The effects of fasting for a single day and during Ramadan upon performance

By

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Abstract

Abstract

Ramadan requires individuals to abstain from food and fluid intake between sunrise and sunset; physiological considerations predict that poorer mood, physical performance and mental performance will result. In addition, any difficulties will be worsened because preparations for fasting and recovery from it often mean that nocturnal sleep is decreased in length, and this independently affects mood and performance.

Previous field studies have indicated that some of these predictions are borne out in practice; in the first study of the present thesis, a field study performed in Libya, these predictions were tested further by adding more physiological measurements and tests of performance. Findings indicated that Ramadan was associated with negative effects upon a wide range of variables, including rising urine daytime osmolality (indicative of progressive dehydration), subjective estimates of amounts of activities actually performed and those wished to be done (indicating less activity in the daytime), and metabolic and subjective responses to a short bout of exercise (increased effort required and metabolism tending towards fat rather than glucose catabolism).

Because of the difficulties of performing a battery of tasks in a field study, two laboratory-based experiments were then performed, the second differing from the first in studying a greater range of variables and more time-points during the daytime. These two studies also differed from the situation in Ramadan in that non-Muslim students were volunteers and fasting was performed for only one day. Many of the changes previously found in Ramadan were duplicated in this work, so justifying the use of laboratory experiments lasting one day and using non-Muslim subjects as a model for some of the problems present in Ramadan.

However, it was also found that preparations before the fast were often less marked than was the case with Muslims in Ramadan, a difference that can be attributed to subjects' lack of experience of fasting as well as the amount of time spent fasting.

A difficulty of interpretation in all these studies was that changes could be due to fasting and/or the length of sleep, which tends to decrease. These two factors were separated in the final experiment, an intervention study performed in the laboratory. This study compared effects of different durations of fasting (4, 8 or 16 h) upon a wide variety of measures (including subjective and objective assessments of performance, dehydration and responses to a short bout of exercise) - but with an unchanged amount of nocturnal sleep and daytime naps not allowed. Many of the negative effects observed in previous studies were present in this experiment also. These findings indicate that fasting was responsible for much of the change previously observed, though some effect of sleep loss, particularly if occurring on successive days (as would occur in Ramadan) cannot be excluded.

One finding common to all studies was that tests of performance that had shown variations due to the combination of circadian influences, time awake and sleep loss in other experiments (including grip strength, the Stroop test and accuracy at throwing darts) seemed little affected. Possible reasons for these negative findings are discussed, together with further experiments to separate out effects of sleep loss and fasting, and the role of subjects' experience in studies of fasting. In addition, more detailed studies to investigate changes in sleep and the type and level of physical activities when fasting are proposed.

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I. A Brief Outline of the Thesis

In part II of this Chapter, an introduction to Ramadan and factors affecting normal food intake is given. This forms the background to the more general literature review of the effects of Ramadan in Chapter 2.

In Chapter 2, a review of the literature describing changes in Ramadan due to food and fluid restriction and changed sleep patterns is given. Any changes in performance and psychology can arise not only from fluid and food restriction during the daytime but also from sleep loss (due to rising early and retiring late after a large meal). The literature has rarely distinguished between these possibilities. Also, many of the studies describe changes during the four weeks of Ramadan rather than changes during the course of single days. Previous work at Liverpool John Moores University (LJMU) has investigated within-day changes in two studies using questionnaires (Waterhouse *et al.,* 2008a, 2008b), and has established that subjective estimates of physical and mental performance indicate deterioration during the daytime (accompanied by increased fatigue) and a recovery in the evening when people eat and drink in the company of friends.

Based on this review, therefore, the two main aims of the present thesis are formulated: first, to build upon previous work at LJMU and investigate in more detail any decrements in performance, including objective measures, that might develop in Ramadan; second, to investigate if laboratory-based simulations, using non-Muslim subjects and only one day of fasting, could simulate the previous changes found in Ramadan.

In Chapter 3, a field study which was performed in Libya of changes in performance and mood in Ramadan is described. It differs from the previous studies at LJMU in two main ways. First, the subjects, taking a full-time course at a Sports College in Libya, were quite young (and so living at home with their parents rather than being self-sufficient). Second, because of their place of study, the students could be tested in a fully equipped sports laboratory. The results enable a fuller picture of the decrements produced by the demands of Ramadan to be obtained. Some differences from the previous work are found, and these can often be attributed to the subjects' not having domestic responsibilities nor having to look after themselves.

In the first part of Chapter 4, the relative advantages and disadvantages of field vs. laboratory studies are considered. It is concluded that, although field studies are "real", they have severe limitations when a variety of measures of performance is to be attempted. In the second part of this chapter, the first laboratory-based simulation of Ramadan, a pilot study, is described. This is a "simulation" of Ramadan for two main reasons. First, subjects undertook fasting between the hours of sunrise and sunset but for one day only. Second, the subjects were non-Muslims and so had no experience of Ramadan. The results show that many of the changes observed in the field study are also observed in this laboratory-based study. Some of the implications of these findings are that decrements in subjective and objective measures of performance during Ramadan can be studied much more simply and upon naïve subjects.

Such positive results opened the way for a more complex study to be performed, and this study is described in Chapter 5. More variables than were measured in the field study and the pilot study were considered, and the variables were measured at more time-points than in the pilot study. The results confirm that objective and subjective measures of performance show decrements during the daytime when fasting, and they also confirm that physiological evidence for dehydration is present. The results add substantially to the information obtained from the field study; taken together, and with the previous work (Waterhouse *et al.* , 2008a, 2008b), they are the fullest account of changes taking place during the course of a day in Ramadan.

One factor that was present in the previous field studies was "fatigue", due to reduced sleep times (subjects rising before sunrise to eat and drink and tending to recuperate from daytime fasting by eating and drinking substantial amounts after the end of the fast). Such sleep causes poorer mental and physical performance (Reilly *et al.*, 1997; Waterhouse *et al.*, 2001) independent of food and fluid restriction (Reilly and Waterhouse, 2009). Therefore, the poorer performance might have reflected effects due to less sleep as well as restricted food and fluid intake.

In Chapter 6, an intervention study is described in which sleep and daytime activities are controlled while the amount of time spent fasting is manipulated. This protocol means that any changes cannot be attributed to changed sleep or daytime activity. The results show decrements in subjective and objective measures of performance and dehydration, as in the previous studies. That is, food and fluid restriction, independent of any sleep loss, have a negative impact upon these variables.

In Chapter 7, the results from the studies are summarized and conclusions drawn with regard to the negative effects of fasting upon some variables. Further, there is a discussion of the value to an understanding of some of the effects of Ramadan of protocols involving only a single day of fasting in laboratory-based studies rather than field studies covering the whole month of Ramadan. In addition, there is a consideration of the choice of subject for such studies – whether naïve or experienced with regard to the practice of Ramadan (non-Muslim vs. Muslim subjects) and whether living independently (Waterhouse *et al.*, 2008a, 2008b) or at home (Chapter 3).

Recommendations for further studies follow: there is need for more sensitive measures of some aspects of physical performance (or for a more extensive training in these measures together with stricter conditions for their performance). There is also need for an investigation of the possible interactions between the effects of sleep restriction and fasting, and for more objective measures of sleep quantity and quality.

The thesis concludes with a section describing the implications of the results that have been found.

II. An Introduction to Ramadan and Factors Affecting Normal Food Intake

1.1. The Timing of Ramadan

The Islamic calendar is a lunar calendar based on 12 lunar months in a year, and lasts 354 or 355 (solar) days. It is used to date events in many Muslim countries, and used by Muslims everywhere to determine when to celebrate Islamic holy days. Ramadan is the ninth month of the Islamic calendar; it is the month of fasting, in which participating Muslims refrain from eating or drinking from dawn until sunset.

As Ramadan is dependent on the moon and a year lasting about 354 days, the dates of Ramadan move forward about ten days each year as judged by the Gregorian calendar (which is based on the solar year, the time taken for the Earth to move around the sun). One effect of this movement of Ramadan relative to the solar year is that the hours between sunrise and sunset vary, particularly when moving from equatorial regions (where the day length varies comparatively little during the course of a solar year) towards the Poles. In the UK, for example, the time interval between sunrise and sunset can be as little as about 8 h (at the winter solstice) and as much as 16 h (at the summer solstice). In the year 2008 (when the field study described in this thesis took place), Ramadan fell between August 31st and September 30th, and the interval between sunrise and sunset was about 13 h.

Ramadan begins with the new (crescent) moon but there are disagreements each year as to the exact day on which Ramadan starts. This stems from the tradition in some countries to sight the moon with the naked eye whereas, in other countries, astronomical calculations are used (so overcoming the problem of poor seeing conditions due to cloud, for example). The crescent moon is typically a day after the astronomical new moon and so different countries might start Ramadan at times differing by a day. More recently, there has been a tendency to use astronomical calculations to avoid this confusion.

1.2. The Requirements of Ramadan

During the holy month of Ramadan, Muslims abstain from food and fluid intake between sunrise and sunset (Zerguini *et al.*, 2007); however, food and fluid intakes are freely allowed from sunset to dawn (Roky *et al.*, 2004). In practice, individuals prepare for the period of fasting by rising earlier and eating a meal similar to their usual breakfast before sunrise, and recover after sunset by eating and drinking a wide variety of foods and fluids to replenish energy and fluid levels (Karaagaoglu and Yucecan, 2000), usually in the company of a group of friends. That is, fluid and food intakes become exclusively nocturnal (Hakkou *et al.*, 1994).

Karaagaoglu and Yucecan (2000) reported on the nutritional habits of 750 adults (320 males and 430 females) aged from 20-75 years. 95% of the subjects stated that they consumed two meals per day, the "Sohor" and the "Ichaa". The "Sohor", consumed shortly before sunrise, consisted mainly of breakfast foods such as cheese, olives, sausage, eggs, jam and/or marmalade and tea. The "Ichaa", consumed shortly before retiring to bed, consisted of soups, vegetable foods, rice, salads or fresh vegetable, fruit and yoghurt. At this meal also, about 62% of the subjects drank tea. Special foods such as "pide", a form of flat bread only baked during the month of Ramadan, was also reported to being frequently consumed by the subjects. That is, not only is the timing of food and fluid intake altered in Ramadan but also there is a change in some aspects of the Muslim's diet.

Fasting during Ramadan displaces energy intake and hydration to the hours of darkness and so also disrupts the sleep-wake cycle. Sleep is curtailed and subjects feel more tired in the daytime and tend to take more naps and rest more at this time (Waterhouse and Reilly, 2007). As a result of the changes imposed by the demands of Ramadan, it is associated with more daytime fatigue and less physical and mental activity than on normal days (Waterhouse *et al.*, 2008a, 2008b; Yacine *et al.*, 2007).

1.3. Effects of Changed Eating Habits in Ramadan

1.3.1. Normal eating habits

The main role for food intake is to satisfy the biological need of obtaining the nutrients required for energy growth and repair. However, humans have invested mealtimes with other purposes including business and social roles - such as working breakfasts, business lunches, meals with friends, and extensive meals to celebrate a special occasion like a wedding feast. By contrast, food intake can at least temporarily fall behind biological requirements when individuals are busy or might even forgo the meal due to lack of time available (Reilly *et al.*, 1997). There are also occasions when food and fluid intake are temporarily altered as during religious fasts. That is, food intake and responses to it are caused by exogenous factors - including general lifestyle, culture, time pressure and social factors - as well as biological need (Bogdan *et al.*, 2001; Waterhouse *et al.*, 2005).

1.3.2. Effects of time of meal upon total daily intake

During a normal nychthemeral lifestyle, daytime activity and nocturnal sleep, food is eaten almost entirely in the daytime (de Castro, 2004). Changes in mealtimes might arise due to work schedules, restricted food availability and social influences (Bogdan *et al.*, 2001). In some circumstances, these changes in food and fluid intake will affect amounts of physical activity that are undertaken. Many studies have indicated that there is a relationship between the time of day when food is eaten and the total daily intake; eating early in the day tends to decrease overall intake, whereas taking in more food later in the day tends to increase the total daily intake (de Castro, 2004). However, in general, meal size increases over the course of the day and the amount of time that the individual waits before eating again, the post-meal interval, decreases (de Castro, 2001).

1.3.3. Factors affecting food intake

Several factors alter what individuals eat and when they do so.

1.3.3.1. Cultural

The distribution of the macronutrients differs between cultures and countries (Bellisle *et al.*, 1998) and the time when the main meal is eaten are influenced by cultural and socio-economic differences (de Castro, 1997, 1999, 2000; Gatenby, 1997; Shepherd, 1989; Wilson, 2000; Winkler *et al.*, 1999). The timing can also differ between days of work and days of rest. In Libya, for example, it is normal to have the main meal at lunchtime on Friday, after prayers; in the UK, by contrast, the main meal is in the evening, except on Sundays when it is at lunchtime.

1.3.3.2. Social factors and time availability

Social factors exert a large influence upon the size of meal that is eaten and when it is eaten (de Castro, 1997, 1999, 2000; Gatenby, 1997; Shepherd, 1989; Wilson, 2002; Winkler *et al.*, 1999). Therefore, meals are larger and are enjoyed more on rest days compared with working days (Waterhouse *et al.*, 2003, 2005), partly because, during rest days, there is more time to prepare and eat a large meal and the meal itself can take on a social role with friends and family (Waterhouse *et al.*, 2005). Conversely, restricted time availability and its social associations can limit the size of meal. Lack of time availability and social factors also affect subjective responses to food, such as hunger before a meal, enjoyment of the meal itself, and satiety afterwards (Marshall and Bell, 2003;Weber et al., 2004).

These factors influencing food intake are also illustrated by eating habits during shift work (for example: Krauchi *et al.*, 1990; Lennernas *et al.*, 1994, 1995; Nikolova *et al.*, 1990; Reinberg *et al.*, 1979; Tepas, 1990; Walker *et al.*, 1985; Waterhouse *et al.*, 1992, 2003, 2005) and after time-zone transitions (for example: Waterhouse et al., 2003, 2004, 2005). Such differences in eating habits will also be reflected in the processes of digestion, absorption and metabolism of food. However, it is known that many of these processes are also affected by the "body clock".

1.4. The Body Clock and Circadian Rhythms

Accepting that food intake might be affected by "internal" (the body clock) as well as external (food availability) factors, the role of the body clock in food intake and its metabolism must be considered.

The circadian clock, located in the suprachiasmatic nuclei (SCN) of the hypothalamus, regulates daily variations in numerous physiological processes such as sleep-wakefulness, temperature, and hormone release, as well as cognitive performance (Reilly et al., 1997). These nuclei are linked neurally to the eyes via the retinohypothalamic tract and they receive a humoral input from the pineal gland. The pineal gland secretes the hormone melatonin during the hours of darkness and there are melatonin receptors in the SCN (Khalsa *et al.*, 2003). These two pathways provide a means of responding to the presence and absence of light.

A daily trough in body temperature occurs about 1.5-2 hours before the usual wake time. This is close to the time of a daily trough in alertness and cognitive performance. Thus the circadian clock is programming the body for maximal sleepiness in the early morning hours (about 03:00-05:00 h for a person on a typical sleep-wake schedule) and alertness during the daytime (Neri *et al.,* 1997). The clock is important also in allowing the future to be predicted, thus allowing preparations for sleep and waking (Moore-Ede, 1986). It is a common experience, for example, that the evening is a time of decreasing alertness and increasing readiness for sleep itself, and many physiological and biochemical changes accompany such progressions. Human subjects cannot suddenly go to sleep, nor can they suddenly awaken and work efficiently; the clock, often aided by our chosen lifestyle, enables us to prepare for these profound changes in neurophysiology (Reilly *et al.*, 1997).

1.4.1. The role of the body clock

Human beings are day-orientated, having evolved to work in the daytime and sleep at night. Our internal body rhythms normally cause regular variations in individual body and mental functions with a period of 24 hours. For instance, our body temperature, heart rate, blood pressure, breathing rate and adrenaline production normally rise during the day and fall at night (Reilly et al., 1997).

The circadian rhythm of core temperature is associated with widespread effects throughout the body, and it is believed to be one of the main determinants of the difference between the active "ergotropic" phase when awake and the "trophotropic" phase when asleep. The rhythm of core temperature is correlated with rhythms of sleep propensity (Dijk *et al.,* 1995), physical performance (Waterhouse *et al.,* 2005), and mental performance (Waterhouse *et al.,* 2001) Also, it must, like melatonin (the hormone released in darkness by the pineal gland), be considered to be an internal Zeitgeber for the organism, synchronizing different circadian functions and, particularly, peripheral oscillators (Brown *et al.,* 2002). The circadian changes of body temperature seem to be involved in the regulation of the circadian sleep–wake cycle (Van Someren *et al.,* 2000). An individual normally falls asleep when core body temperature is decreasing, and the main sleep period ends on the rising part of the circadian temperature curve. It is unlikely, however, that the core body temperature (and, thus, the brain temperature) is causally involved in the modulation of sleep propensity. Skin temperature may, instead, be a better candidate for providing a signal to sleep-related neurons (Van Someren *et al.,* 2000).

These rhythms influence task performance and quality of sleep. Most of the body's basic functions show maximum activity by day and minimum activity by night. The biological rhythms affect the behaviour, alertness, reaction times and mental capacity of people to varying degrees. The risk of car crashes increases when the driver is driving at times when he or she would normally be asleep; there is also an increased crash risk during the mid-afternoon "siesta hours" (Roky *et al.*, 2004).

1.4.2. The role of the body clock in regulating food intake

The role of the body clock in determining food intake can also be assessed directly only by a "forced desynchronisation" protocol (Kleitman, 1939). In this protocol, subjects are required to live on an imposed sleep-wake schedule, associated with which is an altered light-dark cycle, which is beyond the range of entrainment of the body clock. Under these circumstances, measured rhythms show two components - an exogenous component due to the imposed "day'' (the sleep-wake schedule) and an endogenous, circadian component due to the ''free-running'' body clock.

Waterhouse *et al.* (2004) investigated food intake during such a forced desynchronisation routine, using an imposed "day" of 28 h. They found that there was only a very weak endogenous influence on food intake; individuals continued to eat meals of normal size and to enjoy them normally as measured by the imposed daytime. That is, a large meal was eaten and enjoyed towards the end of the day (dinner), whatever the circadian time.

Nevertheless, other aspects of the whole process of food intake are affected more by the body clock. There are many metabolic differences between the day night, including the metabolism of glucose and fat. Thus, in the daytime and due to food intake and insulin and adrenaline secretion, there is a tendency to metabolize glucose and to store fat in adipose tissue; by contrast, at night under the influence of changed levels of insulin, growth hormone and adrenaline, the body tends to metabolize fat instead (Al-Naimi *et al.*, 2004). There are also changes in gut activity. Such rhythmic changes in gut function and metabolism have a strong exogenous component, but there is also some effect due to the body clock (Waterhouse *et al.*, 1990). Therefore, altered food intake at night might be due partly to the effects of a poorly adjusted body clock (Tepas, 1990).

In summary, under normal circumstances, food intake plays many roles and it is influenced by many factors. However, in Ramadan, these factors tend to be overridden by the religious requirement to fast. A summary of the changes produced by fasting in Ramadan will be considered in the literature review of Chapter 2.

Chapter 2

The Demands and Effects of Ramadan:

Review of the Literature

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To summarise the position, fasting in Ramadan during the daylight hours results in many changes (reviewed in Benaji *et al.*, 2006; Leiper *et al.*, 2003; Reilly and Waterhouse, 2007; Roky *et al.*, 2004). During Ramadan, nocturnal sleep is shorter and its architecture is changed (Roky *et al.*, 2003); there is also an increase in the number of naps taken during the daytime (Margolis and Reed, 2004), so reducing daytime somnolence. The change in distribution of the hours of sleep and waking is believed to be responsible for negative effects upon mood and the willingness to work (Karaagaoglu and Yucecan, 2000), and decreased abilities to perform physical and mental activities optimally (Kadri *et al.*, 2000; Roky *et al.*, 2000). An increase in road traffic accidents has been reported (Roky *et al.*, 2004), though this has not always been confirmed (Kammash and Al-Shouha, 2006). There are also several metabolic changes, including a fall in body mass and an increase in the ratio: [high-density lipoprotein cholesterol]/ [low-density lipoprotein cholesterol] (Qujeq *et al.*, 2002; Roky *et al.*, 2004).

These issues will be discussed in more detail in the following sections of this chapter.

2.1. Effects of Ramadan upon Body Weight, Metabolism and Sleep

Over the course of the four weeks of Ramadan, the altered patterns of food and fluid intake change daytime body composition and metabolism (Sweileh *et al.*, 1992). In addition, less sleep (by up to two hours) is taken at night (Roky *et al.*, 2001). This results in decreased daytime alertness (Roky *et al.*, 2004) and has detrimental effects on physical and mental performance. These factors will be discussed in turn.

2.1.1. Body weight, fluid intake and water content.

Water is the largest single component of the human body, accounting for about 50-60% of total body weight. In the short term, the maintenance of body water stores is vital and this will be compromised during the daytime in Ramadan. Water balance depends on the balance between fluid intake and urine excretion; therefore, lack of water intake during the period of fasting causes concentration of all fluids within the body. This is compensated for by water conservation by the kidneys, concentrated urine being produced (Bouby and Fernandes, 2003). Fluid intake is due to thirst but, in Ramadan, such feelings have to be ignored in the daytime (Leiper and Prastowo, 2000).

Some studies have reported that the substantial dehydration is accompanied by raised serum concentrations of uric acid and cholesterol (Toda and Morimoto, 2000). By contrast, Leiper and Prastowo (2000) found that total body content was conserved during Ramadan. The decrease in water intake in the daytime was compensated at other times of the day; individuals increased fluid intake during the night after sunset, and rose before sunrise, to prepare for the daytime restriction in fluid intake (Leiper *et al.*, 2003; Waterhouse *et al.*, 2008a, 2008b).

It has been reported (Leiper *et al.*, 2003) that the stress of dehydration in the daytime during Ramadan is not detrimental to the health of fasting Muslims (as assessed from medical records), and that no ill effects can be attributed to the intermittent negative water balance at the levels that may be produced during Ramadan. However, these observations apply to individuals living normally and need not apply to those who are undertaking heavy exercise during the daytime.

Changes in body mass are controversial. Studies report an increase in body mass (Khatib *et al.*, 1997; Khatib *et al.*, 2004; Mafauzy *et al.*, 1990), a decrease (Khaled *et al.*, 2006; Klöcker *et al.*, 1997; Yarahmadi *et al.*, 2003) or no significant change, but sample sizes were small and activities were not controlled. Most of these authors suggest that a decrease in body weight is related to a decrease in calorie intake, while an increase is correlated with increased food intake coupled with a reduction of daily activities.

2.1.2. Food intake and metabolism.

Food intake is essential for obtaining the nutrients for energy and growth. Abstaining from food intake in Ramadan reduces total energy levels and gives rise to hunger. Normally, the type and size of a meal is influenced by social factors and the environment in which the food is eaten as well as hunger (De Castro, 1997; 2000; Marshall and Bell, 2003; Meiselman *et al.*, 2003; Rappoport *et al.*, 2001; Stroebele and De Castro, 2004; Weber *et al.*, 2004), but these factors change in Ramadan where the main considerations will be preparations for the period of fasting and recovery from it.

Fasting during the daylight hours results in many changes in metabolism (Benaji *et al.*, 2006; Leiper *et al.*, 2003; Roky *et al.*, 2004). Although total energy intake is decreased during Ramadan, so is energy expenditure, this being associated with a lower activity profile in the daytime. Even so, there is a tendency for weight loss and negative energy balance. This decrease in daily calorie intake has been seen as one of the advantages of fasting in Ramadan (Bouguerra *et al.*, 2003; Khaled *et al.*, 2006; Sari *et al.*, 2004), in addition to which, it is reported that levels of free cholesterol fall in Ramadan (Scheen, 1999).

Food intake leads to increased gastrointestinal activity and so patterns of gastrointestinal function are normally in phase with a diurnal eating pattern, with more gut activity in the daytime (Waterhouse *et al.,* 1999).

Figure 2.1. Changes from normal days in hunger, top, and amount of indigestion, bottom, in a group of 12 subjects who underwent a 25-h constant routine. Mean + SE shown. (From Waterhouse et al., 1999a).

Accordingly, the altered pattern of food intake in Ramadan is predicted to change the timing of gut function in a similar way, but no detailed studies of this have been performed. However, this prediction is based on the assumption that gut function follows food intake whatever the time of day, and there is some evidence that this need not be the case. Figure 2.1 shows the mean levels of hunger and indigestion in a group of 12 subjects during the course of a constant routine. In this routine, subjects were kept awake in a constant environment (temperature, humidity and lighting) for 25 hours, starting after a full night's sleep that ended at 08:00 h. At the end of each hour awake, the subjects were given a small sandwich and drink of fruit juice. Immediately before these snacks, they were asked to rate their hunger on a scale from –5 (not at all hungry), through 0 (as hungry as normal) to +5 (very hungry).

They were also asked to rate any feelings of indigestion or feeling overfull from 0 (none) to +5 (marked). It is clear that during their normal waking hours (the first 16 h of the constant routine) their hunger was normal and they suffered little from indigestion or feeling overfull. However, during the night (hours 17+), their hunger fell and their sense of indigestion and feeling over-full increased. That is, it seems that the ability of the gut to absorb ingested food might be compromised at night.

Since the normal pattern of eating and drinking is altered in Ramadan, many changes in metabolism result, mainly those associated with the metabolism of glucose and fat. Thus, in the daytime under normal (non-fasting) conditions, food intake and the increase in insulin secretion produced by it promote the uptake of glucose by the cells of the body and its metabolism, and also to increased fat storage in adipose tissue; by contrast during nocturnal fasting the body tends to metabolize fat instead (Al-Naimi *et al.*, 2004). This pattern will be substantially altered during Ramadan, and increased metabolism of fat stores in the daytime and increased metabolism of glucose during the night after the evening meal is predicted. This change will require the secretion of hormones associated with metabolism (insulin, growth hormone, glucagons, cortisol, for example) to change also. Details of these changes remain to be investigated. However, it is known that the different hormones are influenced to different extents by the body clock, and so it cannot be assumed that all will change their pattern of secretion to the same extent (Waterhouse *et al.,* 1999a).

2.1.3. Sleep

Studies have demonstrated that the average adult human requires 8-8.25 h of sleep for optimal performance and alertness (Dinges, 1995), but there is a range of individual sleep requirements around this amount (about 6-10 h). Episodes of acute sleep loss can build into a cumulative sleep debt; it is important to provide adequate opportunity to minimize such a debt, and to provide adequate recovery sleep if this loss does occur (Neri *et al.*, 1997).

Because, during Ramadan, all meals are consumed at night, they tend to affect the amount of sleep fasting Muslims are able to obtain. Many studies indicate that nocturnal sleep is reduced in length. Hakkou *et al.* (1994) suggested that sleep was reduced by approximately 3 h due to the time taken to eat meals before sunrise and after sunset. Roky *et al.* (2003, 2004) also showed that the percentage of people who went to sleep after midnight increased during Ramadan; sleep duration was less than 6 h in 68% of 150 workers during Ramadan but in only 37% before Ramadan. Sleep loss can degrade many aspects of human performance, including memory, vigilance, decision-making, mood, and reaction time (Waterhouse *et al.*, 2001). Bahammam (2003, 2004) investigated the effect of Ramadan on sleep architecture, daytime sleepiness and sleep patterns in 8 healthy Muslims. Compared to baseline (non-fasting days), bedtime was delayed in the first and third weeks of Ramadan with delays of 1 h

18 min and 1 h 36 min, respectively. These delays were coupled with a constant wake up time, which caused a significant reduction in nocturnal sleep time. This reduction in sleep causes daytime fatigue but there is also an increase in the amount of sleep taken during the daytime (Bahammam, 2003; Margolis and Reed, 2004; Roky *et al.*, 2003), so reducing the total sleep loss.

During Ramadan, many people alter their sleeping habits considerably, staying awake most of the night and sleeping much of the daytime (Wilson *et al.*, 2009). This has the advantage that food and fluid can be taken in during the waking period but the subjects are then similar to night workers and this produces other problems due to lack of adjustment of the body clock (Waterhouse *et al.*, 1992).

Changes in the pattern of food intake in Ramadan are the main cause of reduced nocturnal sleep at this time, but there are further interactions between food intake and sleep loss. First, going to bed with a full stomach might cause discomfort and hinder sleep. In addition, drinking copious amounts of fluid and caffeine-containing drinks could affect the quantity and quality of the sleep (Roky *et al.*, 2001). Second, sleep deprivation seems not to increase appetite (it might even make individuals lose interest in food) but rather leads to a preference for foods containing more energy (Spiegel *et al.*, 2004).

2.2. Ramadan and Physical and Mental Performance, Mood and Social Activity

Mental and physical performance, mood and social activity are affected by many factors including sleep loss and fluid and nutritional status (Reilly and Waterhouse, 2007). As a result, it is to be expected that these performance and cognitive variables will change during Ramadan.

2.2.1. Mental performance and fatigue

Many aspects of mental performance normally improve in the hours after waking, reach a peak around the middle of the day and fall in the evening. These profiles are caused by an interaction between core temperature and time awake (Minors and Waterhouse, 1981; Waterhouse *et al.*, 2001). Core temperature rises in the morning after awakening and peaks in the later afternoon before falling in the evening. In principle, mental performance follows this rhythm, cognitive processes being improved by a rise of temperature in the physiological range (Reilly *et al.*, 1997). In addition, however, the factor time awake tends to cause a decrement in performance. This factor is minimal after awakening and increases progressively while the subject remains awake. The effects of increasing time awake depend upon the type of task. For simple measures of mental performance (simple reaction time, for example), the time-awake effect is small and so the rhythms of core temperature and performance are similar. By contrast, for mood and mental tasks which have a larger cognitive component (decision-making, for example), the time-awake effect is greater. As a result of this, the time of peak performance at such measures is earlier in the daytime, around noon (Folkard, 1990).

Optimal sleep, both in quantity and quality, is necessary for maximal performance and alertness. Reduced (of as little as 2 h) or degraded sleep can significantly decrease or impair alertness and mental performance, including memory, vigilance, decision-making, mood and reaction time tasks (Waterhouse *et al.*, 2001).

The change in distribution of the hours of sleep and waking in Ramadan is believed to be responsible for negative effects upon mood and the willingness to work (Karaagaoglu and Yucecan, 2000) and decreased abilities to perform mental activities optimally (Kadri *et al.*, 2000; Roky *et al.*, 2000). Lack of concentration, tiredness, irritability, sleepiness and other undesirable symptoms during the daytime, which may have negative effects on the working and school life of individuals, have been described, as have a decreases in alertness and cognitive ability and an increase in irritability (Afifi, 1997; Kadri *et al.*, 2000; Karaagaoglu and Yucecan, 2000; Taoudi *et al.*, 1999). An increase in road traffic accidents during Ramadan has also been reported (Bener *et al.*, 1992). Cognitive function has also been shown to deteriorate (Roky *et al.*, 2000). In addition, a frequently cited problem of Ramadan fasting is an increased incidence of headaches; however, the effects of restrictions on smoking tobacco ingesting caffeine and energy and fluid intake must also contribute to this general negative feeling. It has been noted that most of these changes do not show a progressive deterioration during the four weeks of Ramadan (see, for example, Kadri *et al.*, 2000; Leiper *et al.*, 2003; Roky *et al.*, 2004), suggesting that Muslims rapidly adapt to the demands of Ramadan.

It is not only decreased sleep that is responsible for these changes; Roky *et al.* (2004) concluded that daytime subjective alertness during Ramadan, evaluated by a visual analogue scale (in which subjects scored how alert they felt on a scale), decreased at 09:00 h and 16:00 h and showed an increase at 23:00 h. They concluded that reduced energy intake was responsible as well as sleep loss. The role of decreased food intake is supported by the observations of improved mood in the evening after fasting has ended. An additional factor contributing to this improvement is likely to be the increase in social activity that also occurs at this time.

It is generally accepted that there is also a strong relationship between eating, alertness and mood (De Castro, 1997; 2000; Marshall and Bell, 2003; Meiselman *et al.*, 2003; Rappoport *et al.*, 2001; Stroebele and De Castro, 2004; Weber *et al.*, 2004). Mental performance is affected by a wide variety of factors, including energy levels or tiredness, tension, anxiety, depression, relaxation, stimulation/excitement and motivation. Published research increasingly supports a role for food in maintaining mental performance and mood – particularly a role for carbohydrates (Bellisle, 2002). Food and fluid restriction, therefore, might contribute to negative effects on mood, mental performance and irritability observed during Ramadan.

Part of this mood change is believed to be caused also by alterations in normal circadian rhythms. These alterations are due to individuals becoming more active through the evening and night (Taoudi Benchekroun *et al.,* 1999) and to their changed sleep patterns (Bogdan *et al.,* 2001; Husain *et al.,* 1987; Roky *et al.,* 2001). In addition, the effects of restrictions on smoking tobacco and ingesting caffeine must also contribute to this general negative feeling.

Proper hydration is important for optimal cognitive functioning, as it plays a vital role in neural conductivity (Kleiner, 1999), as well as for efficient digestion, elimination of toxins, thermoregulation, joint lubrication and energy production (Felesky-Hunt, 2001). In support of this role of hydration, Sharma *et al.* (1986) found that dehydration of 2-3% of body mass resulted in poorer performance on tasks requiring concentration and speed of psychomotor processing. Similar results were also reported by Cian *et al.* (2000), subjects showing decrements in performance during test of short-term memory, attention and psychomotor speed tests when dehydration reached levels of 2% or more of body mass.

2.2.2. Physical performance

In contrast to mental performance, muscular performance in general is little affected by sleep loss (Reilly and Waterhouse, 2009). However, to the extent that physical performance includes, in addition to muscle contraction, elements of mood, perceived exertion, sensori-motor co-ordination, cognitive performance and motivation, it will also be negatively affected by sleep loss. The effects of fluid restriction on performance have been much researched over recent years. A water deficit of only a few percent will impair physical performance (where integrated activity between muscle groups is required) but not muscular strength (Barr, 1999; Maughan, 2003); a slightly larger loss will bring about symptoms of tiredness, headache and general malaise (Maughan, 2003).

