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THE EFFECT OF FASTING AND FLUID RESTRICTION ON PERFORMANCE

by

JAVAD FALLAH SOLTANABAD

A Doctoral Thesis

**Submitted in Partial Fulfilment of the Requirements for Award of
Doctorate of Philosophy at Loughborough University**

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Abstract

Hypohydration and fasting are used as means to achieve body mass loss and Ramadan fasting is practised by millions of Muslims as a religious custom. Although both hypohydration and fasting have been studied extensively, the effect of hypohydration and Ramadan style fasting on many aspects of human performance is still unclear.

In Chapter 3, the effect of exercise-induced hypohydration on muscle performance was evaluated. The protocol was developed to eliminate masking and exacerbating factors such as changes in muscle glycogen storage, muscle temperature, fatigue and fluid distribution change and acid base status. Muscle strength and endurance decreased due to hypohydration equivalent to 2% of body mass. This suggests that hydration status itself can affect adversely athletes' performance.

In Chapter 4, the effect of one day (11 h) of Ramadan style fasting on some aspects of exercise performance during the day was investigated. Participants were tested three times throughout the day: at 7:00 am, 12:00 noon and 7:00 pm. 11 h of Ramadan style fasting decreased body mass by 2.1%. No change was found in exercise performance measurements. The results of this study suggest that this short period of fasting has no measurable effects on performance and/or that any effects are compensated by the circadian rhythm throughout the day. However, most athletes use more intense fasting or several consecutive days of fasting to achieve their weight reduction goal.

In Chapter 5, the effect of Ramadan fasting (one month long intermittent fasting) on some aspects of exercise performance of athletes (weight category and non-weight

category sports) and recreationally active individuals was studied. Participants were tested before, during (after the first week and in the last week) and after the month of Ramadan. Ramadan style living (involving a change in timing of food intake and sleep pattern) affects most of the anthropometric and physical performance parameters of the athletes and of the recreationally active subjects, with no difference in the pattern of change between groups. Almost all of the differences were recovered a week after the end of fasting.

Chapter 6 contains the results of three studies focused on the effects of fasting on cognitive function (study A- breakfast elimination; study B- one day (11 h) of Ramadan style fasting; and study C- one month of Ramadan fasting). Results of these studies make a logical conclusion that fasting has a detrimental effect on memory, reaction time and accuracy of responses. The pattern of the effects showed differences that may be related to various participants' characteristics (age group, gender) and the nature of intervention.

In summary, Ramadan style fasting may affect performance of some mental and physical tasks in some, but perhaps not in all individuals.

Keywords: hypohydration, Ramadan, fasting, cognitive function, performance.

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Publications

The results of this thesis have been orally presented in conferences as follows:

Chapter 3: Javad Fallah, Susan M. Shirreffs and Ronald J. Maughan. Effect of hypohydration on muscle performance. International Sports Sciences & Sports Medicine Conference (ISSSMC 2009). 20-22nd August 2009. Newcastle, UK (abstract).

Chapter 4: Javad Fallah, Ronald J. Maughan and Susan M. Shirreffs.

Effect of 11 h Ramadan style fasting on some aspects of exercise performance.

11th Asian Federation of Sports Medicine Congress. 5-9th October 2008. Tehran, I.R.Iran (abstract).

Chapter 5: Javad Fallah, Ronald J. Maughan and Susan M. Shirreffs. Effect of Ramadan fasting on some aspects of exercise performance in Iranian athletes.

The 2008 International Convention on Science, Education and Medicine in Sport (2008 ICSEMIS). 1st-5th August 2008. Guangzhou, China (abstract).

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Chapter 1

General introduction

1-1 Introduction

Athletes in different sports use various body mass loss methods to achieve different goals. Athletes choose various methods depending on how well are they informed about body mass loss techniques, available facilities (e.g. sauna), time for weight loss and recovery. The best-known methods for body mass loss are: food and fluid restriction; exercising in rubber sweat suits; passive sauna exposure; use of medication (laxatives and diuretics); and auto-induced vomiting. Commonly, the health-threatening methods (such as use of laxatives and diuretics, uncontrolled severe dehydration in heat, auto-induced vomiting) are chosen when available time is limited. However, amongst athletes the most popular methods for body mass loss are daily food and fluid restriction and periodic fasting (Steen & Brownell, 1990; Tipton & Tcheng, 1970).

There are different classifications of body mass loss methods, but the most popular are those of Fogelholm (1999) and Wilmore (2000). In Fogelholm's classification, methods of body mass loss are divided in two types: rapid and gradual. He considers body mass loss rapid if it occurs in seven days or less, and gradual if exceeds 7 days. However, in Wilmore's classification there are three methods of body mass loss: rapid if it occurs in 24-72 hours; moderate if in more than 72 hours up to one week; and gradual if in more than one week up to one month.

Athletes intend to achieve different goals by body mass loss depending upon the specific demands of their sport. The aim of a wrestler or a boxer to be achieved by body mass loss is to participate in a lower weight category group in a competition. For a gymnast or a diver body mass loss aims to improve body composition and shape for a higher performance and for aesthetic reasons (O'Connor et al., 2006). However, it has been always questioned whether body mass reduction makes any real differences to an athlete's performance and success (Fogelholm, 1994).

Many people in population largely practise fasting in order to achieve weight loss and also for social, cultural and religious reasons. In Muslim communities, every year athletes and non-athletes alike experience an intermittent dawn-to-dusk food and fluid complete abstinence as a religious duty during the one month long Ramadan fast. The effect of this lifestyle during Ramadan month on athletic performance (during training as well as competition) is still not well known. This special Islamic custom is popular amongst 1.5 billion Muslims around the globe, and the fasted competitor might be at disadvantage when competing against a non-fasting opponent. In spite of the great number of Muslim competitors around the world, no attention is paid to this issue in the international sport events calendar.

1-2 Hydration

Water content of the body represents approximately 50-70% the body mass for a young average adult male (Sawka et al., 2005). Differences in total body water percentage in different groups of people are due to differences in body composition. As lean body mass contains ~73% water and fat body mass only ~10%, total water

percentage in the body vary greatly with gender, age, ethnical background, physical activity, fitness, and metabolic status (Van Loan, 1996).

Water in the body is distributed throughout different fluid compartments, intra- and extra-cellular which contains almost 65% and 35% of total body water, respectively.

The extracellular fluid compartment is segmented in interstitial and plasma spaces. Therefore, an average 70 kg male has almost 42 L of body water which is divided into 28 L of intercellular and 14 L of extracellular water which contains 3.2 L of plasma and 10.2 L of interstitial fluid (Sawka et al., 2005). Water content of different compartments changes due to a dynamic exchange (Sawka et al., 2005).

Hydration status has well recognized effects on thermoregulatory and cardiovascular systems (Maughan et al., 2007) as well as providing a perfect medium for intracellular biochemical reactions. Water is the main part of thermoregulatory system responsible for shedding the extra heat in the body generated by the muscles during exercise. The human body cannot tolerate wide variations of temperature which therefore should be always kept within very narrow limits. Normal core temperature of the human body at total rest is between 36.7° - 37.0° C (Guyton and Hall, 2006). Body temperature in an extremely cold environment can drop to 35.6° C while in hot conditions or as a result of intense exercise could rise up to 40° C. However, body temperature follows a circadian rhythm with its minimum in the early morning and its maximum in the early evening. Although body tolerance for decrease of deep body temperature is 10° C and 5° C for increase (McArdle et al., 2006) the effects of body temperature fluctuations on physical performance in athletes become evident at more limited ranges. Any uncontrolled interference in hydration status can be fatal. The deaths of three

American collegiate wrestlers in 1997, in their attempt to rapidly lose weight for competition, was related to severe dehydration with clinical features of irreversible increased core temperature. This is an example of potentially serious consequences of uncontrolled manipulation of hydration (American Medical Association, 1998).

Three levels of hydration status (hydro- in Greek means water) have been described as: euhydration (eu- in Greek means good, well); hypohydration (hypo- in Greek means under, below) and hyperhydration (hyper- in Latin means above, excessive). There is another term oftenly used in describing hydration status, namely dehydration, commonly used as a synonym for hypohydration. Dehydration actually means loss of body water leading to a change from hyperhydration to euhydrated state, or from euhydration to a hypohydrated state. In present work these terms will be used as described below.

Euhydration means a level of total body water that is in normal physiological range. Maintaining euhydration is important especially in highly demanding physical tasks including exercise, because even a moderate loss of body water (~2%) may detrimentally affect physiological functions and performance. In normal daily life preserving euhydrated status is achieved by ad libitum fluid and food intake, but in athletic exercise especially in hot weather, water homeostasis may not be so easy to achieve (Kenefick, 2007). Daily water turnover is approximately 5-10% of total body water and water homeostasis is controlled via fluid loss routes, such as respiratory water loss, urine output, and sweat losses (Sawka et al., 2005). Thirst and hunger, and on the other hand, ad libitum water and food access, regulates daily hydration status resulting in net body water balance (Appel et al., 2004).

However, a marked imbalance between loss and gain of water may occur during illness, exercise, and physical work, taking many hours of rehydration to establish water balance again, via electrolyte and water intake (Adolph & Dill, 1938; Morimoto, 1990; Nielsen et al., 1993; Shirreffs et al., 1998). Daily water adequate intake for adults was calculated to be 3.7 L for males and 2.7 L for females. Water intake shows great variations between lower and upper limits (minimum 1.2 L; maximum > 6 L) in accordance with physical activity, weather conditions, relative humidity, air motion, solar load, and protective clothing (Newburgh et al., 1930; Welch et al., 1958; Greenleaf et al., 1977 a, b; Gunga et al., 1993; Ruby et al., 2002; Dietary reference intakes released by Institute of Medicine Washington, DC written by Appel et al., 2004).

Hydration status can be assessed by measuring serum osmolality as a gold standard test (Armstrong, 2005). However it could be roughly estimated by repeating body weight measurements, in combination with observation of urine colour and subjective feeling of thirst (Kenefick, 2007). Assessment of changes in hydration status is used for different purposes. Maughan et al. (2007) after evaluation of possible errors in hydration status estimation using body mass, stated that body mass measurements for assessment of hydration status changes is the only realistic method for athletes and in field based practice. However, physiologists cannot rely on one single measure. Body fluid osmolality may be as valuable as changes in volume.

Hypohydration is the status of less than normal body water content, and is a common consequence of many sports and exercise activities, particularly in hot and humid environments. It is also a common method for rapid body mass loss.

A great available amount of literature regarding hydration status shows that people in general have not an efficient subjective response to hypohydration and on how much fluid should they drink in order to maintain an adequate water balance (Greenleaf, 1992). The term “involuntary dehydration” has been well explained as being the situation in which a person enters hypohydration status due to lags in voluntary water intake. However, physiology of this process remains less known.

In a recent study was found that the feeling of thirst increases in the first 13 hours of fluid deprivation (body mass loss equal to 1.5%) but do not continue to increase significantly after that (Shirreffs et al., 2004). After approximately 13 hours of fluid deprivation subjects had to strongly control themselves to avoid drinking, showing that subjects could not become hypohydrated to this extent by accident (Shirreffs et al., 2004). It took up to 37 hours to achieve a body mass loss equal to 2.7%.

Physiological responses to hypohydration attempt to preserve homeostasis via fluid redistribution to maintain cardiac output, increase heart rate to counter decreased stroke volume, increase water reabsorption in renal distal tubules and intestines, decrease sweating rate, and decrease peripheral vascular flow. Some alterations caused by hypohydration (e.g. decreased sweating rate, decreased peripheral blood flow) may affect sport performance via decreased thermoregulatory capacity, which is

not always desirable for athletes. Moreover, regardless thermoregulation mechanisms, hypohydration may have direct effects on sport performance.

It was clearly demonstrated that even moderate hypohydration (~2% body mass loss) induces detrimental influence on endurance sports both in laboratory environment (Montain et al., 1992) and field conditions (Saunders et al., 2005; Edwards et al., 2007; Walsh et al., 1994). However, because of the interferences of exacerbating and masking factors (such as differences in glycogen storage, muscle temperature, muscle performance test protocols, muscle acid base level, and differences in choosing the participants) the influence of hypohydration on other components of physical performance (e.g. strength and power) is still controversial (Judelson et al., 2007 a; Shirreffs, 2009).

Majority of studies support the concept of detrimental effect of hypohydration on exercise performance in particular endurance performance. There are many studies on this topic such as works of: Saltin (1964 a), Greenleaf (1967), Costill (1976), Torranin et al. (1979), Nielsen (1981), Caldwell (1984), Sawka (1984), Armstrong (1988), Burge (1993), Gonzalez-Alonso (1997), and Cheuvront et al. (2005). Decrease of maximal isometric muscular strength was demonstrated in a progressive hypohydration trial with water loss up to 3.1% of total body mass (Bosco et al., 1968). Sawka and Pandolf (1990) demonstrated that dehydration decreased muscular endurance, maximal aerobic power and physical work capacity. Jacobs (1980), Guastella et al. (1988) and Webster et al. (1990) found that hypohydration in a range of 2-5% of total body mass loss, decreased Wingate peak power tests and cycling power by 2.2-21%. There have been published literature reviews covering the

majority of existing articles on this subject. Judelson et al. (2007 a) concluded that hypohydration seems to have a detrimental effect on muscular strength, power and high intensity endurance. Shirreffs (2009) pointed out that hypohydration has a consistent negative effect on muscle force generation.

However, there are some findings opposing this statement. Chevront et al. (2006) reported that a hypohydration of 2.7% of total body mass did not affect peak power output. Armstrong et al. (2007) demonstrated that hypohydration equal to 5% of total body mass resulted in no change in VO_{2max} and running economy. Saltin (1964 b) showed that changes in $VO_{2 max}$ induced by a dehydration of up to 5% of total body mass depend on environmental conditions. Heat accompanied by sweat loss reduced VO_2 by 27% while a cool environment did not change it.

Table 1.1 Review of results of studies on effect of hypohydration on muscular performance.

Reference	Number & gender of subjects	Weight reduction methods	% of body mass loss	Tests applied	Results
Greenleaf et al. (1967)	12 female	Resting and walking in hot (49°C)	3.3%	Knee extension & elbow flexion	NC
Torranin et al. (1979)	20 male	Heat exposure	3.9%	Isometric endurance	NC
Serfass et al. (1984)	10 male	Rapid weight loss	5%	Hand grip strength	NC
Viitasalo et al. (1987)	12 male	1-Sauna	3.4%	Maximal strength;	Strength was decreased by 1&2
		2-Water deprivation + diuretic	5.8%	Vertical jumping	
		3-Diuretic alone	3.8%		
Webster et al. (1990)	7 male	36 h fasting + exercise in sweat suit	4.9%	Isometric strength test	Reduction in upper body strength
Greiwe (1998)	7 male	Sauna induced	4%	Isometric knee extension and elbow flexion; Strength endurance	NC
Montain et al. (1998)	10 male	Exercise in warm chamber	4%	Single leg knee extension strength and endurance	Endurance decrease by 15-17% Strength NC
Schoffstall et al. (2001)	10 male	Water deprivation+ sauna induced hypohydration	1.5%	One repetition maximal bench press	Fall by 5.2%
Bigard et al. (2001)	11 male	Sauna induced	2.9%	Isometric knee extension strength	NC
Gutierrez et al. (2001)	6 female	Sauna induced	1.8%	Hand grip	NC
Watson et al. (2005)	9 male	Diuretic induced	2.5%	50,200,400 m; Vertical Jumping	NC
Judelson et al. (2007 b)	7 male	Exercise induced	2.5% 5%	Jump squat; Isometric back squat	NC

NC, No Change.

Hypohydration status affects cognitive function, increasing fatigue, tracking errors, reaction time of decision, and decreasing short-term memory (Maughan et al., 1994; Reilly, 1997; Cian et al., 2000; Kenefick, 2007). However, it was not completely clarified whether hypohydration alone causes detriments to cognitive performance. Or that is the thirst which induces psychological responses via which the subject reduces physical effort (Noakes et al., 2004 a; Noakes et al., 2004 b; St Clair Gibson et al., 2006). Hypohydration, however, may have direct effects on brain. This can account for the observed effects on cognitive function such as brain ventricular volume as a marker for CSF (cerebrospinal fluid) volume changes in different hypohydration levels. Dickson et al. (2005) tested six participants on different hydration levels using exercise-induced hypohydration with MRI scanning. They reported the effect of hydration on brain ventricles volume was peculiar. At 2.2% of body mass loss the brain ventricular volume decreased, but at 2.7% of body mass loss, ventricular volume increased. However, there was no change in total brain volume. Kempton et al. (2009) in a research on seven male subjects reported no change in total brain volume at $2.2 \pm 0.5\%$ of body mass loss achieved by exercise induced-hypohydration, while volume of brain ventricles expanded. A possible explanation for expansion of brain ventricles may be that blood brain barrier permeability increased in hypohydrated status, a fact that was emphasised by Watson et al. (2005) and Maughan et al. (2007).

1-3 Fluid and food restriction (Fasting)

The use of term fasting is not identical over literature. The term fasting has been used with different meanings to explain either food deprivation or food combined with fluid deprivation. In the present thesis, the term fasting will be used to explain only

the status of not eating and not drinking (food and fluid deprivation) for a period of time.

Fasting is the most commonly used method for body mass loss. Furthermore every year during Islamic dawn-to-dusk (~ 12-17 hours) Ramadan fasting, fluid and food abstinence is practised amongst Muslim communities as a common religious requirement, lasting for one lunar month (28-30 days).

Effects of short term food and water deprivation on exercise performance have been investigated previously: Tuttle (1943), Bosco et al. (1974), and Oliver et al. (2007). However, the results of previous researches show marked controversies, demonstrating fasting may induce various changes in exercise performance. The contradictions in the results of different studies can be attributed to a number of various reasons, such as different length of periods of food and water deprivation, different performance test protocols, and failing to eliminate masking and exacerbating factors.

Bosco et al. (1974) showed in a trial of three days acute starvation and dehydration that maximal isometric strength decreased. However, three days is too short a period to distinguish between the effects of hypohydration and starvation, as the main component of weight loss in the first days of fasting is body fluid loss. Oliver et al. (2007) in a 48 h energy and fluid restriction trial reported a body mass loss of $3.6 \pm 0.3\%$, a decrease by almost 5% in plasma volume, and a decrease in plasma glucose.

1-4 Food

The main role of food in the body is to provide the macronutrients that provide energy for survival. Another important role of food is supplying micronutrients (iron, zinc, copper, calcium, sodium) and macronutrients that serve in the body as structural units for synthesis of vital molecules such as proteins, lipids, enzymes, etc. Food deprivation/ restriction is a challenging situation experienced by athletes throughout most body mass loss protocols. In various researches different levels of food deprivation/ restriction during different number of days have been studied (Henry, 2001). However, the effects of early and intermediate periods of food deprivation (early stage from 24 hours to 3 days; intermediate from 4 to 7 days) (Aragon-Vargas, 1993) on different exercise components are still controversial. Literature on studies of food deprivation between 24 hours and 4 days showed a detrimental effect on endurance performance (Aragon-Vargas, 1993). In long term fast the main difficulty is to supply glucose for energy, especially for central nervous system (CNS) (Aragon-Vargas, 1993).

Taylor et al. (1954) demonstrated in an acute starvation experiment (food deprivation with ad libitum water) 1.4% body mass loss during the first 24 h. Henschel et al. (1954) reported that blood lactate concentration measurements indicate no decline with a fixed anaerobic task after one day of fasting (ad libitum water). However, maximum O₂ intake decreased by 266 ml/min (7.7%) after 5 day of starvation.

In a ten day fasting trial (water ad libitum) Consolazio et al. (1967 a, b) demonstrated: 2% (1.44 kg) of body mass loss after the first 24 h; no changes in blood calcium and

sodium levels; and increase in blood potassium and magnesium levels. However, after 5 days they found a decrease in plasma volume and cell volume and at the end of 10 days a decrease in all electrolytes levels.

A decrease in plasma glucose concentration, no changes in VO_{2max} , and a decline in endurance capacity in cycling at 70% of O_2 consumption after 23 h of complete food deprivation (water ad libitum) were reported by Dohm et al. (1986).

In a 7 day semistarvation study (40% of normal energy intake equalling 705-810 kcal/day, daily multivitamin intake, and unlimited ingestion of energy-free liquids) Bender and Martin (1986) found a decrease in plasma glucose level and time to volitional exhaustion walking (8-20 min) on treadmill, but no changes in absolute O_2 consumption and haematocrit, when compared to control values.

Knapik et al. (1987) in a study of 3.5 day of fasting (participants consumed only distilled water and selected herbal teas) pointed out no significant difference for isometric upper torso and hand grip strength, but isokinetic strength declined at two different velocities (0.52 and 3.14 rad. s⁻¹ (Nm)).

Nieman et al. (1987) found plasma glucose level was the same in pre-exercise test and throughout exercise in both fed and fasted groups in a 27 h fasting trial (~2.83 L water intake/day) followed by exercise. Although they reported an average decline of 44.7% in endurance performance of running on treadmill at 70% of $VO_{2 max}$, no significant changes in pre-exercise muscle glycogen and hydration values were found.

In a 36 h of food deprivation (water ad libitum) experiment Maughan & Gleeson (1988) reported no changes in blood glucose; no changes in VO_2 during exercise; and lower respiratory exchange ratio when compared to 12 h overnight fasting (water ad libitum) trial.

In a 24 h food deprivation trial Gleeson et al. (1988) found no changes in blood glucose concentration but a detrimental effect on maximal cycle exercise performance at 100% of $\text{VO}_{2\text{max}}$.

In a 36 hours of fasting (water limited) when compared to fed trial, Zinker et al. (1990) reported a 38% decrease in exercise endurance time tested by cycling on cycle ergometer at 50% of $\text{VO}_{2\text{max}}$ time to exhaustion.

Aragon-Vargas (1993) reviewed almost all of studies in the field of fasting. He concluded that previous studies on humans reported a decrease of time to exhaustion in endurance exercise performance (Gleeson et al., 1988; Loy et al., 1986; Maughan and Gleeson, 1988; Nieman et al., 1987; Zinker et al., 1990) or no difference (Dohm et al., 1986; Knapik et al., 1988). However, studies in rats have demonstrated increase (Dohm et al., 1983) or no change (Koubi et al., 1991) in time to exhaustion endurance exercise test. Discrepancies in results may be due to differences in physiological responses in rat and human; also to mood, intensity of the exercise, duration of fasting and placebo effects due to awareness of participants in fasted trial (Aragon-Vargas, 1993).

A particular principle of early fatigue is been encountered during food deprivation/restriction, which is still unclear (Aragon-Vargas, 1993). In previous literature, it was concluded that none of blood glucose, branched-chain amino acids (BCAA), lactate level, muscle glycogen content, cardio-respiratory parameters, and pre-exercise acid-base status, has central role in inducing fatigue in time to exhaustion tests, after a period of fasting. However, it seems that all of these factors are somehow implicated in the process (Aragon-Vargas, 1993). Filaire et al. (2001) tested eleven judokas throughout one week of food restriction leading to a significant body mass loss. They concluded that a week of food restriction adversely affected the psychology, physiology and physical performance of the participants.

Since publication of Aragon-Vargas review article in 1993 the general concept of effect of food deprivation/ restriction on metabolic and performance factors had not been changed. Oliver et al. (2007) reported a detrimental effect of 48 h of energy restriction (a daily deficit of 2600 kcal) on 30 min treadmill time-trial test in 10 male subjects. However, there was no effect of 48 h fluid restriction (a daily deficit of 2.9 L). Ferguson et al. (2009) and Friedlander et al. (2005) in two studies with similar calorie restriction of 40% of daily need for 3 weeks showed no effect on $VO_{2\max}$ test results. Friedlander et al. (2005) reported a decrease in time to complete lift and carry task and also decline in arm curl endurance after more than 6% loss in body mass. Lieberman et al. (2007) in a double-blind, placebo-controlled study of 2 days calorie deprivation in 27 young healthy subjects (25 male and 2 female) observed no change in ambulatory vigilance, activity, sleep, and cognitive performance and mood status.

Table 1.2 Review of articles on influence of food deprivation/ restriction on metabolic and performance parameters during rest and in response to exercise (Aragon-Vargas, 1993, with some updates).

Metabolic parameters during rest	Decrease	No change	Increase
Blood glucose	Avakian et al., 1987; Bjorkman & Eriksson, 1983; Dohm et al., 1986; Elia et al., 1984; Galbo et al., 1981; Knapik et al., 1987; Zinker et al., 1990; Oliver et al., 2007.	Loy et al., 1986; Friedlander et al., 2005.	
Ketone level			Dohm et al., 1986; Galbo et al., 1981; Gleeson et al., 1988; Loy et al., 1986; Knapik et al., 1987; Maughan and Gleeson, 1988; Zinker et al., 1990. Bjorkman & Eriksson, 1983.
Glucagon level			
Muscle/liver glycogen level	Knapik et al., 1987; Loy et al., 1986; Nieman et al., 1987; Koubi et al., 1991.		
Plasma glycerol level		Bjorkman & Eriksson, 1983; Dohm et al., 1986.	Gleeson et al., 1988; Maughan and Gleeson, 1988; Zinker et al., 1990; Knapik et al., 1987.
Plasma free fatty acid level		Friedlander et al., 2005.	Avakian et al., 1987; Bjorkman & Eriksson, 1983; Dohm et al., 1986; Elia et al., 1984; Knapik et al., 1987; Zinker et al., 1990. Loy et al., 1986; Nieman et al., 1987; Gleeson et al., 1988; Loy et al., 1986; Knapik et al., 1987; Maughan and Gleeson, 1988 ; Oliver et al., 2007.
Heart rate		Oliver et al., 2007.	Avakian et al., 1987; Dohm et al., 1986; Knapik et al., 1987; Nieman et al., 1987.
RPE (Rating of perceived exertion)		Ferguson et al., 2009; Oliver et al., 2007.	Avakian et al., 1987; Elia et al., 1984; Nieman et al., 1987.
Blood glucose	Bjorkman & Eriksson, 1983.	Dohm et al., 1986; Elia et al., 1984; Knapik et al., 1987; Maughan and Gleeson, 1988; Loy et al., 1986; Nieman et al., 1987.	
Plasma free fatty acid			Avakian et al., 1987; Bjorkman & Eriksson, 1983; Dohm et al., 1986 ; Friedlander et al., 2005. Nieman et al., 1987.
Oxygen consumption			
Insulin level	Bjorkman & Eriksson, 1983; Dohm et al., 1986; Galbo et al., 1981; Knapik et al., 1987. Friedlander et al., 2005.	Elia et al., 1984; Loy et al., 1986.	
Glucagon level		Elia et al., 1984; Loy et al., 1986.	Bjorkman & Eriksson, 1983; Galbo et al., 1981.
E, NE, GH, C level			Galbo et al., 1981.
Blood lactate		Elia et al., 1984; Maughan and Gleeson, 1988; Zinker et al., 1990; Friedlander et al., 2005.	Avakian et al., 1987; Bjorkman & Eriksson, 1983; Dohm et al., 1986; Galbo et al., 1981; Knapik et al., 1987; Nieman et al., 1987.

E, Epinephrine; *NE*, Norepinephrine; *GH*, Growth Hormone; *C*, Cortisol.

1-5 Ramadan style fasting

Most healthy adult individuals in Muslim communities practise fasting in the holy month of Ramadan. This kind of fasting is abstinence from food and water during day light period, from sunrise to sunset, approximately 12 to 17 hours, depending on season and geographical latitude. Ramadan is a lunar month lasting for 28 to 30 days that changes along the solar year.

Rather than three common meals consumed in normal life, during the month of Ramadan only two meals are consumed, one before dawn and the other after dusk. In some Muslim communities energy intake during Ramadan decreases and alteration in time and number of meals may be the main explanation. A decline in energy intake during Ramadan was reported by: Hallak & Nomani (1988), Bouhleb et al. (2006), and Reilly & Waterhouse (2007). However, there are other reports with opposite results demonstrating increase in energy intake during Ramadan (Frost & Pirani, 1987). Furthermore, some studies have demonstrated even the energy intake during Ramadan did not change (Afifi, 1997; Ramadan et al., 1999).

Ramadan fasting cannot be simply considered as only a different diet because during Ramadan not only the food composition, but also the pattern and timetable of eating, drinking, and sleeping are changed. In most Muslim countries in the month of Ramadan, sport activities and competitions, including semi-professional and professional leagues, are postponed from morning and midday, to early evening (after sunset) and even late night. Fasting (food and water deprivation) and competition time

table rescheduling (via circadian rhythms) affect athlete's performance during the month of Ramadan. Sporting competitions continue throughout the month of Ramadan (e.g. Olympic Games in 2012 London will run from 27 of July to 12 of August, while Ramadan will start July 21 and last up to August 20). There is a currently limited knowledge pertaining to the physiological and performance consequences of Ramadan fasting on athletes.

