if:

- You use performance enhancing or recreational drugs,
- You have health conditions incompatible with regular training and exercise,
- You are uneasy with any aspect of the study after it has been explained to you.

Confidentiality, Anonymity and Your Right not to Participate and to Withdraw

Your anonymity will be protected. All information will be treated as completely confidential. If you participate your name will be allocated a random number code, which will be used to label all of the samples and data sheets related to you. Once all data have been collected and your personal results discussed with you a second random

number code will be aligned with the first code so that the researcher is not able to link any results and participants.

You have a right to decline to participate in this study.

If you participate in this study, you have the right to:

- Withdraw from the study at any time;
- Prior or during the study have any questions you have about the study answered in full.
- Refuse to answer any particular questions;
- Provide information on the understanding that you name will not be used unless you give permission to the researcher;
- A summary data sheet of all of your individual results;
- Be given access to a summary of the findings of the study when it is concluded.

Cultural Issues

If you have any cultural concerns about sample collection, analysis and disposal, or with any other part of this study we would be happy to discuss them with you to clarify your requirement, and if possible comply with them, before you decide to participate.

Compensation

In the unlikely event of a physical injury as a result of your participation in this study, you will be covered by the accident compensation legislation with its limitations. If you have any questions about ACC please feel free to ask the researcher for more information before you agree to take part in this trial.

Other notes

The researcher will refund any travel expenses incurred by you in order to meet your appointments as a participant, so long as you discuss your situation with the researcher prior to taking part in the study.

Appendix Four

Hormones, Sleep and Athletic Training
(Entry Medical Examination)

Date _____ **GP** _____

Patient Name _____ **Age**

Consent Form Signed **Yes** ☐ **No** ☐

History

Are you currently in good health? Yes ☐ No ☐
If no, give details

_____

_____

Are you sufferning from any illness? Yes ☐ No ☐
If yes, give details

_____

_____

Are you taking any medication? Yes ☐ No ☐
If yes, give details

_____

_____

Are you taking any mineral, vitamin or nutritional supplement? Yes ☐ No ☐
If yes, give details

_____

_____

Have you suffered any significant illness in the past? Yes ☐ No ☐
If yes, give details

_____

_____

Have you ever been hospitalised? Yes ☐ No ☐
If yes, please give details

_____

_____

Do you drink alcohol? Yes ☐ No ☐

If yes, in what form _____

In what quantity ... ...per day ... ...per week ... ...occassionally

Do you smoke? Yes ☐ No ☐

If yes, in what form _____

In what quantity ... ...per day

Do you have any allergies at all? Yes ☐ No ☐
If yes, give details

_____

Exclusion Criteria Questions

Are you currently engaged in regular sports training? Yes ☐ No ☐

Are you currently suffering from any injury that might interfere with training? Yes ☐ No ☐

Are you taking any performance enhancing or recreational drugs? Yes ☐ No ☐

Examination

Height (cm) ☐ Weight (kg) ☐ BMI ☐

Pulse Rate ☐ Rhythm ☐

Blood Pressure ☐ Waist (cm) ☐

Skin: Any severe skin disorders present Yes ☐ No ☐

Head: - ears - any sign of ear disease/clinical deafness Yes ☐ No ☐
If yes, give details

_____

- eyes - VA: Right ☐ Left ☐
(corrected/uncorrected)

- mouth/throat, any abnormality? Yes ☐ No ☐

If yes, give details _____

Neck: - weakness Yes ☐ No ☐

- carotid bruit Yes ☐ No ☐

- neck glands, any abnormality? Yes ☐ No ☐

- thyroid gland, any abnormailty? Yes ☐ No ☐

Upper Limbs - upper limb strength, any abnormality? Yes ☐ No ☐

Heart - heart sounds, any abnormality? Yes ☐ No ☐

Lungs - breath sounds, any abnormality? Yes ☐ No ☐

PEFR ☐ Expected PEFR ☐

Abdomen - organomegaly? Yes ☐ No ☐

- hernia? Yes ☐ No ☐

- inguinal lymphadenopathy? Yes ☐ No ☐

- palable masses? Yes ☐ No ☐

Genitalia - testes, any abnormality? Yes ☐ No ☐

- secondary sexual characteristics, any abnormality? Yes ☐ No ☐

Lower limbs - strength in legs, any abnormality? Yes ☐ No ☐

Spine - any restriction of movement? Yes ☐ No ☐

Urinalysis (MSU) Sugar ☐ Protein ☐ Blood ☐

Positive findings comment:

_____

_____

_____

Disposal

Fit to participate in trial? Yes ☐ No ☐
If no, Mark Morris to be advised

Any significant abnormality, that patient is unaware of or patients doctor is likely to be unaware of, detected in examination Yes ☐ No ☐

If yes, what action taken

_____

_____

_____

Doctors name: _____

Doctors signature: _____

Date: _____

Please return this completed form to Mark Morris

Appendix Five

CONSENT FORM

"This project has been reviewed and approved by the Massey University Human Ethics Committee, PN Protocol 01/67"

I have read and I understand the information sheet dated 1st June 2001 for volunteers taking part in the study designed to investigate the relationships that exist between exercise, fatigability, fitness and hormone levels and the effect of sleep quality/quantity on these parameters. I have had the opportunity to discuss the study. I am satisfied with the answers I have been given.

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time or decline to answer any questions at any time and this will in no way affect my position in the sports team.

I understand that my participation in this study is confidential and that no material, which could identify me, will be used in any reports on this study.

I understand that the investigation will be stopped if it should appear harmful to me.

I have had time to consider whether to take part.

I know whom to contact if I have any questions about the study.

I consent to blood samples being destroyed by incineration at the end of the study.
YES/NO

I consent to blood samples being disposed of by sterilisation and burial at the end of the study. YES/NO

I consent to the blood samples being sent to Hort Research in Hamilton for analysis.

I hereby consent to take part in this study.

Signed: .....

Name: .....

Witness Name: .....

Witness Signature: .....

Date: .....

Appendix Six

Sleep Log

Participant code: *(number filled in by researcher)*

Date: *(filled in by researcher)*

Day of trial: *(filled in by researcher)*

Time of entry: *(filled in by participant)*

1. What time did you go to bed?

   _____

2. How long did it take you to go to sleep?

   _____

3. What time did you wake up?

   _____

4. Did you have any sleep disturbances during the night?

   _____

5. Do you remember having any dreams?

   _____

6. On a scale of 0 to 10 (0 being no sleep and 10 being the best ever) how would you rate your overall sleep quality for the night?

   0 1 2 3 4 5 6 7 8 9 10

7. Did you have any naps during the day? If yes please state for how long.

   _____

8. Have you consumed any alcohol in the last 24 hours? If yes please note how much and what you drank in terms of standard drinks (defined on next page).

   _____

Standard Drink:

These limits are based on a measurement called the 'standard drink'. Each standard drink contains 10 grams of alcohol. Here's how many standard drinks there are in typical servings:

1 can or stubbie of beer (at 5%)	1.5
1 pub measure of spirits (whisky, gin, vodka)	1
1 glass of fortified wine (sherry, martini, port)	1
1 glass of table wine	1
1 bottle of fortified wine (sherry, martini, port)	11.5
1 pint of beer (a 'handle')	2
1 jug of beer	4
1 bottle of table wine	7.5
1 750 ml bottle of spirits (whisky, gin, vodka)	25

Appendix Seven

TREADMILL PROTOCOL

Speed (KPH)	Grade (%)	Acceleration (KHH/s)	Duration (s)
3	0	0.15	20
6.5	5	0.15	20
6.5	5	0	20
6.5	5	0	20
6.5	5	0	20
6.5	5	0	20
6.5	5	0	20
7	8	0.025	20
7.5	8	0.025	20
8	8	0.025	20
8.5	8	0.025	20
9	8	0.025	20
9.5	8	0.025	20
10	8	0.025	20
10.5	8	0.025	20
11	8	0.025	20
11.5	8	0.025	20
12	8	0.025	20
12.5	8	0.025	20
13	8	0.025	20
13.5	8	0.025	20
14	8	0.025	20
14.5	8	0.025	20
15	8	0.025	20
15.5	8	0.025	20
16	8	0.025	20
16.5	8	0.025	20
17	8	0.025	20
17.5	8	0.025	20
18	8	0.025	20
18.5	8	0.025	20
19	8	0.025	20
19.5	8	0.025	20
20	8	0.025	20
3	0	5	300