Restricted food intake also has negative effects, and the general view is that altered eating and drinking schedules negatively impact upon the duration and intensity of physical activity that can be performed (Benaji *et al.* , 2006; Leiper *et al.* , 2003; Reilly and Waterhouse, 2007; Roky *et al.*, 2004). In Ramadan, training or a competitive event scheduled at 14:00 h could well be more than 8 h after the player's last meal and several hours before their next meal, which is outside the window for optimal glycogen replenishment (Reilly and Waterhouse, 2007). Oliver *et al.* (2006) investigated 48h of food, fluid or food and fluid restriction on 30-min running performance. Compared with the control group (who received normal levels of fluid and food during the 48h), the restricted group completed 15% less distance.

Comparatively few studies have directly examined the effects of fasting during Ramadan on physical performance and the results conflict. Meckel *et al.* (2008) investigated the effect of the Ramadan fast on various physical performance measures in adolescent soccer players. They found a significant reduction in jump height performance, the time taken to run 3,000 m and speed endurance and attributed the changes to the lack of daytime fluid ingestion. Decreases in maximal voluntary contractions (MVC) have been measured (Bigard *et al.*, 1998); maximum isometric strength fell by 10-12%, and muscular endurance at 35% and

55% VO_2max caused MVC decreases of 28% and 22%, respectively. Levels of insulin, blood glucose, low energy levels and mood are potential reasons for these decreases in performance (Reilly & Waterhouse, 2007). Other studies have shown there to be no decrease in performance in Ramadan. Zerguini *et al.* (2008) summarised the 2006 F-MARC Ramadan Study in which various endurance and anaerobic performance variables were measured in athletes undergoing training in Ramadan. It was found that performance was not negatively influenced by the restriction of food intake during testing. Similarly, Kirkendall *et al.* (2008) tested subjects for speed, power, agility, endurance and soccer specific skills and found no performance decrements in Ramadan.

Such a divergence of findings has been found also in investigations into the effects of fasting on strength and maximal power output. Karli *et al.* (2007) investigated short-term high-intensity exercise using the Wingate anaerobic test and observed that there was no influence of Ramadan fasting on anaerobic capacity. Conversely, Souissi *et al.* (2007) demonstrated that Ramadan fasting was associated with reduced anaerobic power. It is clear that more data are required to resolve these differences.

It is of interest to note that restrictions of energy and fluid intakes frequently occur intentionally when athletes are required to achieve weight standards (Horswill *et al.*, 1990) or, in wrestling, to adhere to weight classifications (McMurray *et al.*, 1991). Many Judo athletes compete at a weight class 5-10% below their natural weight, achieved through restricted calorie intake before competition (Degoutte *et al.*, 2004). However a later study (Degoutte *et al.*, 2005) concluded that muscle strength declined, thereby decreasing performance. Also, Hall and Lane (2001) reported that boxers restrict fluid and energy intake in the week leading up to a fight in order to lose mass and compete in a lower weight class.

The evidence, though ambiguous, is sufficient to indicate that physical performance might be compromised in Ramadan, and there is a need to explore strategies to optimise physical performance during Ramadan (Yacine *et al.*, 2007).

2.3. Clinical Implications of Ramadan

The changed pattern of food intake in Ramadan raises the possibility of medical problems in susceptible individuals, particularly in patients with, for example, hypertension, hypercholesterolemia, hyperglycaemia and heart, liver or kidney disease (Al-Naimi *et al.*, 2004). It has also been argued that eating certain types of food at inappropriate times may be a factor in gastrointestinal complaints and may predispose individuals to becoming overweight (Iraki *et al.*, 1997).

During the fast, the individual must abstain from oral medication (Azizi and Siahkolah, 1998), and this might lead to problems in diabetic individuals (Benaji *et al.*, 2006). In type 2 diabetes, patients change their treatment regimen in accord with the altered pattern of food intake in order to reduce the risk of hypoglycaemia (Fereidoun and Siahkolah, 1998). Few studies have assessed the effects of changed food intake in diabetics, and those that have been performed deliver conflicting results. Even so, it is generally stated that fasting in Ramadan does not pose a risk to diabetics whose blood sugar and general metabolism are under control, just as there are no problems for those who are at increased cardiovascular risk (Khaled *et al.*, 2006; Sulimani *et al.*, 1991).

2.4. Changes During the Course of the Day

This brief review of the scientific literature relating to some of the effects of fasting during Ramadan indicates that the effects are widespread. However, most of the literature describes only the changes in Ramadan in comparison with normal, non-fasting days, comparing results obtained at the same time of day; most studies do not consider what happens *during the course of the passage of the day* in Ramadan. Information on this issue requires within-day changes to be investigated.

Very little literature exists on this aspect of the problem. However, there are two studies that have been performed by the group in Liverpool. These studies have considered in much more detail how individuals respond to the demands of Ramadan during the course of single days (Waterhouse *et al.*, 2008a, 2008b). In both these studies, individuals were compared before, in the first and fourth weeks of Ramadan and in the week after Ramadan. Assessments of their subjective assessments of fatigue and activities (actually performed and the amount of these activities wished for by those fasting), together with the reasons for sleeping, eating or drinking or not doing so were performed both in England and, as a comparison, in Libya. The general findings were that activities in Ramadan were decreased during the daytimes and increased in the evening after the fast had ended. This is illustrated by Figure 2.2. Moreover, the reasons for sleeping/eating/drinking or not sleeping/eating/drinking differered between fasting and non-fasting days; during Ramadan, subjects' decisions were influenced less by appetite and more by fatigue (due to sleep loss) and the needs to prepare for, and recover from, fasting. Such differences were very similar in both countries (incidentally validating the concept of performing studies on Ramadan in a non-Muslim country).

Figure 2.2. Mean (+SE) profiles of physical activity scores during Control days and Ramadan. (From Waterhouse et al., 2008b)

The value of this work was in determinining how fasting during Ramadan influenced physical and mental activities and social contact at particular times of the day. It indicated that individuals tried to "spare" themselves in the daytime and yet "recover" in the evening while eating and drinking with friends. However, the limitation of such studies is that physiological changes and changes in objective measures of physical performance were not investigated. In the first new study (Chapter 3), some of these limitations will be addressed in a field study performed in Libya.

2.5. Aims and Objectives of the Experiments to be performed

In summary, previous work by the group (Waterhouse *et al.,* 2008a, 2008b) indicates that there are marked changes during Ramadan in individuals' subjective assessments of types of activity (physical, mental and social) performed in the daytime, before sunrise and after sunset. Also, there are changed intakes of food and fluid before sunrise (in preparation for the fasting period) and after sunset (to recuperate from the fasting period), as well as altered sleep patterns and daytime fatigue. These results indicate that the questionnaire developed by us can be used in the field to obtain useful information about these changes, and this tool will continue to be used, but with slight modifications as required, in the experiments to be performed. However, objective measures of activity are lacking, and more details of changes in sleep patterns and the quantity and quality of sleep are required.

Such studies can take two general forms, field studies and laboratory-based studies, and both of these approaches will be used in four new studies that are described in Chapters 3-6.

Chapter 3

Effects of Ramadan upon Physical Activity: a Field Study

Liverpool John Moores University

This study has been published in Biological Rhythms Research:

Waterhouse, J., Alabed, H., Edwards, B. and Reilly, T. (2009). Changes in sleep, mood and subjective and objective responses to performance during the daytime in Ramadan. Biological Rhythm Research; 40: 367-383.

The following account is a slightly modified version of this publication.

3.1. Introduction

Devout Muslims abstain from food and fluid intake between sunrise and sunset during the month of Ramadan. Such a change produces many effects upon an individual's physiology, biochemistry and behaviour (reviewed in Chapter 2). It has been shown that sleep is shorter at night during Ramadan (Roky *et al.*, 2003), and increased amounts of sleep are taken during the daytime to reduce sleepiness (Margolis and Reed, 2004). Mood and the willingness to work decrease (Karaagaoglu and Yucecan, 2000), as do abilities to perform physical and mental activities optimally (Kadri *et al.*, 2000; Roky *et al.*, 2000). Increased frequencies in road traffic accidents have sometimes been reported (Roky *et al.*, 2004), and there are several metabolic changes (Roky *et al.*, 2004; Qujeq *et al.*, 2002). Many of these changes – including those to the circadian rhythms of core temperature (Roky *et al.*, 2000) and several hormones (Bogdan *et al.*, 2001) – have been attributed to the altered distribution of the hours of sleeping and waking.

Most of these studies have concentrated on changes taking place during the course of the four weeks of Ramadan rather than the course of individual days (Reilly and Waterhouse, 2007). However, it is known that individuals tend to prepare for the period of fasting during Ramadan by rising earlier and eating a meal before sunrise; also, after sunset, a wide variety of food and fluids is taken in as individuals replenish energy and fluid levels and then retire later than normal (Karaagaoglu and Yucecan, 2000). These changes to individuals' feeding habits as well as their sleep–wake cycle would be predicted to affect their physiology and metabolism.

Previous work by our group on changes during Ramadan (Waterhouse *et al.*, 2008a, 2008b) investigated individuals' subjective feelings and responses to food and fluid during the course of individual days. Less physical, mental and social activities were performed in the daytime, but there were significant increases after sunset (see Figure 2.2). Similarly, daytime fasting was compensated for by increased fluid and food intakes in the hours before sunrise and, in particular, after sunset. In addition, sleep at night was altered in Ramadan, daytime sleepiness increased and more naps were taken in the daytime, the reason often cited for this being to catch up on lost sleep.

The limitation of these studies is that physiological changes and changes in objective measures of physical performance were not investigated. The present study has repeated the investigations of subjective estimates of daytime activities, sleep and fatigue that were made in these two studies but it has added measures of hydration status, grip strength and responses to a set bout of mild exercise. Such measurements can be difficult to perform accurately in the field but it was possible for the subjects to visit a fully-equipped sports laboratory for the main test sessions and to perform the other test sessions in the comfort of their own home.

3.2. Methods

3.2.1. Subjects and familiarisation

The experimental testing was performed at the Alahli Football Club (Tripoli, Libya), an academy for training in Sports Sciences. It took place in the weeks before, during and after Ramadan, 2008. (Ramadan in 2008 was from August 31st to September 30th). Subjects were recruited by advertisement in the University and by word-of-mouth. The aims and a general outline of the protocol were explained to them, and any questions were answered. The study was approved by the Research Ethics Committee of Liverpool John Moores University.

Potential subjects who showed an interest in the project were then required to take part in a familiarisation day at the Academy. During this occasion, anthropometric data were collected, the subjects were asked which their dominant hand was, the questionnaires that would be answered and subjective information that would be required were explained, and subjects practised on the cycle ergometer, adjusting the saddle height, and so on.

Twenty subjects agreed to participate and signed informed consent forms in the presence of a third, disinterested party. None was a smoker or taking chronic medication. All were male and aged 18; their mean (SD) height was 1.74 (0.06) m and body mass 66.4 (6.2) kg.

During the experiment itself, the subjects were tested during four separate days. For the 48 h before each of these days, subjects were required to follow their normal times of sleep and waking, and not to undergo any period of severe or extended exercise. The four experimental days were: once in the week before Ramadan, twice during Ramadan (once in the first week and once in the last week), and then a final time during the week after Ramadan (after Eid).

3.2.2. Protocol and measurements

On each experimental day, subjects were asked to answer a questionnaire at four times (09:00 h, 12:00 h, 17:00 h and 21:00 h). The questionnaire is shown in Appendices 3.2 and 3.3; translations are to be found in Appendices 4.1. and 4.2. Subjects also gave urine sample at

09:00 h, 12:00 h and 17:00 h. At 12:00 h and 17:00 h, the subjects undertook these tasks in the laboratory, whereas they undertook them at home at 09:00 h and 21:00 h. If subjects wished, they could return to bed after answering the questionnaire and giving a urine sample at 09:00 h.

On the occasions when subjects were tested in the laboratory, the protocol was as follows. (1). Subjects rested quietly for 30 min, during which time the questionnaire was answered and resting heart rate (HR) was measured. (2). Grip strength was measured and subjects undertook the cycling exercise. (3). Immediately after the exercise (within 1 minute), HR was measured again and a sample of blood was taken by pin-prick.

3.2.2.1. Questionnaires

Appendices 3.2 and 4.1 (based on Waterhouse *et al.*, 2008a) shows the questionnaire for 09:00 h, which required information from the subjects about the previous night's sleep (section A), their fluid intake (section B), food intake (section C) and activities (section D) before sunrise. The questionnaires at 12:00 h, 17:00 h and 21:00 h asked the same information but the subjects were instructed that their answers should apply to the interval since answering the previous questionnaire. That is, the questionnaires at 12:00 h and 17:00 h included periods of fasting (in Ramadan) and that at 21:00 h, information about activities after sunset. For these last three times, section A was modified (Appendices 3.3 and 4.2) and questions about daytime naps rather than nocturnal sleep were asked. For many of the questions, subjects were required to state not only if they had/had not performed a particular action (taken a nap, for example) but also their reasons for this choice.

Interpretation of the exact meaning of any question was left to subjects to decide for themselves, although it was explained that the interpretation should be consistent throughout the study. Strict anonymity was guaranteed with regard to the answers given. Subjects were asked not to refer back to previous answers and the results from all questionnaires were collected (by HA) at the end of the study.

"Fluid scores" and "food scores" were calculated for each subject and time interval as previously devised by Waterhouse *et al.* (2008a, 2008b). These scores ranged from 0-4 and were semi-quantitative estimates of total fluid or food intake during this interval. They were calculated as follows. For the total fluid intake score: "0" represented no intake; "1" indicated a "sip"; "2", less than 1 glass/cupful; "3", 1 glass/cupful; and "4", more than 1 glass/cupful. . For the food intake score: "0" represented no intake; "1" indicated a "snack"; "2", a "small meal"; "3", a medium-sized meal; and "4", a large meal.

The two control weeks (before and after Ramadan) did not show any significant differences (paired t-test and Wilcoxon tests), and so they were pooled for each subject ("Controls"). The results from the two weeks of Ramadan also showed no significant differences and were pooled ("Ramadan").

For many of the variables (whether or not a sleep was taken, whether or not a drink was taken, and the reasons for making such choices, for example), the "fraction of possible occasions" was calculated for each subject (see Waterhouse *et al.*, 2008a, 2008b). "Fraction of possible occasions" was calculated for each subject and time interval as follows:-

Suppose that a subject had slept in the interval 12:00-17:00 h on one of the two days of investigation during Ramadan. Then the fraction of possible occasions when a sleep was taken was 0.50. Further, when the fraction of possible occasions that a particular reason for sleeping was calculated, the frequency of occurrence of this reason was expressed as a fraction of the single occasion (as a sleep was taken during this interval on only one day), that is 0/1 (this reason not given) or 1/1 (this reason given). Additionally in this example, when the fraction of possible occasions that a particular reason for not sleeping was calculated, the frequency of occurrence of this reason was expressed as a fraction of the occasion (the single day when a sleep had not been taken in this interval). This approach could not be used if an individual did not perform an action during a particular time interval on either of the 2 days (never slept between 12:00-17:00 h, for example); in such cases, it would be meaningless to assess the reasons for sleeping, although it would be possible to use the results from both days to assess the reasons for not sleeping.

3.2.2.2. Physiological measures

The choice of measures of physical activity, as well as the exact times of testing, was restricted by the fact that the participants were all students undertaking a full schedule of studies.

Heart rate was monitored at 12:00 h and 17:00 h, before and immediately after the bouts of exercise. A heart rate monitor (Polar, Kempele, Finland) was strapped to the chest and a data logger attached to the subject's wrist. A resting HR was measured over a 1-min period at the end of the 20 min, after the subjects had answered the questionnaire (Appendix 3.2, 3.3) and before they gave the urine sample and measured grip strength. Heart rate was also measured a second time in the 30 s immediately after finishing the bout of exercise.

Urine samples were given three times per day (at 09:00 h, 12:00 h and 17:00 h). An aliquot (50 ml) was stored at 10°C until later analysis for osmolality (Osmocheck pocket pal OSMO, Vitech Scientific Ltd, Japan), this being used as a measure of general dehydration.

Grip strength was measured at 12:00 h and 17:00 h (Handgrip Dynamometer TRK5106. Jump MD, Tarek Scientific Instrument Score, Japan). Three measurements were taken from the dominant and non-dominant hand, and the highest of each set of three values was used for subsequent analysis. (Edwards *et al.*, 2005).

The bout of exercise consisted of cycling for 15 minutes on a braked cycle ergometer (Monark Ergo medic 828E Test Cycle, Electronic Company, Sweden) at a constant load of 120 watts. The rate of turning the pedals, the cadence (defining a single turn of the pedals as a cadence of 2), was left to individual choice (the higher the cadence, the lower the force that had to be exerted on the pedals). After the exercise, an estimate of perceived exertion was made on the Borg scale (Borg, 1998).

At the end of the bout of exercise, blood lactate was analysed using a Lactate Pro Test Meter (Model LT7010, Arfray Inc, Kyoto Japan). This apparatus required a drop of blood to be taken from the finger tip by pin-prick, and then it recorded immediately the concentration of lactate in the sample.

3.2.3. Statistics

Comparisons between control days and Ramadan of nocturnal sleep times were made by paired t-tests. Wilcoxon's z was calculated for comparisons between the non-parametric variables: sleep quality, frequencies of taking different types of drink, and fractions of possible occasions given for reasons when not sleeping, drinking or eating.

Analysis of variance with repeated measures was used for parametric measures. The main factors were Day (2 levels, Controls vs. Ramadan) and Time of Day (up to 4 levels, the 4 intervals, t_1-t_4, namely: until 09:00 h, 09:00-12:00 h, 12:00-17:00 h, and after sunset). The additional factor Hand (dominant vs. non-dominant) was used for grip strength. Greenhouse-Geiser corrections were used, and significant differences within the main factors were assessed using Bonferroni corrections. The SPSS package, version 14, was used. Significance was set as $P<0.05$, though occasions where $0.05<P<0.10$ have been reported as "marginally significant". Exact P values have been given; results given as "0.000" in the statistics output have been reported as "<0.0005".

3.3. Results

3.3.1. Results from questionnaires

3.3.1.1. Nocturnal sleep

Estimates of nocturnal sleep times indicated that subjects went to bed and rose later in Ramadan. Mean (±SE) times of retiring on Control days were 23:25 h (13 min) and, in Ramadan, 02:16 h (±20 min); $t_{19} = 8.8$, $P<0.0005$ (paired t-test). Rising times were 09:30 h (±11 min) and 10:15 h (±7 min) for controls and Ramadan, respectively ($t_{19} = 3.9$, $P = 0.001$).

Total time spent in bed fell from 10 h 05 min ($\pm$12 min) on control days to 7 h 59 min ($\pm$17 min) in Ramadan (t_{19} = 7.2, P<0.0005).

Subjective estimates of sleep indicated that subjects went to sleep later in Ramadan (z = 2.09, P = 0.036). Wake times were later also but the difference was not significant statistically (z = 0.32, P = 0.75). The ease of getting to sleep was felt to be greater in Ramadan (z = 2.54, P = 0.011) even though sleep was not felt to be significantly more refreshing (z = 0.27, P = 0.79). Alertness 30 min after waking was lower in Ramadan, but not significantly so (z = 1.62, P = 0.11). Food and fluid intakes just before retiring tended to increase in Ramadan (z = 1.88, P = 0.061) and subjects felt that intake of food at this time was more valuable in promoting sleep than on control days (z = 2.01, P = 0.044).

3.3.1.2. Diurnal naps

During the daytime, naps were taken too infrequently (a total of 3 episodes) to be analysed statistically. However, the reasons for not sleeping could be analysed (Figure 3.1). In the daytime (09:00 h - 17:00 h), the reasons "Not Tired" and "Never do" were given less frequently in Ramadan than on the control days (z = 2.65, P = 0.008 and z = 2.54, P= 0.011 respectively) whereas the reason "Too busy" was given most commonly, particularly in Ramadan (z = 2.88, P = 0.004). In the evenings (17:00 h – 21:00 h), the reasons "Not tired" and "Never do" were cited most frequently, but not with significantly different frequencies on control days and during Ramadan. However, the frequency of citing "Too busy" was marginally significantly greater in Ramadan (z = 1.93, P = 0.053), even though it was still cited on only about 20% of possible occasions.

3.3.1.3. Fluid and food intake

Since fluid and food intake were not allowed between sunrise and sunset in Ramadan, comparisons at 12:00 h and 17:00 h between intakes on control days and in Ramadan would have been meaningless. By contrast, comparisons at 09:00 h and 21:00 h would have been possible and would have indicated preparations for, and recovery from, the period of fasting. However, the responses at 09:00 h indicated that the subjects had included only intakes after rising but before sunrise. Therefore, investigations of preparations for fasting could not be performed. However, it was possible to compare intakes at 21:00 h, when eating and drinking behaviour to recuperate from fasting were taking place. All subjects drank in the evening, with mean (SE) fluid scores of 3.85 (0.05) and 3.73 (0.09) on controls days and during Ramadan, respectively. These scores reached towards the maximum possible of 4.0, indicating more than one glass or cup of fluid was drunk. Figure 3.2, top, shows that water, fruit juice and fizzy drinks were consumed most frequently, and coffee or tea was rarely drunk. In Ramadan, the frequency of drinking water increased significantly ($z = 2.14$, $P = 0.032$) and of drinking fizzy drinks decreased significantly ($z = 2.81$, $P = 0.005$). There was a rise in drinking milk in Ramadan but it did not achieve conventional levels of significance ($z=1.56$; $P=0.12$). The main reasons for drinking on control days (Figure 3.2, bottom) were thirst and being sociable; thirst became a more important reason during Ramadan ($z = 2.89$, $P = 0.004$), at the expense of being sociable ($z = 3.42$, $P = 0.001$). All subjects ate in the

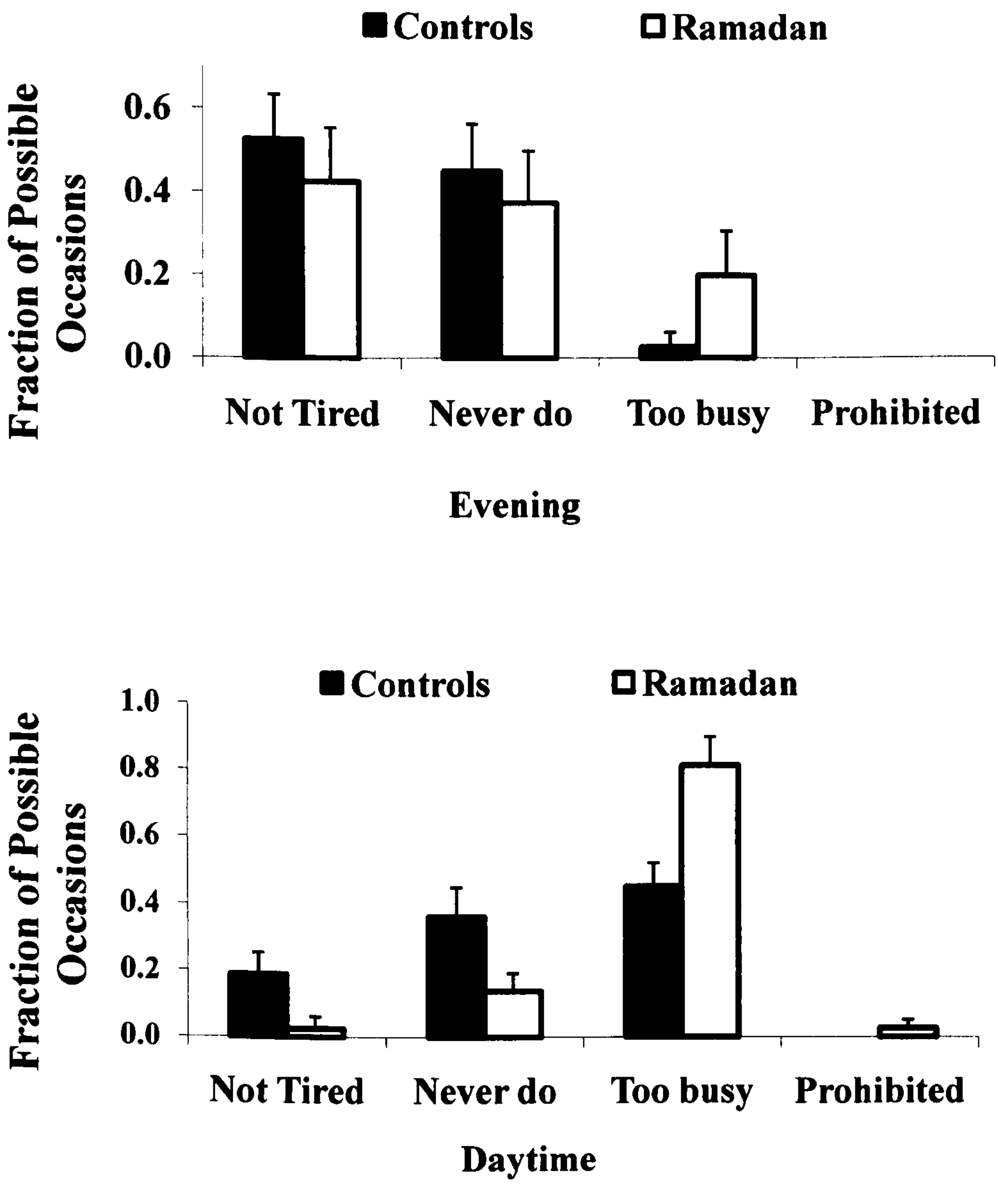
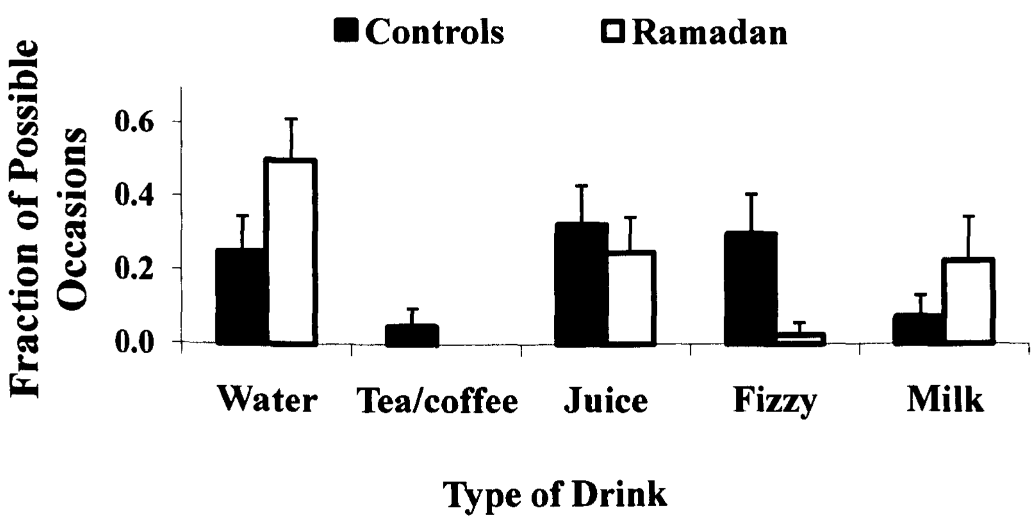


Figure 3.1 . Reasons given for not napping in the evening (top) and in the daytime (bottom). For explanation of "Fraction of Possible Occasions", see text.

evening, with mean (SE) food scores of 3.58 (0.13) and 3.08 (0.13) on controls days and during Ramadan, respectively. These scores indicated that a medium-to-large meal was the norm. Figure 3.3 shows that hunger and being sociable were both cited quite frequently on control days. However, being sociable decreased significantly during Ramadan ($z = 2.84$, $P = 0.005$) and recovery from fasting increased significantly and became cited more frequently that being sociable ($z = 2.07$, $P = 0.038$).



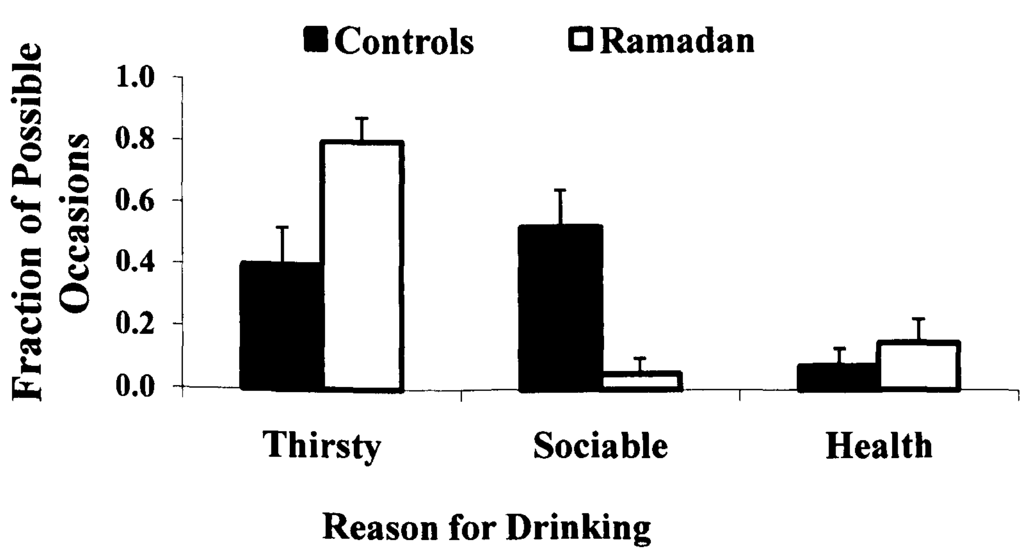


Figure 3.2. Top, type of drink taken in the evening on control days and during Ramadan. Bottom, reasons given for drinking. For explanation of "Fraction of Possible Occasions", see text.

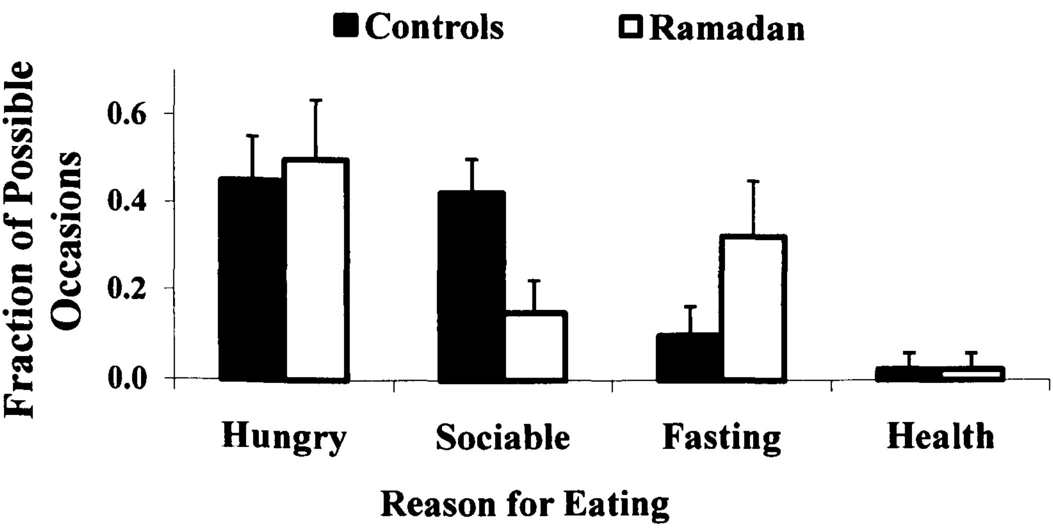


Figure 3.3. Reasons for eating in the evening on control days and during Ramadan. For explanation of "Fraction of Possible Occasions", see text.

3.3.1.4. Subjective estimates of activities and sleepiness

Figure 3.4 shows the mean physical activity (top), social activity (middle) and sleepiness (bottom) scores at the four times of testing on control and Ramadan days. Physical activity was least before sunrise (t_1), and activity during the daytime (t_2-t_3) was greater than after sunset (t_4). Statistically (Table 3.1), there was no significant effect of Day but a highly significant effect of Time and interaction between Time x Day. The significant interaction reflects the tendencies for lower activities in Ramadan during the daytime and higher activities before and after sunset. Similar profiles were observed for mental activities and for the desires to perform physical or mental work. In all cases, scores were higher in the daytime than in the early morning and tended to fall in the evening; the effect of Ramadan was to decrease daytime values and increase the evening value (Table 3.1).

The social activity scores showed a slightly different profile (Fig. 3.4, middle, and associated statistics in Table 3.1). Activity was lowest in the morning and tended to rise throughout the day. This profile was more marked in Ramadan, with peak activities in the evening (the interaction between Time x Day being marginally significant). Sleepiness (Figure 3.4, bottom, and Table 3.1) tended to show a profile that was the inverse of the other variables; values fell in the daytime and rose towards evening. However, the evening rise in sleepiness was much less marked in Ramadan, accounting for the significant interaction between Time x Day.

3.3.2. Urine osmolality

On control days, the osmolality of the urine showed similar values throughout the daytime and indicated (a normal degree of) mild dehydration (Figure 3.5). During Ramadan, by contrast, whilst the values were very similar at 09:00 h, they increased throughout the day to reach a value indicative of a greater degree of dehydration by 17:00 h. This difference in profile between the two types of day accounts for the significant statistical differences that were observed (Table 3.1).

3.3.3. Physical performance measures

Mean grip strength of both the dominant and non-dominant hands showed no significant change between control days and Ramadan but a significant time-of-day effect, with higher values at 17:00 h than 12:00 h (Table 3.1). When the two hands were compared (Figure 3.6), the dominant hand was significantly stronger ($F_{1, 19} = 14.6$, $P = 0.001$). There was a significant interaction between Hand x Occasion ($F_{1, 19} = 5.3$, $P = 0.033$), and Figure 3.6

indicates that the non-dominant hand tended to be slightly weaker in Ramadan - unlike the dominant hand, which became slightly stronger.

The effects of the bout of exercise are summarised in (Table 3.1 and Figures 3.7-3.10). Exercise always produced a highly significant increase in HR (about 75 beats/min, bpm) and this was higher on control days (Figure 3.7). There was also a significant time-of-day effect, the rise being greater at 17:00 h. However, the significant interaction term (Table 3.1) indicates that the detailed changes were more complex; the rise in HR between 12:00 h and 17:00 h observed during Ramadan (about 7 bpm) was replaced by a slight fall (about 2 bpm) on control days.