The literature shows no consistent pattern of change in body mass and body fat content during Ramadan. There is great controversy in research results: Husain et al. (1987), Hallak & Nomani (1988), Adlouni et al. (1998), and Ziaee et al. (2006) showed a decrease in body mass and fat mass during Ramadan; Yucel et al. (2004) showed no change; and Frost & Pirani (1987), Gharbi et al. (2003), and Meckel et al. (2008) reported an increase in energy intake and weight and fat gain.

Effect of fasting on blood biochemistry and lipid profile has been well studied in sedentary healthy people and in patients with hyperlipidaemia (secondary hyperlipidaemia seen in diabetes mellitus and primary hyperlipidaemia Buerger-Gruetz syndrome; Afrasiabi et al., 2003 a; Barber & Wright, 1979). However, there are limited studies addressing the effect of Ramadan fasting in athletes.

Studies show no consistent results for lipid profile in fasting subjects during Ramadan. Weight change, change in energy and food composition and intake, initial fat percentage, season, sex, smoking, moderate alcohol intake, using hypolipidaemic drugs have been named as factors that could affect lipid profile (Sarraf-Zadegan et al., 2000; Toda & Morimoto, 2000; Maislos et al., 1993; Adlouni et al., 1998). However,

is not clear which factor has the major effect on lipid profile. In literature total cholesterol changes during weight loss and/ or Ramadan fasting were contrastingly reported as decrease (Asgary et al., 2000; Scheen et al., 1982; Jackson, 1969; Bolzano et al., 1979); no change (Cesur et al., 2007; Argani et al., 2003; Filaire et al., 2001) and even as increase (Yarahmadi et al., 2003).

Almost all recent studies and some previous ones showed an increase in HDL- Ch level (high density lipoprotein-cholesterol) (Cesur et al., 2007; Yarahmadi et al., 2003; Argani et al., 2003; Afrasiabi et al., 2003 a, b; Maislos et al., 1998; Uysal et al., 1998) during Ramadan fasting and weight loss period. However, others reported a decrease in HDL- Ch level throughout weight loss and Ramadan fasting (Thompson et al., 1979; Larosa et al., 1980; Weisweiler & Schwandt, 1981; Nelius et al., 1982; Tokunaga et al., 1982; Rosenthal et al., 1985; Markel et al., 1985) or no change (Follick et al., 1984; Zimmerman et al., 1984; Sorbris et al., 1982; Widhalm & Zwiauer, 1983).

Research demonstrated different findings on LDL- Ch level (low density lipoprotein-cholesterol) changes during Ramadan as: decrease (Schwartz, 1987; Bosello et al., 1985; Davis et al., 1985; Van Gaal & De Leeuw, 1986; Kesaniemi et al., 1985 b); no change (Cesur et al., 2007; Argani et al., 2003); and increase (Sopko et al., 1985; Markel et al., 1985; Yarahmadi et al., 2003).

Triglycerides has been shown to decrease during Ramadan (Asgary et al., 2000; Yarahmadi et al., 2003 in male subjects); not to change (Bosello et al., 1985; Davis et

al., 1985; Maislos et al., 1993; Cesur et al., 2007); and to increase (Stout et al., 1976; Markel et al., 1985; Filaire et al., 2001; Yarahmadi et al., 2003 in female subjects).

During Ramadan fast a number of metabolic and physiologic changes occur. It has been shown that at the end of Ramadan vs. pre-Ramadan values there was a decrease in serum iron in sedentary and physically active participants (17.7 ± 1.2 vs. 14.7 ± 0.9 $\mu\text{mol/l}$) (Ramadan et al., 1999). However, no significant change in white blood cell count (WBC) was reported throughout Ramadan (Sarraf-Zadegan et al., 2000, Argani et al., 2003). Sarraf-Zadegan et al. (2000) reported no changes in WBC count on 26th day of Ramadan and 2 months after the end of Ramadan. Also Argani et al. (2003) showed no change in WBC count throughout Ramadan amongst renal transplant recipients.

There is a discrepancy in the results of studies on effect of Ramadan fasting on RBC (red blood cells) related factors such as Hb (Haemoglobin), Hct (Haematocrit), MCV (mean corpuscular volume) and MCH (mean corpuscular concentration of haemoglobin). Chronic food deprivation or poor mineral intake (particularly low iron intake which is common worldwide) can lead to different levels of iron deficiency anaemia (microcytic anaemia). Iron deficiency anaemia is characterised by an increase in RBC number and decrease in RBC size and their haemoglobin content. Bouhlel et al. (2006) reported a significant increase in Hb and Hct throughout Ramadan in rugby players in Tunisia. However, Argani et al. (2003) showed no change in Hb in renal transplant recipients during Ramadan.

The immune system can be altered by a whole month of energy restriction due to altered hydration status, hormonal status and serum iron level (Meckel et al., 2008). However, the opposite was also reported namely acute energy restriction can enhance antioxidant capacity and therefore improve immune system (Rankin et al., 2006).

Glucose homeostasis throughout a day of fasting is a big challenge for athletes exercising during Ramadan. However, research has shown that some adaptation occurs after repeated sessions of fasting and exercise, with a better glucose homeostasis in comparison to the first session (Taylor et al., 1945) as this is the case with Ramadan fasting which lasts for 29-30 consecutive days.

Stokholm et al. (1991) and Suwaidi et al. (2006) showed that fasting causes catecholamine inhibition and reduction in venous return, and lead to a decrease in sympathetic tone, which in turn causes a decrease in heart rate, blood pressure and cardiac output.

While Moosavi et al. (2007) reported lung volume increase in Ramadan and therefore possible improvement in pulmonary function, the mechanism was not explained by the authors. Subhan et al. (2006) concluded that Ramadan fasting does not affect expiratory flow rates in healthy participants. However, they reported an increase in forced expiratory flow rates at 75% of vital capacity (FEF75) in post-Ramadan values. Gleeson et al. (1988) showed that 24 hours of fasting has no effect on plasma pH when compared with a fed trial. However, blood P_{CO_2} , plasma bicarbonate concentration, and blood base excess were significantly lower in fasted subjects. It appears that fasted subjects were hyperventilated in comparison with fed subjects.

Although in Gleeson's et al. (1988) research the duration of fasting was longer than a day of Ramadan style fasting (24 h vs. 15 h) food and fluid deprivation was the main component of both.

The influence of short periods of food and water deprivation on different aspects of exercise performance has been investigated previously (Tuttle, 1943; Bosco et al., 1974; Oliver et al., 2007). However, there is limited information on exercise performance throughout either one day or whole month of Ramadan fasting.

Ben Salama et al. (1993) showed that fasting induced a reduction in physical performance in 100 and 800 meters running. Zerguini et al. (2007) reported a significant decrease in anaerobic (speed in 20 m running) and aerobic (12 min run distance) capacities after 2 weeks of Ramadan fasting. However, Meckel et al. (2008) observed a significant decrease in vertical jumping height after Ramadan vs. before Ramadan in soccer player.

The influence of Ramadan fast, regarded as a different life style with certain specific components, including altered food intake, timing, and composition, on various biological and performance factors, has not yet been well studied, moreover keeping in mind cultural, traditional and climatic differences encountered in various Muslim communities around the world.

Table 1.3 Review of results of literature on Ramadan fasting effects on some physiological and exercise parameters.

Author(s)	Subjects	Measurements	Points of testing time	Results
Maughan et al., 2008	young healthy male football players, 59 fasting and 36 not fasting	BM, body composition, dietary intake	before RA; week 2 and 4 of RA; post RA	BM ↓, BF→, BMI →, energy intake →, CHO intake →, Pr intake ↑, fat intake →, Iron intake ↓
Maughan et al., 2008	78 male junior soccer players	some biochemical and haematological parameters	3 weeks before RA; after 2 and 4 weeks in RA; 3 weeks after RA	compared with before RA: Hb↓, Hct↓, mean circulating leukocyte count↓, serum: glucose↓ in the morning, urea ↓, creatinine ↑, uric acid →, Na →, K ↑, transferrin ↑, ferritin ↓, C-reactive protein ↓, cortisol →
Mahdavi et al., 2009	60 female student volunteers from campus	daily fluid, energy, fiber and Ca intake	during RA; after RA	energy intake ↓, % of energy from fat ↓ during RA compared with after Ramadan (1400±571 vs. 1629±589 kcal and 23% vs. 32%), daily intake of fluids was higher than after Ramadan (2392±800 vs. 1685±802 ml)
Zerguini et al., 2008	64 football fasting players; 36 football not fasting players	anthropometric measures (BM, height, skinfold measurements)	3 weeks before RA; 2 and 4 week of RA; 3 weeks after RA	during RA: BM ↓, water intake ↑ by 1.3 l/day; daily Na intake ↓, training load ↑; conclusion: biochemical, nutritional, subjective well-being and performance variables →, physical performance generally ↑
Leiper et al., 2008	79 young male soccer players (48 fasting, 31 non- fasting)	heart rate	third week of RA	heart rate ↑, subjective rating of perceived fatigue →
B. Khaled et al., 2009	89 obese type 2 diabetic women	BM, nutritional record		BM ↓, saturated fat intake ↑, glucose intake ↓, impaired lipid metabolism balance
Ramadan et al., 1999	6 active men, 7 sedentary men	body and plasma composition, haematology, responses to steady state submaximal exercise	one week before RA; two weeks after the start of RA; fourth week of RA	in sedentary men at the end of RA: osmolality ↑, Na ↑, bicarbonate ↑, serum iron ↓; at the end of RA in both group platelet count ↓; during RA submaximal exercise heart rate ↓ in active men, respiratory exchange ratio during steady state submaximal exercise ↓
Indra, 2009	19 male	serum lipid profile and urea		during RA: serum urea ↓, triglycerides ↓, total cholesterol ↓, LDL-Ch ↓
Kirkendall & Donald, 2008	85 male athletes	speed, power, agility, endurance, passing and dribbling skills	1 st and 26 th day of RA	during RA physical performance → (speed, power, agility, endurance, passing and dribbling skills)
Shirreffs et al., 2008	92 young male football players (55 fasting, 37 non-fasting)	water and salt balance	before and during RA (week 3)	sweat parameters → in fasting vs. non-fasting, (sweat loss F 1.41 L, NF 1.61 L; sweat sodium concentration F 20 mmol/ L, NF 17 mmol/ L; total sweat sodium loss F 0.67 g, NF 0.65 g; salt loss F 1.7 g, NF 1.7 g); total body water content →
Yucel et al., 2004	31 (17 women, 21 men)	waist, hip, thigh circumference, BM, assessment of total fat distribution	before and after RA	visceral fat area ↓ in women and young men, all other measurement →, BM →
Zerguini et al., 2007	55 men of professional soccer teams	performance on fitness and skill tests (speed, VP, dribbling, 5m, 10m, 20m, 4-line, 12 min run, heart rate)	2 weeks before, at the end and 2 weeks after RA	EC ↓ and HR ↑ during RA and regained 2 weeks after; performance ↓ during RA (speed, agility, dribbling, endurance)
Stannard & Thompson, 2008	8 men	submaximal exercise test., VO ₂ max and body composition parameters	one week before RA, at the end of the first week of RA, the final week of RA	exercise RER ↓ during RA, RER ↑ during RA with increased power output; rate of lipid oxidation ↑ calculated from non-protein RER by the first week of RA and reversed by the last week; BW ↓; percentage BF unchanged
Asgary et al., 2000	50 men	plasma levels of MDA, serum levels of TG-s, Ch, CD-s, FBG	before and after RA	during RA: TG-s and Ch ↓; CD-s ↑, (suggesting a protection from atherosclerosis offered by a month of fasting);

Table 1.3 (continued)

Leiper et al., 2008	87 (54 fasting and 33 not fasting) football players	feelings questionnaire	pre-RA (week 1,2,3); during RA (week 4, 5,6,7); post RA (week 8,9)	the feeling of being thirsty, hungry, tired all ↑, ability to concentrate before training ↓
Bouhleb et al., 2006	9 trained male	steady-state substrate oxidation on electromagnetically braked cycle ergometer	control period (immediately before RA), at the end of the first week of RA, during the fourth week of RA	at the end of RA : BM and BF ↓, Hb c% and Hct ↑, lipid oxidation ↑, fat utilization ↑ during exercise maybe due to BM and BF loss during RA
Adlouni et al., 1997	32 male	serum levels of: 1- apolipoproteins: apo AI, apo B, apo AIV; 2- lipoprotein particles: apo AI-containing lipoprotein particle (Lp AI), apo AI and apo AII lipoprotein-containing particle (Lp AI:AII)	pre- fasting, each week of RA, post-fasting	at the end of RA : serum apo B ↓, serum apo AI ↑, serum apo AIV →, serum Lp AI:AII →, Lp AI ↑; conclusion: in RA diet beneficially affects apolipoproteins metabolism, preventing CV disease
Saile et al., 1997	32 male	serum lipids, apolipoproteins, lipoprotein particles	pre- fasting, each week of RA, post-fasting	during RA: serum TC ↓ and TG-s ↓ up to one month after RA; at the end of RA: serum HDL-Ch. ↑ up to one month after RA, serum LDL-Ch. ↓, serum apo B ↓, serum apo AI ↑, serum apo AIV →, serum Lp AI:AII →, Lp AI ↑, BW ↓; conclusion: in RA diet beneficially affects apolipoproteins metabolism, preventing CV disease
Maislos et al., 1993	24 (16 male, 8 female)	plasma lipids and lipoproteins	before the end of RA and one month after	at the end of RA : plasma TC, TG-s, LDL-Ch, VLDL-Ch, all →; HDL-Ch and apo AI ↑, both ratios of TC to HDL & LDL to HDL ↓, BW & BMI →; these results are supposed to be due to eating one large evening meal a day during RA
Sarraf-Zadegan et al., 2000	50 (22 male, 28 female)	c% of different plasma lipoproteins, lipoprotein Lp A, apo AI & apo B, fibrinogen, factor VII activity, some selected hematological factors	during RA (on the 26 th day) & two month after RA	during RA: apo B, Lp A, ratio of LDL-Ch to HDL-Ch, fibrinogen level, factor VII all ↓; TG-s, TC, LDL-Ch, HDL-Ch, FBG all →
Cesur et al., 2007	65 male	FBG, PBG, Hb A1c, fructosamine, lipid metabolism	pre-RA, post-RA, one month after RA	at post-RA point and one month after RA: FBG, PBG, HbA1c all →; one month after RA in fasting group: fructosamine ↑, BMI →; TC, LDL-Ch, TG-s all →, HDL-Ch ↑
El Ati et al., 1995	16 female	anthropometric & metabolic variables	2 days before RA, second day of RA, 28 th day of RA, one month after RA	BM →, BF →, plasma insulin c% ↓, fat oxidation ↑, CHO oxidation ↓
Gumaa et al., 1978	16 male	fluid intake, urine output, evaporative water loss, plasma osmolality, urine osmolality	during weeks 1-5 of fasting, on the 10-th day after RA	fluid balance initially ↓, compensation occurred after week 3 of RA by ↑ urine, ↓ urine volume & salt retention, plasma osmolality →; conclusion: healthy adults maintain a good fluid & electrolytes balance during RA

BM, Body Mass; **RA**, Ramadan; **BF**, Body Fat; **BMI**, Body Mass Index; **CHO**, Carbohydrate; **Pr**, Protein; **Hb**, Haemoglobin; **Hct**, Haematocrite; **Na**, Sodium; **K**, Potassium; **LDL-Ch**, Low Density Lipoprotein- Cholesterol; **F**, Fasting; **NF**, Non- Fasting; **VP**, Vertical jump; **EC**, Endurance capacity; **HR**, heart rate; **RER**, respiratory exchange ratio; **BW**, Body Weight; **MDA**, Malondialdehyde; **TG-s**, Triglycerides; **Ch**, Cholesterol; **CD-s**, Conjugated Dienes; **FBG**, Fasting Blood Glucose; **CV**, Cardiovascular; **TC**, Total Cholesterol; **HDL-Ch**, High Density Lipoprotein- Cholesterol; **VLDL-Ch**, Very Low Density Lipoprotein- Cholesterol; **PBG**, Post Prandial Blood Glucose.

1-6 Circadian rhythm

Circadian rhythm can be defined as a set of persistent recurrent intra-individual fluctuations during a 24 h cycle in some hormonal secretions, physiological variables and physical performances, which are not related to the environmental changes and are endogenously controlled. However, theories about origins of circadian rhythm are controversial. Circadian rhythm and its effects on hormonal secretion, general physiology and exercise performance have been previously studied.

The effect of circadian rhythm on exercise performance has been studied by Monk & Leng (1982), Gifford (1987), Reilly and Down (1992), Coldwells et al. (1994), Atkinson et al. (1995), Atkinson & Reilly (1996), and Hobson et al. (2009). Although researches on effect of circadian rhythm on exercise performance are limited, the majority of studies show that most of exercise performance components (e.g. muscle performance) increase in early evening compared with early morning. According to Atkinson and Reilly (1996) this mechanism could be related to variations in daily body temperature, reaching its maximum in early evening.

Different studies have shown that isometric hand grip (Gifford, 1987), back strength (Atkinson et al.,1995), elbow flexion strength (Coldwells et al., 1994) and vertical jumping (Reilly and Down, 1992) performances were significantly higher in the afternoon and early evening (almost by 10- 15%).

1-7 Cognitive function

Brain is responsible for emotional, behavioural and cognitive function in human. It is closely concern to bodily processes and to the mental phenomena and the mind is dependent on brain and body (Bickhard, 2009). Cognition consists of all mental processes and activities that are used in thinking, remembering, perceiving, learning and understanding, and the act of using these processes (Ashcraft, 1998). Cognitive function is described also as a set of processes that produces a set of events and has the ability to store and recover information (Best, 1999). Specific cognitive functions are attention, learning, memory, and reasoning, and there are several tests available for cognitive performance assessment (Lieberman, 2003 and 2005). The science of cognition developed as a reaction against behaviourism mainly inspired by the computer structure (Bickhard, 2009). “Cognitive psychology refers to all processes by which the sensory input is transformed, reduced, elaborated, stored, recovered and used” (Best, 1999).

Cognition has the important role to make people able to perform difficult tasks that would be impossible to perform without it (Best, 1999) and this is vital especially in sports that require maximum performance from brain and body such as table tennis, tennis, badminton, and running target shooting. The ability of a tennis player to anticipate the exact trajectory and position of tennis ball in the air, which is moving at a velocity of more than 30 m/s, and his/ her accurate and fast reaction involves skill, physical fitness and cognition. The amount of cognition involvement is different in different sports (e.g. table tennis vs. swimming). In sports that involve any kind of

reaction to a moving object (e.g. ball) and also in combat sports, an accurate and on time reaction seems to have a substantial share in performance.

Beside the accuracy of response, reaction time is another of the most commonly used measurements in cognitive performance. It is obvious that impairment of cognition in sports has a detrimental effect on performance. However, literature shows scarce research studies in this domain.

Similarly, the effect of various factors that may affect cognition, such as hypohydration, food deprivation, Ramadan fasting, sleep deprivation, food consumption timing and some physiological changes, have also been poorly studied. Previous studies reported that depriving humans of food has a deleterious effect on cognitive performance. Nevertheless, the findings are still not completely clear and there are not enough studies covering concomitantly several cognitive fields (Doniger et al., 2006). It was shown in some studies that human cognitive performance is affected by sleep alteration, hypohydration and heat (adverse effect; Lieberman et al., 2005), quantity of food (restriction adversely affects cognition), composition of food (carbohydrate containing food improves the results), and timetable of meals (Pollitt & Matthews, 1998; Leigh Gibson & Green, 2002).

There are few studies about the effect of Islamic Ramadan style fasting on cognitive function. Due to routine religious fasting in Muslim societies which includes at least annually one month of intermittent fasting (see Chapter 5 for details about Ramadan style fasting) and regarding some common activities (e.g. driving), some professions (e.g. piloting aircrafts, working with sharp tools, military tasks), and speed sports (e.g.

racing) which depend on high cognitive sharpness, is very important to find and accurately describe the effects of fasting on cognitive function. Ramadan fasting includes complete food and water deprivation for a period of 12-18 hours, leading to a short term hypohydration and a drop in blood glucose concentration.

Hydration is one of the essential conditions for homeostasis, including functioning of the brain, and investigating the effect of hypohydration on cognitive function has a great importance. However, investigating the effect of hypohydration on cognitive function is demanding, as there is poor literature on the issue and scarce principles to be readily applied (Lieberman, 2007). It is also very difficult to achieve an accurate assessment of hypohydration (Armstrong et al., 2007).

Severe hypohydration (loss of 10-15% of body fluid) causes successively delirium, coma and death and therefore there is no doubt prior to these extremes a mild to moderate amount of hypohydration will cause a decline in cognitive function performance and mood (Lieberman, 2007). Achieving a precise level of hypohydration is very complex and demanding as there are two physiologically distinct types of hypohydration, namely hypotonic and isotonic. Hypotonic hypohydration happens when electrolytes are lost along with water from either bowel system (in diarrhoea), urinary system (using anti-diuretic agent), or from traumatised skin lesions (burned skin). This leads to a hypohydration with no rise in body fluid osmolality or even with decrease in osmolality due to massive electrolyte loss that makes the issue more complicated (Armstrong et al., 2007).

In literature there is not enough information about what duration and level of hypohydration would cause a specific degree of impairment in cognitive performance and which of the cognitive functions is most affected (Armstrong et al., 2007). A great amount of information deals with the effect of moderate up to severe dehydration and the resulting hypernatraemia (increased plasma osmolality) on cognitive function (Vieweg et al., 1994; Warren et al., 1994), while the effect of mild hypohydration on cognitive function is less known. Due to the limitation of number of published studies and different flaws occurring in almost all the studies regarding this issue, is not possible to reach a definite conclusion about the effects of hypohydration on cognitive function (Lieberman, 2007).

Another aspect of Ramadan style fasting is a short-term drop in blood glucose concentration. Glucose represents the main source of energy for the brain and is indispensable for the normal function of CNS (Sieber & Traystman, 1992). In the literature a great amount of documentation shows that circulating blood glucose concentrations controls several brain functions, especially learning and memory (Sunram-Lea et al., 2001a, b). When blood glucose concentrations decrease to hypoglycaemic levels changes in EEG (electroencephalogram) can be seen, brain function is very rapidly affected, neuronal activity is disrupted, and cortical cellular function is altered (Thompson, 1967; Holmes et al., 1983). Therefore is logical to expect that fall in blood glucose during fasting (Larijani et al., 2003) will affect cognitive performance as well.

An important feature in cognitive function is the effect of circadian rhythm. It has been proven that in cognitive performance ability, there is a circadian rhythm during

the day similar to the rhythm in some physiological variables (e.g. body temperature, melatonin levels) (Monk, 2005). This is consistent with the findings of Potter & Keeling (2005) which consider that it is completely obvious there is a circadian rhythm in memory performance, reaching its highest output at 12:30 midday and poorest at 3:30 pm.

It has been demonstrated that circadian rhythm influences levels of cortical arousal (Carrier & Monk, 2000). On the same theme, another study (Blake, 1967) demonstrated there is an evident post-lunch decrease in cognitive performance measured by reaction time, serial search, and signal detection. Supporting these findings two other investigations show there was an increase in traffic accidents around lunch time (Mitler et al., 1987; Lavie, 1991). It is supposed the post-lunch short decline in cognitive performance may be caused by 12-hour temperature circadian rhythm (Potter & Keeling, 2005). It has been shown that circadian rhythm in body temperature and time-of-day effects have an impact on performance of simple repetitive tasks (Kleitman, 1963).

In spite of all research, the effect of circadian rhythm on cognitive function is still controversial. It was stated that peak performance of working memory happens at midday, while peak performance of short-term memory happens in late morning (Folkard et al., 1975). However, the numeric calculation short-term memory and subjective alertness all are influenced by circadian rhythm of body temperature, with the lowest performance in early morning when body temperature is the lowest (Johnson et al., 1992).

Present theories suggest that there is an interaction between circadian rhythm and time elapsed from awakening (Johnson et al., 1992; Monk & Carrier, 1998; Wright et al., 2002 a). If a person stays awake for 60 hours, body temperature and cognitive performance follow these circadian rhythm. The longer the person stays awake, the more will be the decline in body temperature and cognitive performance (Johnson et al., 1992; Wright et al., 2002 b).

On the other hand, it was reported that the awareness and behavioural efficiency were influenced by levels of dopamine, serotonin, norepinephrine, and acetylcholine and fluctuations of these levels are related to long term memory (Robbins & Everitt, 1995; Potter et al., 2000). Lack of these hormones leads to impairment of the brain function.

Memory (short and long term), sensory competence (visual accuracy), judgemental bias, analytical skills, and attention are some of the important components of cognitive function that can be assessed by the mean of various tests: Californian Verbal Learning Test (CVLT), Stroop test, Sternberg test, RVIP test (Rapid Visual Information Processing), and Visual Search Reaction Time test. The output of these tests is represented by the accuracy of response and the reaction time. However, an accurate assessment of cognitive function is very difficult to achieve (Lieberman, 2007). In research methods the measurement techniques widely vary from case to case, depending on the priorities, purposes, and objectives of the research. Every set of measures has its own advantage and there is no information available on which is the best.

Methods of measurement comprise primary-task measures, formal measures, secondary-task measures, physiological measures and at last subjective measures of mental workload, while memory, vigilance, self-reported mood, speed, accuracy, and perceived fatigue are cognitive measures. Since 1950 various tests for measuring cognitive function have been developed. In recent years, batteries of computer-based cognitive function tests have been used. Many factors and variables contribute to complexity of mental workload, which makes measures to be task specific in the way that each measure reflects a certain task (Moray, 1982). Nowadays, computer-based cognitive function tests allow evaluation of different cognitive function components more accurately than ever before. Therefore, it seems logical to conduct more research in this area.

Regarding the consequences of fasting on cognition, the majority of studies examined only a narrow spectrum of cognitive fields, and differences in study methods and planning make the conclusions ambiguous (Doniger et al., 2006).

1-8 Aim of the study

The aim of this thesis was to examine whether fasting and hydration status have influence on physical and mental performance of athletes and physically active individuals.

Chapter 2**Methods****2-1 Introduction**

In this chapter all methods that have been used in the studies will be described. Unless otherwise stated all four studies in this thesis were conducted in the laboratories of School of Sports and Exercise Sciences in Loughborough University, United Kingdom. The experimental protocol for each study is mentioned in the method section of each chapter.

2-2 Ethical approval

All studies that were conducted on the University premises were approved by Loughborough University Ethical Advisory Committee. The studies conducted in the Islamic Republic of Iran were approved by Ethical Committee of Sports Medicine Research Centre in Tehran University of Medical Sciences. All participants were informed separately about the study protocol for each study in writing and verbally, about what they will be asked to do and the potential health risks. All participants completed a health screen questionnaire, signed an informed consent form and agreed verbally to take part in the studies. They were also made clear about their right to withdraw anytime from the study if they wished so.

2-3 Subjects

The participants (male and female) were recruited through e-mails, advertising posters and verbal invitations. They were all 18 years old or over and had no history of either chronic or acute present illness at the time of the study.

2-4 Familiarisation trials

All participants took part in at least one familiarisation trial before the experimental trial. Familiarisation trials were conducted during day time and 7 to 10 days before the experimental trial. For study on effect of fasting on cognitive function (Chapter 6) two familiarisation trials prior to the test were done.

2-5 Pre-trial standardisation

Participants were asked to follow the same nutritional intake, exercise activity and sleep time and duration during the 24 hours prior to the tests.

In study on the effect of 2% hypohydration on muscle performance (Chapter 3) participants were tested as baseline measurement for knee extension isometric strength and endurance three times in the morning, after having an overnight (almost 8 h) fast from midnight. They were also asked to avoid caffeine intake and intense exercise 48 hours prior to all baseline and experimental tests.

In studies on effect of Ramadan style fasting on some aspects of exercise performance (Chapter 4) and effect of one month of Ramadan fasting on blood biochemistry and some aspects of exercise performance in Iranian athletes and recreationally active individuals (Chapter 5) participants fasted overnight and were asked to avoid intense exercise and keep the same food intake and sleep time and duration 24 hours prior to experimental trials.

In studies on effect of fasting on cognitive function (Chapter 6) participants were banned from eating and drinking 4 hours prior to the sessions 1 and 4 and fasted 12 hours prior to the test. They were asked in all sessions to avoid intense exercise such as cycling and running during the 48 hours prior to the experiments.