Appendix Eight

BUDGET

Sample Analysis

	Quantity	$ Per Unit			
Plasma Testosterone	112	6	672		
Plasma Cortisol	112	6	672		
Plasma Growth Hormone	112	9	1008		
ESP Testosterone	280	6	1680		
ESP Cortisol	280	6	1680		
ESP Growth Hormone	168	9	1512		
Saliva Testosterone	280	6	1680		
Saliva Cortisol	280	6	1680	10584	
Add GST (12.5%)				1323	12000

Consumables

Alcohol skin wipes	8		
Heparinised capillary tubes	13		
Gloves	30		
Saliva sample containers	200		
Sharps container	5		
Lancet refills	45		
Tissues	5		
Lancet Pens	42		
Stationary	100		
Courier	170	618	
Add GST (12.5%)		77	700

Other (GST included)

Reward for Club	500		
Computer Courses	30		
Health checks for participants	400		
Interloans	100		
Travel costs (participants)	700		
Travel costs (researcher)	100		
Printery costs	200		
Sundries	270	2300	2300
			15000

Appendix Nine

Hello again,

Firstly I would like to thank you all for your involvement in my research. You would have seen me stressed and tense at certain times due to equipment failure and the fact that I was responsible for constantly pricking you with pins and putting you through awful tests. However your group made the experience very interesting and enjoyable for me.

Just so you all know I am still writing away at Massey in Palmerston North. My office has moved into the PD Hut located off the runway from the gravel pit to concourse just past Colombo Hall. My name is on my door so if anyone would like to contact me or come and see me to discuss any results further I am easy to find. My Massey extension number is now 2[redacted]

Summary of Results from Trial

This summary includes comments on the analysis of the group as a whole and some notes on your individual results.

Some General Comments from Dr Christian Cook

Just a reminder Christian designed the ultrasound device and analysed the samples.

- A high degree of uniformity among the participants, expected due to age and lifestyle.
- Testosterone levels lower than expected for age group, unsure why prehaps diet, training intensity/duration, and alcohol.
- Growth hormone levels higher than expected, likely due to method of analysis.

Hormone Levels in the Blood

Overall the concentration of all three hormones in the blood were within the normal range for all of the participants. I will outline the role of the hormones that we measured so you may understand more about the study.

Cortisol

- Referred to as an indicator of stress in humans and other animals, generally the higher the concentration the more stress that the person is experiencing. This could be a physical stressor such as heat, exercise or undernutrition, or an emotional stressor such as studying for exams.
- Cortisol increases the amount of fat used in the production of energy and decreases the amount of glucose, thereby conserving it.
- It also decreases the synthesis of protein, therefore can cause decreases in muscle mass.
- Cortisol levels are likely to increase with increasing intensity and duration of exercise. The more highly trained the individual the smaller the increase.

Testosterone

- Is the male sex-steroid, it is responsible for the development of a male foetus and the secondary sex characteristics such as facial hair that develop at puberty.
- It is also responsible for a higher level of protein synthesis in males and therefore the larger muscle mass that males have in comparison females. It is a very important hormone for sport training, particularly for increasing muscle size.
- Testosterone levels increase with increasing exercise workloads.

Growth Hormone

- Is important for growth in children and adolescents.
- In adults it is responsible for the maintenance of body composition. Like testosterone it increases protein synthesis and therefore the production of muscle tissue. Like cortisol it increases the amount of fat used to produce energy and conserves glucose.
- Growth hormone levels increase with increasing exercise intensity. This response is less in trained individuals.

Effect of Sleep

Sleep is important for secretion of a variety of hormones. While we sleep increased amounts of these hormones are secreted at different stages of the sleep cycle. For example there is an increase in the secretion of growth hormone by the brain during the first half of sleep, therefore if there are disturbances in the sleep at this stage, there may be a decrease in secretion. From this it becomes clear that for training to be effective and muscle growth maximised, sufficient, undisturbed sleep is important.

Use of Ultrasound to Measure Blood Composition

When we compared the hormone concentrations in the ultrasound sample with those of the corresponding plasma sample the correlations were very good.

When comparing the concentration of saliva and plasma the correlations while significant were not as high, therefore telling us that the use of ultrasound is a far superior non-invasive method of measuring hormone concentrations.