As indicated by Figure 3.8, a higher cadence was chosen at 17:00 h, though there was no significant difference between Ramadan and control days.

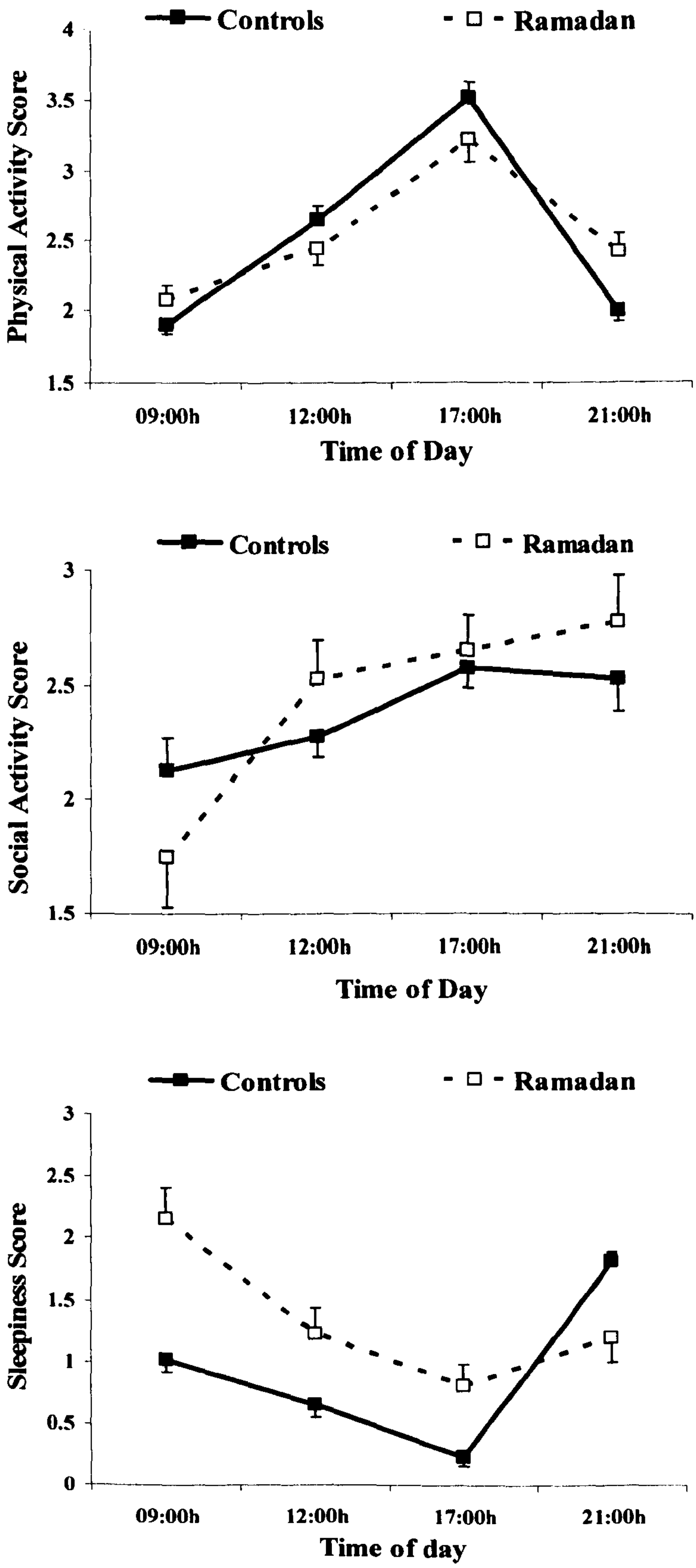


Figure 3.4. Changes with time of day on control days and Ramadan in: top, Physical activity; middle, Social activity; bottom, Sleepiness. For explanation of Scores, see text.

Table .3.1. Statistical results from Analysis of Variance analyses.

		ANOVA		
Variable	**Ramadan vs. Controls**	**Time of Day**	**Interaction**	**Comment**
Subjective Estimates of Activity and Sleepiness:				
Physical Activity	$F_{1,19}$=0.1; P=0.80	**$F_{2.9,\,54.9}$=83.3; P<0.0005**	**$F_{2.5,46.6}$=5.7; P=0.004**	See Fig.3.4, top
Mental Activity	$F_{1,19}$=2.9; P=0.11	**$F_{2.0,38.2}$=29.4; P<0.0005**	$F_{2.2,41.7}$=1.7; P=0.19	Similar profile to Fig. 3.4, top
Social Activity	$F_{1,19}$=0.1; P=0.78	**$F_{2.1,40.2}$=15.9; P<0.0005**	*$F_{2.3,44.5}$=2.7; P=0.071*	See Fig. 3.4, middle
Sleepiness	**$F_{1,19}$=8.3; P=0.010**	**$F_{1.9,35.6}$=25.5; P<0.0005**	**$F_{1.8,34.3}$=12.0; P<0.0005**	See Fig. 3.4, bottom
Desire for Physical Activity	$F_{1,19}$=2.7; P=0.12	**$F_{2.9,55.4}$=18.9; P<0.0005**	$F_{2.8,52.5}$=2.0; P=0.13	Similar profile to Fig. 3.4, top
Desire for Mental Activity	$F_{1,19}$<0.1; P=0.92	**$F_{2.6,49.7}$=19.8; P<0.0005**	*$F_{2.7,51.8}$=2.5; P=0.079*	Similar profile to Fig. 3.4, top
Urine Osmolality:	**$F_{1,19}$=5.3; P=0.033**	**$F_{1.8,33.8}$=16.3; P<0.0005**	**$F_{1.8,34.6}$=44.4; P<0.0005**	See Fig. 3.5
Physical Measures:				
Grip Strength	$F_{1,\,19}$<0.1; P=0.82	**$F_{1,19}$=20.1; P<0.0005**	$F_{1,19}$<0.1; P=0.82	17:00 h >12:00 h
Rise in HR with Exercise	**$F_{1,\,19}$=66.2; P<0.0005**	**$F_{1,19}$=6.6; P=0.019**	**$F_{1,19}$=8.2; P=0.010**	See Fig. 3.7
Cadence	$F_{1,\,19}$<0.1; P=0.86	**$F_{1,19}$=8.4; P=0.009**	$F_{1,19}$=2.2; P=0.15	See Fig. 3.8
Lactate	**$F_{1,\,19}$=15.9; P=0.001**	**$F_{1,19}$=112.1; P<0.0005**	**$F_{1,19}$=9.1; P=0.007**	See Fig. 3.9
RPE	**$F_{1,\,19}$=6.1; P=0.023**	$F_{1,19}$=1.8; P=0.20	$F_{1,19}$=0.3; P=0.59	See Fig. 3.10

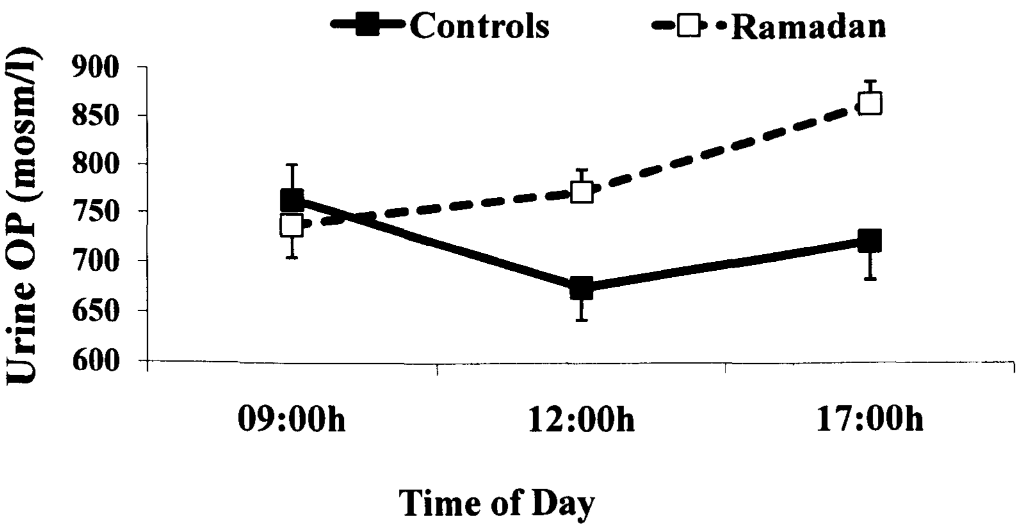


Figure 3.5. Urinary osmotic pressure (mosm/l) during control days and Ramadan.

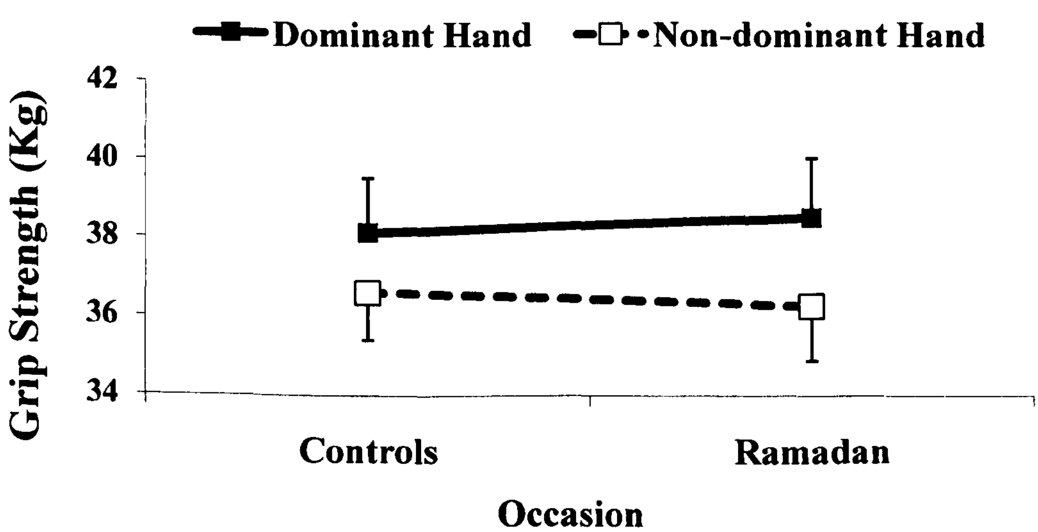


Figure 3.6. Grip strength in dominant and non-dominant hands on control days vs. Ramadan.

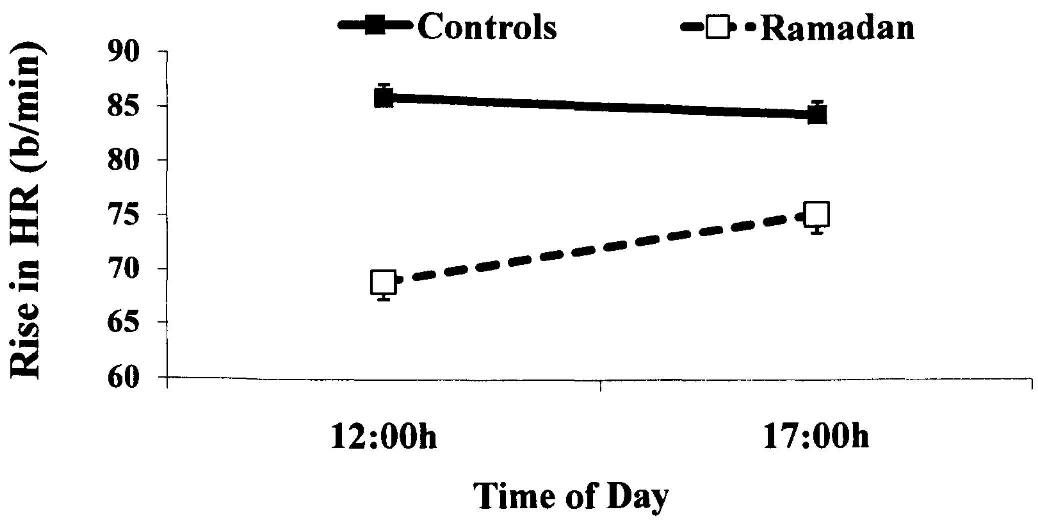


Figure 3.7. Effects of exercise on rise of heart rate, HR, at two times of day on control days and during Ramadan.

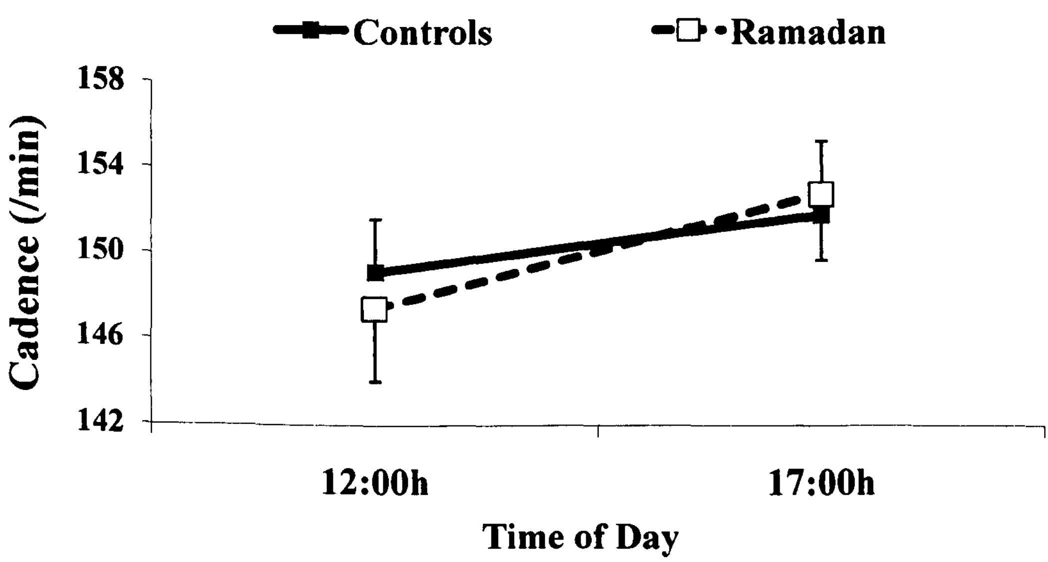


Figure 3.8. Effects of exercise at two times of day on cadence (cycles/min) on control days and during Ramadan.

Also at 17:00 h, post-exercise lactate levels were higher (Figure 3.9). However, Ramadan was associated with lower lactate levels in general (7.3 mM vs. 8.2 mM) and the significant interaction term (Table 3.1) indicates a less marked exercise-induced increase at 17:00 h.

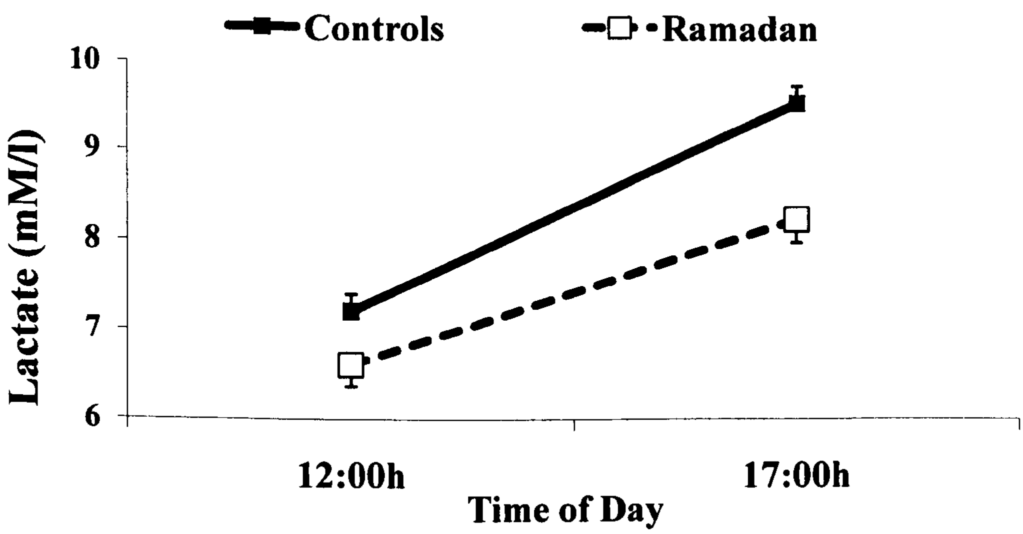


Figure 3.9. Effects of exercise at two times of day on post-exercise lactate (mmol/l) on control days and during Ramadan.

Subjective estimates of exertion (RPE) did not show a significant time-of-day effect, but mean values were higher in Ramadan than at other times (Table 3.1 and Figure 3.10).

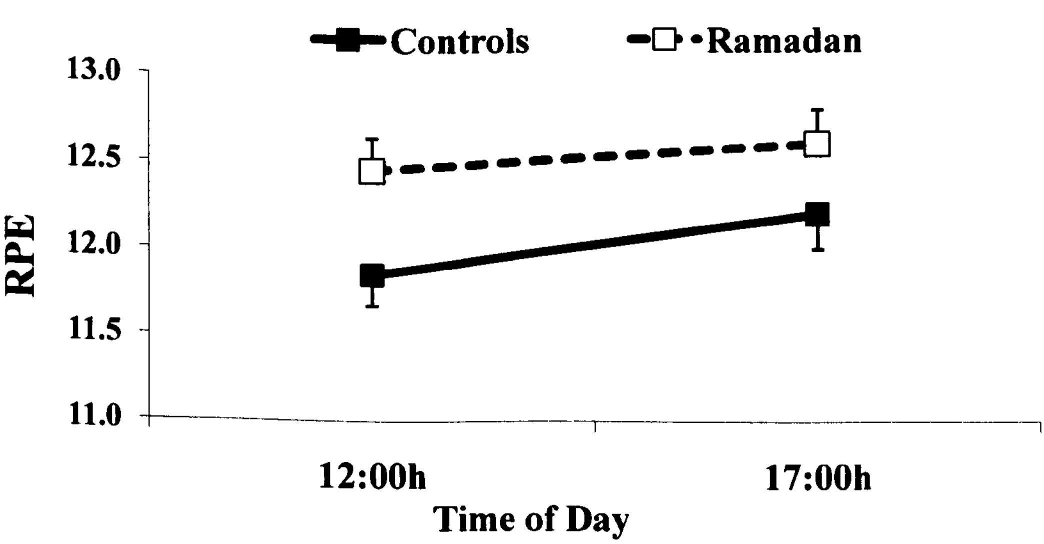


Figure 3.10. Effects of exercise at two times of day rating of perceived exertion (RPE) on control days and during Ramadan.

3.4. Discussion

The results indicate that Ramadan is associated with many changes to an individual's subjective feelings (reasons for eating, general activity, and sleepiness), physiology (urine osmolality and grip strength) and responses to physical activity (HR and blood lactate). Since the results from the first and fourth weeks of Ramadan were not significantly different (and so could be pooled for the statistical analyses), this indicates an important finding – namely, that the subjects adjusted quickly (within the first week) to the demands of Ramadan and that progressive deterioration did not take place. It is likely that such adjustment took place almost immediately since the subjects had previous experience of fasting during Ramadan. Other studies (reviewed in Reilly and Waterhouse, 2007) have found such a rapid adjustment.

A clear indication of the effects of daytime fasting can be seen by considering the intakes of fluid and food after sunset. The osmolality of the urine provided evidence of progressive dehydration during the course of the daytime (Figure 3.5) but, in the evening, all subjects drank large amounts of fluid and ate a substantial meal (drink and food scores approaching maximum values of 4.0). The reasons given for these increased intakes of fluid and food were thirst and hunger and an acknowledgement of the need to recuperate from daytime fasting (Figures 3.2, bottom, and 3.3). The main types of fluid drunk, water, fruit juice and milk (Figure 3.2, top) would all aid rehydration, as would drinking less caffeine-containing beverages (some fizzy drinks, tea and coffee) during Ramadan. This result has been observed before (Waterhouse *et al.*, 2008a) and it is possible that it is part of the culture of those living in a hot country to take less frequently drinks that cause diaphoresis and diuresis, so reducing dehydration.

Subjects went to bed later in Ramadan, and spent less total time in bed (Margolis and Reed, 2004; Roky *et al.*, 2003; Waterhouse *et al.*, 2008a, 2008b). However, the subjects in the present study also tended to raise later in Ramadan, rather than earlier - possibly a reflection of their lack of domestic commitments (they lived at home with parents). The later retiring time might have contributed to the greater ease of getting to sleep in Ramadan. Subjects ate meals in the evening that were larger than normal and also ate just before retiring, claiming that this helped them get to sleep. The quality of sleep has been shown to change in Ramadan (Roky *et al.*, 2003) but possible interactions between time of retiring, amount and timing of food eaten and quality of sleep need further investigation.

Subjects felt sleepier in the daytime but this increase did not extend into the evening (Table 3.1). Even so, they did not nap during the daytime, citing "too busy" as the reason (Figure 3.1). This result differs from some that have been published (Margolis and Reed, 2004), including our own (Waterhouse *et al.*, 2008a, 2008b), and reflected the individual's full commitments at the Sports Academy, with little opportunity to catch up on lost sleep during the daytime. A lack of standardisation of the populations studied here and in the literature (in terms of age, family and work commitments, etc.) probably contributes to the variation in results.

As a result of these changes in fluid and food intakes in the daytime and altered sleep at night, subjects would have been showing effects of sleep, fluid and food deprivation. This combination of factors would be predicted to have negatively affected aspects of mood and mental performance, and some types of physical performance (Reilly and Edwards, 2007; Reilly and Waterhouse, 2007; Waterhouse *et al.*, 2001). Subjective estimates of the amounts of physical and mental performance carried out, and the desire to undertake them (Table 3.1 and Figure 3.4, top), all showed that Ramadan was associated with decreased activities in the daytime but increased values in the evening after sunset. Such results have been found and discussed previously (Kadri *et al.*, 2000; Karaagaoglu and Yucecan, 2000; Roky *et al.*, 2000; Waterhouse *et al.*, 2008a, 2008b). The current results can be interpreted as further evidence of the changes to daily life produced by the demands of Ramadan as well as a validation of the present protocol and subjects. Social activities also changed in Ramadan, being higher throughout the daytime as well as the evening (Figure 3.4, middle); higher daytime values in Ramadan differed from previous findings (Waterhouse *et al.*, 2008a, 2008b). This difference possibly reflects the fact that the subjects were younger and lived at home with few domestic responsibilities, previous studies being upon individuals who were housewives or students living alone.

The results of Figure 3.6 support the view that physical activity is changed in Ramadan, but the effects are complex. As expected, the dominant hand produced greater strength than did the non-dominant hand, and afternoon values were higher than those produced at noon. However, there was a difference between the two hands, the non-dominant hand appearing to be more susceptible to negative effects of Ramadan. A difference between the dominant and non-dominant hands has been observed before using a task requiring hand-eye co-ordination (Edwards *et al.*, 2008). This difference between the two hands might reflect the relative roles played by neural and muscular components and their susceptibilities to fasting and sleep loss, but this possibility needs further investigation.

Time-of-day effects were present in the rise of HR after exercise, the chosen cadence for the exercise and the post-exercise blood lactate level (Table 3.1 and Figures 3.7-3.9, respectively). It should be remembered that the circadian rhythm of HR, which peaks around the early afternoon, is earlier than that of core temperature, which peaks in the late afternoon (Reilly and Brooks, 1982, 1986); as a result, the HR in the current study would have been around its peak at 12:00 h but decreasing by 17:00 h. By contrast, the rise in cadence between 12:00 h and 17:00 h is commensurate with a rise in core temperature and effects of this rise upon metabolic activity (Thomas and Reilly, 1975); such a rise in metabolic activity between 12:00 h and 17:00 h could also explain the rise in blood lactate (Forsyth and Reilly, 2004, 2005).

Ramadan caused changes in the mean rises in HR (Figure 3.7) and blood lactate (Figure 3.9) in response to exercise, but whether these changes arise from the effects of sleep loss and/or the metabolic consequences of dehydration and lack of food intake and/or changes in motivation, for example, cannot be determined from the present results. These factors are likely to interact with one another. The lower rise of HR in Ramadan is compatible with a lower sympathetic drive, commensurate with energy disturbances, during Ramadan, and has been observed also following sleep deprivation (Reilly and Edwards, 2007). By contrast, the greater rise in HR at 17:00 h compared with 12:00 h in Ramadan (Figure 3.7) might reflect the need, as the length of fasting increases, for an increased HR to maintain blood pressure in the face of greater dehydration and falling venous return. The lower lactate values when fasting has been attributed to a greater use of lipids as fuel at this time (Benaji *et al.*, 2006; Leiper *et al.*, 2003; Reilly and Edwards, 2007; Reilly and Waterhouse, 2007; Roky *et al.*, 2004).

The finding that the perception of load was increased (Figure 3.10) in spite of decreased rises in HR and lactate accords with the subjective estimates of a decreased amount of physical work actually performed, and also wished for, in the daytime (Figure 3.4, top and Table 3.1). Also, the statistically significant interactions between Occasion (Ramadan vs. control days) x Time of day for blood lactate and rise of HR (see Table 1 and Figures 3.7 and 3.9) suggest that the effects of Ramadan (whatever their cause) worsen as the day proceeds. These greater "stresses" when performing physical activity later in the day are in contrast to the result that is normally found, where physical performance at 17:00 h is better than at 12:00 h and less demanding physiologically, biochemically and mentally (Reilly and Edwards, 2007; Reilly and Waterhouse, 2007; Reilly *et al.,* 1997).

3.5. Conclusions and Limitations

In summary, Ramadan makes many demands upon those involved, and is responsible for changes in sleep, daytime sleepiness, actual and desired behaviour, and physical performance. The changes cause a decrease in both the amounts of daytime activity actually performed and in the amounts desired. There is also clear evidence that a given bout of physical performance at the end of the daytime fast places a greater burden on the body, and is perceived as being more demanding than is the same bout of activity performed earlier in the daytime. These changes from control days take place within the first week of Ramadan, and further deterioration later in Ramadan does not seem to occur. Individuals recuperate after sunset by eating and drinking more, and their intake of fluid tends to exclude drinks which would promote further dehydration. There are clear implications from these results for individuals performing physically-demanding tasks late in the day in Ramadan, when effects due to fatigue and dehydration will be more marked.

3.5.1. Limitations of the study

The first limitation is that the protocol does not enable possible links between the various changes to be determined. For example, it is reasonable to suppose from the scientific literature that the restrictions of food and fluid intake will be a causative factor in performance and mood deterioration (Bellisle, 2002; Kadri *et al.*, 2000; Karaagaoglu and Yucecan, 2000; Keys *et al.* , 1950; Khammash and Al-Shouha, 2006; Kleiner, 1999; Leiper *et al.* , 2003; Maughan, 2003; Oliver *et al.*, 2006; Ramadan *et al.* , 1999; Roky *et al.* , 2000, 2004; Yacine *et al.*, 2007) but additional effects due to sleep loss (Dinges, 1995; Kleitman, 1939; Margolis and Reed, 2004; Neri *et al.*, 1997; Roky *et al.*, 2001, 2003; Waterhouse *et al.*, 2001) and/or metabolic changes (Bogdan *et al.*, 2001; Bouchared and Touitou, 2001; Consolazio *et al.*, 1967; Karaagaoglu and Yucecan, 2000; Qujeq *et al.*, 2002) cannot be discounted. Also, establishing associations between the various changes (by determining correlations between pairs of variables, for example) does not enable *causal* links to be established. The only way in which causal links can be established is by the use of an intervention protocol in which some of the variables (amount of sleep taken, for example) are kept constant and others (length of fasting, for example) are changed systematically. Such an intervention study will be described in Chapter 6.

A second limitation is that, even though the results from this field study have extended those from previous work by our group (Waterhouse *et al.*, 2008a, 2008b) and shown that physical performance was decreased in Ramadan when measured objectively as well as subjectively, the number of variables measured was comparatively limited. A fuller understanding of the changes in Ramadan requires investigations using a broader range of measurements of physical activity as well as measurements of mental performance. Moreover, making measurements at more times of day (including after the day's fast has ended) is desirable.

Such additions to the protocol are difficult to perform in the field, and they require the studies to be performed in the laboratory. Such studies will be described in Chapters 4 and 5.

Chapter 4

Laboratory-based studies

I. Introduction to Laboratory-based Studies

II. A Pilot Study of Changes in Body Mass and Physical Performance During One Day of Fasting

Liverpool John Moores University

I. Introduction to Laboratory-based Studies

The results from the field study (Chapter 3) extended those from previous work by our group (Waterhouse *et al.*, 2008a, 2008b) and showed that physical performance was decreased in Ramadan when measured objectively as well as subjectively. Even so, the number of variables measured was comparatively limited, and a fuller understanding of the changes during the course of the day in Ramadan requires investigations using a broader range of measurements. Previous work (Chapter 3 and Waterhouse *et al.*, 2008a, 2008b) had also shown that the changes observed were not progressive during the month of Ramadan, differences from control days not being statistically significantly different during the first and fourth week of fasting. This result raises the possibility that the effects of fasting seen in Ramadan might be simulated by a period of fasting lasting less than four weeks, even a single day.

Making further measurements in the field is difficult, and it is not always possible to perform them in a laboratory, as was the case in the study of Chapter 3; one way to overcome such a problem might be to perform the whole study in a laboratory, simulating the demands of Ramadan but for a shorter period of time, a day rather than a month. To investigate if such a protocol would give results of value to an understanding of the changes in Ramadan has been the aim of the pilot study and the study described in Chapter 5. However, before describing these studies, it is necessary to compare the advantages and disadvantages of field- and laboratory-based work.

4.1 Advantages and disadvantages of field- and laboratory-based studies

The advantage of a field study is that it is "real" rather than artificial and, for studies of Ramadan, can investigate any progressive changes during the course of the 4 weeks of fasting. In practice, however, a field study also results in an increased amount of "noise". Such noise results from changes to individuals' daily lifestyles (as a result of domestic and work-related issues) and changes in the weather, for example. There is also a limit to the number of measurements that can be made in such circumstances, due to lack of availability of suitable apparatus and the time taken to perform the tests. The problem can be solved if a fully equipped laboratory is readily available (as was the case in the study of Chapter 3, Waterhouse *et al.*, 2009), but this is not always possible.

If the study takes place in a laboratory, by contrast, some of the advantages are:

There is more control of the environment.

More detailed tests can be carried out (though their application to "real-world" tasks can always be questioned).

The timing of the tests is easier to control.

It is easier to check that the subjects are compliant.

However, one problem that arises when a more detailed study is performed in the laboratory is that of the time required to perform the tests. In practice, therefore, subjects in such studies tend to be restricted in the activities they can pursue when not being tested (needing to be close to the laboratory and to have sufficient time to perform the tests). Also, the investigations cannot last for a whole month (as is the case when effects of Ramadan are being considered) but are restricted to a single day only. This does have a potential disadvantage that adjustments of sleep patterns and the types and timing of food and fluid intakes are not fully comparable in a short-term laboratory-based study to the longer-term changes adopted in Ramadan. Even so, the results from both types of study can be valuable when compared and, in practice, the results from field- and laboratory-based studies complement each other.

4.2 Rational for the three laboratory-based studies

Three laboratory-based studies have been performed. Of these, the first two (the pilot study and the study to be described in Chapter 5) were designed to investigate the value of a single day of fasting in the laboratory as a viable tool for investigating changes that occur during Ramadan. The second study (Chapter 5) was more elaborate, recording more variables at more time-points, than the pilot study. In both studies, subjects were studied on two occasions, a control day (normal food and fluid intakes) and a fasting day (food not being allowed during the daytime). Subjects were free to choose their activities between testing times, their sleep times, and food and fluid intakes before 07:00 h, mimicking the conditions that exist in Ramadan. However, even though long-term changes in sleep and food/fluid intake patterns would be absent from such a protocol, previous work (Waterhouse et al., 2008a, 2008b and Chapter 3) had indicated that such effects were absent.

These two studies confirm the findings of the field study, namely that physical performance deteriorated during fasting, but it was unclear, particularly from the field study, if the decrements in performance were due to food and fluid restriction and/or sleep loss. In the third laboratory-based study (Chapter 6), an attempt was made to resolve this ambiguity. The study was an intervention design that investigated the effects of timed amounts of fasting upon a variety of variables (mood, mental performance, physical performance, activity and dehydration) but when sleep was controlled. This protocol enabled not only effects of specific durations of food and fluid restriction to be investigated but also possible confounding factors, such as changes in ambient temperature and sleep times, to be controlled.

The results from the three laboratory-based studies can be compared with those from the field-based study and published work, to consider the extent to which the effects of Ramadan can be replicated in short-term studies in the laboratory.

II. A Pilot Study of changes in Body Mass and Physical Performance during One Day of Fasting

This pilot study was performed in the UK in November, 2007–March, 2008. The rationale for the study, described above, was to investigate, in more detail than can conveniently be undertaken in the field, changes to a small group of variables during the course of a single day of fasting from 08:00-16:00 h. The variables chosen for investigation were directly based on those measured in the field study (Chapter 3). It was hypothesised that changes observed during the course of fasting for a day would closely mirror those observed in the field in Ramadan; that is, there would be a fall in body weight, deterioration in physical performance, and a rise in urine osmolality as the subjects became dehydrated.

4.3. Methods

4.3.1. Subjects

Twelve subjects (8 males) aged (mean, SD) 26.25, 7.66 years and height 1.74, 0.08 m took part in the study. Females were studied at all stages of their menstrual cycles. Subjects were recruited from the student population at Liverpool John Moores University using day-to-day interactions within the campus community and word-of-mouth. Subjects volunteered to participate in the study with the understanding that participation was not monetarily compensated and they could withdraw at any time without giving any reason.

The general aims and details of the protocol were explained to them (but detailed reasons for the study were not given), and any questions were answered. Volunteers were given a document outlining these procedures and methods and were asked to read and sign a consent document before being allowed to further participate. The study had been passed by the University Ethics Committee.

4.3.2. General design

Before the two experimental days, the subjects were required to come in for a familiarization session. During this session, subjects were informed of the measurements that were going to be made and were able to use the apparatus involved. They were also told which experimental days would be the control and fasting days, the order of which was randomised for the group as a whole.