2-6 Statistical procedure

Statistical analysis was performed using SPSS version 14.0. All variables were checked for normal distribution. In Chapter 3 and Chapter 6 study A the variables were analysed using ANOVA paired t-test and in Chapter 4, Chapter 5, and Chapter 6 study B and C data was analysed using ANOVA with repeated measures. Bonferroni Complex Samples Hypothesis test was used for comparing different sessions. The hypothesis was accepted when $p < 0.05$.

2-7 Exercise-induced dehydration

In the study on effect of 2% hypohydration on muscle performance reported in Chapter 3, exercise-induced dehydration was achieved by intermittent cycling on a

cycle ergometer (Monarch Co. Sweden) in warm and humid environment (approximately 35°C at 60% relative humidity) until achievement of 2% of body weight loss, similar to the protocol used by Shirreffs and Maughan (1998). The duration of exercise session was 10 min followed by 5 min of rest. Exercise intensity was approximately 2 watts per kg of body weight. Room temperature, humidity, general condition, and heart rate of the participants were monitored (Polar Co. Finland) throughout the trial.

2-8 VO_{2max}

2-8.1 *Continuous incremental cycling*

The continuous incremental test was performed using a cycle ergometer (Monarch co, Sweden) in order to measure peak oxygen uptake (VO_2 peak) and power output. The exercise used in this test was a continuous graded exercise starting at 140 W and followed by an increase of 70 W every 2 min until volitional termination. The workload increment was determined based on the experimenter's assessment of the previous stage and subject's feedback. Heart rate was recorded using short-range telemetry (Chapter 3: Polar Sport Tester, Polar Vantage, Polar Electro Oy, Kempele, Finland). Two criteria had to be met in order to make the test valid: 1- respiratory exchange ratio above 1.15; 2- heart rate within 10% of the age predicted maximum (ACSM, 2000).

2-8.2 Expired air collection and analysis

The Douglas bag method was used to determine oxygen uptake. Subjects breathed via a two-way non-rebreathing valve attached to a Falconia tubing and wearing a nose-clip. Douglas bags were used to collect expired gases. Oxygen concentration of expired gases was sampled through a paramagnetic transducer. Carbon dioxide concentration was sampled via an infra-red carbon dioxide analyser (Servomex 1440, Crowborough, UK). Calibration of gas analysers with gases of known concentration (British Oxygen company, London, UK) was carried out three times a day for the study on effect of Ramadan style fasting on some aspects of exercise performance reported in Chapter 4. In order to determine gas volume and temperature, the remaining expired air was evacuated through a dry gas meter (Havard Apparatus Ltd, Cambridge, UK). Standard temperature and pressure for dry gas were the correction points for all gas volumes. A standard mercury barometer was used to measure barometric pressure. Values for respiratory exchange ratio, carbon dioxide (VCO_2) and oxygen uptake (VO_2 peak) were calculated using the assumptions of the Haldane transformation Poole and Whipp (1988).

2-9 Peak power test

During the familiarisation session the settings of the bike used during trials were determined for each participant. The bike used was a Monark Ergonomic 894 E Peak Bike (Vansbro, Sweden), connected to a computer, running the Monark Anaerobic Test Software Programme (ATS) (Vansbro, Sweden). The most comfortable setting for each participant was the guidance to determine the distance of the seat from the

handlebars. In order to set the height of the seat participants were asked to fully extend their knee at the bottom of the downstroke.

The amount of the applied mass chosen in this thesis was the same as in a previous research (Coleman and Hale, 1998). Measurements in previous research showed that the effective applied mass used during the test was 7.5% of the subject's familiarisation body mass. The basket used in test measured 1 kg and the standardised mass was applied to it. When the participants reached 90 revolutions per minute the basket and weights were dropped automatically. Participants were instructed to accelerate as much as possible for seven seconds once the basket had dropped continuing to remain seated on the bike. Using this measurement the peak power in Watts was recorded and used for analysis.

2-10 Flamingo balance test

Participants were asked to stand on the beam with no shoes on and try to maintain their balance by holding the tester's hand. While balancing on the preferred leg, the free leg was fully flexed at the knee with the foot held near to the buttocks. The watch started as the instructor let go. The stopwatch was stopped each time the participants lost balance by falling off the beam or letting go of the foot which was held. The test was repeated until the participant completed 60 seconds. The number of falls in 60 seconds of balancing was counted. If the participants fell more than 15 times in first 30 seconds, the test was terminated and the result considered as unable or zero.

2-11 Sit and reach test (Flexibility test)

Participants were asked to take off their shoes, sit on the floor with legs out straight ahead, place the feet with soles flat against the flexibility box test, with participant's shoulder-width apart. Both knees were held flat against the floor by the instructor. Participants were asked to put hands on the top of each other with palms facing down, and to try to reach forward along the measuring line as far as possible. After three practice reaches, the fourth reach was held for at least two seconds while the distance was recorded.

2-12 Sit up test

The participant was asked to lay down on the mat with the knees bent at appropriate angle in the way that the feet rest flat on the floor being held down by another person. Participant was asked to interlock fingers behind the head. When the instructor commanded "Go", the participant raised the chest so that the upper body was vertical, then returned with the back touching the floor. The process was continued for 30 seconds. The number of sit up-downs during 30 seconds was recorded.

2-13 Arm plate tapping test

Participants stood up in front of a height adjusted table. Two yellow discs were placed with their centres 60 cm apart on the table and a rectangle was placed equidistant between both discs. The participant was asked to place his/ her non-dominant hand on the rectangle in the middle and move the other hand back and forth between the discs

over his other hand in the middle as quickly as possible. This action was repeated for 25 full cycles (50 taps). The time was recorded.

2-14 The Standing long jump (Broad Jump)

Participant was asked to stand on the measured and marked area at the zero point and try to jump as far as he/ she could. Hand movement was allowed and the best jump distance out of three jumps with 30 seconds of rest in between, was recorded.

2-15 Handgrip strength test

Hand grip test was performed using a hand dynamometer (JAMAR hydraulic hand dynamometer, Samoons Preston Co., Chicago). The participant was asked to sit straight and comfortably on a chair and hold the dynamometer in the testing hand in a way that its base was resting on first metacarpal bone. The handle of dynamometer rested on the middle of four fingers, with the arm at right angle and the elbow by the side of the body. The handle of the dynamometer was adjusted if required. Then participant was asked to squeeze the dynamometer as much as possible and maintain it for about 2-3 seconds. No other changes in posture or body movement were allowed. The participant was asked to do 3 efforts with each of his/ her hands with 1 minute of rest in between. The best of 6 attempts was recorded.

2-16 Vertical jumping

The participant was asked to stand on the vertical jumping mat (Smart Mat, FSL Co. Cookstown, Northern Ireland) with the hand placed on the waist. The participants were allowed to bend their knees as much as wanted and then jump as high as possible. The mat measured the time between take off and landing. The jumping mat converted this time to the jumping height and the result was shown in cm on the device display.

2-17 (3 x 20) m shuttle test

The participant was asked to run from the start point to the opposite side and return to the starting point crossing the lines on both sides as fast as he/ she could. This was repeated three times without stopping (covering 60 meters total). The time was recorded.

2-18 Shuttle run bleep test

Participant was asked to run continuously between two lines 20 m apart which signed every 5 meters when an audio CD instructed to do so. A stop watch was used for recording the duration of the test for each participant. A certain beep was made by an audio CD for each line and the speed of bleep controlled the speed of runner. At the start the speed was quite slow (8.5 km/h). After about a minute, a sound indicated an increase in speed, and the beeps became closer together. This continued each minute to increase the speed by 0.5 km/h in each minute. The participant was asked to be at

the right place in accordance to the sounds. The rhythm and harmony was important in this test. When the participant left behind more than 1.5 m from the right line the instructor encouraged him to reach to the appropriate line in 2 more bleeps otherwise the participant was stopped and the time was recorded as his final running duration.

2-19 Isometric knee extension test

Muscular strength and endurance were measured as isometric voluntary contraction of knee extensor muscle group in both legs using an adjustable chair as described by Maughan et al. (1983) using a computer based program (Tracer DAQ, Adept Scientific plc. UK). This allowed the participants to see the graph of their effort on the screen placed in front of them. As appears in the illustration Fig. 2.1 both hip and knee joints were at 90° angle and participant was fixed with a belt in order to avoid any change in position throughout the test. Strength test was performed three times for each leg with an interval of 1.0 min of rest in between. An endurance test was performed on the dominant leg at 50% of maximum knee extension strength during the same test. A vertical line was drawn on the computer display in front of the participants at level 50% of maximum strength. Then participant was asked to make a power at the level of this vertical line and keep it for as long as possible. A fixed time of 5 min rest was given between the end of strength tests and the beginning of endurance test. Fig. 2.3 and Fig. 2.4 show a graph for each of strength and endurance tests respectively.

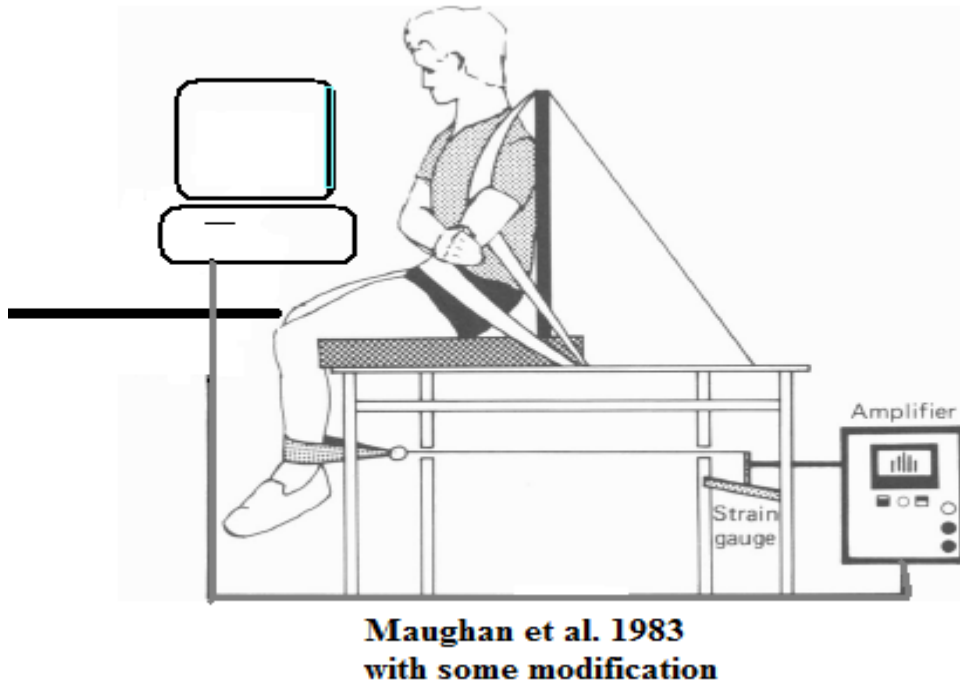


Figure 2.1 Isometric knee extension strength and endurance test chair.

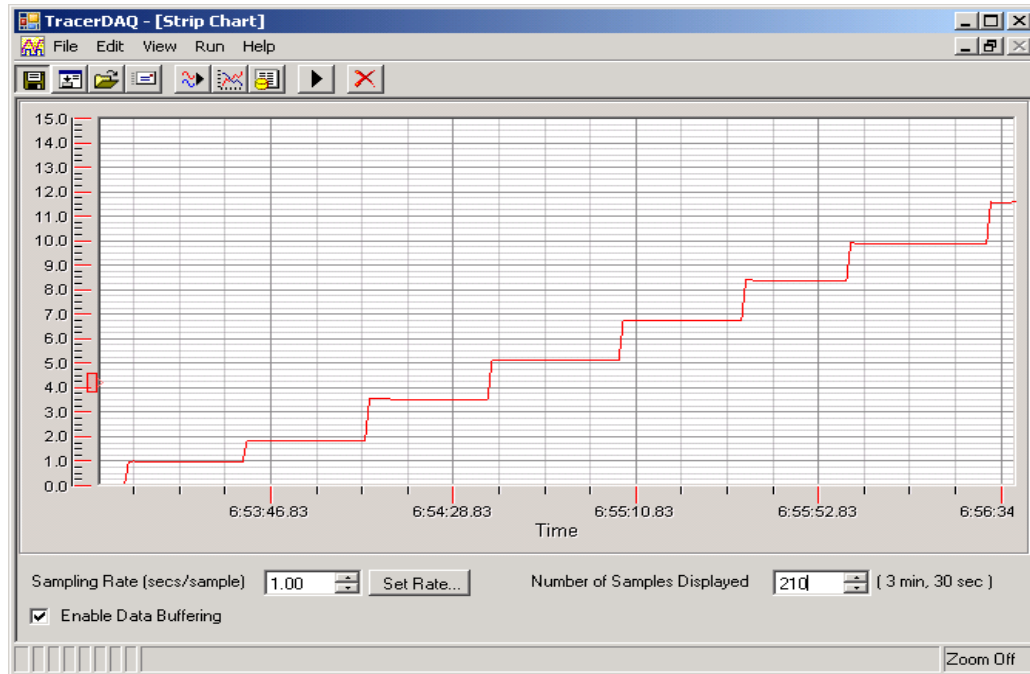


Figure 2.2 A sample of calibration results. The device was calibrated daily for 0, 10, 20.

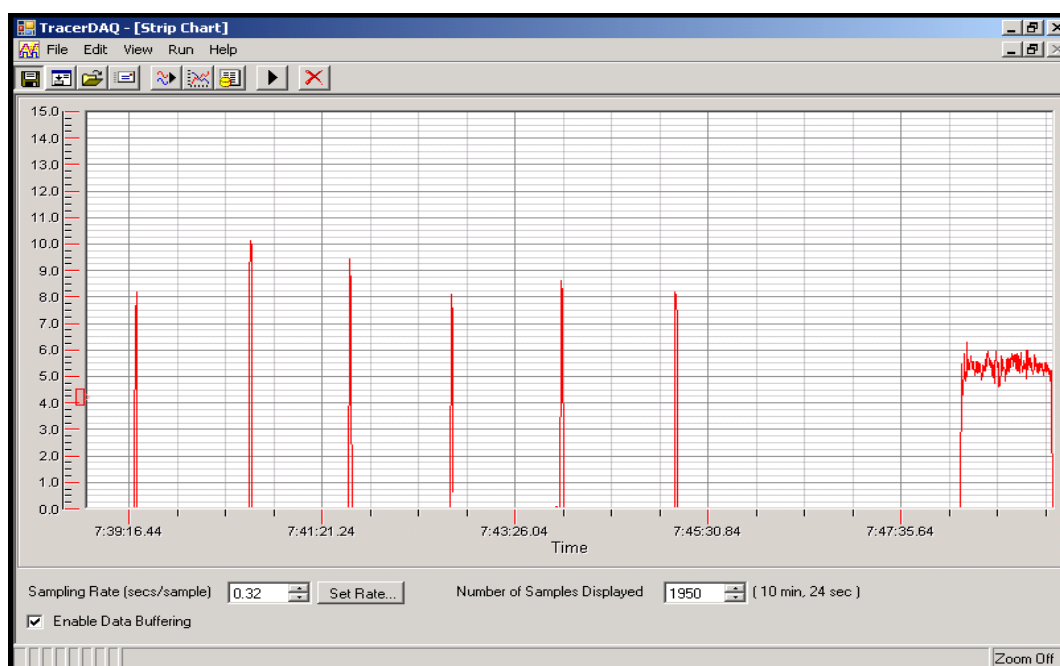


Figure 2.3 A sample graph of strength test result as it could be seen by the participants on the PC screen.

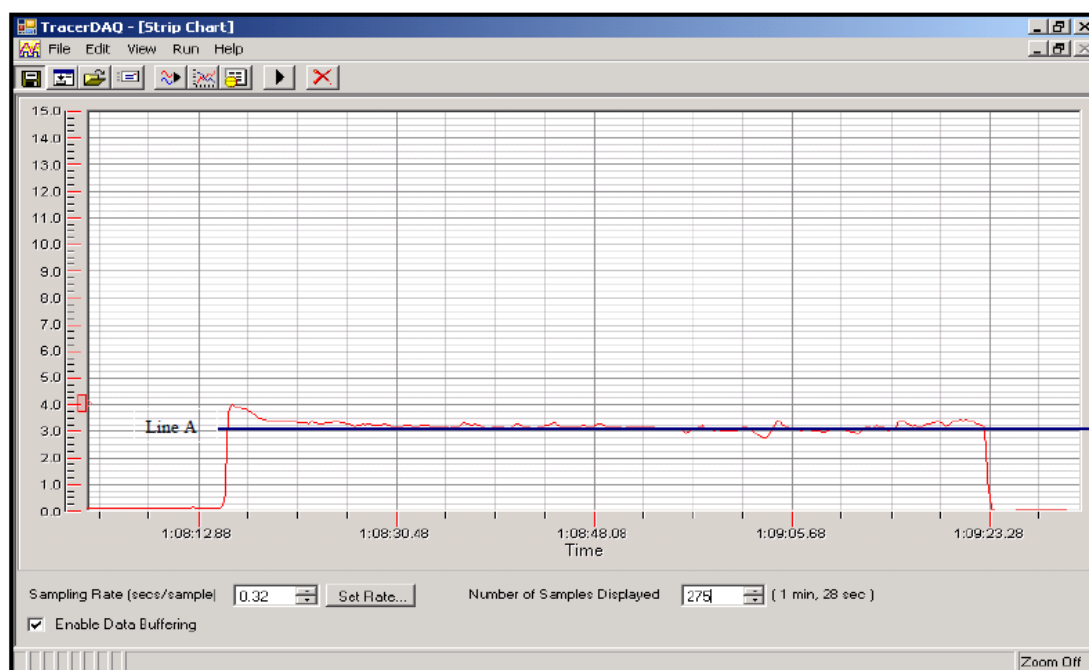


Figure 2.4 A sample graph of endurance test results. Line A was drawn at 50% of maximum strength for the same subject in the same trial. Results are shown as duration in seconds(s) and averages of power (kg) with SD (\pm).

2-20 Shortened Profile of Mood States test (POMS)

A shortened version (37 questions) of profile of mood states questionnaire was used in Chapter 3 study on effect of 2% hypohydration on muscle performance. The questionnaire version was validated by Shacham (1983) and used by many studies including Dilorenzo et al. (1999). Participants were asked to sit on a comfortable chair and complete the questionnaire before performing isometric strength tests. Questions indicate tension, vigour, depression, fatigue, anger and confusion.

2-21 Cognitive function test

All cognitive function tests were applied using a computer based cognitive function battery test HBC (Hogervorst- Bandelow Cognitive) developed in Loughborough University by Hogervorst and Bandelow. Participant was asked to sit on a comfortable chair in front of a laptop and adjust the display in a way that it could be seen easily. Trials began with a memory test using a 16 word slide show, each slide lasting for around 2.5 second on the screen. In order to test the short term memory participants were asked to remember and repeat at the end of the slide show as many words as they could from the word list. Then Visual Search test, Stroop test, Sternberg test and RVIP test were applied. Then the same tests as for short term memory were used to test the long term memory. Throughout this thesis the same order was used for all cognitive function tests. In all tests, room light was almost identical and the noise was minimised in order to have the best performance.

2-22 Laboratories

In Chapter 5 the study on effect of one month of Ramadan fasting on blood biochemistry and some aspects of exercise performance in Iranian athletes and recreationally active individuals was conducted in Abou Raihan Laboratory, a hospital laboratory in Mashhad, Islamic Republic of Iran. However, all other tests were done within the laboratories of the School of Sports and Exercise Sciences at Loughborough University, United Kingdom. Timing details of the samples collection will be mentioned in “Method” section of relevant chapters.

2-23 Blood tests

Blood was tested for: red blood cell count (RBC), haemoglobin (Hb), haematocrit (Hct), RBC mean corpuscular volume (MCV), RBC distribution width (RDW), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), differential white blood cell count (lymphocytes, neutrophils, monocytes, eosinophils), platelets count (PLT), mean platelet volume (MPV), blood glucose (B_{GLUC}), serum sodium (Na), serum potassium (K), serum chloride (Cl), serum calcium (Ca), total blood cholesterol (TC), high density lipoprotein- cholesterol (HDL- Ch), low density lipoprotein- cholesterol (LDL- Ch), triglycerides (TG-s), aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP).

2-23.1 *Blood samples*

Blood samples in all related studies were collected by venepuncture from forearm vein after at least 15 minutes of sitting at rest (Chapter 4 study on effect of Ramadan style fasting on some aspects of exercise performance) or in the supine position (Chapter 5 study on effect of one month of Ramadan fasting on blood biochemistry and some aspects of exercise performance in Iranian athletes and recreationally active individuals).

Hb, Hct and B_{GLUC} analyses were performed from fresh blood samples in less than 4.0 hours from the sampling time. Centrifuged deproteinised blood was used for B_{GLUC} concentration measurement using supernatant (GOD-PAP, Randox, Co. Antrim, UK). Blood sample were poured into a tube containing K₂EDTA and mixed for 15 min before analysis. EDTA mixed blood was triple tested for Hct after centrifusing samples in plastic capillary tubes using Haemato Spin Centrifuge device (Hct, Hawksly, Susses, UK).

2-23.2 *Blood biochemical parameters measurement techniques*

All blood measurements excepting Hb measurement were made in duplicate.

Blood cell count and haematological blood analyses were performed using Automated Haematology analyser AL 820 (AVL, Medical Instrument, Schaffhausen, Switzerland).

Hb concentration was determined using Cyanmethaemoglobin method.

Changes in plasma volume, red cell volume and blood volume were all calculated using the equation developed by Dill and Costill (1974).

Serum osmolality was tested via freezing point depression technique (Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany).

Blood chloride concentration was determined via coulometric titration (PCLM 3, Jenway, Dunmore, Essex, UK).

Serum biochemical concentrations were determined using an auto-analyzer spectrophotometer (Technicon RA 1000TM, Hartwell, LA, USA) and different kits in various wavelengths as follows below.

Serum sodium and potassium levels were measured by flame photometry technique using Corning flame photometer (Corning 410 C, New Jersey, USA). Serum calcium concentration was determined as mg/dl by using Pars-Azmun kits with cresol phthalein complex method at 550-590 nm wavelengths. Serum chloride concentration was determined as meq/l by using Pars-Azmun kits with chem Enzyme method at 500-550 nm wavelengths. Serum cholesterol concentration was determined as mg/dl by using Pars-Azmun kits with chod-pap method at 546 nm wavelengths. Serum glucose concentration was determined as mg/dl by using Pars-Azmun kits with god-pap method at 500-546 nm wavelengths. Serum triglycerides concentration was determined as mg/dl by using Pars-Azmun kits with Gpopap enzymatic method at 546 nm wavelengths.

All normal range mentioned in Chapter 5 is based on information provided by Pars-Azmun laboratory kit and the lab result paper for average male.

2-24 Urine samples

Subjects were taught how to empty their bladder and how to collect the entire sample into a special container. The total volume of the sample was measured and recorded, and after that 5 ml of the sample was retained in order to analyse the urinary electrolytes. Urine samples were kept in a refrigerator for no more than two weeks and all tests were completed in duplicate before placing them in a freezer.

Flame photometry was used for analysis of sodium and potassium concentration (Corning Clinical Flame Photometry 410 C; Corning Ltd., Halstead, Essex, UK) and colorimetry titration for chloride concentration (Jenway Chloride Meter; Jenway Ltd., Dunmow, UK). Urine osmolality was obtained via freezing point depression (Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany). Urine specific gravity was determined via refractometry technique (Digit 0-12 Clinical Pocket Refractometer, Medline, Oxfordshire, UK) and using deep stick Bayer Reagent Strips (Bayer Diagnostic mfg., Ltd., Bridgend, UK). Urine in Chapter 3 study on effect of 2% hypohydration on muscle performance was tested for colour using Urine colour Chart developed by Armstrong et al. (1994).

2-25 Coefficient of variation (CV) of assays

Coefficient of variation (SD/means)*100 calculated from 30 random samples from each assay.

Table 2.1 Coefficient of variation (SD/means)*100 calculated from 30 random samples from each assay.

Assay	CV(%)
Urine osmolality (mOsmol/kg)	0.7
Urine Na concentration (mmol/L)	0.5
Urine K concentration (mmol/L)	0.5
Urine Cl concentration (mmol/L)	0.6
Serum osmolality (mOsmol/kg)	0.5
Serum Na concentration (mmol/L)	0.4
Serum K concentration (mmol/L)	0.5
Serum Cl concentration (mmol/L)	0.7
Blood glucose concentration (mmol/L)	0.7
Hb concentration (g/dL)	0.6
Hct (vol %)	0.01

2-26 Body Fat

Skinfold thickness was determined at four standard body sites namely biceps muscle area, triceps muscle area, subscapular and suprailiac areas using calibrated Harpenden calliper (Harpenden Co. UK). Mean of three recordings was considered. Body fat was then calculated using the equations developed by Durnin and Womersley (1974), as

follows:

$$\mathbf{Body\ fat} = (4.95 / D - 4.50) 100$$

Where D is Body density calculated from the skinfold thickness, as follows:

$$\mathbf{Body\ density} = 1.1765 - 0.0744 (\log_{10} \Sigma S)$$

Where ΣS is the sum of four skinfold readings expressed in mm.

Chapter 3**Effect of hypohydration on muscle performance****3-1 Abstract**

Hypohydration is an inevitable consequence of participation in many sports and also represents a widely used method for rapid body mass reduction in weight category sports. Although there are many studies on the effect of hypohydration on various physiological parameters and on exercise performance, the effect on performance remains poorly understood due to the interference of exacerbating and masking factors. This study investigated the effect of hypohydration on muscle strength and endurance. Nine healthy male volunteers (Means \pm SD: age 26 \pm 4 years; height 176 \pm 7 cm; body mass 71.4 \pm 5.6 kg) were tested in two randomised trials: hypohydration/rehydration (HR) and hypohydration (H). Hypohydration was achieved by intermittent exercise in a warm humid environment (35 \pm 1°C, 61 \pm 3% relative humidity) beginning at 04.00 pm on day 1. After exercise participants were given a standardised meal 10 kcal/kg of body mass: Carbohydrate 71%, Fat 9%, Protein 20% and 250 ml of water in Trial H, and the same meal plus ad-libitum water in Trial HR until 12:00 midnight. At 08.00 the next morning after an overnight fast starting at 12:00 midnight, an isometric knee extension strength and endurance test (IKET) were completed, a urine sample was collected and a profile of mood states (POMS) test performed. Body mass (BM) was recorded before (BM₁) and after exercise (BM₂) on day 1 and also before IKET on day 2 (BM₃). On both trials the hypohydration protocol achieved 2.0 \pm 0.2% BM loss. BM₃ was 3.1 \pm 0.4% less than BM₁ on trial H and 1.1 \pm 0.4% less on trial HR. Muscle strength on the morning of day 2 was lower on

trial H than on trial HR by $8.6 \pm 2.1\%$ (Right Leg 7.2%; Left Leg 9.0%) and endurance time was lower by $12.3 \pm 3.2\%$. This difference in hydration level had no effect on sleep duration and pattern and profile of mood states (POMS) factors.

Keywords: hypohydration, muscle strength, endurance, POMS.

3-2 Introduction

Water loss (hypohydration) is an unavoidable consequence of many sports and exercise activities particularly in hot and humid environments. It is also a method used for rapid body mass loss (Maffulli, 1992). Physiological responses to hypohydration such as fluid redistribution (in order to maintain cardiac output, increased heart rate to encounter decreased stroke volume) attempt to preserve homeostasis. These alterations however, may affect sport performance, which is not always desirable for athletes.

The effect of hypohydration on various physiological parameters of exercise, has been studied by many people including: Saltin (1964 a); Greenleaf (1967); Costill (1976); Torranin et al. (1979); Nielsen (1981); Caldwell (1984); Sawka (1984); Burge (1993); and Gonzalez-Alonso (1997); and have been reviewed by Judelson et al. (2007 a); and Hoffman et al. (1995); and discussed by Shirreffs (2009). While the effect of hypohydration on endurance exercise performance is more clearly known, the effect on other components of physical performance is still poorly understood due to the interference of exacerbating and masking factors (Judelson et al., 2007 a; Shirreffs, 2009). The results of various investigations on hypohydration effect on muscular

strength are controversial, with some of research showing a decrease (Gutiérrez et al., 2003; Evetovich et al., 2002; Moore et al., 1992; Webster et al., 1990; Guastela et al., 1988; Viitasalo et al., 1987; Bosco et al., 1974; Saltin 1964 a); other studies showing an increase (Saltin 1964 a in elbow flexion; Webster et al., 1990 in knee extension; Kraemer et al., 2001 in hip back strength); and some studies showing no effect (Greenleaf et al., 1967; Serfass et al., 1984; Horswill et al., 1992; Montain et al., 1998; Greiwe et al., 1998). Discrepancy in the results may be due to differences in number of subjects included in the study, the level of hypohydration studied and/ or the protocol for the strength used (Sawka, 1990). Regardless of the results however, most past studies in this area appear to have failed to eliminate the effect of interfering factors such as heat stress, glycogen storage changes, and acid- base status of the muscles (Maughan, 2003). It seems more well controlled studies are required to contribute to making clear whether hypohydration could alter muscular performance particularly muscle strength.