No significant relationship was found between alcohol consumption and hormone levels. This does not mean that they don't exist just that the nature of this study did not allow for these to be seen.

Relationships Between Sleep and Hormone Concentrations

At present I am researching a more effective way to analyse the sleep logs. So far no relationship can be established using any of the measures of sleep quality. This is likely to be due to the sleep logs being very subjective measures of sleep and huge differences existing between people's perceptions of sleep.

Overall Levels of Fitness

It was clear to see simply by observation that the group was generally very fit. This was supported by the work loads that were required to have the participants exercising at their theoretical maximum heart rate for age. In most cases each individual did reach

their max heart rate. And this effort was duplicated on successive tests. When max heart rate was not reached the causes are likely to very. One reason could be that the participant's heart could work harder but body could not physically keep up with the treadmill. Other reasons could be that alcohol consumption the previous night left the participant with a "hangover" and their body wasn't up to the task, other factors having an affect include recent food intake, sleep, recent exercise and the list goes on.

The times for the sprints were fairly consistent, I was expecting the times taken to sprint the 40m to increase in general this was not generally seen. Perhaps a few more goes at it were needed to start to slow the group down.

Individual Analysis

Included:

- Individual sprint times, and averages for the group.
- Levels obtained on the treadmill and average for the group.

Thank you again for your involvement,

See you when you return to Massey,

Lara Blackmore

Sprints

	Your Times for Each Sprint			**Average Times for the Week**		
	1st	2nd	3rd	1st	2nd	3rd
Week 2				5.48	5.42	5.41
Week 3				5.14	5.09	5.11
Week 4				5.23	5.22	5.16

Treadmill

	Speed Reached at End of Test (KPH)	Corresponding Power Output (J/s)	Average for the day (KPH)
Week 2			14.2
Week 3			14.4
Week 4			14.1

Appendix Ten

Estimation of plasma hormone levels using low-invasive transdermal electrosonophoresis.

Authors: Lara M. Blackmore, Christian J. Cook, R. Hugh Morton, and David J. Mellor

Lara M. Blackmore, Associate Professor R. Hugh Morton, Professor David J. Mellor
Institute of Food, Nutrition and Human Health, Massey University, Palmerston North New Zealand

Corresponding Author:
Dr Christian J. Cook
Bioengineering Technologies, HortResearch, Ruakura, Private Bag 3123, Hamilton, New Zealand
Phone: +64 7 858 4743
Facsimilie: +64 7 858 4705
Email: ccook@hortresearch.co.nz

Running title: Low-Invasive transdermal collection

Abstract

Purpose: The reluctance of participants to provide blood samples may impede thorough and comprehensive studies in many areas in biomedical science. Transdermal electrosonophoresis, a new technique that collects an ultrasound generated flux of analytes from the bloodstream to the skin surface, may offer a low invasive alternative for sample collection. In young male rugby players, correlations between plasma, transdermal electrosonophoretic and saliva samples were examined for the hormones testosterone, cortisol and human growth hormone, all of which can have an impact on training effectiveness. The purposes of this study were to validate the use of transdermal electrosonophoresis as a non-invasive plasma sampling technique. **Methods:** 14 male competitive club rugby players gave plasma, electrosonophoretic and saliva samples twice weekly for four weeks. These samples were analysed for testosterone, cortisol and growth hormone concentrations, except that growth hormone was not determined in saliva. **Results:** There were highly significant, positive correlations between the concentration of each of the three hormones in the plasma and electrosonophoretic samples (correlation coefficient, $r = 0.965 - 0.977$). The results indicate a single multiplier of 9.891 (SE of estimation 0.041) that can be applied to electrosonophoretic readings in order to obtain plasma concentrations of any of the three hormones measured. **Conclusions:** The high correlations suggest that electrosonophoresis may be a highly accurate method of estimating plasma testosterone, cortisol and growth hormone levels.