During each of the two experimental days, subjects had to attend the laboratory three times, at 08:00, 12:00 and 16:00 h. On each occasion, subjects' body mass was measured and then they rested for about 30 min; during this time, any problems with the protocol were discussed and the apparatus for measurement (see below) was attached. At the end of this period, resting heart rate and grip strength were measured. Subjects then gave a urine sample. Subjects were required to cycle for 5 minutes on a cycle ergometer (Ergo-bike Medical 8, Daun electronic company, Germany) at a constant load of 70 watts. The rate of pedalling was left to each subject to decide upon. Heart rate was taken every minute during the exercise as well as immediately after exercise. After a recovery period of about 10 min, during which the apparatus was removed, subjects were free to leave the laboratory until the next test session.

4.3.3. Measurements made

At each of the three times on the two experimental days, the following measurements were made:

4.3.3.1. Body mass

Body mass was measured to the nearest 0.1kg were recorded using analogue Seca scales (Seca Ltd., Birmingham, UK). Before and after the bout of exercise.

4.3.3.2. Physiological Variables

The variables measured were grip strength (left and right hand) and heart rate. Grip strength was measured by a Handgrip Dynamometer (TRK5106, TAREK Scientific Instrument Score, Japan). Right and left grip strengths were measured alternately, to limit the effects of fatigue. The peak values and averages of the grip strengths were taken.

Heart rate was monitored by a monitor (Polar, Kempele, Finland) strapped to the chest and a data logger attached to the subject's wrist.

For urine osmolality, the bladder was emptied completely and an aliquot (50 ml) of the sample was stored at 10°C until later analysis for osmolality (Osmocheck pocket pal OSMO, Vitech Scientific Ltd, Japan). This variable was used as a measure of general dehydration.

4.3.4. Statistics

Analysis of variance with repeated measures was used. The main factors were Day (2 levels, Controls vs. Ramadan) and Time of Day (3 levels). The additional factors Hand (dominant vs. non-dominant) for grip strength and Exercise (before vs. after) for heart rate, HR, were used in 3-factor ANOVAs. Greenhouse-Geiser corrections were used, and significant differences within the main factors were assessed using Bonferroni corrections.

The SPSS package, version 14, was used. Significance was set as $P<0.05$, though occasions where $0.05<P<0.10$ have been reported as "marginally significant". Exact P values have been given; results given as "0.000" in the statistics output have been reported as "<0.0005".

4.4. Results

4.4.1. Body Mass.

Body mass fell during the daytime in fasting and indicated the loss of about 1 litre (1 kg) of fluid (Figure 4.1). Body mass showed no significant effect of Day ($F_{1, 11} = 2.04$, $P = 0.18$) but a highly significant effect of Time of day ($F_{1.2, 12.9} = 19.73$, $P<0.0005$) and a significant interaction ($F_{1.2, 13.4} = 17.39$, $P<0.0005$) due to the decreasing body mass during the course of the day only when fasting (mass remaining similar on the control day).

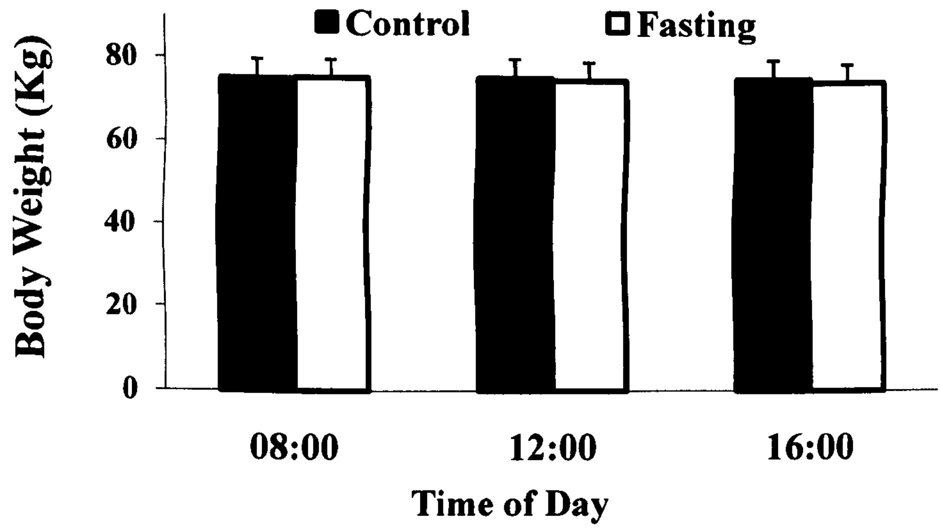


Figure 4.1. Body mass. Mean and SE. Closed bars, Control day; open bars, Fasting day.

4.4.2. Urine osmolality

The osmolality rose on the fasting day but not on the control day (Figure 4.2). There was no significant effect of Day ($F_{1, 11}$ = 2.04, P = 0.18) but there was a significant effect of Time of day ($F_{1.5, 16.3}$ = 6.54, P = 0.013) and a significant interaction between Day x Time of day ($F_{1.7, 18.4}$ = 7.07, P = 0.007), because urine osmolality increased during the daytime in the fasting, but not the control, day.

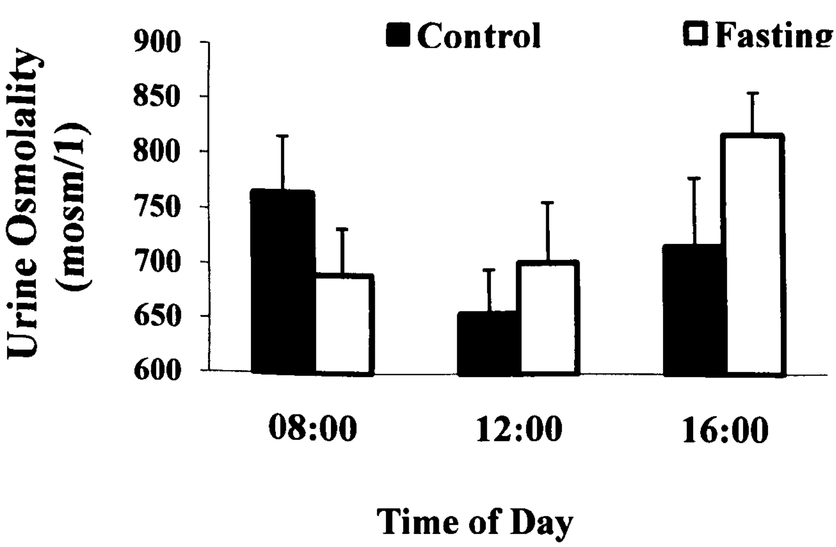


Figure 4.2. Urine osmolality. Mean and SE. Closed bars, Control day; open bars, Fasting day.

4.4.3. Grip strength

For the non-dominant (left) hand, there was no significant difference for Day ($F_{1, 11} = 0.414$, P = 0.53) or Time of day ($F_{1.8, 19.8} = 1.33$, P = 0.29), and no significant interaction ($F_{1.5, 16.8} = 0.60$, P = 0.52) (Figure 4.3). For grip strength of the dominant hand, there was a marginally significant difference for Day ($F_{1, 11} = 4.03$, P = 0.070) but no significant effect of Time of day ($F_{1.7,19.1}$=1.34, P = 0.28) or interaction ($F_{1.9,21.3}$= 2.23, P=0.13) (Figure 4.4). When results from the two hands were compared, the right hand (dominant hand) was significantly stronger ($F_{1, 11}$ = 39.00, P<0.0005) and there was a marginally significant interaction between Hand x Occasion ($F_{1, 11}$ = 3.62, P=0.083) because the fall in grip strength of the dominant hand during fasting was more marked than that of the non-dominant hand.

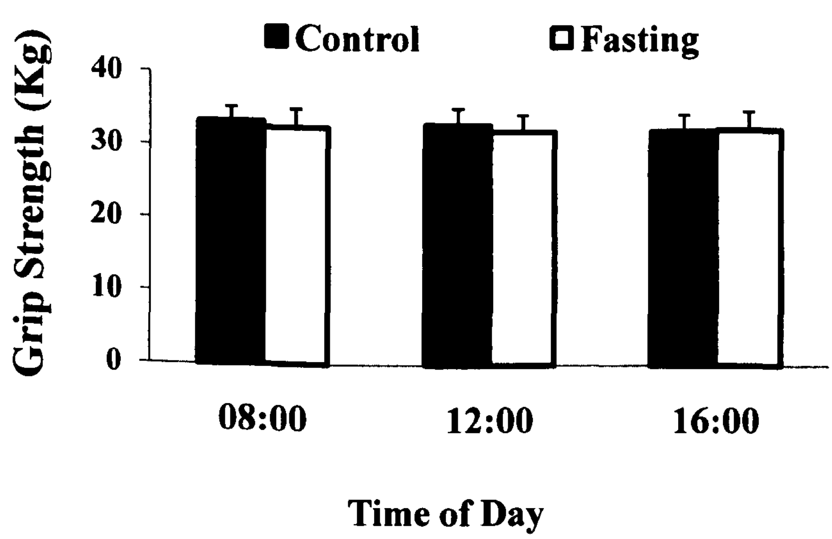


Figure 4.3. Grip strength, left (non-dominant) hand. Mean and SE. Closed bars, Control day; open bars, Fasting day.

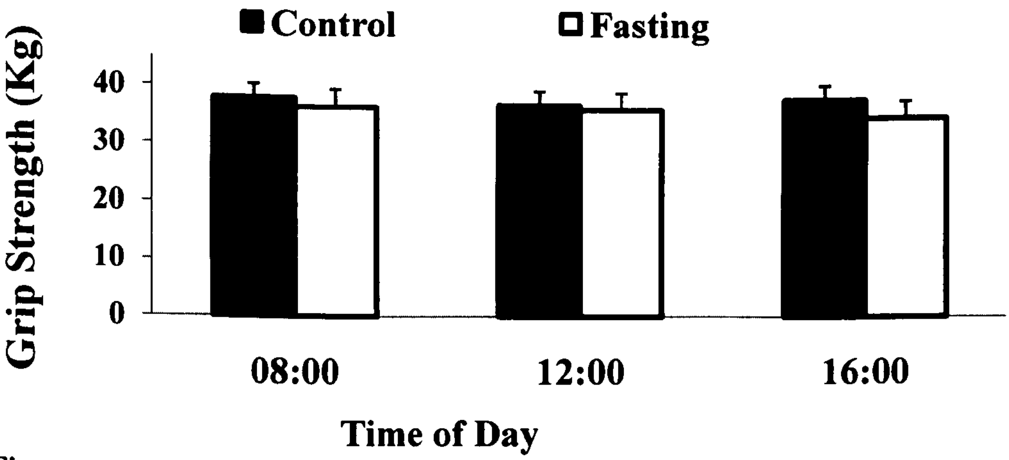


Figure 4.4. Grip strength, right (dominant) hand. Mean and SE. Closed bars, Control day; open bars, Fasting day.

4.4.4. Exercise

There was a rise in HR produced by the 5 min of exercise ($F_{1.8, 20.2} = 7.19$, $P = 0.005$) but this rise was not significantly different between the control and fasting days ($F_{1, 11} = 0.27$, $P = 0.62$) or between the three times of measurement during the course of the day ($F_{1.9, 20.5} = 0.09$, $P = 0.90$). There was one significant interaction, that between the time of day when exercise was taken and whether it was a control or fasting day ($F_{1.7, 18.7} = 4.74$, $P = 0.026$); for the control day, the HR after exercise increased as the day progressed whereas the opposite change took place during fasting (Figure 4.5).

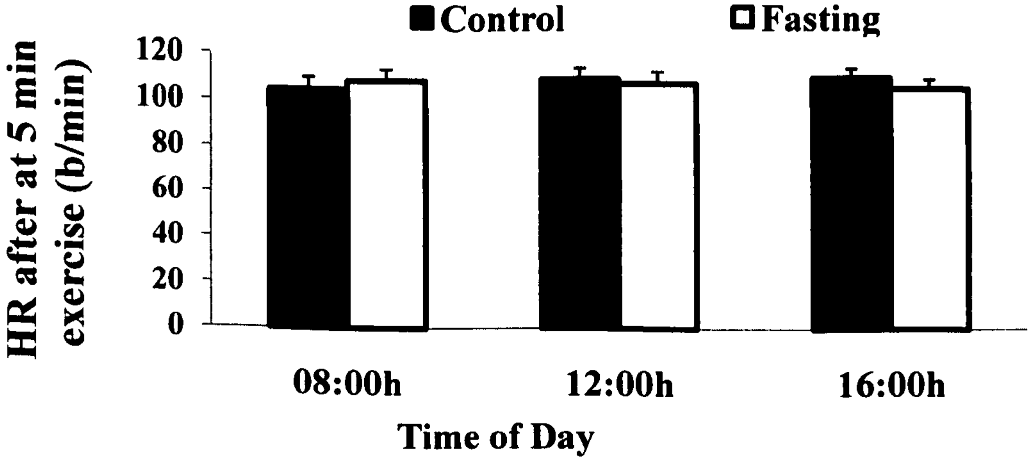


Figure 4.5. Heart rate at the end of the 5-min cycling. Mean and SE. triangles Control days; Squares, fasting days.

4.5. Discussion

The results showed that body mass fell (Figure 4.1) and urine osmolality rose (Figure 4.2), during the fasting but not the control day. These results would be predicted from basic physiology, where dehydration leads to conservation of body fluid and production of concentrated urine. The scientific literature also supports these findings. Thus, body mass has been found to decline during Ramadan and other diet-restriction strategies (Hall and Lane, 2001; Ramadan *et al.*, 1999), and urine osmolality, normally in the range 400-600 milli-osmoles (mOsm), rises towards 900 mOsm as the degree of dehydration increases (Ramadan *et al.*, 1999). Such results have been found previously in Ramadan (Reilly and Waterhouse, 2007) and the rise in urine osmolality also agrees with the results found in the field study (Chapter 3).

Grip strength, a simple and proven test of maximal muscular strength, has been used in many diet-restriction experiments (Keys *et al.,;* 1950Consolazio *et al.,* 1967). Even so, in the current experiment, grip strength (Figures 4.3 and 4.4), showed comparatively little change. There was, however, a marginal decline in grip strength of the dominant hand on the fasting day, and interaction between Hand x [Control vs. Fasting]; both these results point to a greater deterioration of grip strength of the dominant hand during fasting. This greater decrement might reflect a more sophisticated control mechanism, involving neural and muscular elements, in the dominant hand (see discussion in Edwards *et al.*, 2009).

For resting heart rate also, no differences between control and fasting days were observed (data not shown). However, HR immediately after exercise rose during the course of the day on the control day but fell on the fasting day (Figure 4.5). Higher heart rates after exercise during the course of control days would be predicted as a result of the circadian rhythm of heart rate at rest and in exercise (Minors and Waterhouse, 1981); lower post-exercise values during the course of the fasting day accords with the view that physical performance deteriorates with dehydration (Reilly and Waterhouse, 2007).

One explanation of the comparatively few significant differences between control and fasting days is that grip strength and heart rates following a 5-min bout of exercise are not sufficiently sensitive markers to pick up the changes that might be observed during a *single* day of fasting. In support of this view, other studies have indicated that physical performance is quite resilient in the face sleep loss or food and fluid restriction, provided these are limited in extent (Reilly and Waterhouse, 2007, 2009; Reilly *et al.*, 1997; Waterhouse *et al.*, 2001).

What this pilot study does show is that it is possible, in a laboratory-based protocol lasting a single day, to mimic at least some of the effects of fasting in Ramadan. Even so, it is clear that a wider variety of markers of performance would be advantageous. These can include further measures of the response to a bout of exercise and objective estimates of mental performance in addition to the subjective measures that were obtained from the questionnaire used in the field study (Chapter 3). Further, if recuperation in the evening from fasting is to be studied, an important response in Ramadan (Waterhouse *et al.*, 2008a, 2008b and Chapters 2 and 3), then a further time-point, sometime in the evening, needs to be measured.

It seems reasonable to conclude that the current protocol, with the appropriate modifications just described, can be used as a tool to assess in more detail the responses to a single bout of daytime fasting. Such a study will form the basis of Chapter 5.

Chapter 5

A More Detailed Study of Changes in Performance Produced by One Day of Fasting

5.1. Rationale

The results from the pilot study (Chapter 4) indicated that some of the changes found in field studies of Ramadan (Chapter 3 and Waterhouse *et al.*, 2008a, b) could be duplicated in the laboratory in a protocol involving one day of fasting. For example, urine osmolality showed changes indicating the development of dehydration during the daytime. It was concluded that such results indicated that the laboratory-based experiment and the protocol used provided a useful tool for assessing some of the changes produced by fasting and changes of the sleep-wake cycle in Ramadan. By contrast, some of the measurements indicated rather small changes due to fasting, including grip strength and the response of heart rate to a 5-min bout of exercise. It was suggested that such a lack of change in measures of objective performance might indicate that the measures used were not sufficiently sensitive to the effects of only one day of fasting. Another possible problem was that measurements were made only until the end of fasting (16:00 h), as a result of which any recuperation, as previously observed in the field studies, could not be investigated.

The aim of this study is to build upon the results of the pilot study (Chapter 4) and to incorporate two improvements: additional measurements of performance and more frequent times of measurement. These times would span a wider portion of the waking period, including four times per fasting period, rather than three, and a set of measurements after the end of the fast and before retiring, so bringing the protocol closer to those of the field studies of Ramadan (Chapter 3 and Waterhouse *et al.*, 2008a, 2008b).

It is hypothesised that daytime fasting will lead to dehydration and will cause a decrement in performance, measured both subjectively and objectively, in comparison to the control days, when there are no restraints on fluid and food intakes.

5.2. Methods

5.2.1. Subjects

Eighteen male subjects, Liverpool John Moores University undergraduates with an age range of 18-21, were recruited by word of mouth for the study. None was Muslim and none had previously participated in food/fluid-restriction study. The experiments were completed in the months January and February, 2009 and had previously been approved by the University Ethics Committee.

5.2.2. Protocol

Subjects initially underwent a familiarisation session in which all aspects of the testing were explained, including the two conditions (fasting and non-fasting), and the dates on which the two conditions were to be performed. The various tests were described and subjects were required to practise them, to reduce learning effects during the main part of the experiment. Any questions were answered and then the subjects were required to sign informed consent forms.

The main experiment (begun at least one week after the familiarisation session) was in two parts: a non-fasting (control) day and a fasting (intervention) day. The order in which the two experimental days took place was randomly selected by the experimenters, but the subjects knew of this order at least a week in advance. This arrangement gave them the opportunity to eat/drink prior to sunrise (07:00 h) on the testing day and to go to bed/rise earlier if they wished to do so.

On both experimental days, subjects were studied five times, at 09:00, 12:00, 15:00 and 18:00 h and on retiring to bed. On the control (non-fasting) day, subjects were free to choose when to sleep, and what and when to eat and drink. On the fasting day, subjects were free to choose when to sleep and what to eat and drink before 07:00h and after 18:00 h. Food and fluid intake were prohibited between 07:00 h and 18:00 h but subjects were free to nap if they choose to do so.

On each experimental day, subjects attended the laboratory at 09:00, 12:00, 15:00 and 18:00 h, to perform a series of tests and give a urine sample; they also answered the questionnaire again at home, when choosing to retire to bed at the end of the day. The tests and samples were given in the following order: urine sample (analysed for osmolality, see Chapters 3 and 4); questionnaires whilst sitting quietly (used previously, see Chapter 3), hand grip strength (dominant hand only, 3 times each measurement, as performed previously, see Chapters 3 and 4); vertical jumps (performed 3 times; this is an additional measurement); accuracy at throwing darts (as used in previous studies, Waterhouse *et al.*, 2008a, but an addition to this type of study) and the Stroop test (a measure of mental performance that also is new to this type of study). These tests are now described in more detail.

5.2.3. Measurements made

5.2.3.1. Questionnaires

Subjects were required to give information about their daily activities and feelings over the course of the day. The information was gained from a questionnaire which the subjects completed five times during each of the two days they were being investigated. The questionnaire is shown in Appendix 4.1.

The five times they answered the questionnaire were (1) at 09:00 h, which reflected activities after waking, (2) at 12:00 h, which reflected morning activities, (3) at 15:00 h, which reflected activities in the early afternoon, (4) at 18:00 h, which reflected late afternoon activities and (5) on retiring to bed (designated 24:00 h in the Figures), which reflected evening activities after breaking the fast.

For some of the variables (the reasons for drinking/not drinking and eating/not eating), the ''fraction of possible occasions'' was calculated for the group. This calculation was performed as follows. Suppose, for example, that only 12 of the 18 subjects drank on a particular occasion and that 8 of these gave the reason "Thirsty". This means that the fraction of possible occasions that Thirsty was chosen was 8/12, that is, 0.67. Suppose, further, that, of the 6 who did not drink, 2 gave the reason "Too busy". This means that the fraction of possible occasions that Too busy was chosen was 2/6, that is, 0.33.

5.2.3.2. Physiological and performance tests

A. Urine osmolality

This provides a measure of dehydration of the body. The urine sample was collected into a plastic pot and stored at 5°C until analysis. For the analysis, a 5 ml aliquot was taken and its osmolality measured (Osmocheck Pocket Pal OSMO, Vitech Scientific Ltd, Japan).

B. Stroop test

The test used was the Colour-Word Stroop Test (CWST). It is a test that assesses working memory and attention. Therefore, it would be expected to show a daily rhythm peaking around noon (Folkard, 1990; Waterhouse *et al.*, 2001) and, though it has not been used before in studies of Ramadan, might be expected to show deterioration due to restrictions of food and fluid intakes.

The test consisted of the subject viewing 20 separate cards. Each had a colour, "red", "orange", "green" or "brown" typed on it in one of these four colours. However, in many cases, the word was typed in a colour that was different from the meaning of the word – the word "orange" might be typed in green, for example. The participant was required to state the colour in which the word was typed, not the name of the colours itself. (In the example given, the correct answer would be "green" rather than "orange"). Incorrect answers had to be corrected by the subject. The total time taken to answer all 20 questions correctly was recorded on a stopwatch.

C. Throwing darts

Throwing darts can be used as a model for investigating circadian and time-awake factors that influence motor performance (Edwards *et al.,* 2007). The subjects threw darts at a circular target (20 cm diameter) placed on the floor at a distance of 2.37 metres; the target consisted of 10 concentric rings of diameter 2 cm, 4 cm, 6 cm,......20 cm (See Appendix, 5.1). The subjects had 20 attempts to throw the dart as close to the target's centre as possible. After each throw the score for each dart was measured and then the dart was collected for the next throw. The score was determined as follows: a dart in the innermost ring scored 10 points, and darts in the other rings moving out from the centre scored 9, 8, 7...1 point, respectively. When a dart missed the target, the score was recorded as zero (a "miss"), regardless of the size of the miss.

In the analysis, the results from the first and last three darts were ignored (though the subjects did not know this when they were performing the test), due to possible effects of "getting my eye in" and "nearly finished". The total score for the 14 darts, the number of misses, and the mean score per hit (ignoring the misses) were used as measures of throwing accuracy (Edwards and Waterhouse, 2009; Edwards *et al.,* 2007).

D. Hand grip strength

Only the dominant hand of each subject was investigated using a dynamometer (Handgrip Dynamometer TRK5106. Jump MD, TAREK Scientific Instrument Score, Japan). The subject recorded grip strength on 3 occasions separated by 10-sec intervals, the maximum of the three values being recorded. This is a test of muscle strength using a comparatively small group of muscles.

E. Jump height

The jump test is a further example of a test of physical strength but, unlike grip strength, involves the integrated actions of several groups of muscles. Subjects performed 3 maximal vertical jumps using the "stand-and-reach" method (Reilly *et al.*, 1997). Subjects stood side-on to a wall and reached up with the right arm, which was next to the wall. Keeping the feet flat on the ground, the wall was marked by the tip of the fingertips, onto which chalk powder had been placed. The subjects then jumped vertically as high as possible using both arms and legs to assist in projecting the body upwards. The wall was touched at the highest point of the jump. The difference in distance between the standing height and the jump height was the height jumped. The best of three attempts was recorded.

After the vertical jump tests had been completed, subjects were free to leave the laboratory and continue their activities until the next visit to the laboratory. Subjects varied in their experience of having throwing darts before the experiment, but none of them had any previous experience with regard to answering the questionnaires or performing the other tests.

5.2.4. Statistical analysis

The data were analysed by means of the Statistical Package for Social Sciences (SPSS) for windows (version 14). For most data, two-way ANOVA with repeated measures was used. The main factors were Day (2 levels, Controls vs. Fasting) and Time of Day (4 or 5 levels, pre-09:00, 12:00, 15:00, 18:00 and post-18:00h for the results from the questionnaires). When fractions of possible occasions were compared, the data were Arcsin-transformed before ANOVA (a standard method used to make the data distributed more normally). Greenhouse-

Geiser corrections were used, and significant differences within the main factors were assessed using Bonferroni corrections. To compare nominal data (if subjects chose to eat or drink before sunrise, for example) the McNemar and Cochran tests were used.

Correlations between variables were assessed using the method of Bland and Altman (1995), a method which corrects for multiple pairs of values being obtained from each subject.

Exact P values are reported; significance was set as $P<0.05$, though occasions where $0.05<P<0.10$ have been reported as "marginally significant".

5.3. Results

5.3.1. Results from questionnaires.

5.3.1.1. Drinking

Comparisons between drinking and eating on the control and fasting days during the daytime were not made, since they were determined by the requirements of the protocol. However, it was possible to compare results before and after the period of fasting, to investigate preparations for, and recovery from, the period of fasting.

The number of subjects drinking or not drinking before 09:00h was compared on control and fasting days (Figure 5.1). There was a significant increase in the number of subjects drinking before the day of fasting (13 out of 18, compared with only 6/18 on the control day, $P=0.016$).

The reasons given for drinking are shown in Figure 5.2. The four reasons were cited by different numbers of subjects on both control (P=0.010) and fasting (P<0.0005) days. "Thirsty" was cited most frequently on both types of day; "Preparation" was cited only on fasting days, even though this difference was not significant (P=0.25) due to the small sample size.

There were too few occasions of "Not drinking" for statistical analysis of the reasons given to be performed.

All subjects drank after 18:00 h on both days. The reasons given for drinking are shown in (Figure 5.3). The four possible reasons were cited by different numbers of subjects on both control (P<0.0005) and fasting (P<0.0005) days. "Thirsty" was cited most frequently on both types of day; "Recover" was cited significantly more frequently on fasting days (P=0.002).

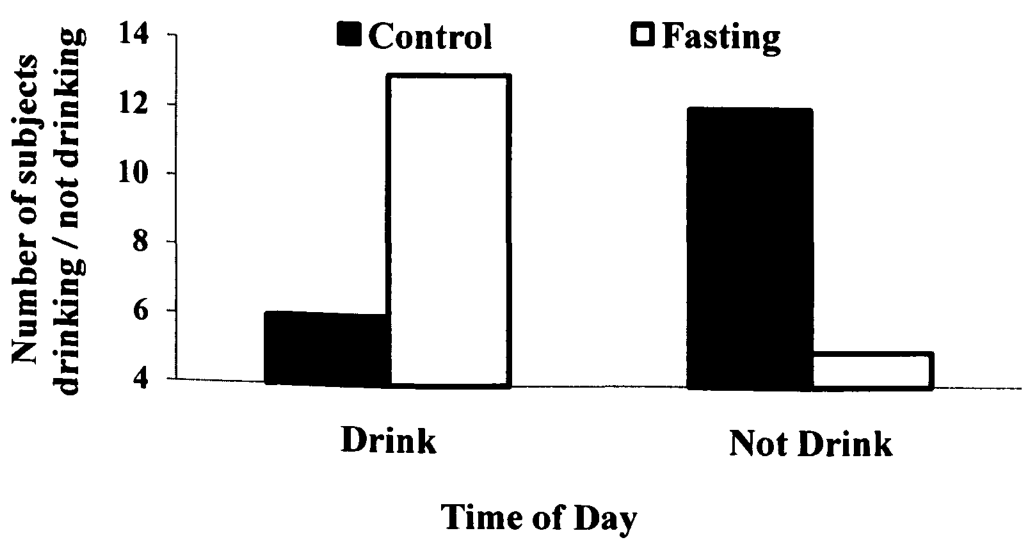


Figure 5.1. Number of subjects drinking/not drinking before 09:00 h

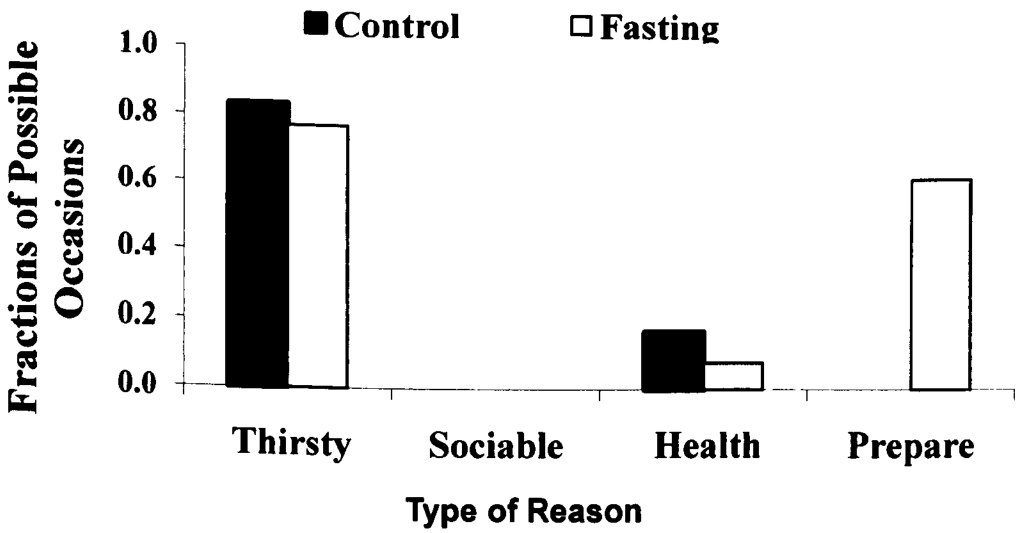


Figure 5.2. Reasons for drinking before sunrise. For calculation of "Fraction of Possible Occasions", see Methods.

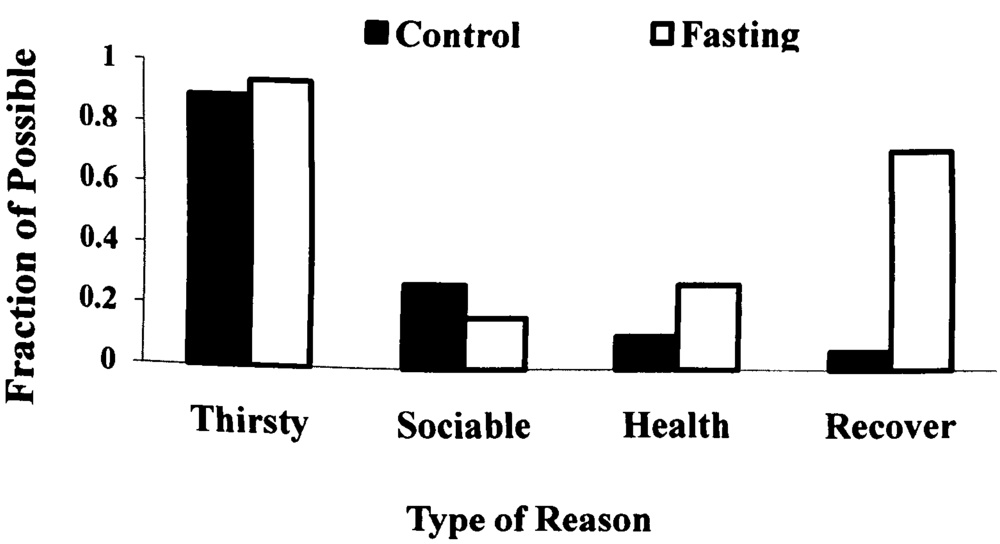


Figure 5.3. Reasons for drinking after sunset. For calculation of "Fraction of Possible Occasions", see Methods.

5.3.1.2. Eating

The number of subjects eating or not eating before 09:00 h was compared on Control and Fasting days (Figure 5.4). There was an increase in the number of subjects before 09:00 h on the day of fasting (12/18 compared with 7/18 on the Control day) but this difference was not significant (P=0.125).

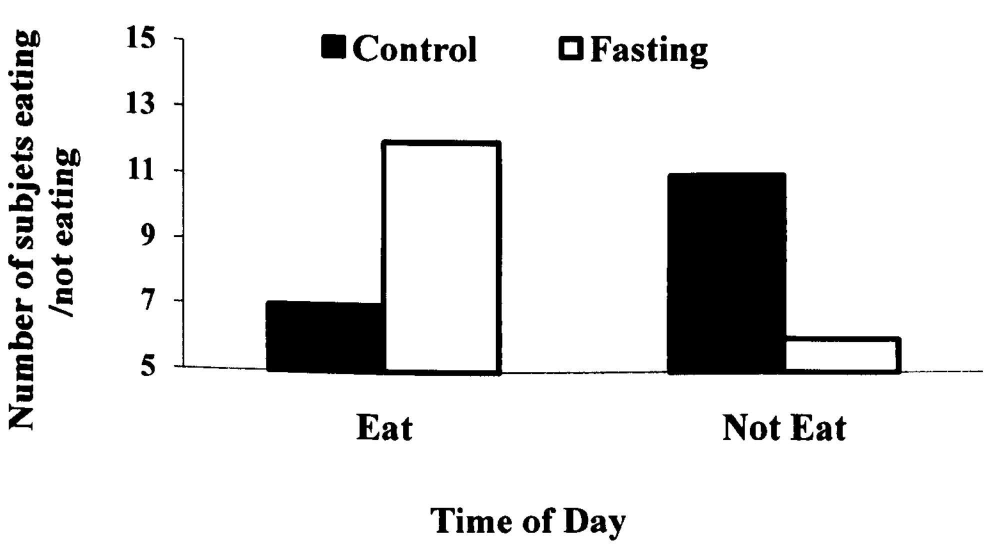


Figure 5.4. Number of subjects eating /not eating before 09:00 h.

The reasons given for eating are shown in Figure 5.5. The four reasons were cited by significantly different numbers of subjects on both control (P<0.0005) and fasting (P = 0.015) days. "Hungry" was cited most frequently on both days; "Prepare" was cited only on fasting days, even though this difference was not significant (P = 0.125).

There were too few occasions of "Not eating" for statistical analysis of the reasons given to be performed.