Therefore, the aim of this study was to investigate the effect of hypohydration on muscle strength and endurance.

3-3 Materials and Methods

To examine the effect of 2% hypohydration on muscular performance, nine healthy recreationally active male volunteers (Means±SD: age 25±4 years; height 176±7 cm; body mass 71.4±5.6 kg) were tested in two randomised trials: hypohydration/ rehydration (HR) and hypohydration (H). Participants did not do strength exercise over the course of the study.

The study was approved by Loughborough University Ethical Advisory Committee. All participants completed a medical screening questionnaire and signed an informed consent form after they were provided full information about the study in writing and verbally and all their questions about the study were completely answered.

Approximately two weeks prior to the first trial participants reported to laboratory for two familiarisation sessions. The first one was for the exercise-induced hypohydration protocol, as described in Chapter 2, and the second one for the POMS (Profile of Mood States) and isometric knee extension test. The time of these visits was chosen in accordance with participant's convenience and the results obtained were not included in the study data.

In order to obtain a baseline measure of muscle strength and endurance, each participant took part in a set of isometric knee extension strength and endurance tests that were repeated on three separate days. The baseline tests were performed at 08:00 am after an overnight fast (from 12:00 midnight). These tests were completed in the week after familiarization visits with at least 24 h interval between them. Trial HR and H were performed with one week in between.

For both trials participants were asked to consume and record their last meal at 12:00 noon on the day of visit and drink 500 ml of water at 02:00 pm. They consumed the same meal 24 h prior to each visit. In addition, they were asked to avoid intense exercise such as cycling and long distance jogging during the 24 h prior to each visit. On the night prior to the isometric test participants were asked to record their sleep duration and comfort. Participants reported to the laboratory at around 04:00 pm. On

arrival, they were asked to empty their bladder and an initial nude BM (BM_1) was measured (Adam Equipment Co- Model: AFW 120 \times 0.02 kg). Participants then entered a hot and humid exercise room ($35\pm 1^\circ\text{C}$ ambient temperature, $61\pm 3\%$ relative humidity) and undertook sets of 10 min intermittent cycling on a cycle ergometer (Monarch Co. LTD Sweden) each followed by 5 min of rest with the aim of achieving 2% of BM loss. The sequence 10 min cycling with 5 min rest was repeated until the 2% BM loss was achieved. During every 5 min of rest participants towelled themselves dry and nude BM was recorded. The exercise intensity was approximately 2 watts per kg body mass (143 ± 11 W). During each 10 min of exercise, room temperature and humidity was recorded once and heart rate was recorded twice at minute 5 and minute 10. When a 2% of BM loss was achieved participants were asked to take a shower and towel dry and nude BM was recorded again as final post-exercise BM (BM_2). The participants were given a standardised high carbohydrate (CHO) meal consisting of 10 kcal/kg body mass.

The meal consisted of a cheese and tomato mini pizza (Tesco Co.UK) and four or five Nutrigrain bars (Kellogg Na CO Warrington. UK). The overall composition of the meal was CHO $72.7 \pm 0.6\%$; protein $9.3\pm 4 \%$; fat $21.8 \pm 0.4 \%$ and the quantity of consumed food was 0.323 ± 0.12 kg from that 42.9 ± 1.6 g was water. Participants in both trials were asked to avoid food till next morning tests. In trial H participants were also given 200 ml of orange juice with no added sugar (Tesco Co. UK) and after that they were asked to stop drinking until next morning visit. In trial HR participants consumed liquids ad libitum and they were encouraged to drink at least a quantity equalling 1.5 their body mass loss until 12:00 midnight, when stopped drinking until next morning.

Participants in both trials reported to the laboratory next day at 08:00 am. They were asked to empty their bladder and a urine sample was retained for analyse of specific gravity, osmolality, and colour chart tests (as described in Chapter 2). Then nude BM was recorded (BM_3) and they completed a POMS test questionnaire. After the POMS test participants performed an isometric knee extension strength and endurance test (IKES). All tests were done as described in detail in Chapter 2.

3-4 Statistics

Statistical analysis was performed using SPSS version 14.0. Data are presented as means \pm SD. All variables were analysed using pair t-test. The hypothesis of significance was set at $p < 0.05$.

3-5 Results

One of the participants failed to perform in the last trial because of knee injury and his data was excluded from data analysis. Therefore all data is presented based on a subject number of 8.

Table 3.1 shows BM data and the differences before and after the cycling test and before the isometric strength test. The exercise protocol was successful in inducing a 2% decrement in BM in both trials as shown in Table 3.2. There was no difference in average BM_1 and BM_2 in trial HR compared to trial H ($p > 0.14$). There was a significant BM loss BM_3 vs. BM_1 in both trials, in trial HR 1.1% and in trial H 3.1%.

Table 3.1 Body mass (BM; Means±SD).

	BM₁ (kg)	BM₂ (kg)	BM₃ (kg)	Δ₁BM (kg)	Δ₂BM (kg)	Δ₃BM (kg)
Hypohydrated/ Rehydrated Trial	71.4 ±5.6	70.0 ±5.5	70.7 ±5.6	-1.4 ±0.2	0.7 ±0.3	-0.8 ±0.3*
Hypohydrated Trial	71.3 ±5.6	69.8 ±5.4	69.1 ±5.3	-1.5 ±0.2	-0.7 ±0.2	-2.2 ±0.4*

BM₁, body mass before hypohydration; **BM₂**, body mass after hypohydration; **BM₃**, body mass before isometric strength test; **Δ₁BM**, body mass change in BM₂ vs. BM₁; **Δ₂BM**, body mass change in BM₃ vs. BM₂; **Δ₃BM**, body mass change in BM₃ vs. BM₁; *, significant difference.

Table 3.2 Percent of body mass change (Means±SD).

	Δ₁(%)±SD	Δ₂(%)±SD
Hypohydrated/ Rehydrated Trial	-2.0±0.2	-1.1±0.4*
Hypohydrated Trial	-2.1±0.2	-3.1±0.4*

Δ₁ (%), percent of BM loss in BM₂ vs. BM₁; **Δ₂ (%)**, percent of BM loss in BM₃ vs. BM₁; *, significant difference.

The urine collected prior to each isometric test was analysed for specific gravity, osmolality, and colour chart. Table 3.3 shows differences in urine osmolality, urine specific gravity, and urine colour chart score in Trial H compared to Trial HR ($p < 0.01$). There is a positive correlation between changes in all measures ($p < 0.01$).

Table 3.3 Urine measurement results (Means±SD).

	U.osmo. (mosmol/kg)	Ref. U_{SG} (gr/ml)	DS. U_{SG} (gr/ml)	U.Col.Chart
Hypohydrated/ Rehydrated Trial	461 ±48	1.017 ±0.002	1.015 ±0.003	3 ±1
Hypohydrated Trial	947 ±31*	1.030 ±0.001*	1.028 ±0.002*	6 ±1*

U.osmo., Urine osmolality; *Ref. U_{SG}*, Urine specific gravity measured by hand held refractometer; *DS. U_{SG}*, Urine specific gravity measured by Dip Stick; *U.Col.Chart*, Urine colour chart score; *, Significant difference ($p < 0.01$); *SD*, Standard Deviation.

Table 3.4 shows the results of a shortened version of Profile of Mood States (POMS) questionnaire test, as described in Chapter 2. There was no significant change in mood states when comparing HR to H trial. Also comparing two trials there was no significant difference in sleep duration and comfort: Trial HR 7.0 ± 0.6 h, Trial H 6.9 ± 0.4 h ($p > 0.3$).

Table 3.4 POMS (Profile of Mood States) test results (Means±SD).

Parameter	Tension	Vigour	Depression	Fatigue	Anger	Confusion
Trial						
Hypohydrated/						
Rehydrated	1.3±1.8	8.0±6.7	1.4±2.5	3.6 ±1.8	1.5±1.3	1.6±1.7
Trial						
Hypohydrated						
Trial	1.1±1.7	8.7±5.6	1.3±2.6	6.0±3.7	1.7±1.5	1.6±1.8

Tension $p > 0.3$; Vigour $p > 0.4$; Depression $p > 0.2$; Fatigue $p > 0.1$; Anger $p > 0.5$; Confusion $p > 0.2$.

Table 3.5 presents the isometric knee extension test results in baseline, H and HR trials. There was no significant difference amongst 3 baseline trials results compared to HR trial. P values were as below: right leg strength test $p > 0.2$, left leg

$p > 0.15$, and muscle endurance time in dominant leg $p > 0.4$. A significant decrease was determined in H trial in right ($p < 0.01$) and left ($p < 0.02$) leg strength tests and endurance time in dominant leg ($p < 0.01$).

Table 3.5 Isometric knee extension strength and endurance tests results (Means±SD).

Test results	RL Stren. (kg)	LL Stren. (kg)	Endurance time (s)	Ave. Endu. Stren. (kg)	Ave. SD.
Baseline 1	51.8±8.9	50.0±8.6	52.0±10.2	28.2±4.5	2.3±0.7
Baseline 2	52.9±8.6	50.6±8.3	51.3±12	28.1±4.8	1.9±0.4
Baseline 3	51.8±8.9	49.8±9.3	54.7±14.9	27.5±4.9	1.9±5
Hypohydration/					
Rehydration	52.5±9.3	51.9±9.2	52.9±15.9	28.7±5.1	2.3±0.71
Trial					
Hypohydration	48.7±9.2*	47.27±6.2*	46.4±11.7*	26.9±4.7	5.6±8.5
Trial	$p < 0.01$	$p < 0.02$	$p < 0.01$		

RL Stren., Right leg strength; *LL Stren.*, Left legs strength; *Ave. Endu. Stren.*, Average of muscle strength during endurance test; *Ave. SD*, Average standard deviation of muscle strength during endurance test; *, Significant difference between Trial H and HR.

3-6 Discussion

Hypohydration can be achieved by a number of different methods, such as exercise (active hypohydration), diuretic drugs, sauna (passive hypohydration) (Schoffstall et al., 2001), water deprivation, and combinations of them. Exercise induced hypohydration has been chosen in this study because it often occurs in exercise activities and sports fields.

This study investigated the effect of hypohydration on muscle performance. BM loss data shows that hypohydration protocol was successful in achieving 2% of BM loss during exercise in hot and humid environment. The rehydration protocol (rehydration fluids equalled 1.5×BM loss according to Shirreffs et al., 1996) was efficient to a great extent in recovering BM loss. However, the 1.1% BM loss difference of Trial HR compared to initial body mass loss was due to overnight fast (from 12:00 midnight) after recovery. In Trial H the exercise induced hypohydration and overnight fast induced a 3.1% decrease in BM loss (2% of it was due to exercise). Considering the weight (323 g) of food consumed in the dinner (after BM₂ measurement) the overnight BM loss was 1.4%.

The results in Table 3.5 show the differences between hydration status in Trials H and HR. In this study BM change was the major hydration marker particularly during exercise-induced hypohydration. Although in this study serum osmolality was not measured, the combination of body mass change, urine osmolality, specific gravity, and colour chart tests results offer enough support to demonstrate hydration status difference between trials prior to the isometric knee extension tests.

In this study special attention was focused on minimizing the effect of some possibly interfering factors such as glycogen storage changes, muscle temperature, body fluid redistribution, sleep duration (Bulbulian et al., 1996), and mood status. High CHO meal (10 kcal/kg body mass consisting of more than 70% CHO) was given shortly after exercise in order to recover the muscle glycogen storage. Almost 14 h elapsed

between hypohydration exercise session and isometric strength test. This interval was enough to normalise muscle temperature and to ensure body fluid redistribution.

Judelson et al. (2007 a) published a review on hypohydration and muscle performance. They covered most published articles from 1940s till 2007. They criticized the methodologies of most of the published studies on muscle performance and particularly muscle strength. They suggested that further investigations should to consider three crucial factors of study design namely: dehydration method, subject selection (to be matched in endurance training history and gender), and performance measure (to use identical workload in all trials). They also suggested achieving dehydration via low intensity exercise in the hot chamber in the evening prior to the morning performance testing. In planning of present study all factors suggested by Judelson and his colleagues were considered and implemented as stated before.

The sleep record of the night prior to the isometric test showed no significant difference between trials. Table 3.4 shows no differences in any component of mood status in Trial HR compared to Trial H. These findings are opposite to the results of Schoffstall & Leutholtz (2006) that demonstrated an adverse influence of 1.5% body mass loss via passive (sauna) induced hypohydration on POMS. The difference in results may be due to the fact they conducted the POMS test immediately after sauna exposure. Therefore it is not clear whether the change observed in their study was due to hypohydration or other possible factors such as increased core temperature.

Hypohydration causes a redistribution of body water amongst the different compartments of the body in order to maintain blood flow in physiologic range. In

other words the share of each body compartment in water loss during hypohydration is not equal and share of each organ in water loss is not the same. Nose et al. (1983) showed that water deficit occurs more in extracellular compartment representing 59% of the entire body water compared to intracellular compartment, which is 41%. Amongst the organs muscle has the biggest share of water loss that is 40%; skin 30%; viscera 14%; and bone 14% (Murray, 1996).

Literature results are controversial regarding the effects of hypohydration on muscle strength and endurance. The key findings of this study show that 2% of body mass loss induced by exercise in hot environment decreases muscle strength by average of $8.6 \pm 2.1\%$ (RL 7.2%, LL 9%). This is in line with studies of Evetovich et al. (2002); Webster et al. (1990); Viitasalo et al. (1987); Bosco et al. (1974); Saltin (1964 a); Gutiérrez et al. (2003); Guastella et al. (1988); and Moore et al. (1992); but disagrees with the results of other's studies: Serfass et al. (1984); Montain et al. (1998); Greiwe et al. (1998); Greenleaf et al. (1967); and Horswill et al. (1992)) which demonstrated no changes in muscle strength with change in hypohydration status.

The present study demonstrates that 2% hypohydration decreased endurance time by $12.3 \pm 3.2\%$. Studies with similar results were performed by Hoffman et al. (1995); Watson et al. (2005); Bijlani and Sharma (1980); King et al. (1985); Jacobs (1980); Guastella et al. (1988); and Webster et al. (1990). However there are some research studies with opposite results showing an increase in endurance such as those of Caterisano et al., (1988) and Fogelholm et al. (1993).

Judelson et al. (2007 a) reported that only 21% (15 out of 70) evaluated studies, showed statistically significant performance reduction after different levels of hypohydration (1.1% to 6%) using different hypohydration protocols such as heat exposure, exercise-induced, water deprivation and combination hypohydration technique. They concluded that hypohydration adversely affects muscular strength, power and endurance.

Shirreffs (2009) published an overview of hydration, fluids and their effect on performance. The author concluded that a hypohydration of 3% - 4% of body mass decreases strength, power and endurance, and the adverse effect of hypohydration on endurance performance is clearer than the effect on muscle strength (Shirreffs, 2009). However there are a number of difficulties in interpreting the results of studies about the effect of hypohydration on muscle strength (Maughan, 2003; Judelson et al., 2007 a).

The interpretation of discrepancies may be made on differences in methods used to achieve various levels of hypohydration, in subjects choice (from weight category athletes familiar with exercise in hot environment to sedentary individuals), in muscle tests techniques (isometric, isokinetic, etc), on masking and exacerbating factors (muscle glycogen level, body temperature, acid-base status, fluid distribution changes and fatigue), and the fact that some of the research was performed as laboratory basic science study while others as field studies with less control on participants. The differences in methods of hypohydration usually includes heat exposure and exercise. Both of these cause an increase in muscle temperature that clearly affects muscle contraction physiology (Maughan, 2003).

3-7 Conclusion

In conclusion exercise induced hypohydration equivalent to 2% BM loss followed by an overnight fast giving 3.1% BM loss decreases muscle strength by an average of $8.6 \pm 2.1\%$ (RL 7.2%, LL 9%) and endurance time by $12.3 \pm 3.2\%$. However, this change in hydration level had no effect on sleep duration and pattern or profile of mood states (POMS) factors. Physiological mechanism of hypohydration and the effect of hypohydration on muscles, neuromuscular system and CNS (Central Nervous System) need to be further investigated.

Chapter 4

Effect of Ramadan style fasting on some aspects of exercise performance

4-1 Abstract

Most healthy individuals amongst Muslim communities follow the custom of fasting in the holy month of Ramadan. Present study was undertaken to assess the effects of one day Ramadan style fasting (11 hours) on some aspects of exercise performance. Twelve young physically active students, seven male and five female (Means±SD: age 22±4 year; height 176±9 cm; body mass 71.2±10.7 kg) participated in two randomised trials F- Fed and NF- No Food (fasted) simulating a Ramadan fasting day. After familiarisation with the experimental measurements, participants were tested in three sessions: S₁ at 07:00 am, S₂ at 12:00 noon and S₃ at 07:00 pm. Prior to S₁ visit they had an overnight fast from 12:00 midnight. All participants had a standardized breakfast (average 428±66 kcal) and only Trial F participants had also a standardized lunch (average 713±110 kcal).

In every session: urine was analysed for specific gravity, osmolality (U.osmo.), sodium concentration, potassium concentration, chloride concentration; body mass (BM) was measured; a computer based cognitive function test was completed; blood was analysed for: haematocrit, haemoglobin concentration, blood glucose concentration (B_{GLUC}), serum osmolality (S.osmo.), serum sodium concentration, serum potassium concentration, and serum chloride concentration; a set of exercise tests was performed: vertical jump test (VJ), isometric knee extension strength

(IKES), isometric knee extension endurance (IKEE), peak power cycling test (PP), and VO_{2max} . U.osmo. and S.osmo. increased ($p < 0.01$) in Trial NF S₃ vs. S₁, and decreased ($p < 0.01$) in Trial F S₃ vs. S₁. BM decreased ($p < 0.01$) in Trial NF S₃ vs. S₂. Blood, plasma and cell volume did not change over trials ($p > 0.05$). B_{GLUC} decreased ($p < 0.02$) in Trial NF S₂ and S₃ vs. S₁. VJ ($p = 0.07$), IKES ($p = 0.06$), PP ($p = 0.3$) and VO_{2max} ($p < 0.08$) did not change significantly.

Conclusion: It can be concluded that 11 h of Ramadan style fasting decreased body mass by 2.1%. However, no changes were found in exercise performance measurements.

Keywords: fasting, Ramadan, exercise performance.

4-2 Introduction

Most healthy adult individuals in Muslim communities practice fasting in the holy month of Ramadan. This kind of fasting is complete abstinence from food and water intake during day light period, from sunrise to sunset, which lasts approximately 11 to 16 hours, depending on the season and geographical latitude. Effects of a short period of food and water deprivation on different aspects of exercise performance have been investigated previously (see Chapter 1). However, there is limited information on exercise performance during a day of Ramadan fasting. In most Muslim countries in month of Ramadan, sport activities and competitions, including semi-professional and professional leagues, are postponed from morning and midday, to early evening (after sunset) and even late night. Fasting (food and water deprivation) and competition time

table rescheduling (via circadian rhythm alteration) affects athlete's performance during the month of Ramadan.

The effect of food and water deprivation (separately and in combination) on physiology and performance, for various durations, has been studied by many investigators including: Tuttle (1943); Saltin (1964 a); Bosco et al. (1968); Bosco et al. (1974); Jacobs (1980); Bender and Martin (1986); Dohm et al. (1986); Nieman et al. (1987); Guastella et al. (1988); Maughan and Gleeson (1988); Gleeson et al. (1988); Webster et al. (1990); Zinker et al. (1990); Armstrong et al. (1995); Chevront et al. (2003 and 2006); Armstrong et al. (2007); Oliver et al. (2007).

Circadian rhythm can be defined as persistent recurrent intra-individual fluctuations in some hormonal secretions, physiological variables and physical performances, which are not related to the environmental changes, during a 24 h cycle that are endogenously controlled. However, theories about origins of circadian rhythms are controversial (see Chapter 2). The effect of circadian rhythm on exercise performance has been extensively studied by: Monk and Leng (1982); Gifford (1987); Reilly and Down (1992); Coldwells et al. (1994); Atkinson et al. (1995); Atkinson and Reilly (1996).

At the best of our knowledge, 11 hours of daytime fasting similar to Ramadan fasting and its effects on exercise performance at different times of the day, has not been studied previously. The aim of this study was to evaluate the effects of one day of Ramadan style fasting (11 hours) on some aspects of exercise performance.

4-3 Material and Method

Twelve young physically active students, seven male and five female (Means \pm SD: age 22 \pm 4 year; height 176 \pm 9 cm; body mass 71.2 \pm 10.7 kg) voluntarily participated in present study approved by Loughborough University Ethics Advisory Committee. After participants were provided full information about the study in writing and verbally, they completed a medical screening questionnaire and signed an informed consent. All their questions about the study were answered in detail. All participants took part in two randomised trials F- Fed and NF- No Food (fasted). In Trial F they had controlled food intake and water ad libitum and in Trial NF they were fasting (complete abstinence from food and water) for approximately 11 h.

A week before the trial participants visited the laboratory for a familiarisation session in which they completed all tests in the same order as in the real trials. Participants were instructed to eat the same food in their last meal the day before the test sessions and avoid for 24 h prior to the trial coffee and alcohol consumption and intense exercise (e.g. running, cycling).

After an overnight fast (from 12:00 midnight abstinence from food and water intake) participants reported to the laboratory for three testing sessions: at 07:00 am (S₁), 12:00 noon (S₂), and 07:00 pm (S₃). On arrival at the laboratory, after emptying their bladder, urine volume was measured and urine sample was retained in a 5 ml Z5 container for analysis. Urine sample was analysed for specific gravity, osmolality, sodium concentration, potassium concentration, chloride concentration as described in Chapter 2. After urine collection body mass (BM) was measured (Adam Equipment

Co- Model: AFW- 120×0.02 kg). Then participants had 20 minutes of seated rest, before a blood sample was collected. During the rest, a computer based cognitive function test was completed which was also used for study in Chapter 6 (Effect of fasting on cognitive function). After rest, 5 ml of blood was collected from forearm vein. Blood was analysed for haematocrit, haemoglobin concentration, blood glucose concentration, serum osmolality, serum sodium concentration, serum potassium concentration, and serum chloride concentration. Laboratory analyses are described in detail in Chapter 2. Then a vertical jump, isometric knee extension strength, isometric knee extension endurance, peak power cycling tests, and $VO_{2\max}$ measurement, with a fixed timetable were performed. The detailed description of the tests can be found in Chapter 2. Average \pm SD exercise room temperature was $23 \pm 1^\circ\text{C}$, relative humidity $40 \pm 8\%$ and barometric pressure 760 ± 11 mmHg. There was no significant difference in room temperature ($p > 0.19$), relative humidity ($p > 0.43$) and barometric pressure ($p > 0.16$) over the sessions or trials. Altogether, tests lasted approximately 50 min from which 11 ± 2 min was the duration of the $VO_{2\max}$ test. Then participants had a shower and immediately after that they were given a standardised breakfast. Breakfast consisted of two to four (optional) Nutrigrain bars (Kellogg Na Co. Warrington, UK) 6 kcal/kg body mass (average 428 ± 66 kcal) with composition: $80.2 \pm 2.3\%$ carbohydrate (CHO), $20.1 \pm 2.4\%$ fat, and $3.8 \pm 0.4\%$ protein. In Trial F 350 ml of water was provided with the breakfast and in Trial NF water was ad libitum. In Trial F participants had access to water ad libitum until 2 hours prior to the next two visits (12:00 noon and 07:00 pm). In Trial NF they had no further access to water and food until the end of the trial. After the breakfast participants left the laboratory.

After S₂ participants in Trial F were given a standardised lunch approximately 10 Kcal/kg body mass, consisting of: 167±30 g brown bread (Tesco Co. UK); 19.4±6.6 g butter; 88.3±18 g jam (average 713±110 kcal), with the following nutritional composition: 81±6 % carbohydrate (CHO); 19±6 % fat; 7±1 % protein.

4-4 Statistics

Statistical analyses were performed using SPSS version 14.0. All variables were analysed using ANOVA with repeated measures. A Bonferroni complex samples hypothesis test was used for comparing different sessions. The hypothesis was accepted when $p < 0.05$.

4-5 Results

Eleven hours of fast (Trial NF) decreased BM by almost 1.5 ± 0.4 % in average (1.11±0.4 kg in absolute amount) (Table 4.1). Considering the weight of food and water consumed in breakfast (88±19 g of food and 350 ml of water) it was calculated that total body mass (TBM) loss was 2.2 ± 0.37 % (1.6±0.4 kg; $p < 0.05$).

Table 4.1 Body mass (BM) (Means±SD) in Trial F and NF, during S₂ and S₃.

Session			
Trial	BM in S₁	BM in S₂	BM in S₃
Trial F (Fed)	71.3±10.7	71.1±10.8	71.0±10.6
Trial NF (No food)	71.0±11.0	70.7±11.0	69.9±10.8

S₁, Session 1; S₂, Session 2; S₃, Session 3.

Table 4.2 shows the results of exercise performance tests. Vertical jump test results did not change over time on either Trial F or Trial NF (F $p < 0.4$; NF $p < 0.7$). There was no difference at any time when comparing Trial F with Trial NF ($p < 0.7$). Isometric knee extension strength did not change over trials ($p < 0.1$) and time trial ($p < 0.3$). However, it was lower in the Trial F ($p < 0.01$) and that could be attributed to daily differences. In both trials isometric knee extension power did not decrease in S₃ vs. S₁ (0.054). However, the absolute amount of decrement was higher in Trial NF compared with Trial F (8.8 kg vs. 3.6 kg). Peak power did not change significantly over the sessions and trials (F $p < 0.2$; NF $p < 0.2$; and F vs. NF $p < 0.1$). VO_{2max} did not change significantly over the sessions and trials (F $p < 0.2$; NF $p > 0.3$; and Trial F vs. NF $p < 0.08$).

Table 4.2 Performance tests results (Means±SD).

Trial sessions	F₁	F₂	F₃	NF₁	NF₂	NF₃
VJ (cm)	28.6±8.7	29.1±8.1	29.3±7.8	28.8±9.2	28.6±7.4	28.4±7.9
IKES (kg)	57.7±16.8	56.3±15.4	54.1±15.5	64.6±20.6	61.5±18.2	55.8±15.7
IKEE (s)	71.8±27	65.0±29	67.5±28	62.4±27	64.8±20	63.7±26
PP (W)	803±224	790±248	786±231	792±221	785±232	778±230
VO_{2max} (ml/kg/min)	42.7±8.3	44.6±12.3	42.7±10.1	42.2±8.3	42.6±9.4	40.7±8.5
VO_{2max} (ml/min)	3042±764	3162±951	3037±833	3053±719	3000±749	2842±732

F, Fed trial; *NF*, Fasted trial; *SD*, Standard Deviation; *VJ*, Vertical Jumping; *IKES*, Isometric Knee Extension Strength; *IKEE*, Isometric Knee Extension Endurance; *PP*, Peak Power; *VO_{2max}*, Maximum Volume Oxygen uptake; None of the parameter changes were significant.

Table 4.3 shows results of the laboratory analysis on urine and blood. Plasma glucose was significantly lower in NF₃ vs. F₃ and NF₁. This shows that plasma glucose decreased over the time and was lower in NF over the trials in afternoon session. Serum and urine osmolality as key laboratory parameters for hydration significantly decreased in trial F and increased in trial NF. Serum and urine osmolality were also lower in trial F₃ vs. NF₃. Urine electrolytes changes followed the urine osmolality changes. Serum and urine Na, K and Cl were higher in NF₃ vs. F₃ and NF₁.

Table 4.3 Blood and urine analyses results (Means±SD) in Trial F and NF, during S₁, S₂ and S₃.