Key Words: cortisol, electrosonophoresis, growth hormone, testosterone

Introduction

The physiological basis of human exercise performance and its enhancement have been investigated for many years. Such investigations in humans are sometimes impeded by the reluctance of participants to provide significant numbers of plasma samples by venepuncture. The recent development of an ultrasound method for non-invasive sampling of plasma (transdermal electrosonophoresis) offers tremendous opportunities for benign monitoring of physiological responses (1) including changes in blood composition associated with exercise. The opportunities in clinical settings are even wider. As this method is still in its infancy the composition of the electrosonophoretic (ES) samples needs to be correlated with the composition of plasma samples in order to validate its further development for widespread use. Given that saliva samples are also often collected as a low invasive method, validation against these samples is also likely to be useful. Cook (1) studied the best method of collecting transdermal exudate in sheep and humans. The result of that study was that high correlations between plasma and ES samples were seen both at rest and during exercise and recovery. In contrast, saliva samples, while showing a high correlation with plasma at rest, showed poor post exercise correlations, possibly due to a time lag in partitioning between the two body fluids (plasma and saliva).

The aim of this study was to further validate the use of ES as a non-invasive sampling methodology in humans. We therefore compared the concentrations of testosterone, cortisol and human growth hormone in plasma and saliva (testosterone and cortisol only) with those derived by ES in young, male, rugby players at rest.

Method

The participants in this study were also participating in another study researching the relationships between hormone levels, sleep and athletic training in which plasma hormone concentration measurements were required. Ethical approval was obtained from the Massey University Human Ethics Committee and potential participants were approached at a local rugby football club. Interested participants underwent a medical assessment and only those who passed fit were included in the study. Informed consent was obtained.

The study ran for four weeks. Participants were weighed and their height measured before the study began. Weight was measured with calibrated scales (Jadever, JPS-2030, Jadever Scale Co Ltd, Taiwan) and height using a stadiometer (Seca 222, Vogel and Halke GmbH Co, Hamburg, Germany). Each participant came into the laboratory twice a week at the same time of day to give blood, ES and saliva samples.

Sterile techniques were used to collect blood, which was taken by lancing the finger tip. Prior to lancing, the area was sterilised with 70% isopropyl alcohol (Sterets pre injection swabs, Seton Prebbles Ltd, England). Once the alcohol had dried, two fingers were lanced using an automatic lancet (Accu-Chek Softclix Pro, Roche Diagnostics NZ Ltd) and the blood was collected into 100µl heparinised capillary tubes (Chase Scientific Glass Inc, Tennessee, USA). Four to six capillary tubes were taken at each time, depending on the flow of blood. The capillary tubes were then centrifuged (IEC Micro Capillary Centrifuge, International Equipment Company, Massachusetts, USA) for five minutes. The plasma was removed from the capillary tube, transferred into a 1.5ml-microtest tube (3810X, Eppendorf, Germany), and stored at -20°C until assay.

During the hour before supplying saliva samples, the participants were advised to avoid eating an apple or other coarse textured food, brushing their teeth, or drinking coffee. Saliva samples (about 5ml) were supplied by spitting into 25ml sterile containers (LBS3534, Labserve, Auckland, New Zealand), which were subsequently stored at -20°C until assay.

ES samples were collected in a manner similar to that described by Cook (1). Briefly, ultrasound transmission gel (Aquasonic, Parker Laboratories Inc, New Jersey, USA) was applied to the area on the medial surface of the arm where the modified ultrasound head was in contact with the skin. This head allowed collection from the skin of any exudates caused by the ultrasound transdermal flux across a semi-permeable size exclusion membrane into a fluid chamber. A 9V generated current was applied across the arm simultaneously with the ultrasound as this increases hormonal flux (1). A continuous ultrasound pulse of 20khz (at skin surface $10Wcm^2$) was applied for 15 seconds, then the arm removed from the ultrasound. Any metabolites that were drawn

out of the arm were collected from the head chamber into an associated removable collection vial in 70% ethanol. To ensure that all of these metabolites were collected, flow through the chamber continued for an additional 60 seconds after the arm was removed. This created a total fluid sample of approximately 0.7-1.0ml. The sample was collected in 1.5ml microtest tubes (3810X, Eppendorf, Germany), which were stored at -20°C until assay.

Testosterone and cortisol were analysed by ELISA methods using manufacturer's instructions (saliva by Salimetric Testosterone or Cortisol EIA kits plasma levels using DRG Diagnostics plasma kits). Growth hormone was assayed by a direct RIA kit for both plasma and ES samples, as per manufacturer's instructions (DRG Diagnostics). For statistical analysis the level of significance was taken at $P \leq 0.05$.