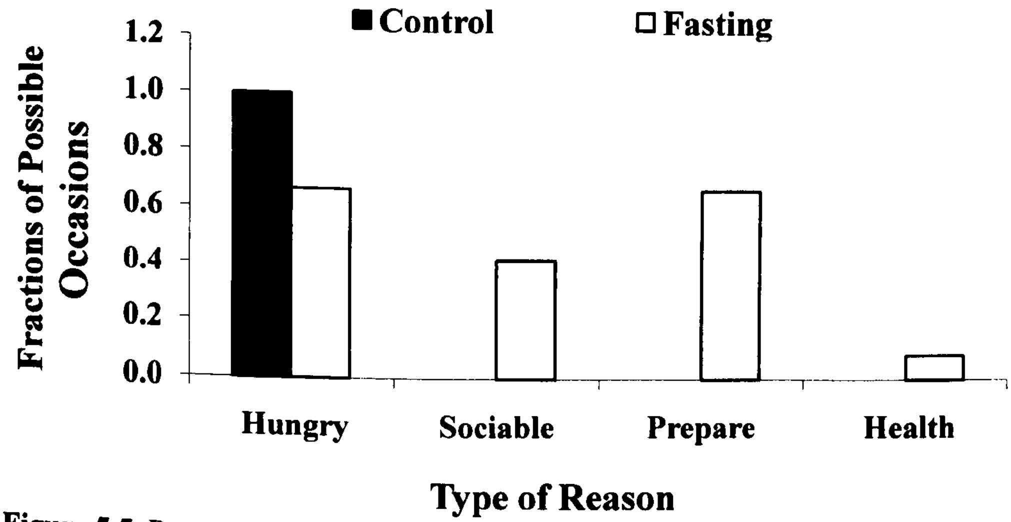


Figure 5.5. Reasons for eating before sunrise. For calculation of "Fraction of Possible Occasions", see Methods.

The reasons given for eating after 18:00 h are shown in Figure 5.6. The four reasons were cited by significantly different numbers of subjects on both control (P< 0.0005) and fasting (P< 0.0005) days. "Hungry" was cited most frequently on both days; "Recover" was cited significantly more frequently on fasting days (P = 0.002).

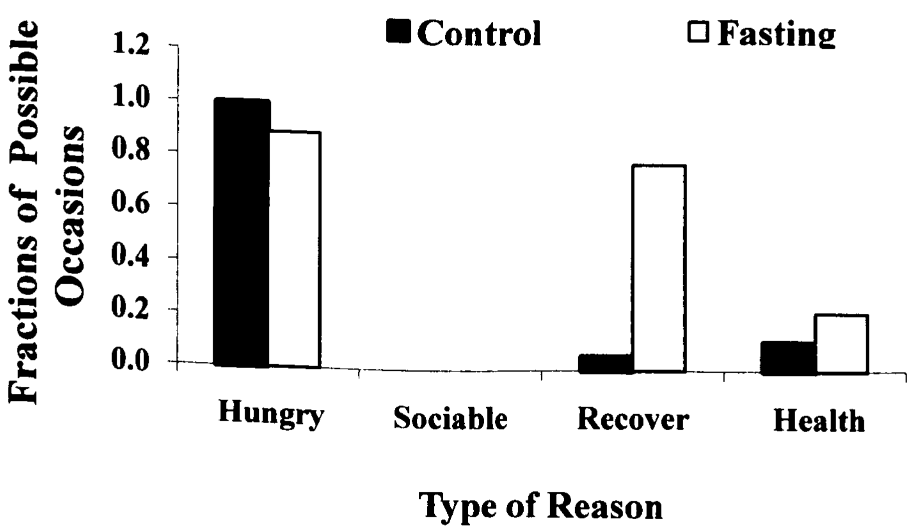


Figure 5.6. Reasons for eating after sunset. For calculation of "Fraction of Possible Occasions", see Methods.

5.3.1.3. Physical activity.

Physical activity done (Figure 5.7) showed a significant difference between the times of day ($F_{3.2, 54.7} = 8.3$, $P < 0.0005$), this increasing throughout the day from the morning to the evening, with low values in the morning and a higher plateau for the rest of the day. There were no significant differences between control and fasting days ($F_{1, 2} = 2.01$, $P = 0.17$) nor any significant interaction ($F_{3.3, 56.6} = 1.5$, $P = 0.22$).

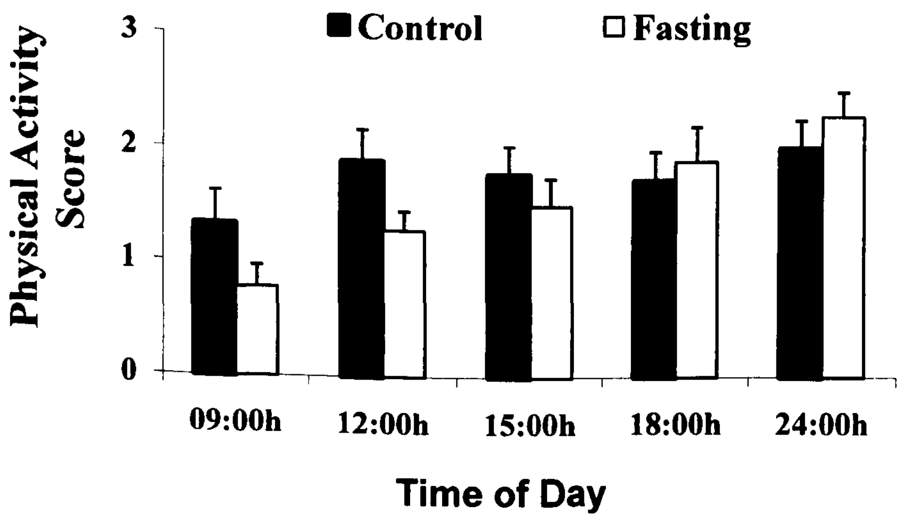


Figure 5.7. Effect of time of day upon physical activity score on control and fasting days. Mean + SE.

5.3.1.4. Mental activity

With mental activity also (Figure 5.8), there was a significant difference with time of day ($F_{2.9, 50.9} = 12.9$, $P < 0.0005$), with low values in the morning and a higher plateau for the rest of the day. There was no significant difference between control and fasting days ($F_{1, 17} = 2.8$, $P = 0.12$) and no significant interaction between the two factors ($F_{2.8, 46.8} = 0.99$, $P = 0.40$).

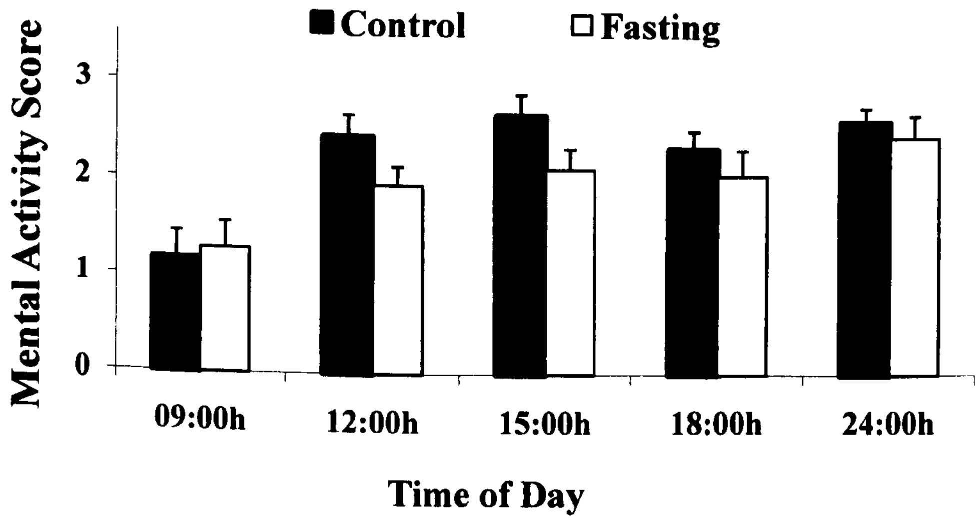


Figure 5.8. Effect of time of day upon mental activity score on control and fasting days. Mean + SE.

5.3.1.5. Social activity

Social activity done (Figure 5.9) showed a significant difference between time of day and of social activity ($F_{2.4, 40.2} = 14.7$, $P < 0.0005$), increasing in the same way as physical activity. Again, there was no significant difference between control and fasting days ($F_{1, 17} = 0.07$, $P = 0.79$) and no significant interaction ($F_{3.1, 53.0} = 0.54$, $P = 0.66$).

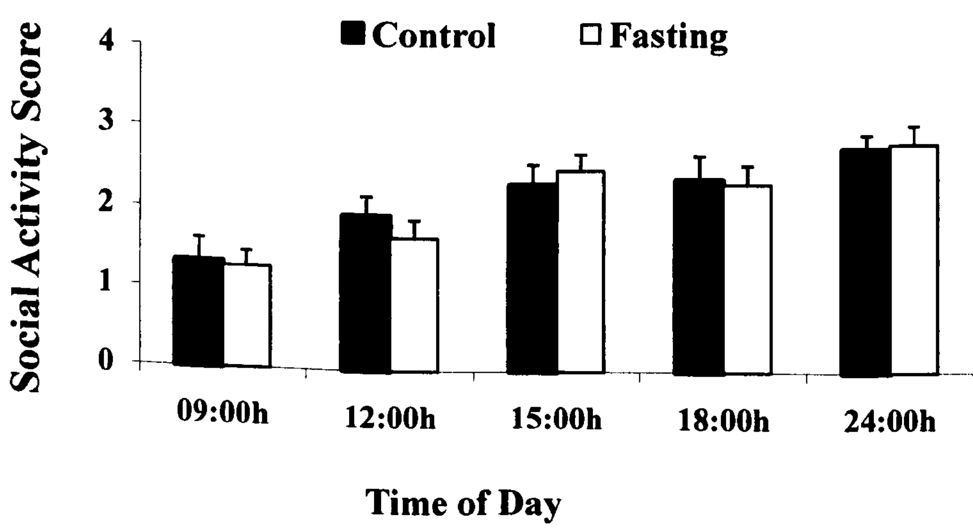


Figure 5.9. Effect of time of day upon social activity score on control and fasting days. Mean + SE.

5.3.1.6. Fatigue

Fatigue (Figure 5.10) showed no significant difference between control and fasting days ($F_{1, 17} = 2.1$, $P = 0.16$). However, there was a significant difference between the times of day ($F_{2.8, 47.6} = 4.8$, $P = 0.006$) with fatigue falling during the morning and then rising throughout the afternoon and later into the evening. Fatigue was significantly lower on retiring. There was no significant interaction between the factors Day x Time of day ($F_{2.6, 44.9} = 1.10$, $P = 0.3$

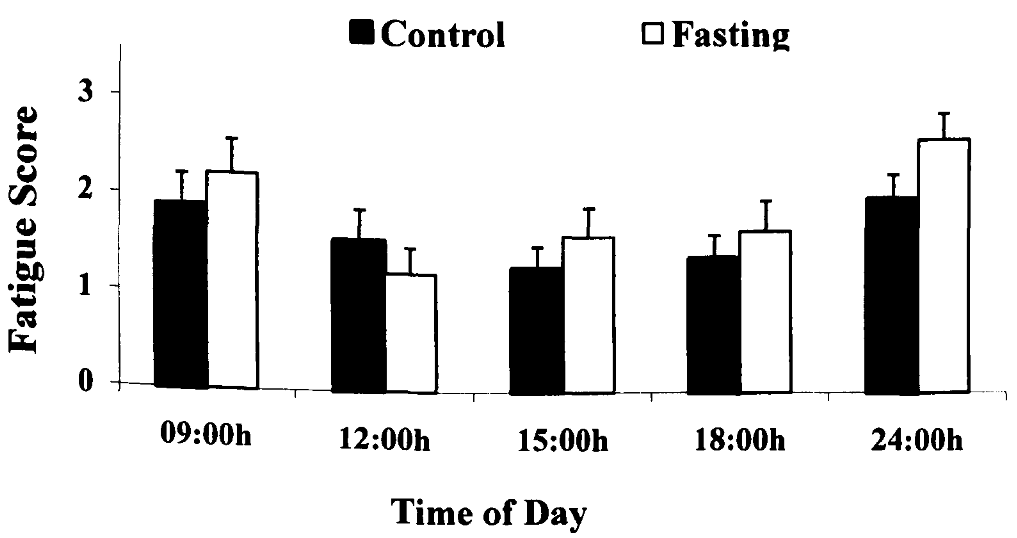


Figure 5.10. Effect of time of day upon fatigue on control and fasting days. Mean + SE.

5.3.1.7. Amount of physical activity wished for

The amount of physical activity that subjects wanted to perform showed a significant difference between control and fasting days ($F_{1, 17} = 5.5$, $P = 0.031$), lower values being associated with the fasting days (Figure 5.11). There was also a significant effect of Time of day ($F_{3.1, 51.8} = 5.1$, $P = 0.004$), values being highest around noon and the early afternoon and then declining after this time. There was no significant interaction ($F_{3.1, 53.0} = 0.76$, $P = 0.52$).

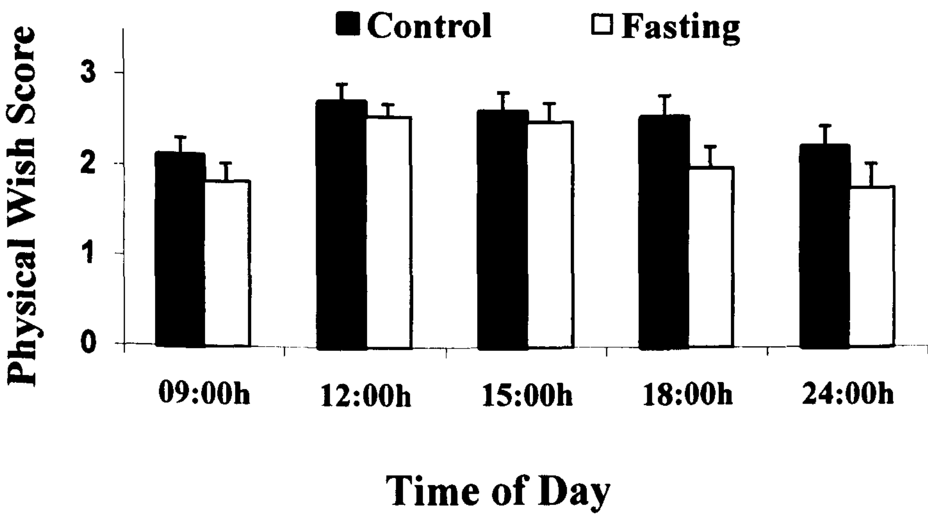


Figure 5.11. Effect of time of day upon the desire to perform physical work on control and fasting days. Mean + SE.

5.3.1.8. Amount of mental activity Wished for

Subjects wanted to do less on fasting than control days ($F_{1,\ 17} = 2.1$, $P = 0.017$), Figure 5.12. There was also a significant difference between the times of day ($F_{2.8,\ 47.2} = 7.5$, $P<0.0005$), the profile being very similar to that of the desire to perform physical activity (Figure 5.11). There was no significant interaction ($F_{3.5,\ 58.9} = 1.8$, $P = 0.15$).

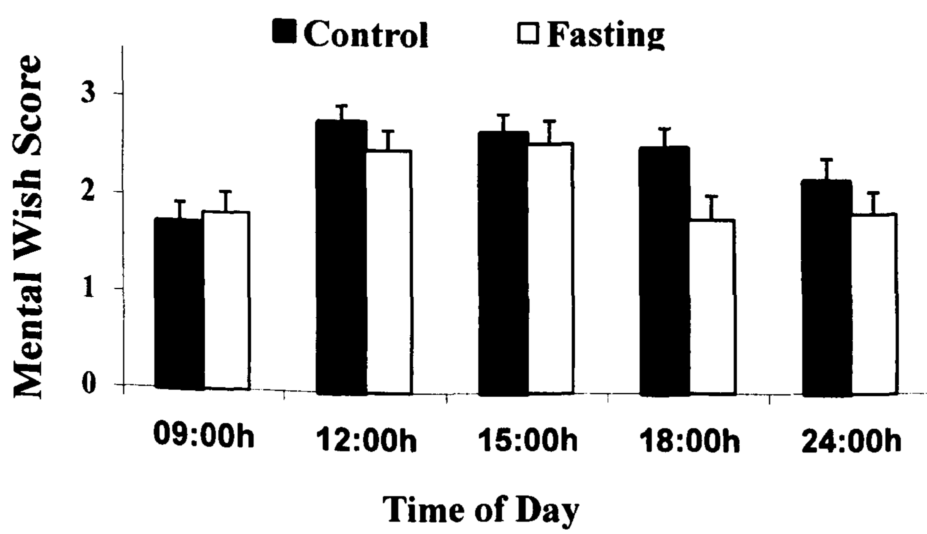


Figure 5. 12. Effect of time of day upon the desire to perform mental activity on control and fasting days. Mean +SE.

5.3.2. Urine osmolality

Figure 5.13 shows mean urine osmolalities during the course of the control and fasting days. There were significant effects of Day ($F_{1, 17} = 30.7$, $P<0.0005$), Time of day ($F_{2.3, 39.8} = 5.6$, $P = 0.005$) and a significant interaction between the Day x Time of day ($F_{2.5, 41.7} = 15.4$, $P<0.0005$). The results show that the urine was fairly concentrated after waking from the night's sleep. Urine osmolality then fell during the daytime on the control days, when daytime fluid intake was allowed, but tended to increase further during the daytime on the fasting day.

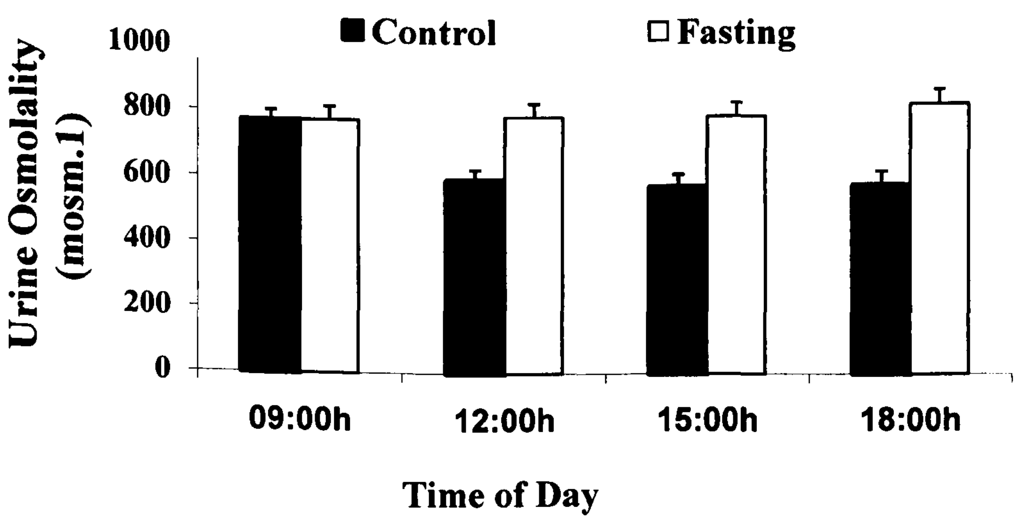


Figure 5.13. Mean +SE of Urine Osmolality under Non-Fasting and fasting conditions.

5.3.3 Physical and mental performance

5.3.3.1. Hand grip strength

For grip strength of the dominant hand, there were a no significant effects of Day ($F_{1, 7} = 1.5$, $P = 0.23$), Time of day ($F_{2.6, 44.4} = 1.7$, $P = 0.19$) or interaction between Time x Day ($F_{2.4, 41.4} = 1.4$, $P = 0.27$) (Figure 5.14).

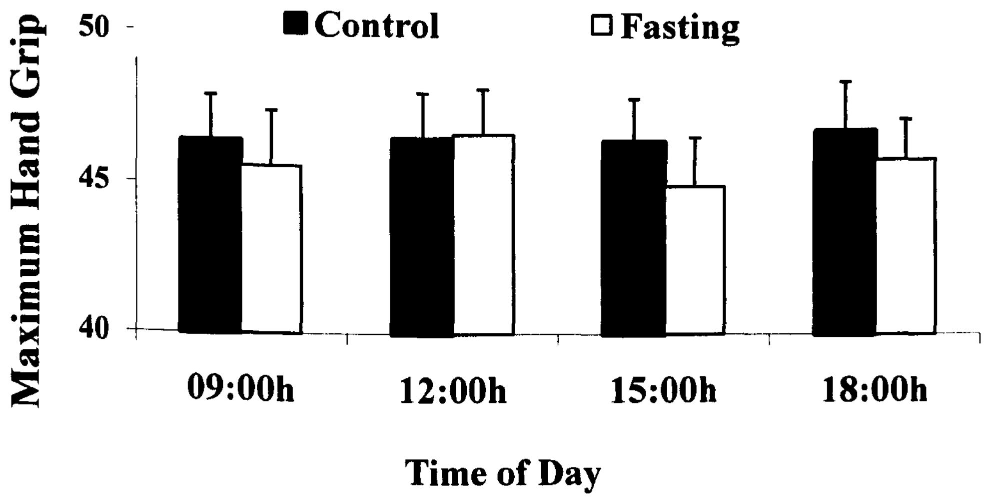


Figure 5.14. Maximum hand grip strength (dominant hand). Mean + SE.

5.3.3.2. Stroop test

For the Stroop test, there was a no significant difference for Day ($F_{1, 17} = 2.5$, $P = 0.13$) but there was a highly significant effect of Time of day ($F_{2.0, 33.7} = 10.8$, $P<0.0005$), with performance improving (time taken decreasing) as the day progressed (Figure 5.15). There was no significant interaction between Time x Day ($F_{2.4, 41.2} = 0.54$, $P = 0.62$)

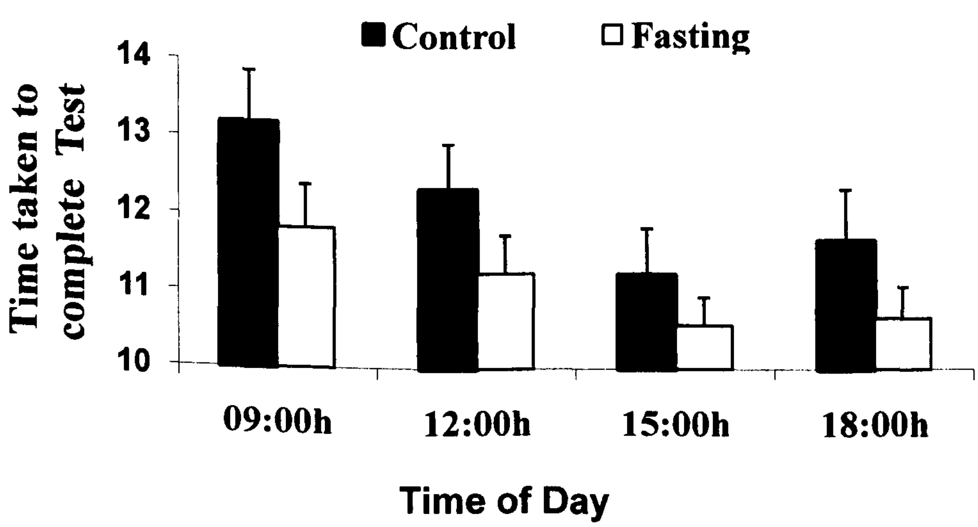


Figure 5.15. Time taken to complete test. Mean + SE.

5.3.3.3. Jump height

Figure 5.16 shows the results for jump height. There was no significant effect of Day ($F_{1, 17}$ = 1.4, P = 0.26) but a significant effect of Time of day ($F_{2.5, 43.2}$ = 11.2, P<0.0005), performance improving throughout the daytime and evening. There was a significant interaction between the two factors ($F_{2.4, 40.1}$ = 4.9, P = 0.009), the improvement during the daytime being less marked on the fasting day, particularly in the late afternoon.

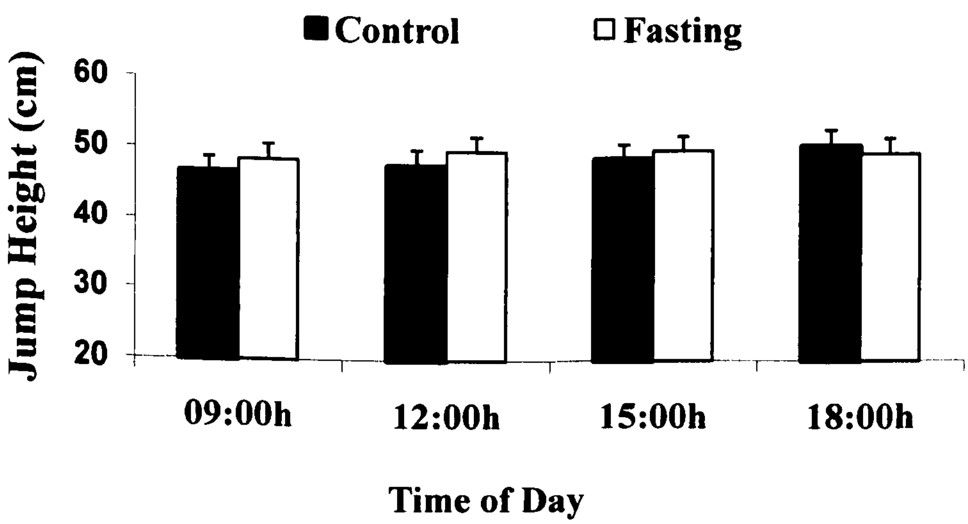


Figure 5.16. Maximum height jumped. Mean + SE.

5.3.3.4. Throwing darts

Total Score for darts 4-17 (see Figure 5.17) showed no significant effect of Day ($F_{1, 17}$ = 0.72, P = 0.41). There was also no significant time-of-day effect ($F_{2.5, 41.9}$ = 0.93, P = 0.42) but an interaction that was marginally significant ($F_{2.6, 44.8}$ = 2.3, P = 0.096), because the scores fell towards the end of the day during fasting only.

The number of misses ("zero" scores) was also analysed (Figure 5.18). Here also, there was no significant difference between Day ($F_{1, 17} = 0.06$, $P = 0.81$) or Time of day ($F_{2.2, 37.4} = 1.6$, $P = 0.21$). There was also no significant interaction ($F_{2.2, 38.2} = 1.12$, $P = 0.36$).

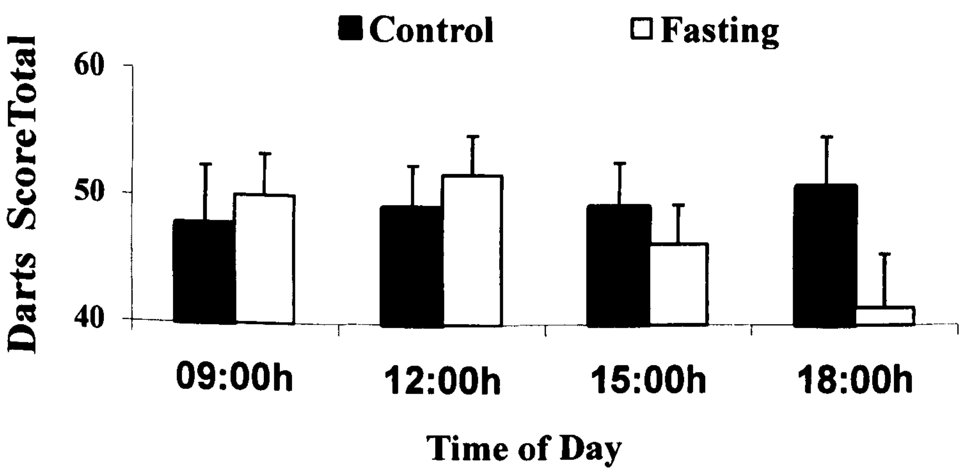


Figure 5.17. Total scores for darts 4-17. Mean + SE. For calculation of Score, see text.

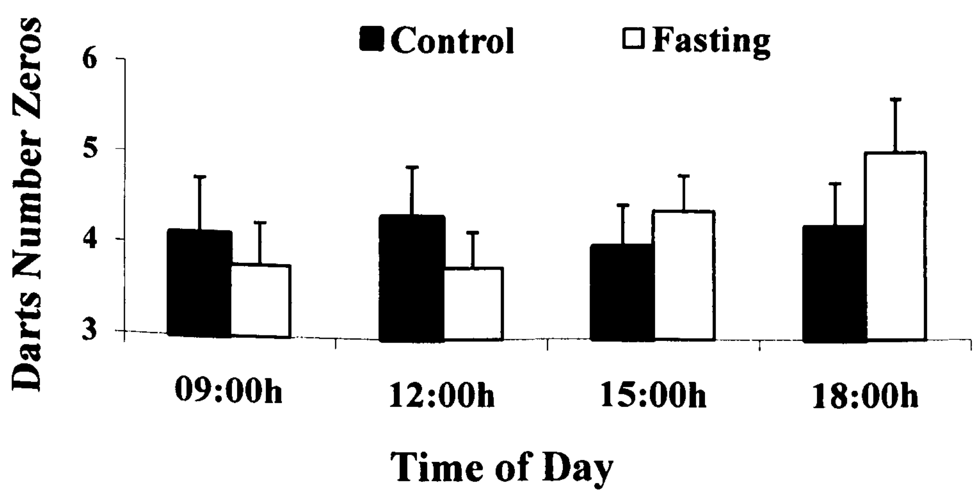


Figure 5.18. Mean number of zeros scored. Mean + SE

As with the other estimates of performance at the task of throwing darts, Mean score per hit (see Figure 5.19) showed no significant effect of Day ($F_{1,17} = 1.9$, $P = 0.19$), Time of day ($F_{2.5,42.3} = 0.58$, $P = 0.60$) or interaction between the two factors ($F_{2.6,44.9} = 1.7$, $P = 0.20$).

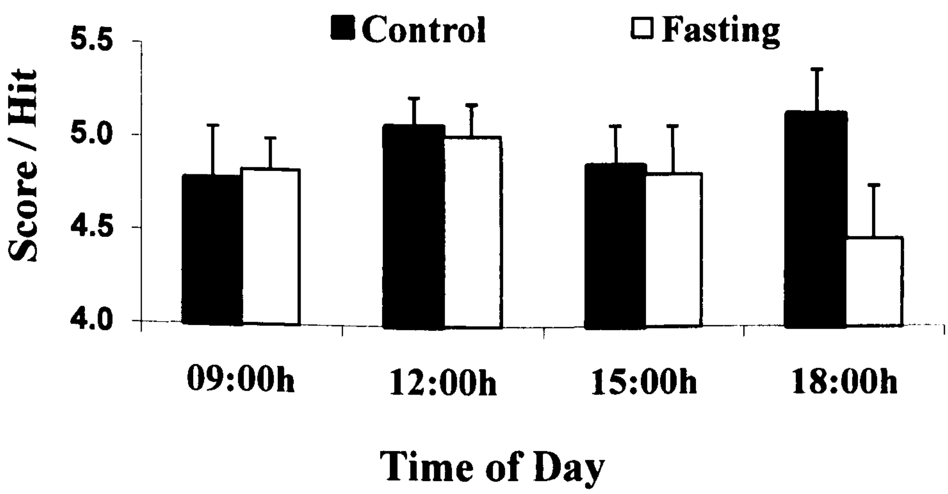


Figure 5.19. Mean score per hit. Mean + SE. For calculation of Score/Hit, see text.

5.3.4. Correlations between the variables.

To calculate these, the three scores for physical, mental and social activities actually performed were combined (ScoreP), as were the two scores for the wished-for amounts of physical and mental activity (ScoreW). In the correlations, all pairs of values from the four times of day and both control and fasting days were used. That is, each subject gave 8 pairs of values (09:00, 12:00, 15:00 and 18:00 h for both control and fasting days). The correlation matrix is shown in Table 5.1. As this Table indicates, the significant correlations were:

ScoreP vs. Fatigue=-0.16; ScoreP vs. ScoreW=+0.39; ScoreP vs. Stroop test=-0.20.

Fatigue vs. ScoreW=-0.62

ScoreW vs. Urine=-0.18

Darts total score vs. Darts number of zeros=-0.74

Stroop test vs. Grip strength=-0.24; Stroop test vs. Jump height=-0.32

It will be noted that there were no significant correlations between performance at darts and the other variables.

Table. 5.1. Correlations between the variables using the method of Bland and Altman (1995). Significant (P<0.05) values given numerically. Values in brackets indicate direction of correlation when marginally significant (0.10 > P > 0.05). Blanks indicate correlations non-significant (P>0.10).

Variables	ScoreP	Fatigue	ScoreW	Urine	DartsTotal	Darts Zeros	Stroop	Grip strength	Jump height
ScoreP	X	-0.16	0.39	(-)			-0.20	(+)	(+)
Fatigue	X	X	-0.62	(+)	(-)			(-)	(-)
ScoreW	X	X	X	-0.18	(+)	(-)	(-)	(+)	(+)
Urine	X	X	X	X	(-)	(+)		(-)	(-)
DartsTotal	X	X	X	X	X	-0.74	(-)	(+)	(-)
Darts Zeros	X	X	X	X	X	X			(+)
Stroop	X	X	X	X	X	X	X	-0.24	-0.32
Grip strength	X	X	X	X	X	X	X	X	(+)
Jump height	X	X	X	X	X	X	-0.32	X	X

5.4. Discussion

Many of the results confirm those obtained in the field study and pilot study; that is, there was clear evidence for changes during fasting to: drinking and eating habits, amounts of wished-for physical and mental activity, and hydration status of the body. By contrast, the changes observed in grip strength, performance at the Stroop test and jump test, and the ability to throw darts accurately was far less marked.

There was limited evidence to support the view that subjects made preparations before the time of fasting. More of them drank and ate before sunrise on the fasting than on the control day (Figures 5.1 and 5.4). The most common reason cited for drinking or eating was "Thirsty" or "Hungry" but "Prepare" was cited almost as frequently before the day of fasting (Figures 5.2 and 5.5), although the increases were not significant statistically. After sunset, all subjects ate and drank. Again, thirst and hunger were cited most frequently but "Recover" was cited significantly more frequently after the day of fasting and almost as frequently as thirst or hunger (Figures 5.3 and 5.6).