Results	Trial sessions					
	F ₁	F ₂	F ₃	NF ₁	NF ₂	NF ₃
Plasma glucose (mmol/L)	5.87 ±0.86	5.96 ±0.89	5.74 ±0.81 ¥	5.76 ±0.67 *	5.43 ±0.64	5.19 ±0.59
Serum osmolality (mOsmol/kg)	0.278 ±0.001*	0.275 ±0.001	0.280 ±0.006 ¥	280 ±0.006 *	282 ±0.004	286 ±0.007
Serum Na (mmol/L)	141 ±2	141 ±3	140 ±4	140 ±2	142 ±3	143 ±3
Serum K (mmol/L)	4.4 ±0.2	4.5 ±0.2	4.4 ±0.4	4.4 ±0.4	4.5 ±0.3	4.6 ±0.2
Serum Cl (mmol/L)	110 ±4	107 ±4	105 ±2	109 ±3	107 ±3	111 ±2
Urine osmolality (mOsmol/kg)	729 ±206 *	358 ±282	312 ±184 ¥	682 ±181*	627 ±223	888 ±160
Urine SG	1.023 ±0.007 *	1.010 ±0.008	1.009 ±0.005 ¥	1.018 ±0.007 *	1.018 ±0.006	1.027 ±0.001
Urine Na (mmol/L)	131.4 ±36.0 *	92.5 ±39.0	76.3 ±40.0 ¥	117.9 ±29.4 *	151.7 ±42.8	185.8 ±43.1
Urine K (mmol/L)	71.4 ±25.0 *	56.6 ±30	40.7 ±25.0 ¥	65.9 ±29.4 *	93.7 ±25.9	119.5 ±24.0
Urine Cl (mmol/L)	100.9 ±56 *	83.0 ±53	64.0 ±32 ¥	121.5 ±65 *	138.8 ±64.5	161.5 ±62.1

SG, Specific Gravity; *, significant change when compared with S₃ in the same trial; ¥, significant difference with the comparable session in Trial NF.

Changes in blood volume, cell volume and plasma volume were not significant neither over the time in both trial F and NF nor between trials (Table 4.4).

Table 4.4 Changes in blood volume (ΔBV), plasma volume (ΔPV), and cell volume (ΔCV) in S_1 vs. S_3 in Trials F and NF (Means \pm SD).

Session 1 vs. 3	$\Delta BV\%$	$\Delta PV\%$	$\Delta CV\%$
F	+1.7 \pm 2.3	+1.7 \pm 1.9	+1.4 \pm 2.0
NF	-1.9 \pm 2.8	-2.1 \pm 2.2	-1.6 \pm 2.5

4-6 Discussion

The aim of present study was to evaluate the effect of one day Ramadan style fasting on some aspects (muscle performance, anaerobic and VO_2 consumption) of exercise performance, in an 11 hours fasting trial (food and water abstinence). The results show there is no significant change in any of the studied parameters of exercise performance during and after 11 hours of Ramadan style fasting. In the present study it has been shown that 11 h of food and water deprivation had no effect on exercise performance. None of the performance parameters tested showed significant fall throughout the trials, nor was any difference found between trials.

Two major factors may have interacted to affect the results of the present study: first, food and water deprivation (namely fasting) and second, the circadian rhythms. We expected that food and water deprivation might decrease performance in the afternoon compared with the morning sessions, while exercise performance is generally better in the afternoon compared with morning sessions because of circadian rhythms interference.

The effect of food and water deprivation on exercise performance, separately and in combination, has been extensively investigated by: Tuttle (1943); Saltin (1964 a); Bosco et al. (1968); Bosco et al. (1974); Jacobs (1980); Bender and Martin (1986); Dohm et al. (1986); Nieman et al. (1987); Guastella et al. (1988); Maughan and Gleeson (1988); Gleeson et al. (1988); Webster et al. (1990); Zinker et al. (1990); Armstrong et al. (1995); Chevront et al. (2006); Armstrong et al. (2007); Oliver et al. (2007). However, the results of previous research show marked controversies demonstrating an increase, decrease or no change in exercise performance due to food and water deprivation. The contradictions in the results of different studies can be attributed to a few reasons: different periods of food and water deprivation, different performance test protocols, failing to eliminate interfering factors. However, amongst researchers there exists more tendencies to consider that food and water deprivation has a detrimental effect on exercise performance.

Circadian rhythm and its effects on hormonal secretion, general physiology and exercise performance have been previously studied. Although research about the effect of circadian rhythm on exercise performance is limited, nevertheless it is widely accepted that most of exercise performance components (e.g. muscle performance) are increased in early evening compared with early morning. In this regard in present study, exercise performance was tested during the day at three different times: 07:00 am, 12:00 noon, 07:00 pm, in order to evaluate any possible changes in performance throughout the day. Choosing a time for training during months of Ramadan is always a challenge for the athletes and their coaches. With results of this study it could be suggested that afternoon (just before dusk) training could be as efficient as in the

morning with an extra advantage that athletes could eat and drink immediately after the end of training.

Circadian rhythm could be defined as persistent recurrent intra- individual fluctuations in some hormonal secretions, physiological variables, and physical performances that are not related to the environmental changes, during a 24 h cycle that are endogenously controlled. Theories about origins of circadian rhythm are controversial. However, many studies confirmed that physical performance tests at different times of the night-day cycle could be influenced by circadian rhythm. The majority of the studies showed that some of physical performance variables oscillate over day time period, with peak in early evening. According to Atkinson and Reilly (1996) this mechanism could be related to variations in daily body temperature, reaching its maximum in the early evening. Different studies have shown that isometric hand grip (Gifford, 1987), back strength (Atkinson et al., 1995), elbow flexion strength (Coldwells et al., 1994) and vertical jumping (Reilly and Down, 1992) performances were significantly higher in the afternoon and early evening (almost by 10- 15%). However, there was a negative relation between speed and accuracy in a simple repetitive task tests in the early afternoon (Monk and Leng, 1982).

Having this in mind, and according to the results of present study, there would have been a possible decrease in isometric knee extension strength and vertical jumping test results in S₃, in both fed and fasted trials. This finding may be due to fasting effect and/ or the fatigue imposed by repeating two times the tests which could have a masking effect on the expected changes.

In present study in Trial NF S₃ vs. S₁ results show urine osmolality, urine specific gravity, urine electrolytes concentrations and serum osmolality increased, while BM decreased. This demonstrates hypohydration in Trial NF S₃ compared with Trial NF S₁, Trial F S₁ and Trial F S₃. Increased serum osmolality during Trial NF S₃ vs. S₁ demonstrates hypohydration was caused not only by fasting that induces an iso-osmotic type of hypohydration but also due to sweat loss and/ or other factors that induces a hyper-osmotic type of hypohydration. Having in mind the ambient temperature and relative humidity of exercise room remained constant during the trial over the day (23±1C°, 40±8% respectively) without need to be adjusted, it can be concluded the ambient parameters outside the building were also constant. At this ambient temperature it is completely logical to accept that participants had lost a tangible fraction of their body water via sweat loss due to the exercise and during the day. In present study the percentage of body mass loss in the Trial F (2.16%) was greater than expected by previous calculation. The reason for this can be partly accorded to sweat loss throughout two sessions of exercise tests (7:00 am and 12:00 am) which each consisted of a number of exercise activity including a continuous VO_{2max} test.

Eleven hours of fasting (Trial NF) caused a decrease in BM by almost 1.54±0.4 % in average (1.11±0.4 kg in absolute amount). Having in mind the weight of food and water consumed in breakfast (88±19 g of food and 350 ml of water) it was calculated the total body mass (TBM) loss should be 2.16±0.37 % (1.6±0.4 kg). A way to estimate the share of water loss out of TBM loss is to subtract from it the substrate loss during the trials. Loss of substrate was calculated using the VO₂ (oxygen

consumption rate) (Maughan et al., 2007). Oxygen consumption rate for an average individual at rest is almost $200 \text{ ml} \cdot \text{min}^{-1}$ (Sherwood, 2006). O_2 consumption in normal daily life with no intense activity is almost $300 \text{ ml} \cdot \text{min}^{-1}$. However, in a given physical activity oxygen needs depend on body composition, food intake composition and basal metabolism rate. Between BM_1 and BM_3 measurements, participants had fasted for 11 hours and took part in two exercise sessions. Cycling peak power test and $\text{VO}_{2\text{max}}$ test lasting altogether for an average of 15 minutes increased the VO_2 more than average of normal daily activity. Considering all this facts calculation demonstrated a 78 g substrate loss during the two exercise tests sessions. Calculation is described below, as follows:

$$11 \text{ h (fasting)} \times 60 \text{ min} = 660 \text{ min} \rightarrow \textit{Fasting period in minutes}$$

Fasting period (min) \times Substrate loss per minute (considering 10% of $\text{VO}_{2\text{max}}$ for the all fasting period except trial exercise time) = *Substrate loss for daily activity*

$$660 \text{ min} \times 0.05 \text{ g} = 33 \text{ g} \rightarrow \textit{Substrate loss for daily activity}$$

$$15 \text{ min (tests duration)} \times 2 \text{ sessions} = 30 \text{ min} \rightarrow \textit{Intense exercise period}$$

Intense exercise period (min) \times Substrate loss per minute (considering 80% of $\text{VO}_{2\text{max}}$) = *Substrate loss for two tests sessions*

$$30 \text{ min} \times 1.5 \text{ g} = 45 \text{ g} \rightarrow \textit{Substrate loss for two tests sessions}$$

Substrate loss for daily life activity + Substrate loss for two tests sessions = *Total substrate loss*

$45\text{ g} + 33\text{ g} = 78\text{ g} \rightarrow \text{Total substrate loss}$

(Calculation method according to Maughan et al., 2007)

This is a rough estimation because defecation was not taken in account; stool weight depends on the food amount and composition that has been consumed on the day before and of the trial.

Subtracting substrate loss (78 g) away from TBM loss (1.6 ± 0.4 kg) shows clearly the greatest remaining part of BM loss in 11 h of fasting within 3 sessions of exercise, is represented by the loss of body water (amount), namely 2% out of 2.16%. James and Shirreffs (2008) in an unpublished study from the School of Sport and Exercise Sciences in Loughborough University compared a 12 h fasting (food and water deprivation) trial with a 12 h water only deprivation trial. Results of their study support those of present study, that BM loss in water-deprivation trial was equal to approximately 70% of TBM loss (1.5% of initial BM) of the fasting trial.

Results in present study confirm that participants were in hypohydration status in Trial F. Having in mind the results of BM loss and substrate loss calculations it is logical to consider that level of hypohydration was almost less than 2% of total body mass (TBM). This level of hypohydration did not affect exercise performance.

Similarly, the results of previous studies showed the same conclusion. Judelson et al. (2007 a) reported that even a higher TBM loss (4.8%) in a water deprivation and exercise-induced dehydration failed to alter isometric back squat and vertical jumping

height tests. In the same topic, most of the previous research reported the same result. Tuttle (1943) reported in a study that a hypohydration of 4.9 % of the TBM did not materially affect strength (measured by right and left hand grips, chest push and pull, and back and leg strength). Saltin (1964 a) demonstrated even at a severe hypohydration as much as 5.5 % of the TBM the maximum isometric muscular strength for elbow flexion and knee extension showed no change.

On the other side, in contrast to the previous studies results, Bosco et al. (1968) showed there was a decreasing trend in maximal isometric muscular strength in muscle groups (knee extension, elbow flexion, leg extension, trunk extension), and a significantly decreased elbow flexion by the third day of a progressive hypohydration trial with a loss of up to 3.1 % of TBM . Bosco et al. (1974) demonstrated a decrease in maximal isometric strength in a trial of 3 day starvation and dehydration, but 3 days was too short a period to distinguish between the effects of dehydration and starvation. Sawka and Pandolf (1990) showed dehydration has an impact on exercise performance, causing a decrease in muscular endurance, maximal aerobic power, and physical work capacity. Jacobs (1980); Guastella et al. (1988); and Webster et al. (1990) showed that a range of 2-5% of TBM loss induced by heat exposure or exercise-induced or combination of the two hypohydration decreased Wingate peak power tests or cycling power by between 2.2 to 21%.

In a review published by Judelson and his colleagues in 2007 (b) the authors concluded that hypohydration seems to have a negative effect on muscular strength, power and high intensity endurance. Moreover, Shirreffs (2009) pointed out that hypohydration of 3-4% TBM seems to have a consistent negative effect on muscle

force generation. However, estimated dehydration level in present study was almost 2%.

Chevront et al. (2006) reported that peak power output (15 s Wingate anaerobic tests) was not affected by 2.7% of TBM passive heat exposure hypohydration. Armstrong et al. (2007) in a 5% of TBM hypohydration study showed no change in $VO_{2\text{ max}}$ and running economy in the competitive distance runner. However, Saltin (1964 b) stated that changes in VO_2 induced by dehydration (up to 5% body weight loss TBM), depends on environmental conditions. A cool environment does not change VO_2 , while heat accompanied by sweat loss reduces VO_2 by 27%.

Previous literature reported muscle performance in the afternoon is better than in the morning. Therefore, this could represent a masking effect for possible decrease throughout the Trial NF. In the same line, in our previous study described in Chapter 3 results show in the hypohydrated trial (13 h of overnight fasting) water deprivation caused a 1.4 % BM loss out of initial BM.

In the literature, there are a number of studies supporting the body mass loss results of present study. Bouhlel et al. (2006) in a study on Ramadan fasting reported almost a 1% decrease the day at the beginning of Ramadan compared with the baseline. Taylor et al. (1954) in an acute starvation (food deprivation with ad libitum water) study found that 1.4% of body mass lost during the first 24 h of the experiment. Consolazio et al. (1967 a, b) in a complete fasting trial reported 2% (1.44 kg) of body mass loss after first 24 h. Oliver et al. (2007) in a an energy and water restriction trial, reported also a body mass loss of $3.6 \pm 0.4\%$ in 48 h. Another study according to Knapik et al.

(1987) in a study of 3.5 day of fasting, after 8 h a slight body weight loss was recorded, but not significant. However, in their study participants did not perform any physical exercise and the duration of fasting was 3 h less than in the present study.

In the present study blood glucose decreased in Trial NF ($p < 0.02$) in both S_2 vs. S_1 (-0.33 ± 0.03 mmol/L) and S_3 vs. S_1 (-0.57 ± 0.08 mmol/L) but remained steady during Trial F. The fall of glucose in the present study might be due to fasting and having two sessions of exercise including VO_{2max} test from session 1 to session 3.

Oliver et al. (2007) found a decrease in plasma glucose in 24 h energy and water restriction trial compared with a control trial, which is similar to the present study's results. Dohm et al. (1986) reported a decrease in plasma glucose after 23 h complete food deprivation compared to fed trial., Bender and Martin (1986) in a 7 day semistarvation study (40% of normal caloric intake) showed a decrease in plasma glucose level compared to control values. They reported no changes in haematocrit in two treatments. Nieman et al. (1987) demonstrated in a 27 h fasting trial followed by exercise, plasma glucose level was the same in pre-exercise test and maintained throughout exercise in both fed and fasted group. However two subjects in the fasted group showed hypoglycaemia. Although they observed no significant changes in pre-exercise muscle glycogen and hydration values, an average decline of 44.7% in endurance performance was reported.

Maughan and Gleeson in 1988 demonstrated no changes in blood glucose after 36 h of food deprivation. Gleeson et al. (1988) in a 24 h food deprivation trial reported no changes in blood glucose concentration. Along the same line Zinker et al. (1990)

reported no changes in plasma glucose after 36 h of fasting. But in a fasted trial during exercise glucose reached, its nadir concentration sooner. The result of the present study demonstrated a decline in plasma glucose throughout the fasting trial from session 1 to session 3 ($p < 0.02$) while it remained steady in fed trial, which is contrast to the result of Nieman et al. (1987); Zinker et al. (1990); and Maughan and Gleeson (1988) study, but in the same line as Oliver et al. (2007); Dohm et al. (1986); and Bender and Martin (1986) study results.

This is in line with Consolazio's et al. (1967 a) results. They also reported an increase in blood potassium and magnesium levels in the first 24 h of fast but no changes in blood calcium and sodium levels. However, all electrolytes levels were significantly reduced at the end of 10 day complete fasting. Increased serum electrolytes levels in fact seem to be due to plasma volume decrease in the first 24 h of fasting. Consolazio et al. (1967 a) reported that blood volume, plasma volume and cell volume decreased by 34.1%, 40.2% and 24.5% respectively after 5 day of fasting. Although figures show a decrease from first day of fasting, the authors did not mention day to day changes throughout the fasting period. Oliver et al. (2007) reported a decrease in plasma volume after 48 h of both energy restriction, and a combination of water and energy restriction, by almost 5% in each trial.

In the present study it has been shown in Trial F S₃ vs. S₁ there was a decrease in serum sodium (-1 ± 2 mmol/L) and serum chloride (-5 ± 2 mmol/L), and no change in serum potassium (0 ± 0.2 mmol/L). However, data correction for plasma volume changes, demonstrated the change was not significant ($p > 0.05$).

In the present study results showed VJ performance did not change over time on either Trial F or Trial NF (F $p < 0.4$; NF $p < 0.7$). There was no difference at any time when comparing Trial F with Trial NF ($p < 0.7$).

In the present study it has been shown 11 hours of fasting caused no significant changes in the isometric knee extension strength (IKES) and isometric knee extension endurance (IKEE). IKES did not change over trials ($p < 0.1$) and time trial ($p < 0.3$). However, it was lower in the Trial F ($p < 0.01$) possibly because of the daily differences. In both Trial F and NF IKE power did not decrease in S_3 vs. S_1 (0.054). However, the absolute amount of decrement was higher in Trial NF compared with Trial F (8.8 kg vs. 3.6 kg). In the same line Consolazio et al. (1967 b) reported no change in isometric handgrip test after 5 day of complete food deprivation and Knapik et al. (1987) in a study of 3.5 day of fasting, pointed out no significant difference for isometric upper torso and hand grip strength, but isokinetic strength declined at two different velocities.

In the present study seven seconds peak power test result did not change significantly over the sessions and trials (F $p < 0.2$; NF $p < 0.2$; and F vs. NF $p < 0.1$). This shows anaerobic performance was not impaired throughout the day in both Trial F and NF. Henschel et al. (1954) reported no deterioration in the physiological response to a fixed anaerobic task after one day of fasting, as shown by blood lactate concentration measurements. Knapik et al. (1987) demonstrated no changes in anaerobic capacity using Thorstensson test that Murphy et al. (1984) showed is closely correlated with the Wingate test.

VO_{2max} did not change significantly over the sessions and trials ($F p < 0.2$; $NF p > 0.3$; and F vs. $NF p < 0.08$).

Dohm et al. (1986) in a 23 h complete food deprivation study demonstrated no changes in VO_{2max} compared to a fed trial, but endurance capacity declined significantly. Taylor et al. (1945) showed that during 5 successive 2.5 day fasting periods separated by 5 to 6 week intervals, at a given work load, ventilation L/minute decreased in fifth vs. first fasting period. This study can not be considered totally reliable because the long time span between trials and the low number of subjects, here it was mentioned as history of articles review.

Henschel et al. (1954) reported a maximum O_2 intake decrease of 266 ml/min (7.7%) after 5 day of starvation, but it remained unchanged when reported per unit of body mass. Bender and Martin (1986) reported no changes in absolute O_2 consumption during a semistarvation study (7 days, 40 % of normal calorie intake). However during calorie intake restriction, time to volitional exhaustion on treadmill exercise was less than on a normal calorie intake. Zinker et al. (1990) reported a 38% decrease in time to exhaustion exercise, after fasting vs. fed trial. Knapik et al. (1987) in a 3.5 day fasting study reported no significant changes in resting or exercising VO_2 and ventilation exchange, while time to exhaustion decreased. Maughan and Gleeson (1988) reported no changes in VO_2 during exercise after 36 h food deprivation. However, respiratory exchange ratio was lower in fasted trial compared to 12 h overnight fasting trial. Gleeson et al. (1988) reported a detrimental effect on maximal cycle exercise performance at 100% of VO_{2max} after 24 h of food deprivation.

Jacobs (1980); Guastella et al. (1988); and Webster et al. (1990) showed that a range of 2-5% of TBM loss, induced by heat exposure or exercise-induced or combination of the two hypohydration decreased Wingate peak power tests or cycling power by between 2.2 to 21%. In a review published by Judelson and his colleagues in 2007 (b) the authors concluded that hypohydration seems to have a negative effect on muscular strength, power and high intensity endurance. Moreover, Shirreffs (2009) pointed out that hypohydration of 3-4% TBM seems to have a consistent negative effect on muscle force generation. However, estimated dehydration level in the present study was almost 2%.

Chevront et al. (2006) reported that peak power output (15 s Wingate anaerobic tests) was not affected by 2.7% of TBM passive heat exposure hypohydration. Armstrong et al. (2007) in a 5% of TBM hypohydration showed no change in $VO_{2\max}$ and running economy in the competitive distance runner. However, Saltin (1964 b) stated that changes in VO_2 induced by dehydration (up to 5% body weight loss TBM), depends on environmental conditions. A cool environment does not change VO_2 , while heat accompanied by sweat loss reduces VO_2 by 27%.

4-7 Conclusion

Eleven hours of Ramadan style fasting (food and water deprivation) decreased body mass by almost 2.16%. The amount of body mass loss was greater than in studies using similar fasting period, due to possible water loss during exercise test bouts over the trial day that included two $VO_{2\max}$ tests. It seems logical to hypothesize that the body mass loss was due in fact to body water loss from different water compartments.

No effect was determined on the exercise performance measures. However, there would have been a possible decrease in isometric knee extension strength and vertical jump test results in S₃, in both Trials F and NF, due to fasting and / or the fatigue imposed by repeating the tests twice that circadian rhythm could have a masking effect on the expected amount of decrease.

Chapter 5**Effect of one month of Ramadan fasting on blood biochemistry and some aspects of exercise performance in Iranian athletes and recreationally active individuals****5-1 Abstract**

The aim of this study was to investigate the effect of one month of Ramadan fasting on blood biochemical parameters and some aspects of exercise performance in Iranian athletes and recreationally active individuals. Thirty-four male volunteers (Mean±SD: age 21±4 year, height 177±9 cm, body mass 76.5±15.3 kg) participated in the study and were divided in three groups: WS- weight category sports (N=12); NWS- non-weight category sports (N=11); RA- recreational activity (N=11). Participants were tested in four sessions: S₁- one week before Ramadan; S₂- one week after the start of fasting; S₃- during the last week of Ramadan; S₄- one week after the end of Ramadan. Urine was analysed for specific gravity and electrolytes; blood was analysed for haematological and profile of lipids tests; body mass (BM), body fat (BF) and body water content (BWC) were measured; high density lipoprotein- cholesterol (HDL-Ch), low density lipoprotein- cholesterol (LDL- Ch), triglycerides (TG-s); total cholesterol (TC) were determined; and Eurofit tests were performed. During the month of Ramadan most of the measured parameters significantly changed, but returned to baseline one week after Ramadan. BM (S₃ vs. S₁ = -1.9±2.0 kg) and BF (S₃ vs. S₁ = -0.1±0.8 kg) decreased progressively during Ramadan but returned to the pre-Ramadan level after the end of Ramadan ($p < 0.01$). A significant correlation was

demonstrated between the change in BM and the change in BF ($p < 0.01$). Performance in the sit up (S_3 vs. $S_1 = -3.5 \pm 2.2$ repetition count), long jump (S_3 vs. $S_1 = -3.6 \pm 3.0$ cm) and endurance shuttle run bleep tests was lower (S_3 vs. $S_1 = -83.5 \pm 61.9$ s) during Ramadan in S_2 and S_3 compared to non-fasting sessions S_1 and S_4 ($p < 0.01$). There was no difference between groups in the pattern of changes over time.

During Ramadan HDL- Ch increased (S_3 vs. $S_1 = +11.1 \pm 8.5$ mg/dL) and TG-s decreased (S_3 vs. $S_1 = +67.3 \pm 32.1$ mg/dL) in all groups ($p < 0.01$), while LDL- Ch decreased only in RA group ($p < 0.01$). TC did not change ($p > 0.2$). All changes recovered one week after Ramadan. **Conclusion:** Ramadan style life, involving a change not only in timing of the food intake but also in the sleep pattern, affects most of the anthropometric and physical performance parameters of athletes and recreationally active individuals. However, most parameters recovered a week after the end of Ramadan. The level of physical activity seems to have no effect on the pattern of performance changes.

Keywords: fasting, Ramadan, exercise performance, anthropometric factors, lipid profile.

5-2 Introduction

Fasting in the holy month of Ramadan is a popular practice amongst Muslims. It is the abstinence from eating, drinking and smoking from about an hour before the sunrise until sunset. Ramadan is a lunar month, which can last for 29 or 30 days according to the season and geographical latitude. Therefore, the length of Ramadan month differs from year to year and from location to location. However, most of the countries with a

majority of Muslim population are located in similar latitudes where the daytime during Ramadan lasts approximately between 12 and 16 hours. Food intake frequency, composition and meals timetable are affected during Ramadan.

In Iran during Ramadan two meals are consumed; one before sunrise and the other after sunset, rather than three meals in the normal daily life. The alteration in the time and number of meals may be the main reason for decrease in energy intake. The fall in energy intake during Ramadan was widely reported by: Hallak and Nomani (1988); Bouhlel et al. (2006) (Reilly and Waterhouse, 2006). However, some other studies demonstrated an opposite results showing the energy intake during Ramadan did not change (Afifi, 1997; Ramadan et al., 1999). Some other researches had even more controversial results showing an increased energy intake during Ramadan (Frost and Pirani, 1987). Ramadan fasting can not be simply considered only a different diet because during Ramadan not only the food composition, but also the pattern of eating, drinking, and sleeping are affected.

Sporting competitions continue throughout the month of Ramadan (e.g. the Olympic games of 2012 in London will run from 27 of July to 12 of August, while Ramadan will last from July 21 to August 20). There is currently limited knowledge pertaining to the physiological and performance consequences of Ramadan fasting for athletes.

The literature shows no consistent pattern of change in body mass and body fat content during Ramadan. There is controversy in the results obtained by Husain et al. (1987); Hallak and Nomani (1988); Adlouni et al. (1998); Ziaee et al. (2006) which showed a decrease in body mass and fat mass during Ramadan; while Yucel et al.

(2004) showed no change; and moreover Frost and Pirani (1987); Gharbi et al. (2003); Meckel et al. (2008) reported an increase in energy intake and weight and fat gain.

During a Ramadan fast a number of metabolic and physiologic changes occur. Long term food deprivation can affect mineral intake including iron intake which is important for red blood cell production in the bone marrow. Moreover, for athletes exercise activity increases some micronutrient needs, particularly iron. Iron needs and turnover of elite and recreational athletes performing strenuous exercise are higher than sedentary people (Deakin, 2006). Therefore, it is likely to expect that iron reserve in fasting athletes can be impaired throughout Ramadan fasting. It is reported that serum iron in 13 participants in overall significantly decreased at the end of Ramadan vs. pre-Ramadan values 17.7 ± 1.2 vs. 14.7 ± 0.9 $\mu\text{mol/l}$ (Ramadan et al., 1999). They also reported that a fall in serum iron in sedentary male subjects was significant. However, the change in physically active subjects was not significant during the same study.

No significant difference in white blood cell (WBC) count was found in 50 participants at the end of Ramadan when compared with a month after Ramadan (Sarraf-Zadegan et al., 2000) and the same result was reported by Argani et al. (2003) in 30 renal transplant recipients throughout Ramadan. However, the immune system can be altered by fasting due to altered hydration status; hormonal status and serum iron level (Meckel et al., 2008). Effect of fasting on blood biochemistry and lipid profile has been well studied in sedentary healthy people and in patients with hyperlipidaemia related health problems such as diabetes mellitus and primary hyperlipidaemia (Buerger-Gruetz syndrome) (Adlouni et al., 1997; Afrasiabi et al.,

2003 a; Salah et al., 2005). However, there are scarce studies addressing the issue in athletes.

Therefore the aim of this study was to examine the effects of one month of Ramadan fasting on blood biochemical factors and some aspects of exercise performance in Iranian athletes and recreationally active individuals.

5-3 Materials and methods

34 male volunteers (Means \pm SD: age 21 ± 4 year, height 177 ± 9 cm, body mass 76.5 ± 15.3 kg) took part in present study in four trials: S₁- one week before Ramadan; S₂- one week after the start of fasting; S₃- during the last week of Ramadan; S₄- one week after the end of Ramadan. Namely, S₁ and S₄ were fed trials, S₂ and S₃ fasted trials (down-to-dusk abstinence from food and water from approximately one hour and half before sunrise until after sunset). Participants were divided in three groups: WS- weight category sports (N=12); NWS- non- weight category sports (N=11); RA- recreational activity (N=11). The study was approved by Ethical Advisory of Sports Medicine Research Centre, Teheran University of Medical Sciences, Islamic Republic of Iran. All participants completed a medical screening questionnaire and they signed an informed consent after they were provided full information about the study in writing and verbally. All their questions about the study were answered in detail. The study was conducted in Mashhad, in north-east of Iran. The city is situated at above 1012 m altitude from sea level. The month of Ramadan lasted from the 14th of September to the 13th of November 2007.