Results

Details of the hormone concentrations in plasma, ES and saliva samples are provided in Table 1.

Table 1. Range of hormone concentrations* in samples.

Hormone	Sampling Method	N	Minimum	Maximum	Mean	SD
Testosterone	Plasma	99	2.1	10.5	5.45	1.75
	ES	130	0.11	1.03	0.511	0.186
	Saliva	155	0.07	0.59	0.238	0.106
Cortisol	Plasma	99	39	217	131.8	36.8
	ES	130	3.5	25.8	13.62	3.97
	Saliva	155	0.73	15.84	3.581	2.151
Growth	Plasma	99	4.5	20.4	12.13	4.16
Hormone	ES	101	0.49	2.09	1.255	0.420

*all measurements are in ng/ml

SD: standard deviation

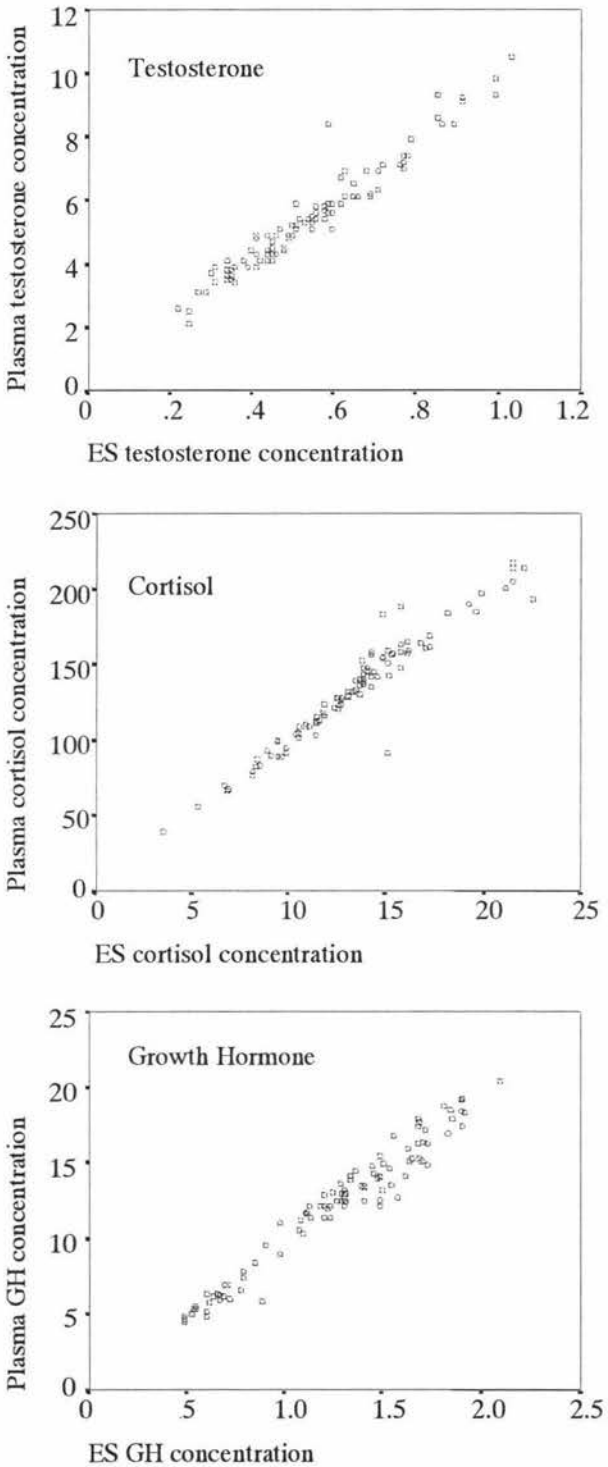
Table 2 shows the correlations between hormone levels measured in the three fluids. Samples taken at all times from all participants were included in these calculations.

Correlations were calculated between plasma and ES, plasma and saliva and saliva and ES hormone levels. The correlation coefficients for the concentrations of testosterone and of cortisol in plasma and ES samples were greater than those for the same hormones in plasma and saliva samples (Table 2). Growth hormone levels in plasma and ES samples were particularly strongly correlated (Table 2). When plotted, they suggest a strong linear relationship through the origin (Figure 1).