These preparations for fasting and recovery from it afterwards agree with the work previously found by us in subjects during Ramadan (Hakkou *et al.*, 1994; Waterhouse *et al.*, 2008a, 2008b, 2009 and also in Chapter 3). Whilst it can be argued that such preparations are intuitively likely, it must also be remembered that the fast lasted for one day only (unlike the case in Ramadan) and the subjects had no prior knowledge of the demands of Ramadan, none of them being Muslim. That is, as argued previously (Waterhouse *et al.*, 2008b), the link between fluid and food intakes and the amount of drink or food ingested tends to break down in these circumstances. In this respect, there is some similarity with intakes when the meal serves an important social function, when intakes might be greater than required (de Castro, 1987; Waterhouse *et al.*, 2005).

The subjects showed decreases in the types of activity in the daytime during fasting (Figures 5.7-5.9), though the differences were not statistically significant. Similar profiles were seen for the amounts of physical and mental activity that subjects wished to undertake (Figures 5.11, 5.12) but in these cases the differences were statistically significant. During the daytime, fatigue tended to be higher on the fasting day (Figure 5.10), even though not significantly so. These results lend limited support to those found by us previously (Waterhouse *et al.*, 2008b), indicating that subjects "spare" themselves when fasting; they support the view that fasting in Ramadan has negative effects upon fatigue (in part due to a fall of glucose intake) and performance in general (see, for example, Kadri *et al.*, 2000; Karaagaoglu and Yucecan, 2002; Leiper *et al.*, 2003; Roky *et al.*, 1999, 2000).

In the evening, the amounts of activity actually performed and wished-for activity tended to increase towards, or even beyond, values on control days, but these increases were not as marked as found previously (Waterhouse *et al.* , 2008a,2008b), the interaction terms not being statistically significant. This lack of increase after the end of the fast is likely to reflect the social role played by food intake (de Castro, 1987, 1997, 2000), a role that is more marked in the Muslim community during Ramadan than in non-Muslim students performing a single day of fasting.

Urine osmolality was quite high on waking on both days (Figure 5.13), indicating the development of dehydration during sleep. The subsequent fall during the daytime on control days is due to the intake of fluids during the daytime. By contrast, osmolality (and dehydration) continued to rise during the daytime on the fasting day, as would be expected. Such results have been found previously in Ramadan (Reilly and Waterhouse, 2007) and also agree with those found in the field study (Chapter 3, Waterhouse *et al.*, 2009) and the pilot laboratory-based study (Chapter 4). The observation that urine osmolality was not lower at 09:00h on the fasting day indicates that fluid intake before the start of the fasting period did not correct overnight dehydration. That is, even though subjects were more likely to drink before the start of the fasting period (see Figure 5.1), the amount of fluid they drank was insufficient. This lack of preparation for the demands of daytime fasting can be contrasted with the extra fluid intake observed in Ramadan in the field study (Chapter 3).

The lack of changes observed with grip strength (Figure 5.14) agrees with the findings from the pilot study (Chapter 4). In addition, no circadian rhythm was demonstrated on either day as well as no significant difference between the fasting and control days. It must be concluded either that this aspect of physical performance (maximal contraction of a limited muscle group) does not change with the amount of fasting undertaken in this study or that, as performed by these subjects, the test was not sufficiently sensitive. Since other studies have shown a circadian rhythm of grip strength (Reilly *et al.*, 1997), the latter reason (lack of sensitivity as performed in the current study) seems more likely.

Unlike grip strength, jump height rose significantly as the day progressed (Figure 5.16). In addition, the two days differed, with the rise on the fasting day being less marked. This difference was most marked at 18:00 h when the effects of fluid restriction are likely to have been most marked. Research involving vertical jumps in conditions of fluid and food restriction is sparse. However, Judelson *et al.* (2007) investigated the effect of hydration state on this measure and found no significant differences in vertical jump height in euhydration and hypohydration at 2.5% and 5% body mass reductions. This result differs from the present one. Possibly, it is because Judelson *et al.* achieved these levels not only by prohibiting water fluid intake but also requiring the subjects to perform an exercise-induced heat stress trial on the previous day. Nevertheless, the present finding has implications for those required to perform physically demanding tasks after an interval of fasting.

Considering physical performance in general, it has often been considered that muscle power is comparatively immune to effects of sleep loss and fasting (Reilly *et al.*, 1997). However, motivation to perform activities and neural control of movement are both affected negatively by sleep loss and fasting (Reilly and Waterhouse, 2009), and these factors might have contributed to the decline in jump height observed later in the day when fasting. Whatever the detailed explanation, the present findings reinforce the implications (see above) for those required to perform physically demanding tasks after an interval of fasting.

The Stroop test showed a clear circadian rhythm (Figure 5.15) with performance improving (time taken to finish the test decreasing) until about 15:00 h. These accords with other tests of mental performance which tend to peak slightly earlier than core temperature (Folkard, 1990). The reason generally given to explain the earlier peak than for core temperature is that mental performance is negatively affected by increasing time awake far more than is core temperature (Adam *et al.* , 2008); as a result, performance begins to fall any time from noon onwards as fatigue due to time awake increases. Mental performance is also adversely affected by sleep loss (Akerstedt *et al.*, 2007) and by fasting (Adam *et al.*, 2008). In the current study, performance was lower on the fasting day (Figure 5.15) but not by amounts that were statistically significant. This lack of statistical significance might reflect sample size.

With regard to throwing darts, no statistically significant rhythms were found in the any of the three methods used for scoring this task (total score, number of misses and score per hit, Figures 5.17-5.19, respectively). This contrasts with previous work (Edwards *et al.*, 2007a) where rhythms peaking in the afternoon have been found. Also, there were no statistically significant effects of fasting. Nevertheless, inspection of the Figures suggests that performance tended to improve during the course of the daytime on control days (total score, Figure 5.17 and score/hit, Figure 5.19) whereas, though rising in the first part of the fasting day (09:00 h and 12:00 h), it then deteriorated at 15:00 h and 18:00 h. This deterioration in comparison with the control day is shown by the falls in total score (Figure 5.17) and score per hit (Figure 5.19), and by the increased number of misses (Figure 5.18). Such differences are unlikely to be due to effects of time awake, which were almost identical on control and fasting days. Instead, the deterioration at the end of the daytime is more likely to reflect negative effects of fasting (Reilly and Waterhouse, 2007) and to bring the findings in agreement with those found in the jump test and discussed above. The high variability in the results (resulting in the lack of statistical significance) might have arisen in part from a learning effect, subjects differing in previous experience of throwing darts.

Whilst it is not possible to deduce causal links from correlations, the results from such analyses can be used to support or refute possible links that have been deduced from other studies of interactions between circadian rhythms, effects of sleep loss and restricted food and fluid intakes. For example, the following possible scenarios can be devised by considering the results in Table 5.1: if individuals are tired (Fatigue), they tend to perform less physical, mental and social activities (ScoreP) and wish to do less (ScoreW); and, if they are dehydrated (Urine osmolality), then one of the general effects of this is to feel less inclined to perform physical or mental tasks (ScoreW). There might also be correlations between different performance tasks, these both being affected by the same demands of a day of fasting. For example: the less activities that have been undertaken (ScoreP), the poorer will performance be at some tests (Stroop test); and poorer performance at one task (Stroop test) is likely to be associated with poorer performance at other tasks (Grip strength and Jump height). Clearly, much further work is required to assess these suggestions.

5.4.1. General conclusions

5.4.1.1. Effects of fasting

The present results accord with the concept that fasting is associated with a general decline in performance and the sense of well-being, a conclusion that has been drawn in previous studies (Waterhouse *et al.*, 2008a, 2008b), the current studies (Chapters 3 and 4 and Waterhouse *et al.*, 2009) and reviews of the field (Reilly and Waterhouse, 2007). Many of the correlations (Table 5.1) suggest that there are general deteriorations in several aspects of performance, a consideration of the correlations that were only marginally significant ($0.10>P>0.05$) adding some further support to the view that there exist many negative effects during food and fluid restriction. There are important implications of these findings for those who are fasting, particularly in Ramadan, and these will be considered in Chapter 7.

5.4.1.2. Relationship between field and laboratory-based studies

Considered as a whole, these results have several important implications for attempts to study some of the effects of Ramadan fasting in the laboratory. It can be concluded, first, that a laboratory-based study lasting only one day gives results that are comparable to those in Ramadan (when at least 28 successive days of fasting are involved); second, non-Muslim subjects often behave like Muslims with regard to preparations for fasting and recovery from fasting. When differences between field- and laboratory-based studies exist, particularly in the evening, it seems to be at least partly due to the lack of a communal social element, something that is strong amongst Muslims in Ramadan.

In conclusion, a protocol which uses a single day of fasting in the laboratory environment is a useful tool for investigating the kind of problems that will arise in Ramadan. Also, subjects can be non-Muslim (and so have no first-hand experience of fasting in Ramadan). The advantages of such a protocol are that the choice of subjects is far less limited and also that more detailed tests can be performed in the laboratory than is possible in the field.

5.4.1.3. Measurement tools

However, some results were not as predicted, particularly those for grip strength (no circadian rhythm nor effect of fasting) and throwing darts, where there were marginal effects of fasting but no circadian rhythm). The lacks of effect of fasting might indicate that the attributes required for such tests were not affected adversely by the amounts of food and fluid restriction that were used. However, this is slightly surprising since many aspects of athletic performance have been found to be worse in Ramadan (Reilly and Waterhouse, 2007). Of more concern, however, is the failure to demonstrate circadian rhythmicity in these variables, since such rhythms have been found in previous studies (Reilly *et al.*, 1997; Edwards *et al.*, 2007). The explanation for this is not certain but it suggests that the variability associated with the tasks was higher than that normally found. Lack of training in the tasks before the experimental trials and/or insufficient supervision are two possible explanations, but these suggestions are speculative.

5.4.1.4. Problems in these types of study

There is another problem that is common to all the studies (both field- and laboratory-based) that have been described so far. It results from the subjects being free to choose many aspects of their lifestyle, including whether or not to drink before sunrise and after sunset and, if drink or food was taken, its type and amount. For example, some subjects drank on both occasions, some on neither and some on only one occasion. Such differences increase the amount of "noise" in the data and also limit some of the statistical analyses that can be performed. Other differences exist; for example, the amount of physical or mental activity actually performed will not have reflected the individuals' desire to perform physical or mental tasks but rather their commitments, whether these are to work, study or child-rearing. This difference between what was done and what would have been preferred to have been done is clear by comparing the results of Figures 5.7-5.9 (actual performance) with those of Figures 5.11 and 5.12 (wished-for activity). Such differences are part of normal living, of course – the "real world" - but they complicate analysis of the results as well as having important practical implications for those fasting.

One improvement would be to allow subjects to behave as they wished (subject to the constraints of the protocol) but to ensure that they behaved in the same way for both the control and fasting days. In addition, more control of the hours before the start of the protocol (the food eaten the night before and the amount of sleep, for example) would be advantageous due to its reducing these further sources of variation. If subjects adhered to such a protocol, this would form the background to interventions in which the length of time spent fasting could be controlled. Such an intervention study would enable a clearer assessment of the effects of fasting to be obtained. In practice, adhering to all these requirements is difficult for subjects living normally. However, at least some of the requirements can be met more easily and such a study is described in Chapter 6.

Chapter 6

An Intervention Study with Controlled Time for Sleep and Variable Length of Fasting

Liverpool John Moores University

6.1. Introduction

The results from previous field studies (Waterhouse *et al.* , 2008a,2008b) and from the field study (Chapter 3) and two laboratory-based studies (Chapters 4 and 5) discussed so far in this thesis have enabled a clearer picture of effects arising from the demands of fasting during the course of a day in Ramadan to be obtained. However, the studies are restricted in that they are descriptive only. Moreover, subjects have been free to choose their activities, times of sleep and naps and, apart from when fasting, the timing and type of food and fluid taken in. Whilst this reflects the position in Ramadan, it produces interpretive difficulties. Thus, it cannot be deduced if a measured change in a variable (mental or physical performance, for example) is due to lack of food and fluid intake and/or changed fatigue due to altered sleep times. As indicated in the literature review, any of these factors could lead to the observed results.

The present study, an intervention study, has been planned to deal with this limitation. A variety of variables will be measured at three times of day after subjects have undergone different lengths of prior fasting. By contrast, food intake during the evening before the test day (supper) and the length of sleep will be unchanged. The intervention will be in three parts, the lengths of fasting being 4 h, 8 h and 16 h, these being administered in random order for the group as a whole.

With such a protocol, any changes in the measured variables with length of fasting cannot result from any change in sleep; rather, they must reflect the duration of prior fasting. The analysis of the results will focus upon the effects of specific durations of fasting upon these variables. It is hypothesised that general performance will deteriorate with fasting, the amount of deterioration being in proportion to its duration. Because there is no change in the amount of prior sleep, sleep loss (found in the other studies and complicating interpretation of the results) cannot be a possible cause in the current study.

6.2. Methods

6.2.1. Subjects

Twelve subjects (8 males and 4 females, mean age 34.4 (SD=3.2) years, height 1.74 (SD=0.09) m took part in the study. Subjects were recruited from the student population at Liverpool John Moores University by word-of-mouth. Subjects volunteered to participate in the study with the understanding that participation was not monetarily compensated and they could withdraw at any time without giving any reason and with no negative consequences.

6.6.2. Protocol

For the two days before each of the three parts, the subjects' normal sleep times were observed and their eating habits were normal (breakfast, lunch and evening meal), with a small supper before retiring. Performance and other variables (see below) were assessed at 08:00, 12:00 and 16:00 h. The intervention, which was designed to change the length of fasting before the measurements made at 16:00 h, consisted of three parts, the order of them being randomised for the group as a whole. The three parts were:

A. Supper before retiring but no breakfast and no lunch, and no fluid intake apart from a glass of water at 08:00 h - a fasting period, by 16:00 h, of 16 hours. This is called the Fasting condition.

B. Supper before retiring and a standard breakfast (at 08:00 h) including a glass of water, but no lunch or other fluid intake - a fasting period, by 16:00 h, of 8 hours. This is called the Breakfast condition.

C. Supper before retiring, breakfast (at 08:00 h) including a glass of water, and lunch (at 12:00 h) - a fasting period, by 16:00 h, of 4 hours. This is called the Control condition.

6.2.3. Measurements and apparatus

On the three days of the experiment, subjects came to the laboratory for testing at 08:00, 12:00 and 16:00 h. On each occasion, many of the same variables as used in Chapter 5 (the second laboratory-based study) were measured but further variables were added to increase the range of the investigation and its value to studies of Ramadan.

6.2.3.1. Actimetry

This was measured from 20:00 h on the day before each experimental day. This is a non-invasive and objective measure of physical activity; it has been widely used to investigate amounts of physical activity, and to compare waking activity with sleeping inactivity (Carvalho-Bos *et al.*, 2003). The apparatus (Actiwatch AW4 Software England) was worn on the non-dominant wrist from just before suppertime on the day before the experiment until 17:00 h at the end of the experiment. It was set to record activity at 1-min intervals. The activity record was down-loaded onto a laptop computer for later analysis each time the subject came to the laboratory.

The other variables measured, and the order in which they were given, were:

6.2.3.2. Questionnaires.

These were modified versions of those used previously (Waterhouse *et al.*, 2008) and assessed food and fluid intakes, sleep, and subjective estimates of mood, mental performance and physical performance.

6.2.3.3. Body mass and urine osmolality.

In addition to body mass (Seca 702, Seca GmbH & Co.KG, Hamburg, Germany), the indirect measure of water content, urine osmolality (Osmocheck Pocket Pal OSMO, Vitech Scientific Ltd, Japan) was measured.

6.2.3.4. Performance measures - throwing darts.

This is a measure of hand-eye co-ordination (see Edwards *et al.*, 2007, 2009). The subjects threw darts at a circular target (20 cm diameter) placed on the wall at a distance of 2.37 metres; the target consisted of 10 concentric rings of diameter 2 cm, 4 cm, 6 cm,......20 cm. Participants were instructed to stand 2.37m away from the target (see Appendix 5.1) and to throw one dart (Unicorn Precision darts, 24 g, Unicorn Products Ltd, England) a total of 20 times at the target. After each throw, the score for each dart was measured (according to which of the rings the dart was in) and then the dart was collected for the next throw. When a dart missed the target, the score was recorded as zero (a "miss"), regardless of the size of the miss (see Appendix 5.1).

6.2.3.5. Exercise.

This was on a cycle ergometer (Ergo Bike Premium 8i from Germany's Doum Dedronic). The trial started at 120 watts and then increased by 10 watts per minute to a final value of 160 watts which was maintained for the last minute, the trial taking 5 min to complete. The rate of pedalling was chosen by the subjects.

Before and immediately after the bout of exercise, the respiratory exchange ratio (RER), blood lactate and heart rate were measured. For the measurement of RER, Douglas bags and standard equipment for measuring gas volume and oxygen and carbon dioxide concentrations (Servomax Gas Analyser, Cranlea and company, Birmingham) were used (see Appendix 6.1). Blood lactate was taken from a finger-prick sample and analysed by a Lactate Pro Test Meter (Model LT7010, Arfray Inc, Kyoto Japan). Standard healthcare precautions were taken for blood sampling and analysis (See Appendix 6.2). Heart rate was measured using a monitor (Polar, Kempele, Finland) strapped to the chest and a data logger attached to the subject's wrist.

Immediately after exercise, subjects were asked to assess their perceived exertion after exercise using the Borg scale (Borg, 1998) - see Appendix 6.3.

6.2.4. Treatment of results

6.2.4.1. Questionnaires

The results were treated as described in Chapter 3.

6.2.4.2. Actimetry

The actimetry record was divided into three sections: the middle three hours of sleep (assessed from the activity record which clearly indicated times of retiring and rising), a two-hour "morning" section (09:00 h to 11:00 h) and a two-hour "afternoon" session (13:00 h to 15:00 h). For each of these, the summed activity count was used.

6.2.4.3. Darts

The first and last three throws were excluded, to allow for any effects due to ''getting going'' and being ''nearly finished'' (Edwards *et al.,* 2007). The participants were not aware that the results from some of the throws would not be analysed. The central 14 scores were used to assess accuracy in two ways: the total score and the number of errors (zero scores). For more details, see Appendix 5.1.

6.2.5. Statistics

Unless stated otherwise, the data were analysed by the means of the Statistical Package for Social Science (SPSS) for Windows, version 17, using a two way Analysis of Variance (ANOVA) with repeated measures model. The main factors were Condition (3 levels: Control, Fasting and Breakfast) and Time of Day (3 levels: 08:00, 12:00 and 16:00 h). To correct for violations of sphericity, the degrees of freedom were corrected by using either Huynh-Feldt (>0.75) or Greenhouse-Geisser (<0.75) in accordance with two way repeated measures ANOVA assumptions (Field, 2000). Significance was set as $P<0.05$.

6.3. Results

6.3.1. Questionnaires

6.3.1.1. Sleep times and food and fluid intakes

The questionnaires were inspected to ensure that the subjects had adhered to the protocol with regard to food and fluid intakes. Also, it was possible to assess that each subject's time in bed was similar on the three occasions (less than 30 min differences for any subject), and that no daytime naps were taken during the experimental days.

By comparing the fluid and food intakes during supper the day before each experiment, it was also possible to assess the types of fluid drunk (Figure 6.1). Water was almost universally drunk, tea or coffee were drunk about 0.6 of possible occasions and fruit juice on about 0.4 of possible occasions. These differences in frequency of intake between types of drink were significant (P=0.01, Cochran) but the frequencies did not differ significantly between the three parts of the experiment (P=0.51, Cochran), again indicating that subjects adhered to the requirements of the protocol.

6.3.1.2. Activities performed

There was a significant difference between Control, Breakfast only and Fasting days in the amount of physical activity performed ($F_{1.7, 19.2} = 9.46$, $P = 0.002$), this decreasing when fasting (Figure 6.2A). There was also a highly significant effect of Time of day ($F_{1.9, 21.3} = 18.4$, $P<0.0005$), activity being least at 08:00 h. There was no significant interaction between Day x Time of day ($F_{2.9, 32.3} = 0.17$, $P = 0.91$).

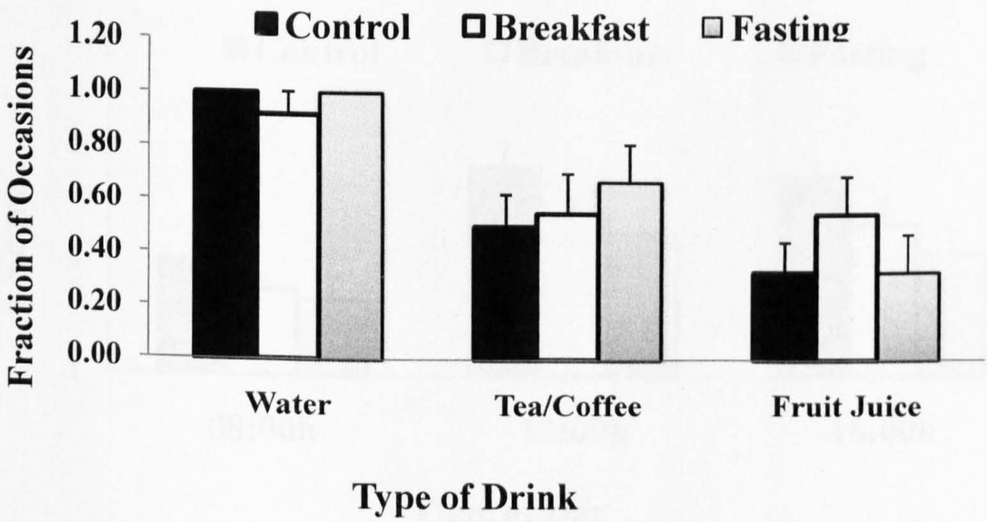


Figure 6.1. Fraction of possible occasions that different types of fluid were drunk during supper just before the Control, Breakfast and Fasting days. For calculation of "Fraction of Possible Occasions", see Chapter 5.

Mental activity also showed a significant effect of Day ($F_{1.5, 16.3} = 5.65$, $P = 0.020$), being marginally less than on control days when no breakfast and/or lunch was eaten (Figure 6.2B). There was a significant effect of Time of day ($F_{1.8, 19.4} = 20.16$, $P<0.0005$), values at 08:00 h being lower than at the other two times of measurement. There was no significant interaction between the two factors ($F_{2.8, 30.9} = 0.14$, $P=0.93$).

Social activity showed a significant difference between the three types of day ($F_{1.7, 18.8} = 4.78$, $P = 0.025$) with values on the control day being greater than when fasting (Figure 6.2C). There was a significant difference between times of day ($F_{1.7, 19.0} = 5.26$, $P = 0.018$), activity at 08:00 h being less than at the other two times of day. There was no significant interaction between the factors ($F_{2.8, 30.6} = 0.23$, $P = 0.87$).

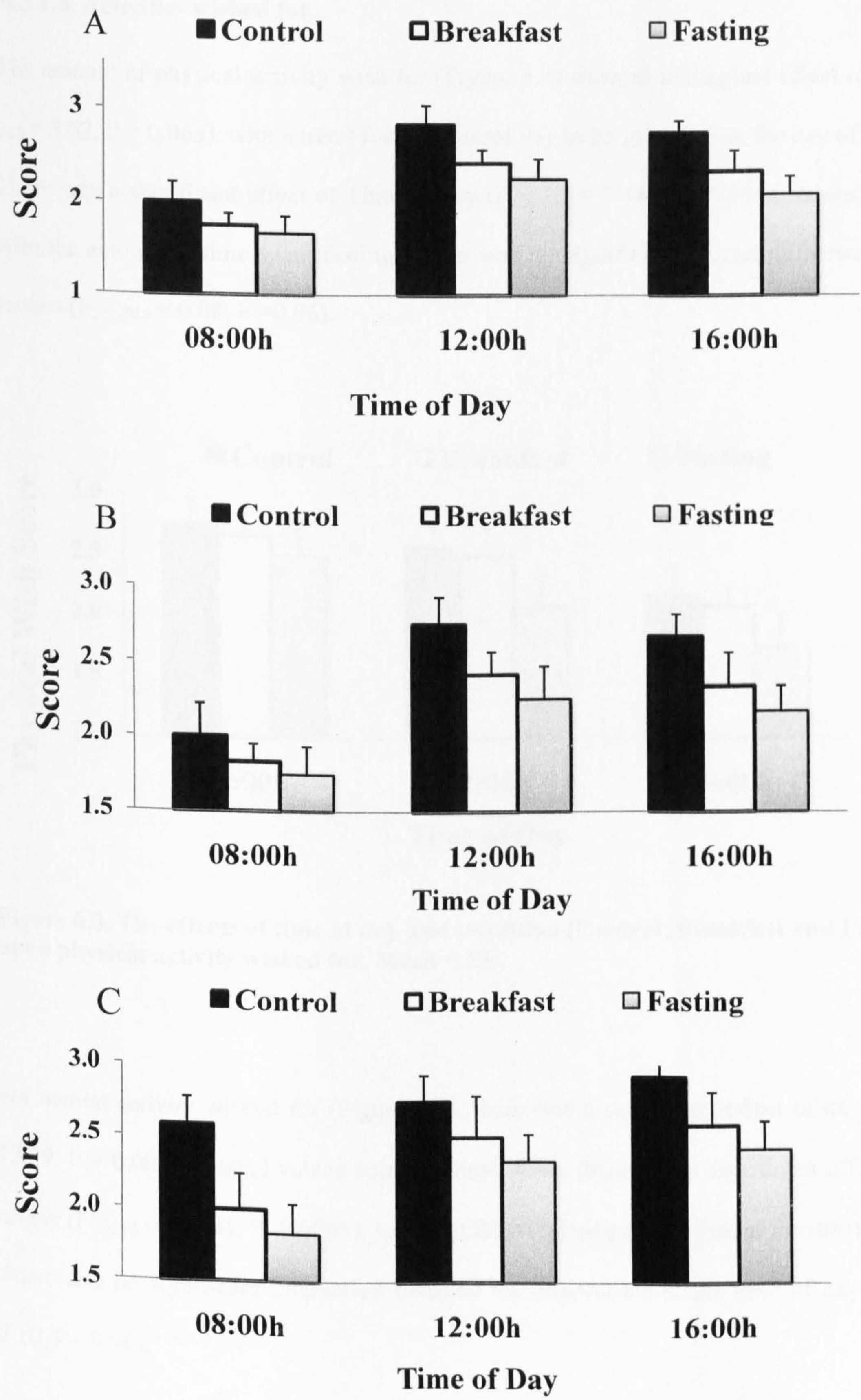


Figure 6.2. Scores for (A) physical activity, (B) mental activity and (C) social activity during Control, Breakfast and Fasting days. Mean + SE.

6.3.1.3. Activities wished for

The amount of physical activity wish for (Figure 6.3) showed a marginal effect of Day ($F_{1.5, 16.9} = 3.52$, $P = 0.063$), with a trend for the control day to be greater than the day of fasting. There was a significant effect of Time of day ($F_{1.2, 13.2} = 7.48$, $P = 0.014$), values decreasing with the amount of time spent fasting. There was no significant interaction between the two factors ($F_{2.7, 29.8} = 0.08$, $P = 0.96$).

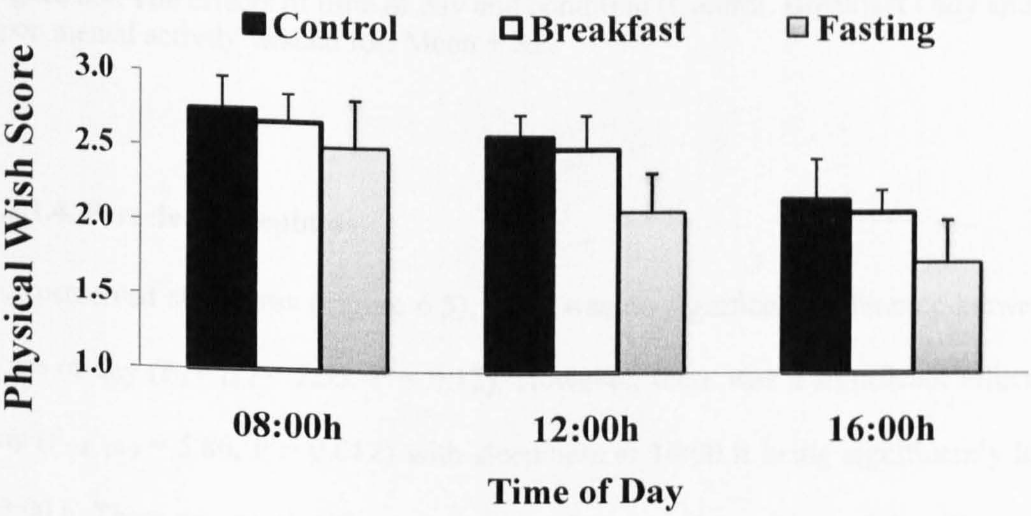


Figure 6.3. The effects of time of day and condition (Control, Breakfast and Fasting) upon physical activity wished for. Mean + SE.

For mental activity wished for (Figure 6.4), there was a significant effect of day ($F_{1.8, 19.5} = 12.29$, $P = 0.001$), control values being highest. Also, there was a significant effect of Time of day ($F_{1.9, 20.7} = 24.04$, $P<0.0005$), values at 08:00 h being lower than at the other two times. There was no significant interaction between the condition and the time of day ($F_{3.0, 33.0} = 0.10$, $P = 0.96$).

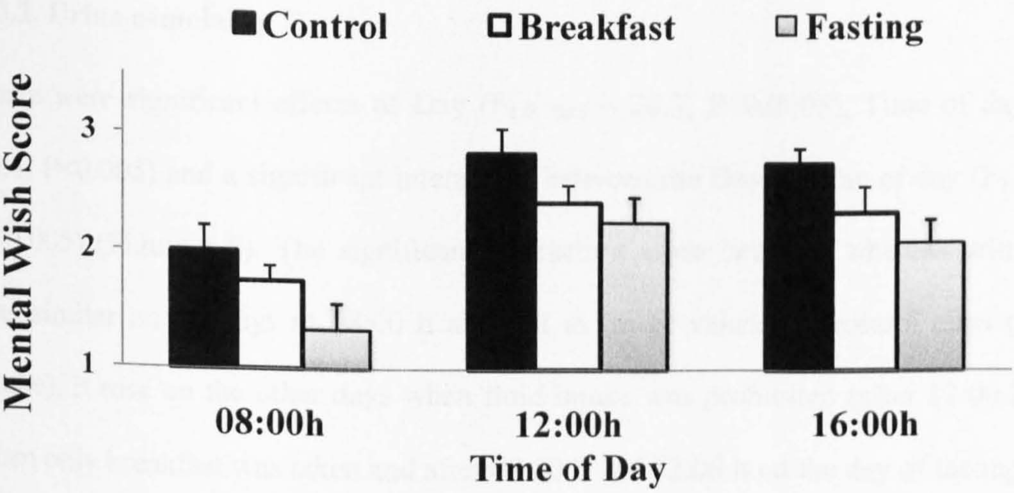


Figure 6.4. The effects of time of day and condition (Control, Breakfast Only and Fasting) upon mental activity wished for. Mean + SE.

6.3.1.4. Perceived sleepiness

For perceived sleepiness (Figure 6.5), there was no significant difference between the three types of day ($F_{1.5, 16.4} = 2.53$, P = 0.12). However, there was a significant effect of Time of day ($F_{1.8, 19.9} = 5.86$, P = 0.012) with sleepiness at 16:00 h being significantly lower than at 08:00 h. There was no significant interaction between Day x Time of day ($F_{2.8, 30.6} = 0.21$, P= 0.88).

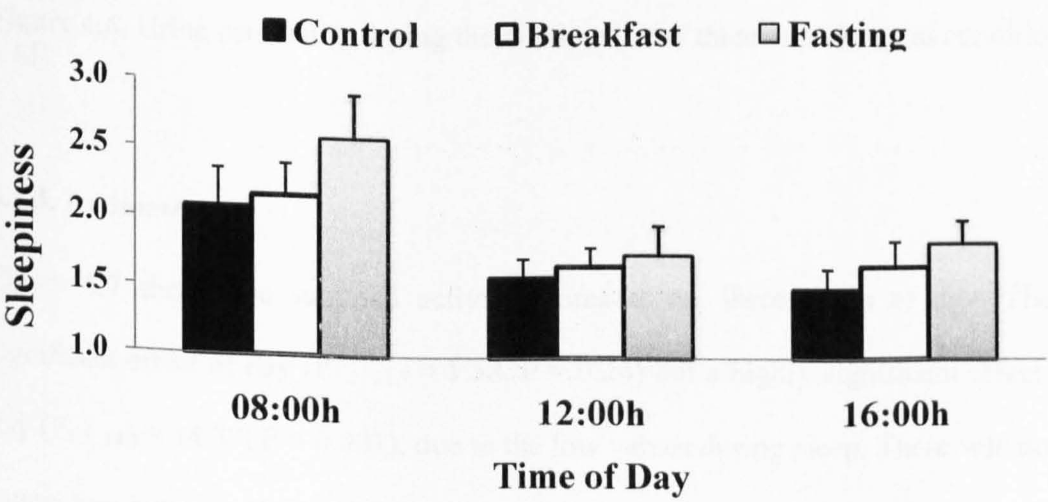


Figure 6.5. The effects of time of day and condition upon perceived sleepiness. Mean + SE.

6.3.2. Urine osmolality

There were significant effects of Day ($F_{1.9,\ 20.8} = 28.3$, $P<0.0005$), Time of day ($F_{1.2,\ 13.5} = 10.4$, $P<0.005$) and a significant interaction between the Day x Time of day ($F_{2.8,\ 30.9} = 28.1$, $P<0.005$) (Figure 6.6). The significant interaction arose because, whereas urine osmolality was similar on all days at 08:00 h and fell to lower values on control days (due to fluid intake), it rose on the other days when fluid intake was prohibited (after 12:00 h on the day when only breakfast was taken and after 08:00 h and 12:00 h on the day of fasting).