In one visit familiarisation session held in the week before the trial, participants were familiarised with the laboratory and the tests, following the same trial timetable and succession as real trials.

The average and SD of maximum (t_{\max}°) and minimum (t_{\min}°) ambient temperature and relative humidity (h) in the course of study were: t_{\max}° 26 ± 5 C°; t_{\min}° 11 ± 5 C°; h 34 ± 10 %. Trials were started two hours before sunset (04:00 pm). Participants were asked to avoid for 24 hours before all visits intense physically activities like cycling, running and walking lasting more than 15 min. In trials S₁ and S₄, they were asked to eat the same food in their last meal prior to visits. In trials S₂ and S₃ they were asked to stop eating 4 hours, and drinking 2 hours, prior to visits. In trials S₂ and S₃ they fasted every day (food and water abstinence) during a lunar month, one hour and half before sunrise to sunset (almost 12 hours). Upon arrival to the laboratory urine sample was collected and afterwards body mass (BM) was measured using Sahand Scale C. (Tehran, Iran), with an accuracy up to ± 20 g. Urine sample was tested for specific gravity (U_{SG}), sodium (U_{Na}), potassium (U_K) and chloride (U_{Cl}). Urine sample collection and testing were performed as described in Chapter 2.

After BM measurement participants took a 15 min rest in a supine position. After 10 min of rest, blood pressure and respiratory rate were recorded. At the end of 15 min of rest, a blood sample was collected by venipuncture from forearm vein. Blood samples were tested for: red blood cell count (RBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), RBC volume distribution width (RDW), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC) and white blood cell differential count

(lymphocytes, neutrophils, monocytes, eosinophils), platelets count (PLT), mean platelet volume (MPV), blood glucose (B_{GLUC}), serum sodium (S_{Na}), serum potassium (S_K), serum chloride (S_{Cl}), serum calcium (S_{Ca}), serum phosphorous (S_P), total cholesterol (TC), high density lipoprotein- cholesterol (HDL- Ch), low density lipoprotein- cholesterol (LDL- Ch), triglycerides (TG-s), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). All blood tests are described in detail in Chapter 2.

After blood sampling, skin fold thickness was measured at four sites, namely: triceps, biceps, subscapular and illiac crest using Harpenden Skinfold Calliper (Harpenden Co, UK). The measurement was repeated three times for each site and the average calculated. Body fat (BF) was then calculated using the equation developed by Durnin & Womersley (1974). Afterwards participants performed a series of exercise performance tests (Eurofit tests; Adam et al., 1998) with some modification. Tests were performed at eight different stations namely: flamingo balance, sit and reach, sit up, plate tapping, standing long jump, hand grip, vertical jumping, 3 x 20 m shuttle test, and shuttle run bleep test. For details on exercise performance tests techniques, see Chapter 2. After the test participants left the laboratory.

5-4 Statistics

For all variables in this study descriptive statistics (means and standard deviation) were calculated. Statistical analysis was performed using SPSS version 14.0. All variables were analysed using ANOVA with repeated measures. Bonferroni complex

samples hypothesis test was used to compare different sessions. The hypothesis was accepted when $p < 0.05$.

5-5 Results

In all groups body mass and body fat percentage decreased progressively during Ramadan (BM decreased in S₃ vs. S₁ by 1.9 ± 2.0 kg; BF decreased in S₃ vs. S₁ by 1.0 ± 0.8 kg) ($p < 0.01$) and returned to the pre-Ramadan level afterwards. There was no significant difference between S₄ and S₁ ($p < 0.01$).

There was a significant positive correlation between BM change and BF change ($p < 0.01$). However, there was no difference in the pattern of BM and BF changes over time between groups.

Table 5.1, 5.2, and 5.3 shows absolute BM loss and BF loss in S₃ vs. S₁. Although the BF loss in WS group (0.8 ± 0.8 kg) was lower than NWS group (0.9 ± 0.7 kg), the absolute BM loss in WS group was higher than in NWS (2.0 ± 1.5 kg vs. 1.3 ± 1.7 kg).

Table 5.1 Body mass (Means±SD) in three groups (WS, NWS, PA) in trials S₁, S₂, S₃ and S₄.

	S ₁	S ₂	S ₃	S ₄
WS	72.7±12.0	71.8±11.9	70.7±11.6	72.4±11.4
NWS	75.0±15.0	74.2±14.4	75.7±14.1	75.0±14.8
RA	81.7±18.2	80.8±17.7	79.3±15	81.2±16.6

WS, Weight category Sports; *NWS*, Non-Weight category Sports; *RA*, Recreational Activity; *SD*, Standard Deviation; *S₁*, one week before Ramadan; *S₂*, one week after the start of fasting; *S₃*, during the last week of Ramadan; *S₄*, one week after the end of Ramadan.

Table 5.2 Body fat percentage changes (Means±SD) amongst three groups (WS, NWS, RA) in trials S₁, S₂, S₃ and S₄.

	S ₁	S ₂	S ₃	S ₄
WS	13.3±5.2	12.9±5.0	12.5 ± 4.6	13.4±5.9
NWS	14.9±2.8	14.6±2.7	14.0± 2.8	14.7±2.9
RA	20.5±6.4	20.1±6.3	19.3±65	20.3±6.2

WS, Weight category Sports; *NWS*, Non-Weight category Sports; *RA*, Recreational Activity; *SD*, Standard Deviation; *S₁*, one week before Ramadan; *S₂*, one week after the start of fasting; *S₃*, during the last week of Ramadan; *S₄*, one week after the end of Ramadan.

Table 5.3 Values (Mean±SD) of absolute body mass loss (kg) and body fat loss in three groups (WS, NWS, RA) in S₃ vs. S₁.

	Body Mass loss	Body Fat loss
WS	2.0±1.5	0.8±0.8
NWS	1.3± 1.7	0.9±0.7
RA	2.4±2.9	1.2±0.9

WS, Weight category Sports; *NWS*, Non-Weight category Sports; *RA*, Recreational Activity; *SD*, Standard Deviation; *S₁*, one week before Ramadan; *S₃*, during the last week of Ramadan.

Table 5.4 Sum of skinfolds in mm (Means±SD) in three groups (WS, NWS, RA) in trials S₁, S₂, S₃ and S₄.

	S ₁	S ₂	S ₃	S ₄
WS	24 ±11	23±10	22±11	24 ±11
NWS	25±6	24±5	23±5	25 ±5
RA	41± 23	40±23	38±21	40±22

WS, Weight category Sports; *NWS*, Non-Weight category Sports; *RA*, Recreational Activity; *SD*, Standard Deviation; *S₁*, one week before Ramadan; *S₃*, during the last week of Ramadan.

As can be seen in Table 5.5 heart rate decreased ($p < 0.01$) in both S_3 vs. S_1 (by 3.7 ± 3.5 /min) and S_2 vs. S_1 (by 3.9 ± 3.0 /min). Respiratory rate increased ($p < 0.05$) in S_3 vs. S_2 (by 0.8 ± 1.4 /min) and S_4 vs. S_2 (by 1.8 ± 1.4 /min). In addition, respiratory rate has an increasing tendency ($p = 0.06$) in S_4 vs. S_1 (by 1.7 ± 2.0 /min). While systolic blood pressure did not change ($p > 0.1$), the diastolic pressure decreased ($p < 0.01$) in S_3 vs. S_1 (by 5.3 ± 8.2 mmHg) and S_2 vs. S_1 (by 5.8 ± 8.2 mmHg).

Table 5.5 Values (Mean \pm SD) of heart rate, blood pressure and respiratory rate in trials S_1 , S_2 , S_3 and S_4 .

	S_1	S_2	S_3	S_4
Heart rate */ min	70 \pm 15	66 \pm 10	66 \pm 7	73 \pm 12
Systolic blood pressure (mmHg)	109 \pm 8	105 \pm 8	106 \pm 7	110 \pm 19
Diastolic blood pressure (mmHg)*	67 \pm 10	61 \pm 9	62 \pm 9	66 \pm 9
Respiratory rate */ min	17 \pm 2	17 \pm 2	17 \pm 2	18 \pm 4

SD, Standard Deviation; S_1 , one week before Ramadan; S_2 , one week after the start of fasting; S_3 , during the last week of Ramadan; S_4 , one week after the end of Ramadan; * $p < 0.05$.

Table 5.6 shows that red blood cell count increased ($p < 0.01$) in trials S_2 vs. S_1 (by $0.2 \times 10^6/\mu\text{L}$), S_3 vs. S_1 (by $0.4 \times 10^6/\mu\text{L}$) and S_4 vs. S_1 (by $0.2 \times 10^6/\mu\text{L}$). Haemoglobin increased ($p < 0.05$) significantly in S_2 vs. S_1 (by 0.5 ± 0.3 g/dL) and then decreased ($p < 0.05$) significantly in S_3 vs. S_2 (by 0.4 ± 0.3 g/dL) and S_4 vs. S_2 (by 0.6 ± 0.5 g/dL). Haematocrit did change during the course of the study ($p < 0.05$) but the significant change could not be located. Over the course of the study, there was a continuous decline in size and haemoglobin content of red blood cells throughout the course of study (MCV, MCH, RDW) while MCHC increased significantly ($p < 0.05$) in S_2 vs. S_1 (by 0.8 ± 1.2 g/dL) and S_4 vs. S_1 (by 2.5 ± 1.1 g/dL).

Table 5.6 Values (Means \pm SD) of haematological factors in trials S_1 , S_2 , S_3 and S_4 .

	S_1	S_2	S_3	S_4	Normal range
RBC ($\times 10^6/\mu\text{L}$)*	5.0 \pm 0.3	5.2 \pm 0.3	5.4 \pm 0.3	5.2 \pm 0.3	4.00–5.50
Hb (g/ dL)*	14.9 \pm 0.7	15.4 \pm 0.7	14.9 \pm 0.8	14.8 \pm 0.8	12.0–16.5
Hct (vol %) ¥	43.7 \pm 2.0	44.2 \pm 2.2	44.9 \pm 2.9	43.8 \pm 2.6	36.0–48.0
MCV (μm^3)*	88.9 \pm 4.0	84.0 \pm 4.1	82.1 \pm 4.0	81.8 \pm 4.1	78.0–96.0
MCH (pg)*	30.4 \pm 1.7	29.6 \pm 1.5	27.9 \pm 1.4	27.9 \pm 1.3	26.0–34.0
MCHC (g/ dL)*	33.8 \pm 1.1	35.2 \pm 0.7	34.2 \pm 0.8	34.4 \pm 0.7	32.0–36.0
RDW (%)*	12.1 \pm 0.7	10.8 \pm 0.5	11.0 \pm 0.4	11.1 \pm 0.6	10.0–15.0

SD, Standard Deviation; S_1 , one week before Ramadan; S_2 , one week after the start of fasting; S_3 , during the last week of Ramadan; S_4 , one week after the end of Ramadan; **RBC**, Red Blood Cell Count; **Hb**, Haemoglobin; **Hct**, Hematocrit; **MCV**, Mean Corpuscular Volume; **MCH**, Mean Corpuscular Haemoglobin; **MCHC**, Mean Corpuscular Haemoglobin Concentration; **RDW**, RBC Volume Distribution Width; * $p < 0.01$; ¥ $p < 0.05$.

In Table 5.7 can be observed that WBC count decreased in fasted trials vs. fed trials: S₂ vs.S₁ (by $1.0 \pm 1.0 \times 10^9/L$); S₂ vs.S₄ (by $1.3 \pm 1.2 \times 10^9/L$); S₃ vs.S₁ (by $1.0 \pm 0.8 \times 10^9/L$); S₃ vs.S₄ (by $1.2 \pm 1.1 \times 10^9/L$) and recovered in S₁ vs.S₄ (by a difference of $0.2 \pm 1.0 \times 10^9/L$; $p > 0.1$). Lymphocytes and Eosinophils fraction increased ($p < 0.01$), and Neutrophil and Monocytes fraction decreased ($p < 0.01$) throughout the course of the study. Platelets count decreased ($p < 0.01$) in S₂ vs.S₁ (by $9.0 \pm 31.3 \times 10^3/\mu L$), and increased ($p < 0.01$) in S₃ vs.S₁ (by $13.4 \pm 25.8 \times 10^3/\mu L$), S₃ vs.S₂ (by $22.3 \pm 24.6 \times 10^3/\mu L$), S₄ vs.S₁ (by $21.2 \pm 32.1 \times 10^3/\mu L$), and S₄ vs.S₂ (by $30.2 \pm 26.5 \times 10^3/\mu L$). There was no change in mean platelets volume ($p > 0.05$).

Table 5.7 Values (Means±SD) of white blood cells factors in trials S₁, S₂, S₃ and S₄.

	S ₁	S ₂	S ₃	S ₄	Normal range
WBC ($\times 10^9/L$)*	7±1	6±1	6±1	7±2	4–10
Lymphocytes (%)*	40.8±5.8	47.8±7.4	46.4±7.2	47.4±10.4	25–33
Neutrophils (%)*	58±6	50±7	52±7	51±11	54–62
Monocytes (%)*	0.8±0.8	0.3±0.5	0.3±0.4	0.1±0.3	3–7
Eosinophils (%)	0.8±1.0	1.8±1.8	1.7±2.0	1.5±2.3	1–3
PLT ($\times 10^3/\mu L$)*	217±30	208±30	230±29	238±33	150–450
MPV (μm^3)	8.6±0.7	9.0±0.7	8.4±0.7	8.8±0.8	7.6–10.8

SD, Standard Deviation; *S₁*, one week before Ramadan; *S₂*, one week after the start of fasting; *S₃*, during the last week of Ramadan; *S₄*, one week after the end of Ramadan; *WBC*, White Blood Cell Count; *PLT*, Platelets Count; *MPV*, Mean Platelets Volume; * $p < 0.01$.

Table 5.8 shows blood chemistry results: blood glucose was lower in fasted sessions (*S₂* and *S₃*) compared with fed sessions (*S₁* and *S₄*; $p < 0.01$). *S₂* vs.*S₁* (-1.2 ± 0.3 mg/dL) and *S₃* vs.*S₁* (-1.3 ± 0.4 mg/dL); serum Calcium was lower ($p < 0.01$) in *S₂* vs.*S₁* (-1.0 ± 0.3 mg/dL) and *S₃* vs.*S₁* (-0.8 ± 0.3 mg/dL); serum Potassium was lower in *S₂* vs.*S₁* (-0.3 ± 0.3 mg/dL) and *S₃* vs.*S₁* (-0.4 ± 0.3 mg/dL); TC did not change ($p > 0.2$); HDL- Ch has increased ($p < 0.01$) in *S₂* vs.*S₁* (by 4.7 ± 7.1 mg/dL) and *S₃* vs.*S₁* (by 11.1 ± 8.5 mg/dL); LDL- Ch was lower ($p < 0.01$) only in recreationally active subjects; TG-s was lower in *S₃* vs.*S₁* = $+67.3 \pm 32.1$ mg/dL in all groups ($p < 0.01$). AST was lower ($p < 0.05$) in *S₂* vs. *S₁* (by 0.7 ± 4.6 mg/dL) and then increased ($p <$

0.05) in S_3 vs. S_2 (by 3.4 ± 7.4 mg/dL); ALT was lower ($p < 0.01$) in S_2 vs. S_1 (by 2.7 ± 10.7 mg/dL) and then increased ($p < 0.05$) in S_3 vs. S_2 (by 10.2 ± 13.1 mg/dL). However, it could not be located over the sessions. ALP did not change ($p > 0.05$) over the course of the study.

Table 5.8 Blood chemistry values in trials S_1 , S_2 , S_3 and S_4 (Means \pm SD).

	S_1	S_2	S_3	S_4	Normal range
4Blood glucose (mmol/L)*	5.3 ± 0.3	4.6 ± 0.3	4.5 ± 0.3	5.1 ± 0.5	4.00–5.50
Serum Ca (mmol/L)*	9.7 ± 0.4	8.7 ± 0.3	8.9 ± 0.2	9.2 ± 0.3	8.6–11.0
Serum K (mmol/L)*	3.9 ± 0.3	3.6 ± 0.4	3.5 ± 0.3	4.0 ± 0.4	2.6–4.5
TC (mmol/L)	4.4 ± 0.8	4.3 ± 0.8	4.4 ± 0.8	4.3 ± 0.9	up to 5.2
HDL- Ch (mmol/L)*	1.5 ± 0.3	1.6 ± 0.3	1.8 ± 0.4	1.5 ± 0.4	over 0.9
LDL- Ch (mmol/L)*	2.3 ± 0.5	2.2 ± 0.5	2.3 ± 0.5	2.2 ± 0.5	up to 3.9
TG-s (mmol/L)*	1.6 ± 0.6	1.1 ± 0.6	0.9 ± 0.5	1.7 ± 1.3	up to 2.3
AST (IU/L)¥	24.6 ± 5.0	23.9 ± 5.2	27.3 ± 7.7	26.6 ± 9.2	0.00–37.00
ALT (IU/L)*	23.8 ± 9.7	21.1 ± 8.9	31.3 ± 18.2	26.3 ± 20.4	0.00–41.00
ALP (IU/L)	279 ± 153	284 ± 169	258 ± 165	283.5 ± 175.3	80.00–306

SD, Standard Deviation; S_1 , one week before Ramadan; S_2 , one week after the start of fasting; S_3 , during the last week of Ramadan; S_4 , one week after the end of Ramadan; *Ca*, Calcium; *K*, Potassium; *TC*, Total Cholesterol; *HDL- Ch*, High Density Lipoprotein- Cholesterol; *LDL- Ch*, Low Density Lipoprotein- Cholesterol; *TG-s*, Triglycerides; *AST*, Aspartate Transaminase; *ALT*, Alanine Transaminase; *ALP*, Alkaline Phosphatase; *IU*, International Unit; * - $p < 0.01$; ¥ - $p < 0.05$.

Values for performance tests are shown in Table 5.9. Hand grip strength did not change during Ramadan ($p > 0.06$). Arm plate tapping performance was lower ($p < 0.01$) in fasting sessions vs. fed sessions: S_2 vs. S_1 (by 3.6 ± 9.0 s), S_2 vs. S_4 (by 7.3 ± 3.8 s), S_3 vs. S_1 (-2.4 ± 9.4 s), S_3 vs. S_4 (-6.1 ± 3.9 s), while there was no significant change in S_1 vs. S_4 ($p > 0.08$). Vertical jumping performance decreased ($p < 0.01$) in S_2 vs. S_1 (by 1.8 ± 2.8 cm), in S_3 vs. S_1 (by 3.6 ± 3.0 cm), and increased ($p < 0.01$) in S_4 vs. S_1 (by 0.3 ± 2.7 cm). Long jump test performance dropped ($p < 0.01$) sharply throughout Ramadan: in S_2 vs. S_1 (by 6.8 ± 8.9 cm), and in S_3 vs. S_2 (by 6.5 ± 9.3 cm) and recovered ($p > 0.05$) almost to the pre-Ramadan level in S_4 vs. S_1 (by 4.5 ± 10.8 cm). Sit up down test performance was lower ($p < 0.01$) in fasted sessions (S_2 and S_3) vs. fed sessions (S_1 and S_4), namely S_2 vs. S_1 by 3.3 ± 2.0 , S_3 vs. S_1 by 3.5 ± 2.2 , S_2 vs. S_4 by 2.2 ± 2.7 , S_3 vs. S_4 by 2.5 ± 2.8 . Sit and reach test performance shows an increase ($p < 0.01$) over the course of the study in S_2 vs. S_1 (by 0.9 ± 2.2), S_3 vs. S_1 (by 1.7 ± 2.3) and S_4 vs. S_1 (by 2.1 ± 2.4). In 3×20 m shuttle run test the performance did not change over the course of study. ($p > 0.05$). Endurance in the shuttle run bleep test decreased ($p < 0.01$) in S_2 vs. S_1 (by 64.7 ± 55.9 s) and S_3 vs. S_1 (by 83.5 ± 61.9) and recovered ($p < 0.17$) afterwards to the initial S_1 level. Flamingo balance test change was not significant ($p > 0.15$) throughout the course of experiment.

Table 5.9 Values associated with performance tests in trials S₁, S₂, S₃ and S₄ (Means±SD).

	S ₁	S ₂	S ₃	S ₄
Hand grip (kg)	45.4±6.3	45.8±6.0	46.2±5.8	45.6±5.7
Arm plate tapping * (s)	16.8±3.8	18.0±3.2	17.5±3.4	15.3±2.3
Vertical jumping * (cm)	39.6±6.8	37.8±7.1	36.1±6.6	38.9±7.0
Long jump * (cm)	227.5±27.3	220.7±25.5	214.1±24.6	223.0±24.9
Sit up down * (count)	31.8±4.6	28.6±4.4	28.3±5.0	30.7±5.0
Sit and reach * (cm)	36.7±6.9	37.6±6.6	38.3±6.3	38.8±6.6
3×20 m shuttle run test (s)	13.6±0.8	13.5±0.8	13.4±0.9	13.1±0.7
Endurance shuttle Run bleep test * (s)	523.4±114.9	458.7±119.3	439.9±113.7	557.6±119.3

SD, Standard Deviation; *S*₁, one week before Ramadan; *S*₂, one week after the start of fasting; *S*₃, during the last week of Ramadan; *S*₄, one week after the end of Ramadan; * *p* < 0.05

5-6 Discussion

The results in present study demonstrated that one month of Ramadan fasting has various effects on blood biochemistry and some aspects of exercise performance in Iranian athletes and recreationally active individuals. The results will be discussed separately on each of the performed tests. Body mass and body fat percentage

decreased progressively during Ramadan and returned to the pre-Ramadan level afterwards. There was a significant positive correlation between BM change and BF change. However, there was no difference in the pattern of BM and BF changes over time between groups.

The literature on fasting shows no consistent pattern of change in body mass and body fat during Ramadan. Various results were found: Zebidi et al. (1990); Adlouni et al. (1998); and Ziaee et al. (2006) showed a decrease in body mass and fat mass; Yucel et al. (2004); and Beltaifa et al. (2002) showed no change. On the other hand Gharbi et al. (2003) reported an increase in energy intake and therefore weight and fat gain during Ramadan. This inconsistency could be justified by differences in food intake habits and daily activities in different Muslim communities due to geographical, cultural and socioeconomic features (Musaiger, 1993; Rashed, 1992).

Our results show that RBC number has increased during the month of Ramadan fasting maybe to compensate decrease in Hb content of the RBC. Haemoglobin concentration also increased after one week of Ramadan fasting compared to the week before fasting and this may be related to the hypohydration induced during the trial. However, haemoglobin concentration decreased afterwards during the last week of Ramadan and one week after the end of Ramadan. Iron deficiency anaemia is a general health problem in Iran and probably the tiny margin of Iron reserve and inadequate Iron intake along with the increased iron needs in athletes, led to this phenomenon. Chronic food deprivation or poor mineral intake below nutritional recommendations which is common worldwide, can lead to different levels of iron deficiency anaemia (microcytic). Iron deficiency anaemia is characterised by an

increase in red blood cells number and decrease in size and haemoglobin contents of the RBC.

There are controversial findings about the effect of Ramadan fasting on red blood cell related factors (RBC, Hb, Hct, MCV and MCH). Bouhlel et al. (2006) reported a significant increase in Hb and Hct throughout Ramadan month in rugby players in Tunisia. However in another study Argani et al. (2003) showed no change in Hb in renal transplant recipients during Ramadan. More controversially, Sarraf-Zadegan et al. (2000) reported a significant increase in RBC, Hct, and Hb two months after the end of Ramadan in comparison to the 26th day of the Ramadan month.

Our data showed a significant decrease of white blood cell (WBC) in fasted sessions (S₂ and S₃) vs. fed (S₁ and S₄) sessions. Changes in WBC differential consist of an increase in Lymphocytes number and decrease in both of Neutrophil and Monocytes number suggesting that athletes tend to be more at risk from infection. However, there was no change in Eosinophils. WBC count recovered one week after the end of Ramadan to normal values. However, there was no recovery of any of the changes in WBC differential. Sarraf-Zadegan et al. (2000) reported no change in WBC count on the 26th day of Ramadan and 2 months after the end of Ramadan. In the same line Argani et al. (2003) showed no change in WBC throughout Ramadan amongst renal transplant recipients.

Result of our study showed that platelets count increased significantly in S₃ and S₄ in comparison to S₁ and S₂. This is similar to the findings of Sarraf-Zadegan et al. (2000)

who reported a higher PLT count at the end of Ramadan vs. two months after Ramadan.

Lipid profile change in our study suggests that Ramadan fasting has a health beneficial effects on individuals with increasing factors which are known as heart protective lipids (HDL- Ch) and decreasing cardiovascular diseases risk factors (LD Ch and TG-s).

Studies showed not consistent results for lipid profile in fasting subjects during Ramadan. Body mass change, change in energy and food composition intake and initial fat percentage could be the factors to possible effect lipid profile. However, is not clear which factor has major effect on lipid profile. Even in matched groups there could be demonstrated a discrepancy in lipid profile changes. It is suggested that response to lipid profile changes is individual to some extent.

Our results showed no significant changes in total cholesterol in all three groups throughout Ramadan. Total cholesterol change during weight loss and/ or Ramadan fasting was reported in different studies: we can find in the literature results showing a decrease (Asgary et al., 2000; Scheen et al., 1982; Jackson, 1969; Bolzano et al., 1979; Wechsler et al., 1981; Nelius et al., 1982; Follick et al., 1984; Zimmerman et al., 1984; Rosenthal et al., 1985; Schwartz, 1987; Schouten et al., 1981; Sorbris et al., 1982; Widhalm and Zwiauer, 1983; Bosello et al., 1985; Davis et al., 1985; Weltman et al., 1980); other results demonstrating no change (Cesur et al., 2007; Argani et al., 2003; Filaire et al., 2001; Maislos et al., 1993; Thompson et al., 1979; Larosa et al., 1980; Weisweiler and Schwandt, 1981; Friedman et al., 1982; Carmena et al., 1984;

Gonen et al., 1985; Kesaniemi et al., 1985 b; Van Gaal and De Leeuw, 1986; Stout et al., 1976; Contaldo et al., 1980 a, b; Rabkin et al., 1981; Sorbris et al., 1981); and even some findings revealed increase (Yarahmadi et al., 2003; El Ati et al., 1995; Fedail et al., 1982; Born, 1979; Markel et al., 1985; Ende, 1962).

This is consistent with result of a study which was done in France on Judokas by Filaire et al. (2001). The subjects in this study seem to match more with our study in terms of age, weight, body fat and also in being involved with exercise and fasting during whole month of Ramadan. Other studies were mainly done on obese or diabetic people who wanted to decrease their body mass. It was reported that the absolute total cholesterol change was not related to body mass change during Ramadan fasting (Hallak & Nomani, 1988).

However, Denis (1993) observed a relation between body mass reduction and TC reduction that was related to the base line of total cholesterol. Therefore, we conclude it could be because of low baseline total cholesterol in our subjects, we did not observe any significant change in our tests.

Our data show a significant increase in HDL- Ch concentration throughout the fasting period.

Although almost all recent studies and some previous ones (Cesur et al., 2007; Yarahmadi et al., 2003; Argani et al., 2003; Qujeq et al., 2002; Maislos et al., 1998; Uysal et al., 1998; Latif et al., 1993; Schwartz, 1987; Gonen et al., 1985; Jauhiainen et al., 1985; Kesaniemi et al., 1985 a; Sopko et al., 1985; Carmena et al., 1984; Wolf and Grundy, 1983; Friedman et al., 1982; Bronwnell and Stunkard, 1981; Rabkin et

al., 1981; Sorbris et al., 1981; Weisweiler and Schwandt, 1981; Streja et al., 1980; Contaldo et al., 1980 b) showed an increase in HDL- Ch concentration during Ramadan fasting and weight loss period, many others reported a decrease (Thompson et al., 1979; Larosa et al., 1980; Weisweiler and Schwandt, 1981 Nelius et al., 1982; Tokunaga et al., 1982; Rosenthal et al., 1985; Markel et al., 1985; Taskinen and Nikila, 1979; Schouten et al., 1981; Bosello et al., 1985; Weltman et al., 1980) or no change (Follick et al., 1984; Zimmerman et al., 1984; Sorbris et al., 1982; Widhalm and Zwiauer, 1983; Davis et al., 1985).