Table 2. Correlations between plasma, ES and saliva hormone concentrations.

Comparison	Testosterone	Cortisol	Growth Hormone
Plasma – ES			
Bi-variate Correlation	0.970	0.965	0.977
Sig. (2-tailed)	<0.001	<0.001	<0.001
Plasma – Saliva			
Bi-variate Correlation	0.791	0.612	-
Sig. (2-tailed)	<0.001	<0.001	-
Saliva – ES			
Bi-variate Correlation	0.698	0.522	-
Sig. (2-tailed)	<0.001	<0.001	-

Figure 1. Relationships between plasma and ES hormone concentrations.



As well as the high correlations in Table 2, we note from the panels of Figure 1 that the slope of any regression line fitted to these data will be approximately 10, irrespective of the hormone being measured. Such a regression should in the first instance allow for possible slope differences. We can fit and examine a regression equation of the form

$$PL = K + \alpha_0 ES + \alpha_1 X_1 ES + \alpha_2 X_2 ES$$

applied to the combined data. In this equation PL is the plasma concentration of the measured variables in ng/ml, K is a constant, ES is the electrosonophoretic concentration in ng/ml. α_0, α_1 and α_2 are parameters. Testosterone was wused as the reference hormone in these calculations so that X_1 and X_2 are indicator variables used multiplicatively for cortisol and growth hormone respectively. To investigate whether any cross reactivity during sample analysis occurs we include the constant; the resulting fitted equation is

$$PL = 1.251 + 7.885ES + 1.921X_1ES + 0.840X_2ES$$

with parameters as detailed in Table 3.

Table 3. Parameters for a regression equation with a constant and indicator variables for each hormone.

Predictor	Coefficient	SE Coefficient	T	P
Constant	1.251	1.084	1.154	0.249
ES	7.885	2.046	3.855	0.000
X_1ES	1.921	1.980	0.970	0.333
X_2ES	0.840	1.479	0.568	0.570

SE, standard error of estimation; T, student's t; P, p-value.

The constant is non-significant and is omitted resulting in the following fitted equation

$$PL = 9.954ES + 0.06054X_1ES - 0.334X_2ES$$

with parameters as detailed in Table 4.

Table 4. Parameters for a regression equation without a constant.

Predictor	Coefficient	SE Coefficient	T	P
ES	9.954	0.985	10.106	0.000
X_1ES	0.06054	0.986	-0.061	0.951
X_2ES	-0.334	1.073	-0.312	0.755

SE, standard error of estimation; T, student's t; P, p-value.

Noting that the coefficients of X_1ES and X_2ES do not approach statistical significance, nor are they large in any numerical relative sense, the data have been refitted without these indicator variables, resulting in

PL = 9.891ES

with parameters as detailed in Table 5.

Table 5. Parameters for a regression equation without a constant or indicator variables for each hormone.

Predictor	Coefficient	SE Coefficient	T	P
ES	9.891	0.041	243.575	0.000

SE, standard error of estimation; T, student's t; P, p-value.

These results provide strong evidence that a single multiplier, estimated at 9.891 with a standard error of estimation of 0.041, is all that needs to be applied to the ES readings in order to obtain PL values with a high degree of accuracy. In effect, 9.891 is the calibration constant to convert ES to PL for this equipment and the protocol described.

We can subdivide the analysis according to each participant to establish whether differences occur between individuals. A regression equation for each participant for each hormone was calculated, and then analysed for variance of slope (Table 6) and intercept (Table 7).

Table 6. Analysis of variance for slope.

Source	DF	SS	MS	F	P
Participant	13	13.554	1.043	0.96	0.508
Hormone	2	1.942	0.971	0.90	0.420
Error	26	28.108	1.081		
Total	41	43.604			

DF, degrees of freedom; SS, sum of squares; MS, mean square; F, f-statistic; P, p-value.

There were no significant differences between the slopes for any of the 14 participants nor for any of the hormones.

Table 7. Analysis of variance for intercept.

Source	DF	SS	MS	F	P
Participant	13	1328.30	102.18	1.09	0.410
Hormone	2	506.42	253.21	2.69	0.086
Error	26	2443.53	93.98		
Total	41	4278.25			

DF, degrees of freedom; SS, sum of squares; MS, mean square; F, f-statistic; P, p-value.