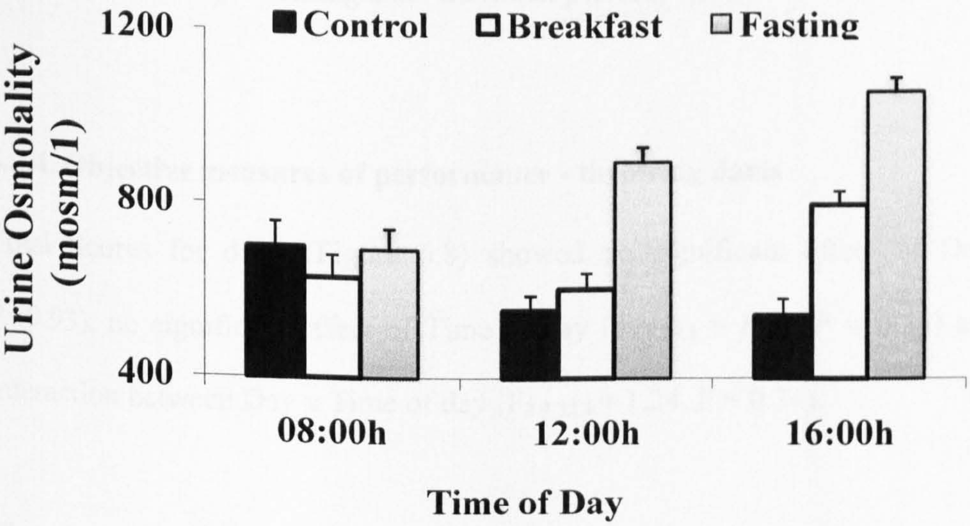


Figure 6.6. Urine osmolality during the daytime in the three experimental conditions. Mean + SE.

6.3.3. Actimetry

Figure 6.7 shows the summed activity scores at the three times of day. There was no significant effect of day ($F_{1.1,\ 11.7} = 1.58$, $P = 0.24$) but a highly significant effect of Time of day ($F_{1.3,\ 14.7} = 14.87$, $P = 0.001$), due to the low values during sleep. There was no significant interaction between Day x Time of day ($F_{2.2,\ 24.3} = 1.08$, $P = 0.36$), indicating that objective measures of activity showed no differences between the three experimental days.

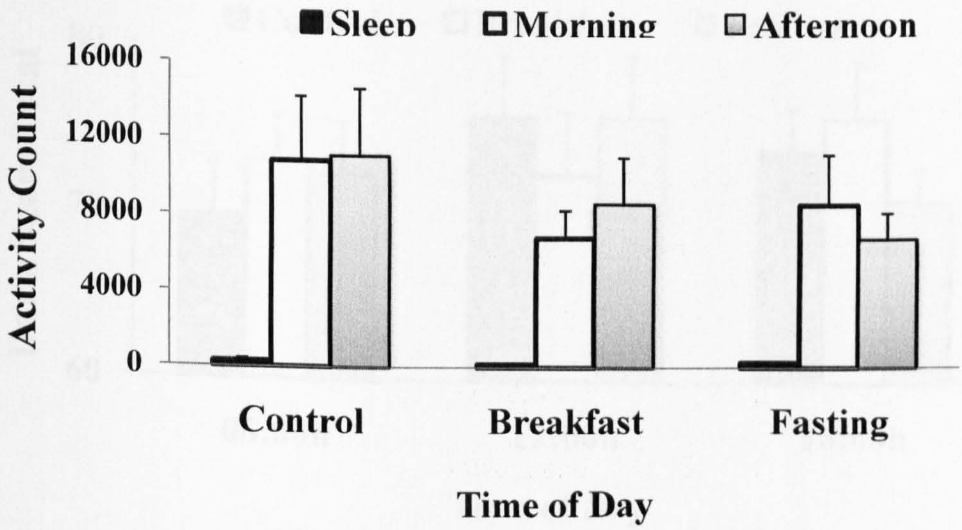


Figure 6.7. Effects of time of day and conditions upon summed activities. Mean + SE. For definitions of Sleep, Morning and Afternoon periods, see text.

6.3.4. Objective measures of performance - throwing darts

Total Scores for darts (Figure 6.8) showed no significant effect of Day ($F_{1.8,19.3}$=0.05; P=0.93), no significant effect of Time of day ($F_{1.7,19.1}$ = 1.12, P = 0.35) and no significant interaction between Day x Time of day ($F_{2.9,32.4}$ = 1.24, P = 0.34).

The number of misses, or 'zero' scores (Figure 6.9), also showed no significant effects of Day ($F_{1.9, 21.6}$=1.07; P=0.34), Time of day ($F_{1.8, 19.9}$ = 0.65, P = 0.52) or interaction ($F_{2.8, 30.4}$=1.70, P = 0.19).

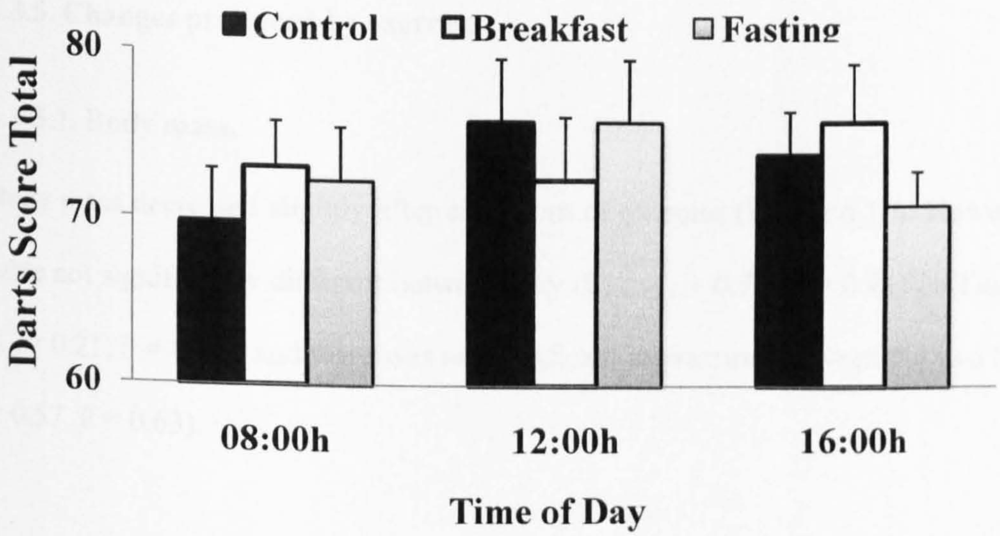


Figure 6.8. Total scores for darts 4-17 on control, breakfast only and fasting days at the 3 times of measurement. Mean + SE.

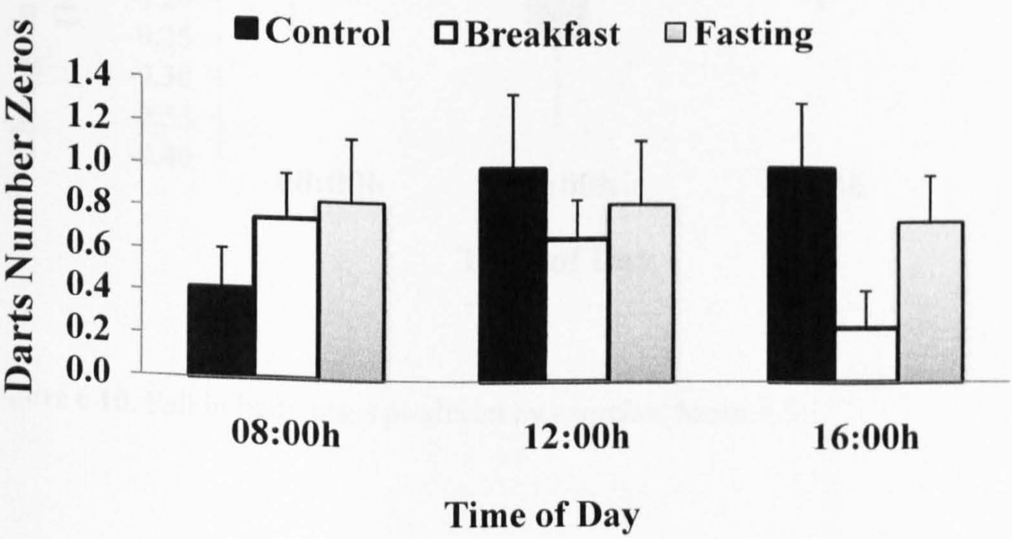


Figure 6.9. Mean number of zeros scored. Mean + SE.

6.3.5. Changes produced by exercise

6.3.5.1. Body mass.

Body mass decreased slightly after each bout of exercise (Figure 6.10). However, these falls were not significantly different between Day ($F_{1.1, 12.2} = 0.78$, $P = 0.41$) or Time of Day ($F_{1.4, 15.8} = 0.21$, $P = 0.74$), and there was no significant interaction between the two factors ($F_{2.7, 30.2} = 0.57$, $P = 0.63$).

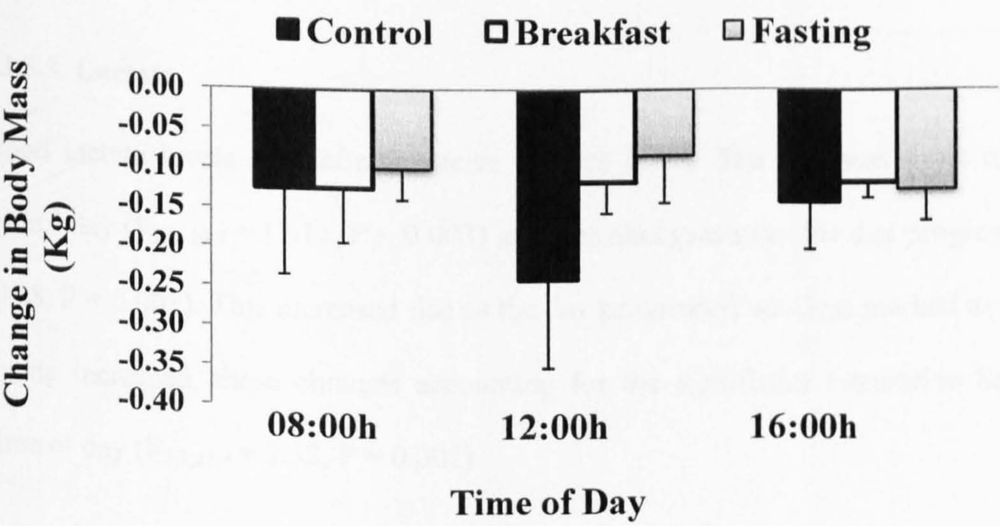


Figure 6.10. Fall in body mass produced by exercise. Mean + SE.

6.3.5.2. Respiratory exchange ratio (RER)

Figure 6.11 shows the increases in RER produced by exercise. The increase depended significantly upon Day ($F_{1.3, 14.2} = 5.29$, $P = 0.030$), and there was a marginal effect of Time of day ($F_{1.4, 15.2} = 2.85$, $P = 0.10$), the rise being least at 16:00 h. During fasting, these effects of time of day were far less marked, as a result of which there was a significant interaction between Day x Time of day ($F_{2.7, 29.6} = 3.49$, $P = 0.032$).

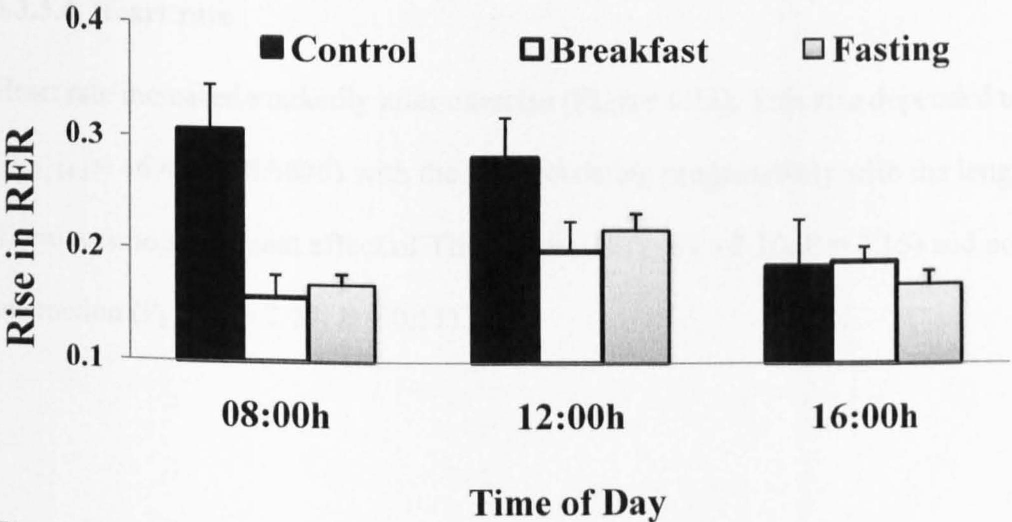


Figure 6.11. Changes in RER produced by exercise. Mean +SE.

6.3.5.3. Lactate

Blood lactate levels rose after exercise (Figure 6.12). The rise was most marked on the control day ($F_{1.2,\ 13.7} = 11.11$, $P = 0.003$) and was also greater as the day progressed ($F_{1.6,\ 18.0} = 10.68$, $P = 0.001$). This increased rise as the day progressed was less marked as the amount of fasting increased, these changes accounting for the significant interaction between Day x Time of day ($F_{2.3,25.4} = 7.32$, $P = 0.002$).

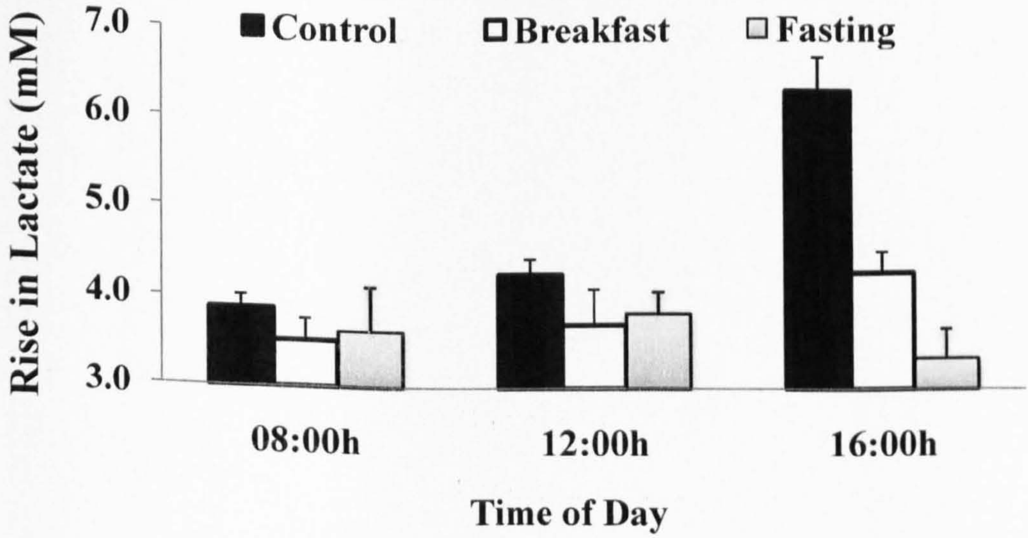


Figure 6.12. Lactate rise following exercise at different times of day and during Control, Breakfast only and Fasting days. Mean + SE.

6.3.5.4. Heart rate

Heart rate increased markedly after exercise (Figure 6.13). This rise depended upon the Day ($F_{1.3, 14.1} = 46.48$, $P<0.0005$) with the rise increasing progressively with the length of fasting. There was no significant effect of Time of day ($F_{1.8, 19.3} = 2.10$, $P = 0.15$) and no significant interaction ($F_{3.1, 34.4} = 2.17$, $P = 0.11$).

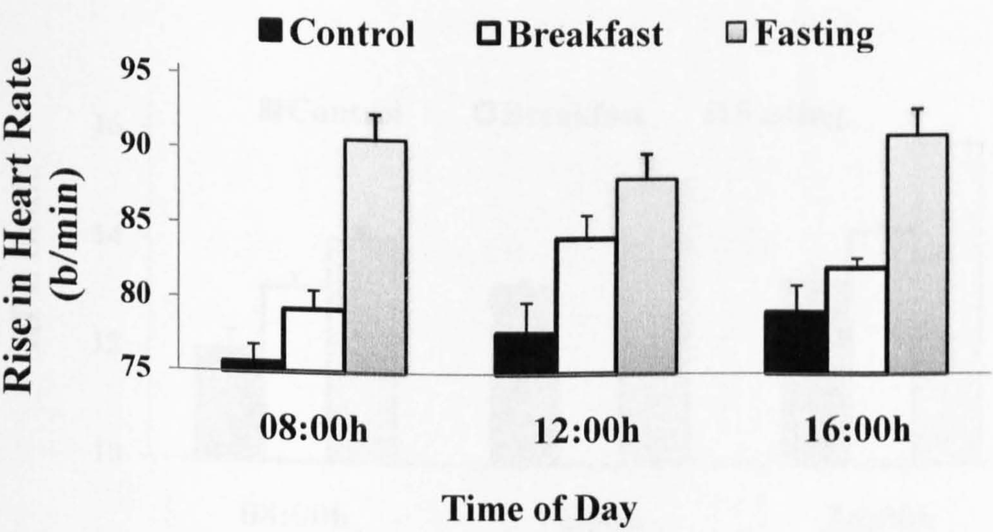


Figure 6.13. Rise in HR produced by exercise at different times of day on Control, Breakfast only and Fasting days. Mean + SE.

6.3.5.5. Rating of perceived exertion (RPE)

Figure 6.14 shows the ratings of perceived exertion after the bout of exercise. There was a significant effect of Day ($F_{1.4,\ 15.9} = 46.6$, $P<0.0005$), with perceived exertion increasing as the length of fasting increased. There was also a significant effect of Time of day ($F_{1.7,\ 18.9} = 50.87$, $P<0.0005$), exertion being perceived as greater later in the day. There was no significant interaction between Day x Time of day ($F_{2.4,\ 26.6} = 0.66$, $P = 0.55$).

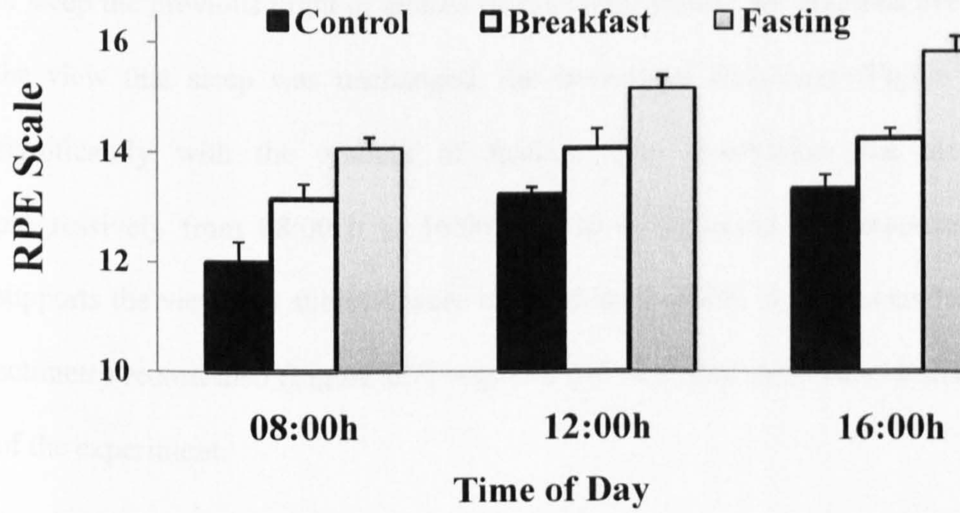


Figure 6.14. Rating of perceived exertion (RPE) after bout of exercise. Mean + SE.

6.4. Discussion and Conclusions

Inspection of the subjects' records indicates that the protocol was adhered to - that is, the duration of fasting varied between the three parts of the experiment (4 h, 8 h or 16 h for the control, breakfast only and fasting day, respectively) but the times of sleeping and the amount of food eaten in the evening before were the same. In addition, the amount of fluid drunk in the evening before the experimental days did not vary (Figure 6.1). As a result of the constancy of these aspects of lifestyle, differences between the three parts of the experiment can be attributed to the lack of food and fluid intakes during the daytime rather than to a lack of sleep the previous night or altered eating habits during the previous evening. In support of the view that sleep was unchanged, the amount of sleepiness (Figure 6.5) did not vary significantly with the amount of fasting. The observation that sleepiness decreased progressively from 08:00 h to 16:00 h is to be expected (Waterhouse *et al.*, 2001) and supports the view that subjects were responding normally to the demands of wake time. The actimetry record also (Figure 6.7) supports the view that sleep was similar in the three parts of the experiment.

Subjective estimates of the amounts of activity performed in the daytime, whether physical, mental or social activity (Figure 6.2), indicate that less activity was performed when fasting, a result in accord with that found previously (Waterhouse *et al.*, 2008a, 2008b and in Chapters 3-5). Moreover, even though there were no statistically significant differences between morning and afternoon activities produced by restrictions of food and fluid intake, inspection of Figure 6.9 suggests that the activity counts during the daytime were lower when fasting. Further work needs to be performed to clarify the reasons for this possible discrepancy between objective and subjective measures of amounts of activity. Wrist actimetry is normally a reliable indicator of general physical activity (Carvalho-Bos *et al.*, 2003), but that there are some circumstances - if an activity is stressful, requires large amounts of isometric contraction, or involves large amounts of hand movements, for example – where this parallelism between actimetry and subjective assessments fails (Waterhouse *et al.*, 1999b). There is also the possibility that the subjective estimates might be affected by fasting (Reilly and Waterhouse, 2007). The issue remains unresolved, there being a need for detailed comparisons between subjective estimates of "physical activity" and objective assessments by wrist actimetry in control and fasting conditions.

Wished-for physical (Figure 6.3) and mental activity (Figure 6.4) were both negatively affected by fasting, a result found previously in the literature (Reilly and Waterhouse, 2007; Waterhouse *et al.*, 2008a, 2008b) as well as in previous chapters in this thesis. The time-of-day effects that were present (wishing for more mental activity and less physical activity as the day progressed) are in accord with the literature on the subject (Reilly *et al.*, 1997; Waterhouse *et al.*, 2001); the rise in mental activity is probably due to effects of "waking up" and the fall in physical activity due to the onset of physical fatigue. It is predicted that both variables would show a decline if measured later in the day, due to the combined effects of increased time awake, general fatigue and preparations for sleep driven by the body clock.

Osmotic pressure (Figure 6.6) showed changes that were very similar to those reported in chapters 3 and 5) and fully supportive of the hypotheses proposed in the Introduction to this study. Thus, values were quite high and very similar in the three parts of the experiment on rising, indicative of mild dehydration due to no fluid intake since suppertime. After this, the values fell on the control day due to fluid restoration. On the day when breakfast only was taken, values also fell initially by a similar amount (due to fluid intake) but then rose after 12:00 h due to fluid restriction. When fluid was not allowed (the fasting day), the osmolality increased at 12:00 h above its rising value with a further rise by 16:00 h. Such results indicate clearly that fluid restriction results in dehydration, the degree of which is quite marked by 16:00 h on the fasting day. Previous studies (Waterhouse *et al.*, 2008a,2008b and Chapter 3) have shown that this dehydration is normally combated in the evening by increased intakes of fluid, particularly water and fruit juice which do not have diaphoretic or diuretic properties. Since dehydration has a negative effect upon physical performance (Reilly and Waterhouse, 2007; Waterhouse, 2010), this would be expected in the present study.

In contrast, the performance at throwing darts (Figures 6.8, 6.9), both the scores achieved and the number of misses, showed no effects of fasting. Moreover, there was no time-of-day effects such as had been shown to exist in previous studies (Edwards *et al.*, 2007, 2009). There are several possible explanations for this result, and they will be discussed in chapter 7. At this stage, it is only necessary to state that such a task, as performed in the current study, appeared to be inadequate for assessing any decrement in performance that might have existed. By contrast, such decrements were present when the effects of a bout of exercise were considered (see below).

Exercise caused small falls in body weight (Figure 6.10), probably the result of sweating, to remove excess body heat, but the rate of sweating was not measured. Even though the changes did not depend significantly upon Day or Time of day, there is the suggestion that the falls were greater on the control days than when fasting. Accepting that the urine osmolality indicated that dehydration was present when fasting (Figure 6.6), such a reduced rate of sweating would be predicted.

As predicted from a consideration of the urinary evidence for dehydration (above), perceived exertion (RPE) following the bout of exercise showed negative effects of restricted food and fluid intakes (Figure 6.14). The greater the fluid restriction (Fasting > Breakfast > Control), the greater was the effort perceived to be, even though the exercise itself was of a constant load. Moreover, RPE increased as the day progressed - that is, as the degree of dehydration due to fluid restriction became more marked (compare the profiles of Figures 6.6 and 6.14). Such results accord with the literature on the effects of dehydration (reviewed in Reilly and Waterhouse, 2007; Waterhouse, 2010). RPE is also likely to rise if core temperature rises more with exercise, as would be the case if sweating were inhibited by dehydration (see above)

The bout of exercise caused rises in RER, lactate and heart rate, all of which would be predicted from basic physiology. However, as found previously (Waterhouse *et al.*, 2009 and Chapter 3), the effects of time of day and fasting were complex. The increase in RER (Figure 6.11) was greatest on control days, consonant with the view that glucose was a more important substrate during exercise than on days with fasting. On days with normal food and fluid intake (Control), glucose metabolism (from the food eaten) tends to increase during the course of the day; by the afternoon, glucose is the main substrate for metabolism. Therefore, the switch to glucose metabolism produced by exercise is likely to be less marked in this circumstance, and this might account for the decreased rise in RER produced by exercise at 16:00 h during the control part of the experiment (see Figure 6.11).

Exercise increased blood lactate levels (Figure 6.12) and this rise was greatest on the control day. Again, given that glucose is likely to have been the main substrate on control days and that fat metabolism would have played a more important role on fasting days, this would be predicted.

Heart rate rose following exercise (Figure 6.13) and the size of this rise was proportional to the amount of fasting. Since the amount of exercise did not change, these rises are an objective indication of the "stress" caused by the bout of exercise. The stress can arise directly, from restrictions to food intake (reducing energy stores) and fluid intake (causing dehydration and reducing venous return, so requiring an increase in heart rate to maintain blood pressure); it can also rise indirectly, due to a fall in motivation of the subject, any task then being perceived as more difficult to perform. In support of this role of stress, the profiles of Figures 6.13 and 6.14 are very similar, objective (rise in heart rate) and subjective (RPE) estimates of stress matching closely. It is widely reported that dehydration and lack of food intake decrease physical and mental performance, cause a given activity to be more difficult to achieve, and result in a workload being perceived as being more difficult (reviewed in Reilly and Waterhouse, 2007, 2009; Waterhouse, 2010).

6.4.1. Conclusions

Taking all results together, it is concluded that restriction of food and fluid intakes for a single day in a laboratory environment produces widespread effects upon the individual with regard to subjective perceptions of daily activities, perceived workload when performing exercise, and objective measures of "stress" and changes to metabolism produced by a bout of exercise. These changes are associated with the amount of dehydration produced by the restriction of fluid intake, as assessed from urine osmolality, and causal links are possible, a possibility that was also considered when the correlations investigated in Chapter 5 were discussed. For other variables, including actimetry and performance accuracy at throwing darts, the effects of fasting were not as clear statistically, even though they also tended to show deterioration later in the daytime.

Even though the changes that have been observed are very similar to those observed in field studies of Ramadan (Waterhouse *et al.*, 2008a, 2008b, 2009 and Chapter 3), the field studies suffered from an interpretive ambiguity. This ambiguity was that Ramadan is associated with sleep loss as well as fasting. In the current study, sleep loss was avoided, and so the changes observed can be attributed to effects of food and fluid restriction only. This is not to say that sleep loss is without effect. There is much evidence that sleep loss causes performance decrements (see reviews in Valdez *et al.*, 2008; Waterhouse *et al.*, 2001) and a study to investigate the interaction between the decrements caused by sleep loss and fasting could be the subject of further study.

Chapter 7

General Discussion, Conclusions, Future Work and Implications

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7.1. General Aims

As indicated in Chapter 2 of the present thesis, the aim has been to build upon previous work performed in the field (Waterhouse *et al.,* 2008a, 2008b). The following extract is taken directly from the Conclusions section of the second paper, a field study performed in Libya and comparing results with a similar study performed in the UK:

"We draw the following conclusions from our results:

1. Ramadan resulted in decreases in several types of daytime activity and increases in fatigue and the frequency with which naps were taken in the first part of the daytime.

2. Based on food and fluid intake, there was evidence for preparing for the fasting period during Ramadan, and strong evidence for changes after the end of the fast, at sunset. These latter changes can be interpreted as "recuperation" from fasting, though social factors and a general increase in activity were likely contributory factors.

3. Before sunrise and after sunset, there were changes during Ramadan in the frequencies with which reasons for taking naps in the daytime were cited, as there were in the frequencies of reasons for drinking or not drinking, and eating or not eating. These changes meant that the links between fatigue and activity and between fatigue and fluid and food intake were all altered in Ramadan.

4. Subjects become dehydrated during the daytime, but this was not reduced in menstruating females who drank during this time.

5. There were several differences between results from the studies in the UK and Libya. Some of these differences (in the amount of activity undertaken, for example) can be interpreted to indicate conservation of energy during the daytime by the Libyan subjects; other differences (in fluid and food intake and the reasons for this, for example) suggest that there were cultural factors in preparing before sunrise for the daytime fast and recovering from it after sunset.

These findings have been extended in two main ways. First, more measurements have been made of mental and physical performance and of physiological variables. Second, laboratory-based studies using non-Muslim subjects and investigating only one day of fasting have been used.

7.2. The Field Study

Subjective assessments of performance obtained in the current field study (Chapter 3) gave results that were essentially the same as those obtained previously; subjective assessments indicated that less mental and physical performance was carried out and wished for during Ramadan. These findings validated the protocol, the subjects used and the measurements made. The measurement of urine osmolality confirmed that the subjects became dehydrated during the period of fasting, and the bout of exercise produced many physiological and biochemical changes that differed during fasting from control conditions. That is, there was clear evidence that exercise was felt to be more difficult in Ramadan and metabolic effects were different from on control days. It is likely, but not proved, that the changes observed resulted directly from the fasting (see Chapter 1).

Some of the conclusions were (taken from Chapter 3):

"In summary, Ramadan makes many demands upon those involved, and is responsible for changes in sleep, daytime sleepiness, actual and desired behaviour, and physical performance. The changes cause a decrease in both the amounts of daytime activity actually performed and in the amounts desired. There is also evidence that a given bout of physical performance at the end of the daytime fast places a greater burden on the body, and is perceived as being more demanding than is the same bout of activity performed earlier in the daytime. Individuals recuperate after sunset by eating and drinking more, and their intake of fluid tends to exclude drinks which would promote further dehydration."

Other points arising from this field experiment will be discussed later, but one further point is immediately relevant. Even though the investigation was a field study (insofar as the subjects were practising Muslims taking part in Ramadan), the tests were carried out in a fully-equipped sports laboratory. It was clear that, without this special circumstance – that is, if it had been necessary for the measurements to be made away from a laboratory - the protocol would have been impracticable. In fact, it was such limitations associated with field studies (Chapter 4, part I) that meant that later experiments were performed in the laboratory.

7.3. The Laboratory-based Studies

The two laboratory-based studies (Chapter 4, part II and Chapter 5) repeated many of the measurements made in the field study but they differed in several respects: more measures of mental and physical performance were made; only one day of fasting was considered (instead of one month); and non-Muslim subjects were used. Many of the results, including the development of dehydration and greater difficulty in performing the bout of exercise, were very similar to those found in the field study. This implies not only that a one-day experiment can give results that are relevant to studies of the effects of Ramadan but also that subjects need not be practising Muslims. However, there were some results that differed from those found previously and other that were unexpected.

One difference was that evidence of preparation for the time of fasting was less clear; subjects did not all drink large amounts of fluid before the start of the fast (though there was evidence of fluid and food replenishment after the end of the time of fasting). Another difference was that daytime naps were taken far less frequently than found in the previous work (Waterhouse *et al.* , 2008a, 2008b). Unexpected results included the observation that many of the tests of mental and physical performance (the Stroop test, grip strength and throwing darts) showed little or no negative change during fasting. This was particularly surprising for the task of throwing darts since this has been used previously to investigate successfully circadian changes and effects of time awake and sleep loss upon the accuracy of performing this task (Edwards and Waterhouse, 2009). The explanation of these differences from results obtained in the field studies and from expectations based upon measurements in other studies is unknown, but possible reasons and ways to assess these will be considered in the section on Further Work.

7.3.1. Separating the effects of sleep loss from fasting – the intervention study

Apart from the negative effects of fasting upon performance in a short bout of exercise, other tests of mental and physical performance, though validated in other studies, showed very little change in the present work. Several possible reasons exist for this: (1) insufficient practice at the task before the experiment; (2) insufficient supervision while performing the task; and (3) lack of sensitivity of the task. Items (1) and (2) could easily be remedied in further studies but the other issue is more difficult, if only because a negative result statistically (no change) could indicate a small (or non-existent) effect or too much variability in the results. One possibility would be to measure accuracy at throwing darts, a task that has been shown to be sensitive in previous studies involving sleep loss (Edwards *et al.*, 2007,2009), at multiple stages during the course of Ramadan. Measurements could be repeated at weekly intervals (see Waterhouse *et al.*, 2008a, 2008b), or more frequently, in conditions which were strictly supervised and highly standardized, using subjects who had practised the task until they had reached a performance plateau. These last conditions would minimize within-subject variation and increase the chance of measuring small changes.

7.4. Further Work

7.4.1. Measurement of performance

Apart from the negative effects of fasting upon performance in a short bout of exercise, other tests of mental and physical performance, though validated in other studies, showed very little change in the present work. Several possible reasons exist for this: (1) insufficient practice at the task before the experiment; (2) insufficient supervision while performing the task; and (3) lack of sensitivity of the task. Items (1) and (2) could easily be remedied in further studies but the other issue is more difficult, if only because a negative result statistically (no change) could indicate a small (or non-existent) effect or too much variability in the results. One possibility would be to measure accuracy at throwing darts, a task that has been shown to be sensitive in previous studies involving sleep loss (Edwards *et al.*, 2007,2009), at multiple stages during the course of Ramadan. Measurements could be repeated at weekly intervals (see Waterhouse *et al.*, 2008a, 2008b), or more frequently, in conditions which were strictly supervised and highly standardized, using subjects who had

practised the task until they had reached a performance plateau. These last conditions would minimize within-subject variation and increase the chance of measuring small changes.