We consider that it may be due to Ramadan specific changes in food contents or meals frequency. However, there were no significant differences amongst groups and between S₁ and S₄. Cesur et al. (2006), in patients with diabetes mellitus type II and Maislos et al. (1998) in healthy young males and females reported a significant fall in HDL- Ch a month after Ramadan vs. the end of Ramadan, that is similar to our finding.

HDL- Ch has protein components in its structure, namely Apolipoprotein (Lp) A-I and A-II. HDL- Ch has mainly two different forms: one includes only Lp A-I and the other includes both Lp A-I and Lp A-II. Parra et al. (1992) reported that the variety of HDL- Ch containing only Lp A-I is involved in reverse cholesterol transport and therefore has a protective role in cardiovascular diseases. Adlouni et al. (1998) and Maislos et al. (1993) reported that Lp A-I increased during fasting in Ramadan. In this sense it could be claimed that Ramadan fasting can have positive influence on cardiovascular status. However, the HDL- Ch concentration falls after the end of

fasting period. Therefore it seems that the possible effects are limited to this course of time.

LDL- Ch was lower during Ramadan (in S_2 and S_3) compared with before and after Ramadan (S_1 and S_2). It returned to the baseline one week after Ramadan. It suggests that Ramadan fasting has limited effect on LDL- Ch only during the period of fasting. Our finding is in the same line with: Schwartz, 1987; Bosello et al., 1985; Davis et al., 1985; Van Gaal and De Leeuw, 1986; Kesaniemi et al., 1985 b; Follick et al., 1984; Zimmerman et al., 1984; Widhalm and Zwiauer, 1983; Friedman et al., 1982; Nelius et al., 1982; Bronwnell and Stunkard, 1981; Sorbris et al., 1981; Contaldo et al., 1980 b. However, it is in contrast with some other studies which showed no change: Cesur et al., 2007; Argani et al., 2003; Maislos et al., 1993; Streja et al., 1980; Wolf and Grundy, 1983; Taskinen and Nikila, 1979; Gonen et al., 1985; or even an increase: Yarahmadi et al., 2003; Sopko et al., 1985; Markel et al., 1985; Weisweiler and Schwandt, 1981; Larosa et al., 198).

TG-s was lower during Ramadan (in S_2 and S_3) compared with before and after Ramadan (S_1 and S_2). It returned to the baseline one week after Ramadan suggesting that Ramadan fasting has limited effect on TG-s only during the period of fasting. TG-s in S_4 was even higher than S_1 slightly but not significantly maybe due to increased food intake at the end of Ramadan celebrations.

Our data was supported by a number of studies (Yarahmadi et al., 2003 in male subjects; Asgary et al., 2000; Uysal et al., 1998; El Ati et al., 1995; Jackson, 1969; Schwandt, 1981). However, it is contradicted by studies which showed no change

(Cesur et al., 2007; Maislos et al., 1993; Bosello et al., 1985; Davis et al., 1985; Widhalm and Zwiauer, 1983; Bronwnell and Stunkard, 1981; Rabkin et al., 1981; Sorbris et al., 1981; Schouten et al., 1981; Contaldo et al., 1980 b; Taskinen and Nikila, 1979) or increased serum TG-s (Yarahmadi et al., 2003 in female subjects; Filaire et al., 2001; Markel et al., 1985; Stout et al., 1976) throughout Ramadan fasting.

We observed in the present study a decline in ALP concentration, but it was not statistically significant. However, AST and ALT increased significantly in S₃ vs. S₁ and did not return to the baseline a week after Ramadan. There was no difference over the groups in the pattern of change. It was expected that Ramadan style intermittent fasting would have some effect on liver enzyme concentration in serum. It seems this is due to a number of changes induced by fasting such as modified hydration and blood glucose status. Opposite to our results Farooq et al. (2006) reported a decrease in alkaline phosphatase (ALP) secretion in rats after 30 days of intermittent fasting for 12 hours followed by 12 hours of feeding.

The present study confirms that, level of physical activity seems to have no effect on the pattern of change in body mass and body fat. Ramadan et al. (1999) also observed no difference in active compared with sedentary men.

Ben Salama et al. (1993) showed that fasting caused a reduction in physical performance in 100 and 800 meters running tests. Zerguini et al. (2007) reported a significant decrease in anaerobic (speed in 20 m running) and aerobic (12 min run distance) capacities after two weeks of Ramadan fasting. In that study, there was no

change in vertical jumping results before vs. the end of Ramadan, but a lower value was observed at two weeks after vs. the end of Ramadan.

Zerguini et al. (2007) concluded that even two weeks after Ramadan many of the decrements in performances had not recovered, but this was not the case in present study. There is no clear evidence to explain this difference. However, it is may be due to cultural differences in observance of Ramadan.

Data in present study showed a significant decrease in heart rate and diastolic blood pressure (Table 5.5). However, systolic blood pressure did not change significantly. This finding shows that body metabolism demand is likely to be decreased in Ramadan compared with normal life style.

Stokholm et al. (1991) and Suwaidi et al. (2006) showed that fasting causes catecholamine inhibition and reduction in venous return, and this leads to a decrease in the sympathetic tone, which causes a decrease in heart rate, blood pressure and cardiac output.

In present study respiratory rate increased slightly throughout and after Ramadan. The increase in session 2 and 3 vs. session 1 could be explained by slightly acidosis due to fasting condition, but respiratory rate increase in session 4 vs. 1 might be due to weight gain and increase in physiologic demand after Ramadan.

While Moosavi et al. (2007) and Subhan & Kabir (2008) reported a lung volume increase in Ramadan and therefore possible improvement in pulmonary function,

Subhan et al. (2006) concluded that Ramadan fasting does not affect expiratory flow rates in healthy participants. However, he reported an increase in forced expiratory flow rates at 75% of vital capacity (FEF75) in post Ramadan values. In a study which was done by Gleeson et al. (1988), they showed that 24 hours of fasting have no effect on plasma pH when compared with fed trial. However, blood P_{CO_2} , plasma bicarbonate concentration and blood base excess were significantly lower in fasted subjects. It appears that fasted subjects were hyperventilated compared with fed subjects.

Our data shows a significant decline in vertical jumping height and long jump length during Ramadan. However, in this study they both recovered one week after the end of Ramadan. While Zerguini et al. (2007) reported no change in vertical jumping during Ramadan and a significant decrease 2 weeks after the end of Ramadan in professional soccer player in Tunisia, Meckel et al. (2008) observed a significant decrease in vertical jumping performance compared to period before Ramadan in soccer player in occupied Palestine.

Our study confirms that there is no significant change in hand grip strength over the month of Ramadan. Hand grip strength has been shown to be unaffected in three days of fasting (Gutierrez et al., 2001).

The result of vertical jumping and long jump suggest a loss in muscle strength at least in the big muscle group which are involved in jumping. However, this effect has not been shown in hand grip strength. This maybe due to a smaller power that is produced by the hand muscles and therefore this small change might not be observed.

Sit and reach test measures the flexibility of hamstring muscles and the lower back flexibility. Increased flexibility in fasted sessions (Table 5.9) could be explained by loss of body mass and fat in our participants during Ramadan. Possible abdominal fat loss could justify the result. Although Yucel et al. (2004) reported no change in abdominal fat during Ramadan. There was no significant change in body mass in that study.

The time for 25 complete cycle of arm plate tapping test (which represents speed and coordination of hand movement) increased in fasted trials in this study. There is no study to evaluate effect of fasting in Ramadan on arm plate tapping test performance. However, cognition (coordination) seems to be the main component of the test. Therefore, our result is in the line with Leiper et al. (2003) studies which reported an impairment of cognitive function during Ramadan.

5-7 Conclusion

Ramadan lifestyle (change in timing and composition of food intake and sleep pattern) affects most of the anthropometric and physical performance parameters of the athletes and of physically active individuals and there is no difference in the pattern of change between groups. However, almost all of the changes recovered a week after the end of fasting.

The discrepancy in results of different studies may be due to differences in climate, length of the day, Ramadan culture, food composition change, sleep pattern and daily life timetable. Therefore, it seems that more nutritional, cultural, climate and

geographical information is needed to be included in Ramadan fasting studies in the future.

Chapter 6

Effect of fasting on cognitive function

6-1 Abstract

Cognition has an important role in helping people able to perform difficult tasks that would be impossible to perform without it (Best, 1999) in particular in sport exercise and activities that need in most cases maximum body and brain performance. Regarding the consequences of fasting on cognition, the majority of studies examined only a narrow spectrum of cognitive fields and differences in study methods and planning make the conclusions ambiguous (Doniger et al., 2006). Although, the effect of food and water deprivation on cognition has previously been studied, the effect of one day and one month of Ramadan style fasting on cognitive function is unknown.

In this chapter three separate studies (A, B and C) are reported. The aim of study A was to evaluate the effect of breakfast elimination on cognitive function; study B the effect of one day of Ramadan style fasting on cognitive function; and study C the effect of one month of Ramadan fasting on cognitive performance. In study A, 31 volunteers (15 female and 16 male) (Means \pm SD: age 20 \pm 1 year; height 174 \pm 10 cm, body mass 66.2 \pm 12 kg) participated in two trials: Trial NF (no food, namely fasted) and Trial F (fed). In Trial NF participants fasted 12 h overnight (food and water deprivation) from 9:00 pm to 9:00 am when the test was performed. In Trial F they fasted 10 h starting in the evening 9:00 pm up to 2 h prior to morning testing. The participants had breakfast at home with ad libitum water and food and without any

recording of the consumed food. After participants reported to the laboratory urine sample was collected, and then body weight with minimal clothes was measured, followed by battery of cognitive tests while they were sitting, and lastly a blood sample was collected. In study B, 12 participants were tested in two trials F- Fed and NF- No Food (fasted), each consisting of three sessions: S₁ at 7:00 am, S₂ at 12:00 midday, and S₃ at 7:00 pm. In study C, 17 male volunteers (Means±SD age 21±4 year, height 177±9 cm, body mass 76.5±15.3 kg) participated in four computer- based cognitive function test trials. Participants were tested on four different sessions: S₁- one week before Ramadan, S₂- one week after the start of fasting, S₃- during the last week of Ramadan, S₄- one week after the end of Ramadan. For all three studies HBC test (Hogervorst– Bandelow Cognitive battery test) was used with identical components and timing.

Results: In Study A, short-term memory was impaired in the fasted trial vs. fed trial. In study B, for Visual Search test there was a significant decrease in reaction time comparing Trial NF with Trial F in 12:00 am session ($p < 0.01$). For Sternberg test, the only significant difference was found comparing the number of correct responses of trial F with trial NF in all three sessions ($p < 0.02$). When comparing reaction time of Trial F with NF results show some changes which tend to be significant: an increase in S₂ ($p = 0.07$) and a decrease in S₃ ($p = 0.08$). In study C, in the Sternberg test there were significant changes in reaction time, namely an increase in S₃ vs. S₁ ($p < 0.02$); increase in S₄ vs. S₁ ($p < 0.01$); and a decrease in S₄ vs. S₃ ($p < 0.03$).

RVIP test results show a significant decrease in number of correct answers only in S₂ vs. S₁ ($p < 0.02$), while in reaction time there were more significant changes: an increase in S₂ vs. S₁ ($p < 0.01$); increase in S₃ vs. S₁ ($p < 0.01$); increase in S₄ vs. S₁ (p

< 0.02). For the Stroop test the results show there were significant changes in the number of correct answers: decrease in S₂ vs. S₁ ($p < 0.02$); increase in S₃ vs. S₂ ($p < 0.01$); increase in S₄ vs. S₂ ($p < 0.05$). Also there were significant changes in reaction time: increase in S₂ vs. S₁ ($p < 0.01$); increase in S₃ vs. S₁ ($p < 0.02$); increase in S₄ vs. S₁ ($p < 0.04$). In conclusion, 11-12 h of food and fluid deprivation had detrimental effects on the accuracy of the response (attention) and slowed the reaction to the stimulus (longer reaction time). Long-term memory performance (recall after 20 min of the first seeing) in noon of three studies and short-term memory (immediately recall after seeing) in study B and C were not altered by this duration of fasting. However, short-term memory was poorer in study A in the no food trial comparing with the food trial. In study C Stroop and Sternberg tests reaction time increased (reaction performance impaired) throughout the month of Ramadan and did not recover even a week after Ramadan.

Keywords: cognitive function, fasting, Ramadan

6-2 Introduction

The brain is responsible for emotional, behavioural and cognitive function in human. The brain is intimately associated with all bodily processes and to the mental phenomena and the mind is dependent to the brain and body (Bickhard, 2009). Cognition consists of all mental processes and activities that are used in thinking, remembering, perceiving, learning and understanding, and the act of using these processes (Ashcraft, 1998). We can describe cognitive function also as a set of processes that produces a set of events, having the ability to store and recover information (Best, 1999).

Specific cognitive functions are attention, learning, memory, and reasoning, and there are hundreds of tests available for cognitive performance assessment (Lieberman, 2003 and 2005). Science of cognition developed as a reaction against behaviourism mainly inspired by the structure of a computer (Bickhard, 2009). “Cognitive psychology refers to all processes by which the sensory input is transformed, reduced, elaborated, stored, recovered and used” (Best, 1999).

Cognition has the important role of enabling people to perform difficult tasks that would be impossible to perform without it (Best, 1999) and in particular in sport, exercise and activities that need in most cases maximum body and brain performance. The ability of a tennis player to anticipate the exact trajectory and position of the tennis ball which is moving in the air with more than 30 m/s speed, and his/ her accurate and fast reaction mainly involves skill, physical fitness and cognition. The amount of cognition involvement differs in different sports (e.g. table tennis vs. swimming).

In sports that involve any kind of reaction towards a moving object (e.g. ball) and also in combat sports, an accurate and on time reaction seems to have a substantial share in performance. Beside the accuracy of the response, reaction time is also one of the most used measurements in cognitive performance. It is obvious that impairment of cognition in sports has a detrimental effect on performance. However, the literature shows scarce number of studies in this domain. Similarly, the effect of various factors that may affect cognition such as: hypohydration, food deprivation, Ramadan fasting, sleep deprivation, food consumption timing and some physiological changes, were also poorly studied previously.

It has been reported in previous studies that depriving humans of food has a deleterious effect on cognitive performance. Nevertheless, findings are not completely clear and there are not enough studies covering concomitantly several cognitive fields (Doniger et al., 2006). Although it was shown in some researches that human cognitive performance is affected by quantity and composition of food and timetable of meals (Leigh Gibson & Green, 2002; Pollitt & Matthews, 1998) there are few studies about the effect of Islamic Ramadan style fasting on cognitive function.

Due to routine religious fasting pattern in Islamic society which includes annually at least one month of fasting (see Chapter 2 for details about Ramadan style fasting) and regarding some common activities (e.g. driving), professions (e.g. piloting aircrafts, working with sharp tools, military tasks) and speed sports (e.g. racing) which depend on high cognitive sharpness, is very important to find and accurately describe the effects of fasting on cognitive function. Ramadan fasting includes complete food and water deprivation, leading to a short term hypohydration (for a period of 10-18 hours) and a drop of blood glucose concentration. In our study one day of Ramadan fasting blood glucose (see Chapter 4) after 11 hours of fasting blood glucose was lowered by 0.57 ± 0.55 mmol/L that is still within the normal range.

Hydration is one of the essential conditions for homeostasis, including functioning of the brain and therefore investigating this matter has a great importance. However, in investigation this domain is difficult as there is poor literature on the issue, scarce principles to be readily applied (Lieberman, 2007), and very difficult to accurately assess hypohydration level (Armstrong et al., 2007). Achieving a precise level of hypohydration is very complex and demanding as there are two physiologically

distinct types of hypohydration: hypotonic and isotonic that make the issue more complicated (Armstrong et al., 2007). In the literature there is not enough information about which duration and level of hypohydration would cause a specific degree of impairment in cognitive performance and which of the cognitive functions is most affected (Armstrong et al. 2007).

Severe hypohydration causes delirium, coma and death and therefore there is no doubt that prior to these extreme hypohydration levels, the cognitive function performance and mood will decline (Lieberman, 2007). A great amount of information deals with the effect of moderate to severe dehydration and the resulting hypernatraemia, on cognitive function (Vieweg et al., 1994; Warren et al., 1994), while the effect of mild dehydration on cognitive function is less known. Due to scarce number of studies regarding this issue and different flaws occurring during the studies, is not possible to make a definite conclusion about the effects of hypohydration on cognitive function (Lieberman, 2007).

Glucose represents the main source of energy for the brain and is indispensable for the normal function of CNS (Sieber & Traystman, 1992). In the existing literature a great amount of documentation shows that circulating blood glucose concentrations control several brain functions, especially learning and memory (Sunram-Lea et al., 2001 a, b). When blood glucose concentrations decreases to hypoglycaemic levels, obvious changes in EEG can be seen, brain function is very rapidly affected, neuronal activity is disrupted and cortical cellular function is altered (Thompson, 1967; Holmes et al., 1983). Therefore it is logical to expect that blood glucose drop during fasting might affect cognitive performance as well.

Another important feature in cognitive function is the effect of circadian rhythm. The influence of circadian rhythm on levels of cortical arousal represents an interfering factor in studying the effect of exercise on cognitive function, making the results controversial (Carrier & Monk, 2000). It has been proven that cognitive performance has circadian rhythm during the day similar to circadian rhythms in some physiological variables (e.g. body temperature, melatonin levels) (Monk, 2005). This is consistent with the findings of Potter & Keeling (2005) which consider that due to circadian rhythm memory performance reaches its highest output at 12:30 midday and poorest at 3:30 pm. Along the same line Blake (1967) demonstrated there is an evident post-lunch decrease in cognitive performance measured by signal detection, serial search, and reaction time. Supporting these findings two other investigations showed there was an increase in traffic accidents around lunch time (Lavie, 1991; Mitler et al., 1987). It is supposed the post-lunch short decline in cognitive performance may be caused by 12-hours temperature circadian rhythm which shows a dip after lunch particularly in morning type individuals (Monk & Leng, 1982; Potter & Keeling, 2005). Kleitman (1963) has demonstrated that the time-of-day has an effect on performance of simple repetitive tasks that is similar to circadian rhythm in body temperature.

It was found that peak performance of working memory happens at midday, while the peak performance of short-term memory (STM) happens in late morning (Folkard et al., 1975). These findings lead to the conclusion that the time of day for best performance for a given task is in fact dependent on the nature of the task (Folkard, 1983).

Present theories suggest that there is an interaction between circadian rhythm and time elapsed from awakening (Johnson et al., 1992; Monk & Carrier, 1998 and 1998; Wright et al., 2002 b). The numeric calculation, short-term memory and subjective alertness all are influenced by circadian rhythm of body temperature, with the lowest performance in early morning when body temperature is the lowest (Johnson et al., 1992). If a person stayed awake for 60 hours, body temperature and cognitive performance follow this circadian rhythm cycle, and the longer the person stayed awake, the more the decline in body temperature and cognitive performance would be (Johnson et al., 1992; Wright et al., 2002 b).

On the other hand, it was reported that the awareness and behavioural efficiency are influenced by levels of dopamine, serotonin, norepinephrine, and acetylcholine and fluctuations in level of these parameters are related to long term memory (Potter et al., 2000; Robbins & Everitt, 1995).

Memory (short and long time), sensory competence (visual accuracy), judgemental bias, analytical skills, and attention are some of the important components of cognitive function that can be assessed by the mean of various tests: Californian Verbal Learning Test (CVLT), Stroop test, Sternberg test, RVIP test (Rapid Visual Information Processing), and Visual Search Reaction Time test. The output of these tests is the accuracy of response and the reaction time. However, an accurate assessment of cognitive function is very difficult to achieve (Lieberman, 2007). In research methods the measurement techniques widely vary from case to case, depending on the priorities, purposes and objectives of the research. Every set of measures has its own advantage and there is no information available on which is the

best. Methods of measurement comprise: primary-task measures, formal measures, secondary-task measures, physiological measures and at last subjective measures of mental workload. Memory, vigilance, self-reported mood, speed, accuracy and perceived fatigue are cognitive measures. Since 1950 various tests for measuring cognitive function were developed. In recent years computer-based cognitive function batteries have been used. Many factors and variables will fully contribute to the complexity of mental workload which makes measures to be task specific (Moray, 1982). Nowadays using computer based cognitive function tests allow to evaluate different cognitive function components more can be evaluated now accurately than ever before. Therefore it seems logical to conduct more researche in this area.

Regarding the consequences of fasting on cognition the majority of studies examined only a narrow spectrum of cognitive fields and differences in study methods and planning make the conclusions ambiguous (Doniger et al., 2006).

In this chapter three separate studies (A, B and C) are reported. The aim of study A was to evaluate the effect of breakfast elimination on cognitive function; study B the effect of one day of Ramadan style fasting on cognitive function; and study C the effect of one month of Ramadan fasting on cognitive performance.

6-3 Materials and Methods

6-3.1 Study A

Thirty-one volunteers (15 female and 16 male) (Means±SD: age 20±1 year; height 174±10 cm, body mass 66.2±12 kg) participated in two trials: Trial NF (no food,

namely fasted) and Trial F (fed). In Trial NF participants fasted 12 h overnight (food and water deprivation) from 9:00 pm to 9:00 am when the test was performed. In Trial F they fasted 10 h starting in the evening 9:00 pm up to 2 h prior to morning testing. The participants had breakfast at home with ad libitum water and food and without any recording of the consumed food. After participants reported to the laboratory a urine sample was collected, then body weight with minimal clothes was measured, followed by a battery of cognitive test in sitting position, and a blood sample was collected. For all tests description details see Chapter 2.

6-3.2 *Study B*

12 participants were tested in two trials F- Fed and NF- No Food (fasted), each consisting of three sessions: S₁ at 7:00 am, S₂ at 12:00 midday and S₃ at 7:00 pm. Detailed information about participants and experimental protocol was presented in Chapter 4.

6-3.3 *Study C*

Seventeen male volunteers (Means \pm SD age 21 \pm 4 year, height 177 \pm 9 cm, and body mass 76.5 \pm 15.3 kg) participated in four computer-based cognitive function test trials. Participants were tested on four different sessions: S₁- one week before Ramadan, S₂- one week after the start of fasting, S₃- during the last week of Ramadan, S₄- one week after the end of Ramadan. The study was approved by Ethical Advisory Committee of Sports Medicine Research Centre, Teheran University of Medical Sciences, and

Islamic Republic of Iran. Participants signed an informed consent form. A familiarisation session was performed a week before the first trial (S₁).

For all three studies the HBC test (Hogervorst– Bandelow Cognitive battery test) was performed with identical components and timing (for more details see Chapter 2).

6-4 Statistics

Statistical analysis was performed using SPSS version 14.0. Variables in study A were analysed using paired t-test. All variables for studies B and C were analysed using ANOVA with repeated measures. Bonferroni complex samples hypothesis test was used for comparing different sessions. For all studies the hypothesis was accepted when $p < 0.05$.

6-5 Results

6-5.1 Study A

Table 6.1 shows the Californian Verbal Learning Test memory test results in study A demonstrating there was a decrease in number of the remembered words in short term memory (STM) vs. long term memory (LTM) in both trials ($p < 0.01$). The results show in trial F the STM performance was better than in trial NF ($p < 0.05$). However there was no difference in LTM over trials.

Table 6.1 Study A- CVLT memory test results (number of words remembered; Means \pm SD) in trial F and NF.

	Trial F		Trial NF	
	STM *	LTM	STM	LTM
Mean\pmSD	9 \pm 2	8 \pm 2	8 \pm 2	7 \pm 2

CVLT, Californian Verbal Learning Test; *STM*, Short Term Memory (first reminder); *LTM*, Long Term Memory (second reminder); *, significant difference in trial F vs NF.

Table 6.2 shows the results of study A cognitive function battery. For Visual Search Reaction Time test no significant difference was found when comparing Trial F with NF ($p > 0.1$). Sternberg test results show no significant difference in the number of correct answers ($p > 0.4$) and no significant increase in reaction time ($p > 0.1$) comparing trial NF with F. For RVIP test the results showed no significant difference between trials F and NF in the number of correct answers ($p > 0.3$) and reaction time ($p > 0.2$).

The results demonstrate for Stroop test there was no significant difference in number of correct answers in trial NF vs. F ($p > 0.5$), while the reaction time shows a significant increase in trial NF vs. F ($p < 0.01$).

Table 6.2. Study A- results of Visual search, Sternberg, RVIP, and Stroop tests in Trial F and NF (Mean±SD).

	Visual search	Sternberg		RVIP		Stroop	
	ART(ms)	NCA	ART (ms)	NCA	ART (ms)	NCA	ART (ms)
Trial F	1294±130	77± 2	555±69	24±8	513±76	68±3	769±124*
Trial NF	1323± 165	77± 2	576±83	24±9	502±67	68±2	801±116

ART, Average Reaction Time; *NCA*, Number of Correct Answer; *ms*, milliseconds; * significant difference.

Table 6.3 shows the results of some measurements. Body weight changes were not significant ($p > 0.05$). S.osmo. (Serum Osmolality) showed an increase in Trial NF vs. F ($p < 0.01$). U_{SG} (Urine Specific Gravity) changes were not significant ($p > 0.16$). U.osmo. (Urine Osmolality) showed no significant changes ($p > 0.15$).

Table 6.3 Study A- measurement results for Body Mass (BM), Urine Specific Gravity (U_{SG}), Urine Osmolality (U.osmo.), and Serum Osmolality (S.osmo.) (Means±SD).

	BM (kg)	U_{SG} (gr/ml)	U.osmo. (mosmol/kg)	S.osmo.* (mosmol/kg)
Trial F	66.2±12	1.019±0.01	626±300	286±4
Trial NF	65.5±12	1.021±0.005	677±193	289±3

*, significant difference.

6-5.2 Study B

Table 6.4 shows the results of memory test in study B demonstrating that the difference between STM and LTM results in each Trial F and NF in three different sessions during the day were statistically significant ($p < 0.05$). There was no difference between Trial F and NF for both STM and LTM ($p > 0.05$). In Trial F there was a significant decrease in both STM and LTM performance when S_1 compared with S_3 ($p < 0.03$), while between S_1 and S_2 there was no difference ($p > 0.3$). In Trial NF there was no significant difference in both STM and LTM ($p > 0.05$).

Table 6.4 Study B- CVLT memory test results in trial F and NF (Number of words remembered; Means \pm SD) in sessions S_1 , S_2 and S_3 .

	S_1		S_2		S_3	
	STM	LTM	STM	LTM	STM	LTM
Trial F	10 \pm 3	9 \pm 3	10 \pm 3	8 \pm 3	9 \pm 2	7 \pm 2
Trial NF	10 \pm 3	9 \pm 3	9 \pm 3	7 \pm 3	10 \pm 2	7 \pm 3

CVLT, Californian Verbal Learning Test; *STM*, Short Term Memory (first reminder); *LTM*, Long Term Memory (second reminder).

Table 6.5 shows the results of Study B the effect of one day Ramadan fasting on cognitive function. For the Visual Search test there was a significant decrease in reaction time comparing S₂ Trial NF with S₂ Trial F ($p < 0.01$). For the Sternberg test the only significant difference was found when comparing the number of correct responses of trial F with trial NF for S₁ $p < 0.02$, S₂ $p < 0.02$, S₃ $p < 0.01$. For RVIP test when comparing sessions within trial F with each other it was shown that the only significant change was an increase in the number of correct answers in S₃ vs. S₁ ($p < 0.02$). However an increased reaction time in S₃ vs. S₂ ($p = 0.09$) tended toward statistical significance. Within Trial NF for the number of correct answers there was a significant increase in S₃ vs. S₁ ($p < 0.04$). When comparing reaction time of sessions within trial NF with each other the results showed an increase in S₂ vs. S₁ ($p < 0.02$) and a decrease in S₃ vs. S₂ ($p < 0.04$). When comparing reaction time of Trial F with NF results showed some changes which tended toward significance: an increase in S₂ ($p = 0.07$) and a decrease in S₃ ($p = 0.08$).

For the Stroop test there were no significant changes nor differences throughout trials and neither between Trial F and NF, excepting in reaction time which was shorter during trial F in midday session (S₂) compared with the morning session (S₁) ($p < 0.03$). However, the Stroop test reaction time during Trial F showed decrease that tended to be significant in S₂ vs. S₁ ($p = 0.06$) and S₃ vs. S₂ ($p = 0.06$).

For more details of the laboratory results see Chapter 4.

Table 6.5 Study B- results of Visual search, Sternberg, RVIP, and Stroop tests in Trial F and NF (Means±SD) during sessions S₁, S₂, and S₃.