There were no significant differences between the intercepts for any of the 14 participants nor for any of the hormones.

Discussion

Herein testosterone, cortisol and human growth hormone concentrations in plasma and ES demonstrated strong correlations (Table 2). This supports and extends the results of previous human studies for other analytes. Kost et al (2) used this technology to demonstrate that glucose, theophylline, creatinine, urea and calcium can be extracted from the blood stream through the skin. Cook (1) monitored glucose, insulin, estradiol, cortisol and testosterone successfully in both humans and sheep. Clearly ES may be used to monitor many constituents of plasma quickly and safely in a non-invasive manner. This provides a range of opportunities for research in sport and exercise where a low invasive method would maximise full participation in such trials.

Some of the positive effects of this new technology include increasing the number of suitable participants available for research. Due to the reduced need for blood sampling by venepuncture it is likely that management staff of elite athletes and the athletes themselves would be more likely to agree to be involved in studies that would otherwise have required blood sampling by venepuncture. This technology also increases the number of samples that could be taken from human participants in a set time period, as the method is non-invasive, painless and the small volume required will not measurably alter blood composition.

Monitoring training effectiveness may also be possible with transdermal electrosonophoresis. For example blood composition can be examined for potential hormonal markers of overtraining such as low testosterone: cortisol ratios.

Transdermal electrosonophoresis may also provide a method of sampling during competition, due to its speed and low invasiveness. This could be applied at all levels of competition and has applications in many areas. For example analysis of blood composition could provide trainers with a clear picture of what bioenergetic systems are being used and in what capacity. They could then apply a training programme that would improve these systems in relation to their use during competition.

Other potential uses including drug testing, nutritional mornitoring and general health monitoring are all possible applications of this technology. Areas that need to be investigated to allow the use of this technology in all situations include extreme hormonal ranges, temperature extremes (including skin, body and environment), effects of perspiration, body fat differences, skin property, age and gender differences.

References

1. Cook CJ. Rapid noninvasive measurement of hormones in transdermal exudate and saliva. Physiology and Behavior 2002;75(1-2):169-81.

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Appendix Eleven

USE OF A LOW-INVASIVE TRANSDERMAL COLLECTION TECHNIQUE TO APPROXIMATE PLASMA HORMONE LEVELS.

Lara M. Blackmore*[†], Christian J. Cook[‡], R. Hugh Morton[†], David J. Mellor[†].
[†]Institute of Food, Nutrition and Human Health, College of Sciences, Massey University, Palmerston North, New Zealand
[‡]Bioengineering Technologies, HortResearch, Ruakura, Private Bag 3123, Hamilton, New Zealand

Lara Blackmore is a Master of Science student at Massey University. At the time of presentation she will have taken up employment at Bioengineering Technologies in Hamilton. For her thesis Lara studied the relationships between the hormones, testosterone, cortisol and growth hormone, sleep and athletic performance in young, competitive rugby players. During the study she used transdermal electrosonophoresis as well as blood sampling and saliva collection to measure hormone levels.

The reluctance of participants to provide blood samples may impede thorough and comprehensive studies in many areas in biomedical science. Transdermal electrosonophoresis, a new technique that collects an ultrasound generated flux of analytes from the bloodstream to the skin surface, may offer a low invasive alternative for sample collection. In young male rugby players, correlations between plasma, transdermal electrosonophoretic, and saliva samples were examined for the hormones testosterone, cortisol and human growth hormone, all of which can have impact on training effectiveness. The purposes of this study were to validate the use of transdermal electrosonophoresis as a non-invasive plasma sampling technique. In this paper we describe the results and discuss application of electrosonophoresis in the areas of sport science research and training. Fourteen male competitive club rugby players gave plasma, electrosonophoretic and saliva samples twice weekly for four weeks. These samples were analysed for testosterone, cortisol and growth hormone concentrations, except that growth hormone was not determined in saliva. There were very highly significant, positive correlations between the concentration of each of the three hormones in the plasma and electrosonophoretic samples (correlation coefficient, $r = 0.965 - 0.977$). The high correlations suggest that electrosonophoresis may be a highly accurate method of estimating plasma testosterone, cortisol and growth hormone levels.