7.4.2. Subjective vs. objective measurements of activity

While actimetry is an objective measure of movement, it does not enable types of movement to be distinguished (Carvalho-Bos *et al.*, 2003). Even though subjects report that they do less and wish to do less when fasting, it is not clear whether this is, in fact, true, whether the same types of task are performed but more slowly, or if different types of task are undertaken. Observation of subjects and "scoring" them for the different types of activity they perform (and the amount and speed of performing these activities) could be compared with simultaneous measurements of actimetry from multiple sites (wrist, ankle, and back, for example).

In the relevant studies performed so far (Waterhouse *et al.*, 2008a, 2008b, 2009 and Chapters 3-5), one limitation has been that subjects have had commitments (whether work, study or child-rearing, for example) and this will have influenced their activity patterns and restricted their option to be less active. Using subjects who have none of the above commitments would overcome this difficulty. Such subjects could be investigated in short studies performed in the laboratory as well as in longer field studies during Ramadan.

7.4.3. Role of naps

In the same way as daily commitments might influence activity, even though changes in the length of sleep at night might increase daytime sleepiness, the subjects are often not free to nap during the daytime. Again, this is the case when the majority of the subjects are students or workers with work schedules to keep. To investigate the true "cost" of loss of sleep at night, a similar suggestion to that made for measuring daytime activities in the absence of commitments is offered. It is suggested that subjects should have little structured commitments but rather be free to do what they wished when they wished to do it (see Waterhouse *et al.,* 2005). It is predicted that this protocol would result in the frequency of daytime naps on fasting days being greater than that on control days, this difference better reflecting the "stress" caused by sleep loss at night in Ramadan. Moreover, subjective estimates of sleep are inferior to objective measures (polysomnography) and this latter method could also investigate sleep architecture at night as well as during daytime naps (Bahammam, 2003, 2004: Roky *et al.,* 2003; Margolis and Reed, 2004).

7.4.4. Possible interaction between sleep loss and fasting

As discussed in Chapter 6, many of the negative results observed in Ramadan and in the laboratory-based studies of Chapters 3-5 seem to be due to fasting rather than sleep loss since they were replicated when sleep was controlled (Chapter 6). Even so, the intervention study could be modified in such a way that sleep loss is manipulated and the amount of fasting is standardized (using a protocol based on that used for Chapter 6). It would be predicted that, if sleep loss itself does not contribute significantly to the negative effects observed in Ramadan, changing the amount of sleep lost would be without effect. An alternative hypothesis is that sleep loss and fasting together combine to produce decrements that are greater than those due to either intervention alone. Results from the proposed protocol, combined with those from Chapter 6, could be used to investigate this alternative hypothesis.

It is interesting to note that many athletes delay training until the evening, after the fast has ended (Wilson *et al.*, 2009). Moreover, some can delay retiring and rising times so that the duration of daytime fasting is curtailed. (In this respect they are showing a more extreme version of the delayed habits shown by the subjects in the field study, Chapter 3). The detailed nature of their sleep patterns and their circadian rhythms of performance and metabolism are all areas that deserve further investigation.

7.4.5. Subjects' experience

The results from the first field study of this thesis (Chapter 3) indicated that sleep times were delayed in Ramadan, subjects not only retiring later but also rising later; indeed, total sleep time was increased, unlike changes observed in the first two field studies (Waterhouse *et al.* , 2008a, 2008b) and in general (Reilly and Waterhouse, 2007). This might reflect the facts that, unlike the case in the first two field studies, the individuals were not independent, still living with their parents. Also, the laboratory-based studies (Chapter 4, part II and Chapter 5) indicated that non-Muslims, who had not previously fasted in the daytime, made less preparation for the fast. These results indicate that a subject's experience of the demands of fasting is an important factor in studies such as these. This issue could be tested as follows. Subjects would be required to undergo a series of fasting days, either sequentially or at weekly intervals, for example. Both Muslim (experienced in the demands of fasting) and non-Muslim subjects (initially inexperienced) would take part separately, and the results would be compared. It is predicted that any differences with regard to preparation for fasting would disappear during the course of the experiment as experience was gained by the non-Muslims.

7.4.6. Metabolism

The results from all four studies indicate that fasting causes a bout of exercise to seem more difficult to perform. Fasting also alters metabolism at rest and following the exercise. Many other aspects of metabolism could be investigated, including hormones associated with the short-term (insulin and cortisol, for example) and long-term (leptin, for example) metabolism of food. Further, although the studies have shown that food and fluid intakes before and after fasting can be altered, more detailed information on the type and amounts of food and fluid taken in would be valuable; the present questionnaires are only a first approach to this whole problem.

7.5. Concluding Comments and Implications of the Findings

In my transfer report, I wrote: "Taken together, the four studies, together with the results from the previous two field studies (Waterhouse *et al.*, 2008a, 2008b) will enable a far more detailed understanding of the changes produced by fasting as undertaken in Ramadan to be understood."

I believe that the results from the present studies have added to our knowledge of the effects of fasting upon several aspects of physiology and biochemistry. The results have also raised some problems relating to differences that might arise due to the type of measurement made, the subjects used and the detailed protocol employed. The further experiments, described above, should clarify some of these issues.

The findings have several implications for those undertaking fasting, particularly for extended periods of time during Ramadan.

7.5.1. Implications of the findings

The present findings have several implications for those undertaking fasting, particularly for extended periods of time as during Ramadan. As indicated in the literature survey (Chapter 2), there is some evidence for decreased performance ability during Ramadan but the effects upon routine daily tasks seem to be comparatively slight. By contrast, when a greater degree of physical exertion is required and for a more extended period of time (as in sports training, for example), the negative effects seem to be more marked (Reilly and Waterhouse, 2007).

However, most studies have considered changes on a weekly basis rather than during the course of the waking day. The present studies have investigated changes during the weeks of Ramadan and have confirmed that these are not progressive, changes during the fourth week of Ramadan not being significantly different from those in the first week. Adjustment to the demands of Ramadan is rapid, therefore, probably reflecting the fact that the vast majority of Muslims will have experienced the demands of Ramadan on previous occasions.

What is new about this work is that decrements during the course of the waking day have been investigated, and these have been found to be more marked towards the end of the period of fasting. These decrements include: subjective estimates of the amount of physical activity actually performed and of the amount of work that individuals wish to perform; and objective estimates of responses to exercise, indicating that it is more "stressful" at the end of a period of fasting. There is also evidence suggesting that objective measures of activity decrease and performance at tasks involving neuromuscular co-ordination deteriorates towards the end of the fasting day. This general deterioration is associated with the progressive amount of dehydration and loss of energy stores that occur during fasting.

As a result of these changes, the individual is more disadvantaged at the end of the fasting day than in the morning. It is predicted from this result that road traffic accidents, for example (see Roky *et al.*, 2004), will be increased more when individuals are returning home at the end of their work period compared with going to work in the morning. The findings also suggest that tasks that are physically demanding (and also tasks that are mentally demanding, since mental performance is also worse later in the day when fasting) should be performed earlier in the daytime.

An alternative to performing such tasks earlier in the daytime is to perform them after breaking the fast, having drunk and eaten and so having begun to replenish lost stores of body fluid and energy. In support of this suggestion, some training sessions are conducted after sunset in Ramadan (Wilson *et al.*, 2009). However, whilst delaying these sessions might be acceptable in countries where the majority of team members are Muslim, it is unlikely to be acceptable when only a minority of the team undertakes fasting in Ramadan.

Chapter8

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Chapter 9

Appendices

JMU

Liverpool John Moores University

Appendix 3.1. Consent Form

LIVERPOOL JOHN MOORES UNIVERSITY
CONSENT FORM

Title: The effects of fasting for a single day and during Ramadan upon sleep and waking performance

Researchers: Hadhom Alabed
Supervisor: Jim Waterhouse

Faculty: School of Sport and Exercise Sciences

1. I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and that this will not affect my legal rights. ☐
3. I understand that any personal information collected during the study will be anonymised and remain confidential. ☐
4. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
______________________	__________	______________
Name of Researcher	Date	Signature
______________________	__________	______________
Name of Person taking consent *(if different from researcher)*	Date	Signature
______________________	__________	______________

Appendix 3.2. (استمارة الإستبيان هذه عند تمام الساعة التاسعة صباحا)

الرقم......... اليوم................ الوقت............ صائم أو فاطر ....

أ - النوم

1) هل تمكنت من النوم بسهولة ليلة البارحة ؟
أ) بأقل سهولة (بعض الصعوبة)
ب) بشكل عادي
ج) بأكثر من سهولة

2) متى كان موعد ذهابك للنوم ليلة البارحة ؟
أ) مبكرا.
ب) توقيت عادي.
ج) وقت متأخر.

3) كيف كان نومك ليلة البارحة ؟
أ) إشتيقض مرات أكثر من المعتاد
ب) بشكل عادي
ج) إستيقض مرات أقل من المعتاد

4) متى كان موعد إستقاضك ؟
أ) باكرا.
ب) عادي .
ج) متأخر.

5) كيف كان شعورك بعد 30 دقيقة من إستفاضك (درجة نشاط جسمك) ؟
أ) أقل إنتباه من العادي .
ب) عادي .
ج) أكثر إنتباه من العادي .

6) هل تناولت أى طعام أو شراب خلال ساعة قبل الذهاب الى النوم؟
أ) لا
ب) شرب
ج) خفيفة
د) وجبة صغيرة

7) إذا كنت قد تناولت طعام أو شراب أى شى هل تعتقد ان ذلك ساعدك على النوم؟

أ) لا
ب) قليل
ج) كثيرا

ب- الشرب

8) هل تناولت أي سوائل (ماء أو غيره) منذ إستقاضك حتى الأن؟ (إختر إجابة واحدة برسم دائرة حولها)
نعم لا

إذا كانت إجالتك بنعم إذهب إلي السؤال رقم9, 10 , 11 إذا كانت إجابتك بلا إذهب إلي السؤال رقم12.

9) ما هي الكمية التي شربتها؟ (إختر إجابة واحدة)
أ) رشفة واحدة ملء الفم.
ب) أقل من كوب واحد .
ج) ملء كوب.
د) أكثر من كوب واحد .

10) ماذا شربت؟ (إختر أي عدد من الإجابات التي قد تنطبق معك)
أ) ماء
ب) شاي
ج) عصير فواكه
د) شراب غازي
ه) حليب

11) لماذا شربت ؟ (إختر أي عدد من الإجابات التي قد تنطبق)
أ) أحسست بالعطش
ب) كنت اجتماعيا ودعيت للشرب
ج) لأسباب صحية (يما في دلك دورتي)

12) لماذا لم تشرب ؟ (إختر أي عدد من الإجابات التي قد تنطبق)
أ) لم أحس بالعطش .
ب)لا أشرب عادتا في هذا الوقت.
ج) كنت منشغلا كثيرا.
د) لا يسمح لي بالشرب خلال هذا الوقت.

ج – الغداء

13) هل أكلت أي شيء منذ 'إستقاضك حتى الأن ؟ (إختر إجابة واحدة برسم دائرة حولها)
نعم لا

إذا كانت أجابتك بنعم اذهب إلى السؤال 14,15 إذا كانت اجابتك بلا اذهب إلي السؤال 16.

14) ما هي الكمية التي أكلتها؟ (إختر اجاية واحدة)
أ) وحية خفيفة جدا .
ب) وحية صغيرة
ج) وحية يحجم متوسط
د) وجبة يحجم كبير

15) ماذا أكلت ؟ (إختر أي عدد من الإجابات التي قد تنطبق عليك)
أ) أحسست بالجوع ,
ب) كنت اجتماعيا ودعيت للأكل .
ج) كنت استعد للأوقات التي لا أكل فيها
د) لأسباب صحية (يما في دلك دورتي)

16) لمادا لم تأكل؟ (أحط أي عدد من الإجابات التي قد تنطبق)
أ) لم أحس بالجوع
ب) لا أكل أبدا في هدا الوقت
ج) كنت منشغلا كثيرا
د) ليسمح لي بالأكل خلال هدا الوقت

د- الأنشطة والشعور

الإجابات المتطلبة للأسئلة من 20 إلى 25 هي الأرقام من 0 إلي 4

0 تعني مطلقا
1 تعني قليلا
2 تعني بشكل معتدل
3 تعني شيء ما كثيرا
4 تعني كثير جدا

17) إلي أي مدي كنت نشط بدنيا؟ (الإجابة 0-4)
18) إلي أي مدي كنت نشط ذهنيا ؟ (الإجابة 0-4)
19) إلي أي مدي كنت نشط اجتماعيا؟ (الإجابة 0-4)
20) إلي أي مدي تشعر بالنعاس؟ (الإجابة 0-4)
21) إلي أي مدي تشعر بالقدرة علي أداء مهام بدنية الآن ؟ (الإجابة 0-4)
22) إلي أي مدي تشعر بالقدرة علي أداء مهام ذهنية الآن ؟ (الإجابة 0-4)

Appendix 3.3. إستمارة الإستبيان هذه عند تمام الساعة الثانية عشر ظهرا, الساعةالخامسة بعد ظهرا و الساعة التاسعة مساء

الرقم.......... اليوم.................. الوقت............ صائم أو فاطر ....

أ- النوم

1) هل نمت خلال هدا الوقت؟ (أحط اجلبة واحدة)

نعم لا

ادا أجبت نعم اذهب إلي السؤال رقم 2 ادا أجبت لا اذهب إلي السؤال رقم 4

2) ما هي المدة التي نمتها ؟ (أجب بعدد الساعات)..........

3) لمادا نمت؟ (أحط أي عدد من الإجابات التي قد تنطبق)

أ) شعرت بالتعب

ب) كنت منزعج

ج) أردت استدراك ما فقدت من النوم

4) لمادا لم تنم؟ (أحط أي عدد من الإجابات التي قد تنطبق)

أ) لم أشعر بتعب

ب) لا أنام أبدا في هدا الوقت

ج) كنت منشغلا كثيرا

د)ليسمح لي بالنوم

ب- الشرب

5) هل تناولت أي سوائل (ماء أو غيره) منذ إستقاضك حتى الأن؟ (إختر إجابة واحدة برسم دائرة حولها)

نعم لا

إذا كانت إجالتك بنعم إذهب إلي السؤال رقم 6, 7 , 8 إذا كانت إجابتك بلا إذهب إلي السؤال رقم 9 .

6) ما هي الكمية التي شربتها؟ (إختر إجابة واحدة)

أ) رشفة واحدة ملء الفم .

ب) أقل من كوب واحد .

ج) ملء كوب .

د) أكثر من كوب واحد .

7) ماذا شربت؟ (إختر أي عدد من الإجابات التي قد تنطبق معك)

أ) ماء

ب) شاي

ج) عصير فواكه

د) شراب غازي

ه) حليب

8) لماذا شربت ؟ (إختر أي عدد من الإجابات التي قد تنطبق)
أ) أحسست بالعطش
ب) كنت اجتماعيا ودعيت للشرب
ج) لأسباب صحية (يما في دلك دورتي)

9) لماذا لم تشرب ؟ (إختر أي عدد من الإجابات التي قد تنطبق)
أ) لم أحس بالعطش .
ب)لا أشرب عادتا في هذا الوقت .
ج) كنت منشغلا كثيرا .
د) لا يسمح لي بالشرب خلال هذا الوقت .

ج – الغداء

10) هل أكلت أي شيء منذ 'إستقاضك حتى الأن ؟ (إختر إجابة واحدة برسم دائرة حولها)
نعم لا

إذا كانت أجابتك بنعم اذهب إلى السؤال 11, 12 إذا كانت اجابتك بلا اذهب إلي السؤال 13 .

11) ما هي الكمية التي أكلتها؟ (إختر اجاية واحدة)
أ) وحية خفيقة جدا .
ب) وحية صغيرة
ج) وحية يحجم متوسط
د) وجبة يحجم كبير

12) لماذا أكلت ؟ (إختر أي عدد من الإجابات التي قد تنطبق عليك)
أ) احسست بالجوع ,
ب) كنت إجتماعيا ودعيت للأكل .
ج) كنت استعد للأوقات التي لا أكل فيها
د) لأسباب صحية (يما في دلك دورتي)

13) لماذا لم تأكل؟ (أحط أي عدد من الإجابات التي قد تنطبق)
أ) لم أحس بالجوع
ب) لا أكل أبدا في هدا الوقت
ج) كنت منشغلا كثيرا
د) ليسمح لي بالأكل خلال هدا الوقت

د- الأنشطة والشعور

الإجابات المتطلبة للأسئلة من 14 إلى 19 هي الأرقام من 0 إلي 4

0 تعني مطلقا
1 تعني قليلا
2 تعني بشكل معتدل
3 تعني شيء ما كثيرا
4 تعني كثير جدا

14) إلي أي مدي كنت نشط بدنيا؟ (الإجابة 0-4)
15) إلي أي مدي كنت نشط ذهنيا ؟ (الإجابة 0-4)
16) إلي أي مدي كنت نشط اجتماعيا؟ (الإجابة 0-4)
17) إلي أي مدي تشعر بالنعاس؟ (الإجابة 0-4)
18) إلي أي مدي تشعر بالقدرة علي أداء مهام بدنية الآن ؟ (الإجابة 0-4)
19) إلي أي مدي تشعر بالقدرة علي أداء مهام ذهنية الآن ؟ (الإجابة 0-4)

Appendix 4.1. Questionnaire given at 09:00 h. For more details, see text.

A. SLEEP:

1. Estimated time of retiring....................
2. Estimated time of rising.....................

(For questions 3a-3e, place a mark on the lines to indicate your responses):

3. Last night's sleep, when compared with normal:

a. How easily did you get to sleep?

-5 -- 0 ------------------------------------- +5

much less easily	normal	much more easily

b. What time did you get to sleep?

-5 -- 0 ------------------------------------- +5

earlier	normal	later

c. How well did you sleep?

-5 -- 0 ------------------------------------- +5

more waking episodes	normal	fewer waking episodes

d. What was your waking time?

-5 -- 0 ------------------------------------- +5

earlier	normal	later

e. How alert did you feel 30 min after rising?

-5 -- 0 ------------------------------------- +5

less	normal	more

f. Did you eat or drink anything in the hour before going to bed? (Ring one)

1. No
2. A drink only
3. A snack
4. A small meal.

g. If you ate or drank anything, do you think it helped you sleep? (Ring one)

1. No
2. A little
3. A lot

B. DRINK:

4. Did you drink anything since waking time? (Ring one answer)
YES NO

If you answered YES, go to question 5. If you answered NO, go to question 8.

5. How much did you drink? (Ring as many answers as apply)
a. one sip or mouthful
b. less than one glass or cupful
c. one glass or cupful
d. more than one glass or cupful

6. What did you drink? (Ring as many answers as apply)
a. water
b. tea or coffee
c. fruit juice
d. fizzy drink
e. milk

7. Why did you drink? (Ring as many answers as apply)
a. I felt thirsty
b. I was with a friend and being sociable
c. For health reasons (including having my period)

Go to Section C, FOOD

8. Why did you NOT drink? (Ring as many answers as apply)
a. I did not feel thirsty
b. I never drink at this time
c. I was too busy
d. I am not allowed to drink during this time

C. FOOD:

9. Did you eat anything during this time? (Ring one answer)
YES NO

If you answered YES, go to question 10. If you answered NO, go to question 12.

10. How much did you eat? (Ring one answer)
a. snack
b. small meal
c. medium sized meal
d. large meal

11. Why did you eat? (Ring as many answers as apply)
a. I felt hungry
b. I was being sociable
c. I was preparing for times when I would not eat
d. for health reasons (including having my period)

Go to Section D, Activities and Feelings.
12. Why did you not eat? (Ring as many answer as apply)
a. I did not feel hungry
b. I never eat at this time
c. I was too busy
d. I am not allowed to eat during this time

D. ACTIVITIES AND FEELINGS:
The answers to questions 13 - 18 are a number from 0 to 4, where:
0 means "not at all"
1 means "slightly"
2 means "moderately"
3 means "quite a lot"
and 4 means "very much"

13. How physically active have you been? (ANSWER 0 - 4).....

14. How mentally active have you been? (ANSWER 0 - 4).....

15. How socially active have you been? (ANSWER 0 - 4).....

16. How sleepy do you now feel? (ANSWER 0 - 4).....

17. How able to perform physical tasks do you now feel? (ANSWER 0 - 4).....

18. How able to perform mental tasks do you now feel? (ANSWER 0 - 4).....

Appendix 4.2.VSection A of questionnaire given at 12:00, 17:00 and 21:00 h. For more details, see text.

A. SLEEP:

1. Did you sleep during this time? (Ring one answer)

YES NO

If you answered YES, go to question 2. If you answered NO, go to question 4.

2. About how long did you sleep? (answer in hours)...........

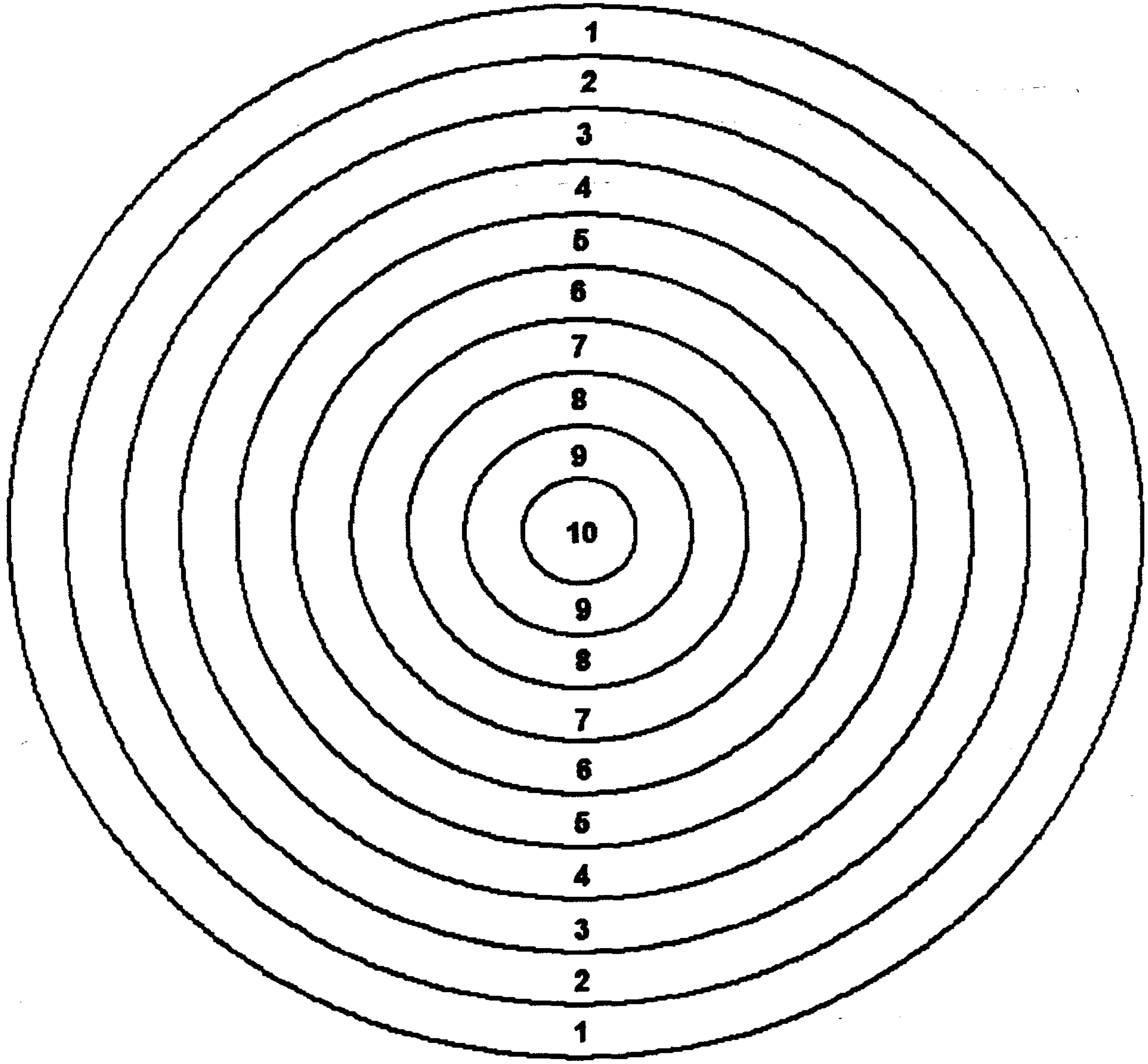
3. Why did you sleep? (Ring as many answers as apply)
a. I felt tired
b. I was bored
c. I wanted to catch up on lost sleep

Go to question Section B, DRINK

4. Why did you NOT sleep? (Ring as many answers as apply)
a. I did not feel tired
b. I never do sleep at this time
c. I was too busy
d. I am not allowed to sleep

Appendix 5.1. Accuracy of throwing darts

Hand-eye coordination test
20 shots
Dominant hand
Performance
Total score
Average score
Distance (2.37m)

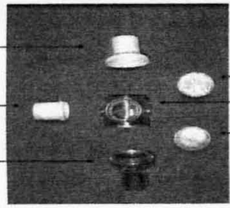


Appendix 6.1. Gas Analysis Using the Douglas Bags

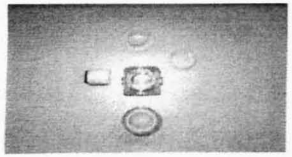
1

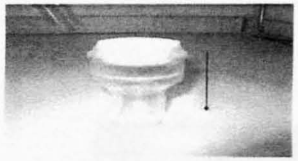
How to Construct the Hans Rudolph Mouth Piece for Expired Gas Analysis

Step 1) Collect the individual pieces.

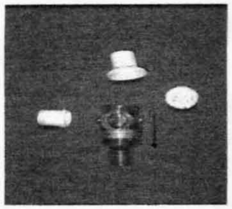


Step 2) Connect Valve 2 to the bottom of the Middle connector

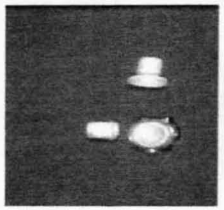




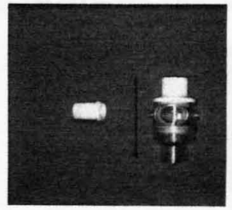
Step 3) Screw together the middle connector to the gas hose connector with valve 2 in between them. You must ensure that the gas can flow in a downwards direction!



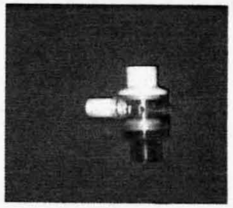
Step 4) Place valve 1 onto the top of the middle connector



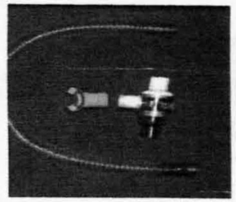
Step 5) Screw together the top piece to the middle connector with valve 1 in between them. Again you must ensure that the gas can flow in a downwards direction!



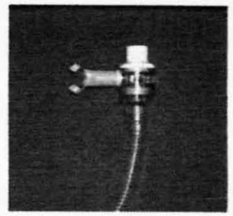
Step 6) Screw the mouth piece connector to the middle connection piece



Step 7) Collect the mouthpiece and gas hose ready for connection



Step 8) Connect the mouthpiece and the gas hose to complete the setup.



School of Sport and Exercise Science – Liverpool John Moores University

2

Gas Analysis using the Douglas Bags

Step 1.) Collect all the relevant equipment
i.e. Douglas bags, mouthpiece, hoses, nose clips)

Step 2.)Record the following measurements before you start: -

Ambient temperature, Humidity and Barametric pressure (mmHg) (see sheet 4)

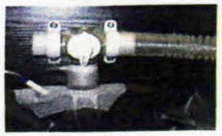
Step 3.) Collecting Expired gas in a Douglas Bag

-Evacuate all the gas from the bags before you start

-Ensure all valves are closed so no air can get into or out of the bags

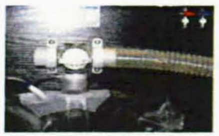
-Ensure Mouthpiece and hose air securely fitted to the Douglas bags

-Attach the mouthpiece to the subject and fit the nose clip



-Open the valve on the Douglas Bag to start expired gas collection. Duration should be 1 minute.

-Close valve when finished



Step 4.) Calibrate the Gas Analyser (Servomax)

-Turn on pumps to allow a reading for ambient conditions – O_2 = 20.9, CO_2 = 0.04
You may need to tweak the values to get an accurate result

-Fill a gas bag with calibration gas and run it through the analyser – Cal gas = O_2 = 16.0, CO_2 = 4.0

Step 5.) Analysing the gas with the Gas Analyser (Servomax)

-Attach the Douglas Bag tubes to the connection at the back of the analyser

-Open the valves that correspond with the Douglas Bag and turn pumps on

-Wait for %O2 and %CO2 to stabilise and record the values

-Close the valves and disconnect from the analyser

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Measuring Minute Ventilation and Expired Gas Temperature from the Douglas Bags

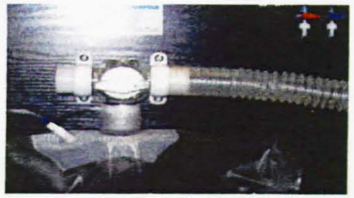
Step 6) Attach Douglas Bag to the Harvard Dry Gas Meter

Step 10) Turn on the Harvard Dry Gas Meter

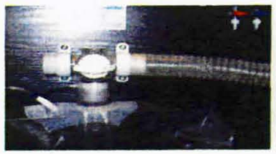
Step 7) Reset volume meter dial to zero

Step 11) Record the volume and gas temperature when the bag is emptied

Step 8) Read expired gas temperature from the top reading and room temperature from the bottom reading



Step 12) Close Douglas Bag, turn off Harvard Dry Gas Meter



Step 9) Open the Douglas Bag valves

For the appropriate Douglas Bag

Step13) Reset dial to zero after use.

<u>**Step 14) Wash all pieces of equipment you have used in the Milton solution provided – i.e. mouthpiece, nose clip, hoses**</u>

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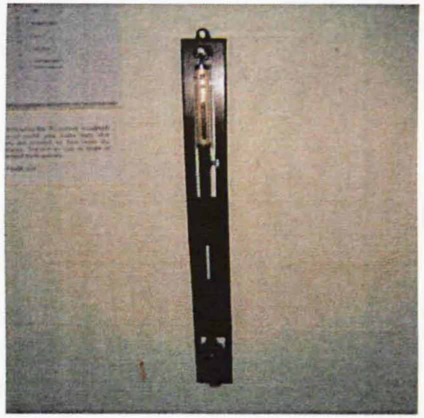
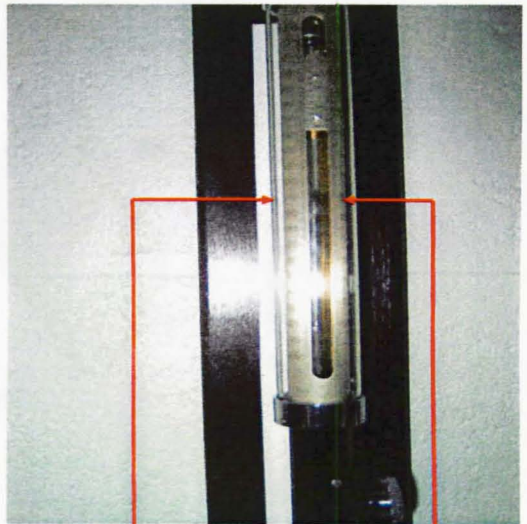
4

How to Measure Barametric Pressure (mmHg)

Barometer





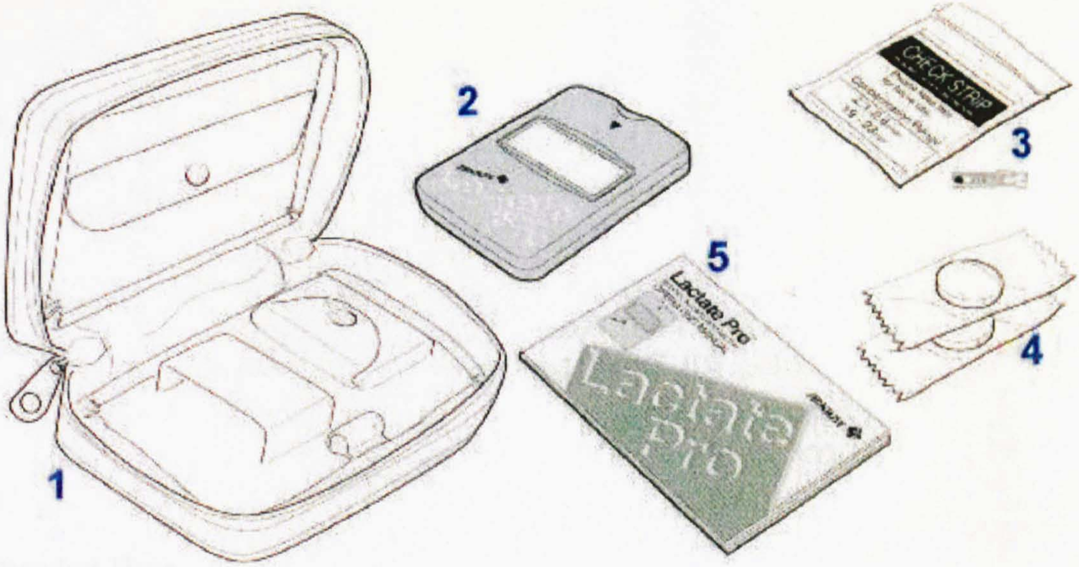
Check that the mercury is Just touch the white knob.

If the mercury is too high or too low, twist the screw to increase or decreases the mercury until it touches.

Then simply read off the results in either mmHg or mbar

Appendix 6.2. Blood lactate

Lactate Pro Meter Set



Speedy measurements and simple operation - Blood Lactate Testing

Insert the test strip into the strip inlet of the meter.

Collection of the blood.

Blood is automatically aspirated and measurement begins.

The measurement result is displayed in 60 seconds

Appendix 6.3. BORG SCALE (Rating of Perceived Exertion)

6- No Exertion at all

7- Extremely Light

8-

9- Very Light

10-

11- Light

12-

13- Somewhat Hart

14-

15-Hart

16-

17-Very Hart

18-

19-Extremely Hart

20- Maximal Exertion