		Visual search*	Sternberg		RVIP		Stroop	
		ART (ms)	NCA*	ART (ms)	NCA*	ART* (ms)	NCA	ART* (ms)
Trial F	S₁	1559±222	77±1*	526±53	24±7	528± 91	67±3	782±146
	S₂	1534±179*	77±3*	525±79	25±7	509±58*	67±2	747±103*
	S₃	1483±218	77±2*	519± 103	26±7*	572±139*	67±3	717±88*
Trial NF	S₁	1508±127	76±3*	524±93	25±7	503±72	66±4	729±108
	S₂	1462±146*	75±3*	514±94	26±10	574±153	66±3	710±101
	S₃	1450±179	76±2*	505±62	27±7*	504±89	66±3	703±101

ART, Average Reaction Time; *NCA*, Number of Correct Answer; *ms*, milliseconds; * significant difference.

6-5.3 Study C

Table 6.6 shows the results of study C for the short term memory (STM) and long term memory (LTM) tests. There was no significant difference ($p > 0.1$) between STM and LTM in all sessions. However the average words remembered in every session were less in LTM vs. STM. Comparing STM in all sessions, there were a significant increase only in S₃ vs. S₂ ($p < 0.04$) while in other sessions the changes were not significant: S₁ vs. S₂ ($p > 0.6$); S₁ vs. S₃ ($p > 0.36$); S₁ vs. S₄ ($p > 0.4$); S₂ vs. S₄ ($p > 0.15$); S₃ vs. S₄ ($p > 0.19$). LTM did not change significantly over sessions: S₁ vs. S₂ ($p > 0.1$); S₁ vs. S₃ ($p > 0.3$); S₁ vs. S₄ ($p > 0.4$); S₂ vs. S₃ ($p < 0.5$); S₂ vs. S₄ ($p > 0.09$); S₃ vs. S₄ ($p > 0.22$).

Table 6.6 Study C- CVLT memory test results (number of words remembered; Means±SD) in S₁, S₂, S₃, and S₄.

	S ₁		S ₂		S ₃		S ₄	
	STM	LTM	STM	LTM	STM*	LTM	STM	LTM
Mean±SD	9 ±3	7±4	8±3	6±3	9±2	7±2	9±2	7±2

CVLT, Californian Verbal Learning Test; *STM*, Short Term Memory (first reminder); *LTM*, Long Term Memory (second reminder); *, significant difference.

Table 6.7 shows the results of cognitive battery of tests for study C. For the Visual search test there was no significant changes between the sessions. For Sternberg test there were significant changes only for reaction time, namely an increase in S₃ vs. S₁ ($p < 0.02$); increase in S₄ vs. S₁ ($p < 0.01$); and a decrease in S₄ vs. S₃ ($p < 0.03$). RVIP test results show a significant decrease in the number of correct answers only in S₂ vs. S₁ ($p < 0.02$), while in reaction time there were more significant changes: an increase in S₂ vs. S₁ ($p < 0.01$); increase in S₃ vs. S₁ ($p < 0.01$); increase in S₄ vs. S₁ ($p < 0.02$). For the Stroop test the results show there were significant changes in the number of correct answers: a decrease in S₂ vs. S₁ ($p < 0.02$); an increase in S₃ vs. S₂ ($p < 0.01$); an increase in S₄ vs. S₂ ($p < 0.05$). Also there were significant changes in reaction time: an increase in S₂ vs. S₁ ($p < 0.01$); an increase in S₃ vs. S₁ ($p < 0.02$); an increase in S₄ vs. S₁ ($p < 0.04$).

The results of laboratory tests performed in study C are described in detail in Chapter 5.

Table 6.7 Study C- results of Visual search, Sternberg, RVIP, and Stroop tests (Means±SD) during sessions S₁, S₂, S₃, and S₄.

		Visual search	Sternberg		RVIP		Stroop	
		ART (ms)	NCA	ART* (ms)	NCA*	ART* (ms)	NCA*	ART* (ms)
Sessions	S ₁	1638±236	75±4	537±69	23±9	569±94	65±3	911±158
	S ₂	1668±183	75±4	603±65	17±5	700±129	61±6	1056±29
	S ₃	1613±301	75±3	615±69	19±5	645±91	66±3	1018±135
	S ₄	1573±290	74±3	604±65	19±6	723±108	65±4	970±156

ART, Average Reaction Time; *NCA*, Number of Correct Answer; *ms*, milliseconds; *, significant change.

6-6 Discussion

In this chapter a set of three different studies has been presented. The components and timing of all cognitive function batteries used in these studies were identical, using different pre-test interventions. In all three studies the results of tests in the no food condition (Trial NF) were compared with a fed condition (Trial F). The duration of fasting in the present studies were almost similar: study A- 12 h; study B- 11 h; Study C- 12 h, but the time of day was different: in study A overnight and in study B and C during the day.

The overall discussion and conclusion will be based on the results of all three studies. The results will be discussed focusing on three main measures of cognitive function assessed in current studies, namely: memory, reaction time, and the number of correct answers.

Regarding memory performance, the results of all three studies showed that generally speaking 11-12 hours of fasting had no detrimental effect on cognitive performance (tested using CVLT). However, the only change was found in study A in Trial NF vs. F in STM performance which was poorer ($p < 0.05$). In all studies in both fasted and fed conditions the number of recalled words in LTM (second reminder) was less ($p < 0.05$) than in STM (first reminder) demonstrating that fasting had no effect for the recognised difference, but the time distance was responsible in this regard. In study B in both trials NF and F memory performance in the morning and midday session were better than in evening session that does not support the findings of previous studies which reported a peak of memory performance in midday (Potter & Keeling, 2005).

Based on the findings of all tests in present studies it can be concluded that fasting has detrimental effect on reaction time performance. Longer reaction time in fasted subjects compared with the fed trial showed that stimulus recognition and/or pre-chosen response initiation were slowed. Reaction time is one of the most important factors in athletes' performance. For the stimulus to be recognized the visuospatial sketchpad should be switched on, while the central executive in this process is very rarely demanded (Barbas, 2000). Prefrontal cortex principally controls the reaction time tasks, but instead tasks with a great demand of executive control involve much more neural activity (Barbas, 2000). When the response in reaction time tasks requires a motor action (e.g. touching an object as in present studies' tests) prefrontal cortex is

assisted by basal ganglia (Bares et al., 2003). It can be hypothesised that in fasting slower reaction time indicated that different parts of the brain are affected by food and water deprivation.

Generally speaking the accuracy of response (number of correct answers) declined in the fasted trial (NF) compared with the fed trial (F) as found in the majority of tests in cognitive battery of tests in studies B and C, suggesting that attention in the fasting condition is impaired (see table 6.5 and 6.7). However, there was one contradictory result in study C (one month of Ramadan fasting) in the Stroop test which shows an increase in the number of correct answers ($p < 0.05$) in S₄ (one week after Ramadan) when compared with S₂ (first week of Ramadan). This discrepancy in the results may be due to the impossibility to randomise the participants in study C (for more details see Chapter 5) which could intensify the learning effect.

The results of current study are consistent with the findings of other researchers demonstrating that human cognitive performance is affected by quantity and composition of food and timetable of meals (Leigh Gibson & Green, 2002; Pollitt & Matthews, 1998) which is in fact the main change in lifestyle of Muslims during the month of Ramadan (for further details see Chapter 5). Two important aspects of fasting are fall in blood glucose and hypohydration. In the present studies data confirmed that both hypoglycaemia and hypohydration occurred throughout fasting. For more details see Chapter 4 and Chapter 5.

Adverse effects of hypohydration on cognitive function may be present even at a level less than 1.3% body weight loss (Lieberman, 2007). Grandjean et al. (2007) has

reviewed a number of studies dealing with the effect of hypohydration on cognitive function dated between 1972 up to the time of the review. They concluded that a hypohydration up to 2% of body mass loss achieved by different hypohydration methods (water restriction, heat exposure, and/or physical exertion) has a detrimental effect on cognitive performance. Similarly mild dehydration (i.e. equivalent to 1– 2% of body mass) for even a brief period, leads to a reduction of the subjective perception of alertness and ability to concentrate, and an increase of self-reported tiredness, and an increase of headache (Armstrong, 2005). Several studies have shown that the response to dehydration was a significant reduction in cognitive performance for various abilities such as decisional or perceptual tasks (Gopinatham et al., 1988, Cian et al., 2001). In a recent study it has been shown that 24 hours of water deprivation causes a decline in executive performance, attention, information processing speed and motor skills (Szinnai et al., 2005). The reduction in performance is proportionate to the degree of dehydration (Gopinathan et al., 1988) and becomes significant with a 2% body weight loss (Sharma et al., 1986; Gopinathan et al., 1988). In one study it has been reported that a decline in cognitive function is caused by as little as 1-2% hypohydration (Armstrong & Epstein, 1999).

It has been demonstrated that administration of glucose can improve learning and memory in old people (Gonder-Frederick et al., 1987; Hall et al., 1989; Craft et al., 1994; Messier et al., 1997) and can provide some recovery of cognitive function in people with Alzheimer's disease (Craft et al., 1992; Manning et al., 1993) and Down's syndrome (Manning et al., 1998). Glucose ingestion increases working memory performance after an overnight fast (Martin & Benton, 1999) and in another study it has been shown that it improves memory function in general (Sunram-Lea et al., 2001

a, b). The results of current study are in the same line with some other studies which have shown memory and attention are affected in food deprivation (Green et al., 1995 and 1997; Gutierrez et al., 2001; Liebermeister & Schroter, 1983) and executive function, verbal function and information processing speed either, but less significantly (Bryan and Tiggemann, 2001; Liebermeister & Schroter, 1983).

Heat and hypohydration cause changes in cardiovascular and thermoregulatory systems and via these changes may negatively affect mental task performance (Maughan et al., 2007). The effects of heat and hypohydration are well known to decrease both physical and mental performance (ibid). In a study it was found that arithmetic task performance, short-term memory, and visual tracking were adversely affected by hypohydration induced by exercise in the heat and water restriction (Gopinathan et al., 1988). It appears that the decrement caused by exercise-induced hypohydration is greater on mental tasks involving cognitive effort than on repetitive tasks which do not need to much attentional resources (Tompsonski, 2006). Cian et al. (2001) showed that dehydration affects the performance only for tests evaluating predominantly cognitive function, while Gopinathan et al. (1988) and Szinnai et al. (2005) found that predominantly motor function tests are influenced detrimentally by hypohydration. Similarly there is another study showing that food deprivation has a detrimental effect on executive performance, attention and motor skills (Green et al., 1995 and 1997; Pönickeet al., 2005; Roky et al., 2000).

On the other hand, the current study's results do not support the results of other research which showed that even in acute starvation the intellectual capacity has been preserved (Keys et al., 1950; Mays, 1995). In the same line and opposite to current

study it was proved that exercise-induced hypohydration up to 2% did not adversely affect cognitive performance, but a higher level of hypohydration causes a decrement in working memory and psychomotor performance (Tomporowski, 2006). Szinnai et al. (2005) in their study found no significant effects of 2.6% hypohydration on cognitive performance, while there were effects on tiredness, alertness, effort and concentration ($p < 0.05$). Also the present study did not support the hypothesis of Green et al. (1997) and Azari (1991) which showed that the brain is not affected by short periods of food deprivation demonstrating there is no correlation between blood glucose concentrations and cognitive function.

Another study which has contradictory result to the present study is that reported by Green et al. (1997) who showed that missing a meal has no negative effect on cognitive function. In the body there are complex mechanisms which aim at preventing the fall in blood glucose concentration in order to protect brain function (Amiel, 1994). Only a decrease of glucose concentration to about 3 mmol/L can produce some cognitive function impairment be recorded (Pramming et al., 1986; Richardson, 1990). However, Green et al. (1997) showed that low blood glucose concentrations did not affect task performance that supporting the hypothesis that the brain is not sensitive to short term food deprivation. In spite of that lowered blood glucose concentrations decreases glucose supply to the brain, and consequently impairs brain function, but the body has complex mechanisms to prevent such oscillations (Amiel, 1994). Only a critical concentration of blood glucose as low as about 3 mmol/L or lower can induce measurable deteriorations in cognitive function (Pramming et al., 1986; Richardson, 1990; Amiel, 1994). Normally blood glucose concentration is maintained above 5 mmol/l even after an overnight fast. The

mechanisms involved in maintaining a constant blood glucose concentration includes liver, muscle and adipose tissue making the brain relatively invulnerable to short food deprivation (Frayn, 1996).

6-7 Conclusion

In conclusion, 11-12 h of food and fluid deprivation had detrimental effects on the accuracy of the response (attention) and slowed the reaction to the stimulus (longer reaction time). Long-term memory performance (recall after 20 min of the first seeing) in none of the three studies and short-term memory (immediately recall after seeing) in study B and C were not altered by this duration of fasting. However, short-term memory was poorer in study A in the No food trial compared with the food trial. In study C the Stroop and Sternberg tests reaction time increased (reaction performance impaired) throughout the month of Ramadan and did not recover even a week after Ramadan.

Chapter 7

General discussion and conclusion

7-1 Overview

In this Chapter the results of the studies reported in Chapters 3, 4, 5 and 6, will be discussed.

For various reasons athletes use different body mass loss methods. The most common methods for body mass reduction are: food and fluid restriction; exercising in rubber or plastic sweat suits; passive sauna exposure. Sometimes these methods are used alone but sometimes a combination of these methods is used. However, the less time that is available for body mass loss, the more extreme and possibly health threatening the methods are that are being chosen (e.g. use of laxatives and diuretics, uncontrolled severe dehydration in heat, auto induced vomiting). Amongst athletes the most popular methods for body mass loss are daily food and fluid restriction, and once a week one day of fasting (Steen & Brownell, 1990; Tipton & Tchong, 1970).

There are different classifications of body mass loss methods, but Fogelholm's (1999) and Wilmore's (2000) are the most well known. In different sports, athletes perform body mass loss aiming for different goals (see Chapter 3). However, the question remains as to whether body mass loss does actually provide any advantage in sport performance and success?

In a specific context, in Muslim communities, Ramadan fasting is a challenge for the athletes as they participate in training and competitions while fasting dawn-to-dusk (food and fluid abstinence). However, the effect of the altered life style during Ramadan on sport performance (training and competition) is still controversial. While fasting could lower sport performance, no considerations are made in scheduling international sport events. The most important issue in order to have fairer competitions is to clarify the effect of fasting (particularly Ramadan fasting) on exercise performance.

The aim of experiments conducted in this thesis was to contribute data to assist in identifying whether fasting and hydration status has an effect on physical and mental performance of recreationally active individuals and athletes.

7-2 Effect of hypohydration on physical performance

Effect of hypohydration on muscle performance was more controversial in 2006, the year when the study presented in Chapter 3 was designed. In 2009 however, there is more evidence to confirm that to a certain extent hypohydration can alter muscle performance. Judelson et al. (2007 a) concluded that hypohydration has a detrimental effect on muscle strength and endurance. Also Shirreffs (2009) concluded in her overview that a hypohydration exceeding 3% of body mass consistently decreases strength, power and endurance.

Amongst different hypohydration methods that have been used for body mass loss purposes, such as exercise induced hypohydration (active hypohydration), water

deprivation, heat induced hypohydration, using diuretics (passive hypohydration) (Schoffstall et al., 2001), or combinations of them, the exercise-induced hypohydration was chosen in this study which reported in Chapter 3 because of its greater similarity to real situation in exercise activities and sport.

The study reported in Chapter 3 evaluated the effect of hypohydration on muscle performance. The study protocol managed to achieve 2% of body mass loss during exercise in a warm and humid environment and also for recovery in the hypohydration-rehydration trial. In the hypohydration trial the overall decrease in body mass loss achieved by exercise induced hypohydration and overnight fast was 3.1% (of which 2% was due to exercise). Considering the weight (323 g) of food consumed during dinner (after BM₂ body mass measurement), the overnight body mass loss was 1.4%.

A large number of studies on the effect of hypohydration on muscle performance have been performed in the last decades using hugely different protocols. The study reported in Chapter 3 was designed in such a way to be able to minimize the effect of known interfering factors such as change in glycogen storage, muscle temperature, body fluid redistribution, sleep duration (Bulbulian et al., 1996), and mood status (for more detail see Chapter 3).

A review of the studies investigating the effect of hypohydration on muscle performance (Judelson et al., 2007a) was published covering most of articles on this subject dating from 1940 to 2007. This review criticizes the protocols used in some of these studies and the authors give some suggestions for further investigation (for

details see Chapter 3). In the present study reported in Chapter 3 all the factors suggested by Judelson and his colleagues were considered. Although in Judelson's et al. (2007) opinion only 21% (15 out of 70) of the studies evaluated showed statistically significant performance reduction after different levels of hypohydration (1.1% to 6%) using different hypohydration protocols (heat exposure, exercise-inducement, water deprivation and combinations of these techniques), they concluded that hypohydration adversely affects muscular strength, power and endurance.

The key findings of Chapter 3 showed that 2% of body mass loss induced by exercise in warm environment decreased muscle strength by an average of $8.6 \pm 2.1\%$ (Right Leg 7.2%, Left Leg 9%). A considerable number of studies support these findings: Evetovich et al. (2002); Webster et al. (1990); Viitasalo et al. (1987); Bosco et al. (1974); Saltin (1964 a); Gutiérrez et al. (2003); Guastela et al. (1988); and Moore et al. (1992). Contrary to our study some previous studies showed no change in muscle strength in different hydration states: Serfass et al. (1984); Montain et al. (1998); Greiwe et al. (1998); Greenleaf et al. (1967); and Horswill et al. (1992).

Although in the most recent review (Judelson et al., 2007 a) the number of studies that showed no difference in strength after hypohydration is more than those which showed decrease, after evaluation of the study methods it was concluded that hypohydration decreases muscle strength. Our results confirm this conclusion maybe due to the study protocol that succeeded in limiting masking and exacerbating factors (see Chapter 3).

Other findings demonstrated in Chapter 3 are that 2% hypohydration decreased endurance time by $12.3 \pm 3.2\%$. Previous studies showing similar results were performed by: Hoffman et al. (1995); Watson et al. (2005); Bijlani and Sharma (1980); King et al.; (1985), Jacobs (1980); Guastella et al. (1988); Webster et al. (1990). However, there are research studies with opposite results, showing that hypohydration caused an increase in endurance time maybe due to less body weight: Caterisano et al. (1988) and Fogelholm et al. (1993). Shirreffs (2009) pointed out that negative effect of hypohydration on muscle endurance is less controversial than muscle strength. Our finding in Chapter 3 contributes in strengthening Shirreffs (2009) statement in this regard.

A review of the literature showed that the adverse effect of hypohydration on endurance performance is clearer than its effect on muscle strength (Shirreffs, 2009). However, there are a number of difficulties in interpreting the results of studies about the effect of hypohydration on muscle strength (Maughan, 2003; Judelson et al., 2007 a). The discrepancies in results seems to be the consequence of different criteria in subject choice (a wide range of subjects from weight category sport athletes familiar with exercise in hot environment, to sedentary individuals), differences in hypohydration level and methods (usually including heat exposure and exercise increasing muscle temperature that affects muscle contraction physiology (Maughan, 2003) recovery phase, muscle performance test techniques (isometric, isokinetic, etc), the interval between hypohydration and muscle performance test, interference of masking and exacerbating factors (muscle glycogen level, body temperature, acid-base status, fluid distribution changes and fatigue) and the fact that some of the

researches was performed as laboratory basic science studies while others as sport field studies with less control of the participants.

Finally our data confirms that exercise induced-hypohydration equivalent to 2% body mass loss followed by an overnight fast giving 3.1% body mass loss, decreased muscle strength and endurance performance. This makes clearer the importance of a good hydration status and a wise recovery protocol after hypohydration in athletes. In Chapter 3 we did not test participants for brain function performance. However, our data confirmed that changes in hydration level had no effect on sleep duration and pattern and profile of mood states (POMS) factors. Our findings in Chapter 3 are in the line with the conclusion which made by Judelson et al. (2007 a).

7-3 Effect of fasting on physical performance

In Chapter 4 and Chapter 5 the effects of one day and one month respectively of Ramadan fasting on different aspects of exercise performance were studied.

In Chapter 4 the investigation was focused on exercise performance changes over a whole day. As such, it was logical to speculate that along with fasting, the circadian rhythm might play a significant role in performance changes as well. The Chapter 4 findings confirm that one day of Ramadan style fasting had no effect on the tested parameters of exercise performance tests, namely vertical jumping, knee extension isometric strength, knee extension isometric endurance, peak power cycling and VO_{2max} . In the fasted trial (Trial NF) 11 h of fasting led to 2.2% loss of body mass (mainly body water loss). It was expected that this amount of body water mass loss

should have affected the performance during each trial session and in comparison of trials with each other, but it did not. One possible explanation for finding no change in different sessions of each trial may be the effect of circadian rhythm. The data showed no difference between fed and no food trials suggesting that 11 hours of fasting had no effect on anaerobic power, explosive power, and muscle strength and muscle endurance.

To the best of our knowledge, there were no previous studies on the effect of dawn-to-dusk Ramadan fasting on exercise performance, with an identical design to our study. However, studies with other designs on fasting and exercise performance showed various results on some aspects of exercise performance, such as no change after 24 h (Henschel et al., 1954) or even up to 3.5 days of fasting (Knapik et al., 1987), whereas other have shown a decrease in physical performance after three days of fasting (Bosco et al., 1974).

In summary, Chapter 4 results showed that 11 h of Ramadan fasting did not lower the exercise performance over the day (as measured at different times during the day). However, it can be speculated that an increase in performance due to circadian rhythm might mask any possible decrease, resulting in no noticeable changes (Monk & Leng, 1982; Gifford, 1987; Reilly and Down, 1992; also see Chapter 4).

The study presented in Chapter 5 was performed in Iran, investigating the effect of one month of Ramadan fasting on some aspects of exercise performance in field conditions. Participants performed nine different field tests namely: flamingo balance,

sit and reach, sit up, plat tapping, standing long jump, hand grip, vertical jumping, 3x20 m shuttle test and shuttle run bleep test.

The results of Chapter 5 show that overall exercise performance decreased during the months of Ramadan in all three groups of participants (weight category athletes, non-weight category athletes and recreationally active individuals) but recovered a week after Ramadan.

Performance in the endurance bleep running test, long jump test, sit up test, vertical jumping and arm plate tapping test decreased during Ramadan consistently from week one to week four. However, performance did not change in the hand grip strength and flamingo balance test.

Our data is in the same line with that of other research such as the study of Ben Salama et al. (1993) who demonstrated that physical performance was affected detrimentally by Ramadan fasting. Also Zerguini et al. (2007) reported similar results showing a significant decrease in anaerobic and aerobic capacities after 2 weeks of Ramadan fasting. In Zerguini's et al. study, vertical jumping did not change significantly, when comparing the period before and after the end of Ramadan, but a lower value was obtained two weeks after Ramadan vs. immediately at the end of Ramadan.

Chapter 5 results confirmed that level of physical activity seemed to have no effect on the pattern of change in body mass and body fat. Ramadan et al. (1999) also observed no difference in active men compared with sedentary men.

Meckel et al. (2008) demonstrated that Ramadan fasting in youth soccer player (aged 14 to 16) caused a significant decrease in vertical jumping height. Data in the study reported in Chapter 5 showed a significant decrease in vertical jumping height and long jump length during Ramadan, of which both recovered one week after the end of Ramadan.

Gutierrez et al. (2001) demonstrated hand grip strength stayed unaffected in three days of consecutive fasting. These findings are similar to the present study's results which confirm that there is no significant change in hand grip strength over the month of Ramadan.

The results of vertical jumping and long jump suggest a loss in muscle power at least in the big muscle group which are involved in jumping. However, this effect has not been showed for hand grip strength, and it can be supposed the reason for no effect is less strength produced by the hand muscles and therefore a very small change could exist but might not be observed.

In summary, results of Chapter 5 confirm that a month of Ramadan fasting has a detrimental effect on different aspect of physical performance. However, all parameters recovered a week after Ramadan.

The findings of Chapters 4 and 5 show that Ramadan style fasting during one isolated day may have intangible effects on different aspects of exercise performance while 30 consecutive days of Ramadan life style including intermittent food and fluid

deprivation result is a significant decline in physical performance. However, most of the altered parameters have almost recovered during one week after Ramadan when eating and drinking have returned to normal. In the literature there are obvious discrepancies in the results of different Ramadan fasting studies due to different factors, such as study protocol, participants choice criteria, climate, length of the day, Ramadan culture, food composition change during Ramadan, sleep pattern and daily life time table. Therefore, it seems that nutritional, cultural, climate and geographical details need to be taken in account in future Ramadan fasting studies.

7-4 Effect of fasting on cognition performance

Chapter 6 presents the results of cognitive function test of three different studies (see Chapter 6). The brain has a major role in sports as well as in daily life tasks. Cognitive function tests can be used as a practical ways of evaluating brain function. Well-designed computer based software was used for cognitive function tests with the same test protocol used in all three interventions (see Chapter 6).

The results of all three studies demonstrated that 11-12 hours of fasting had no detrimental effect on cognitive performance. This is different from findings of previous studies which reported a peak of memory performance in midday relative to the morning and afternoon (Potter & Keeling, 2005). Based on the findings of all tests presented in Chapter 6 it can be concluded that fasting also has detrimental effect on reaction time performance.

Also generally speaking the accuracy of response (number of correct answers) declined in the fasted condition compared with a fed condition suggesting that fasting impairs attention. The results of current study are in line with some other studies which have shown that memory and attention are affected by food deprivation (Green & Rogers, 1995, Green et al., 1995 and 1997; Gutierrez et al., 2001; Liebermeister & Schroter, 1983). It was also reported that executive function, verbal function and information processing speed impaired after a period of food deprivation. However, the change was not as severe as it was in memory and attention (Bryan and Tiggemann, 2001; Liebermeister & Schroter, 1983).

The current studies do not support the findings of previous researches which demonstrated that even in acute starvation the intellectual capacity is preserved (Keys et al., 1950; Mays, 1995). Also the present studies demonstrate findings opposite to those of Green et al. (1997) and Azari (1991) which showed that the brain is not affected by short periods of food deprivation.

In summary, our data shows that food and fluid deprivation has detrimental effects on the accuracy of the response (attention) and slowed the reaction to the stimulus (longer reaction time). Long-term memory performance (recall after 20 min of the first seeing) in the non in all three studies and short-term memory (immediately recall after seeing) neither during one isolated day of fasting nor during one months of consecutive fasting were altered. However, short-term memory performance was poorer when participants did not consume the breakfast (study A) compared with fed trial.

During one month of Ramadan fasting, reaction time as tested in the Stroop and Sternberg tests, increased (reaction performance impaired) and did not recover even one week after Ramadan. Our findings show that cognitive function seems to be impaired by food and fluid deprivation demonstrating the importance of food and fluid consumption in all sport tasks in particular more concentration demanding sports and skills. It is also important to emphasise that sport events held during Ramadan cannot provide a fair platform for competition between those who are fasting during the day and those who are not. Postponing the competition to after dawn seems not to eliminate the entire fasting burden on fasted individuals. One way to have a fair competition might be to have competition timetable at least one week away from the end of Ramadan.

7-5 Conclusion

In conclusion, the results of studies presented in this thesis demonstrated that a hypohydration level of 2% and over and also fasting can in some circumstances adversely affects physical and mental performance.

Data in Chapter 3 shows that exercise induced-hypohydration reaching 2% of body mass, followed by an overnight fast reaching 3.1% body mass, cause a decline in muscle strength and endurance time performance by $8.6 \pm 2.1\%$ and $12.3 \pm 3.2\%$ respectively. Findings of Chapter 4 and 5 show that Ramadan style fasting for one isolated day may have an intangible effect on different aspects of exercise performance while 30 consecutive days of Ramadan life style including intermittent

food and fluid deprivation decreased exercise performance. However, most of the altered parameters were recovered up to one week after Ramadan.

According to our data in Chapter 6, cognitive function seems to be impaired by food and fluid deprivation. Accuracy of the response (attention) and reaction time decreased significantly. Long-term memory performance (recall after 20 min of the first seeing) in none of three studies and short-term memory (immediately recall after seeing) neither in one day of fasting nor during one month of fasting were altered. However, short-term memory was poorer when participants did not consume the breakfast (study A Chapter 6) compared with fed trial.

During one month of Ramadan fasting, reaction time as tested in the Stroop and Sternberg tests increased (reaction performance impaired) and did not recover one week after Ramadan.

The results of this thesis firmly emphasise the significance of good hydration status and food and fluid consumption in preserving physical and cognition performance.

Ramadan fasting is practised by almost one in five persons in the world, therefore it seems logical to emphasise that in scheduling important international sport events, month of Ramadan should be excluded from competitions time table. Postponing the competition to after dawn seems not to eliminate the entire fasting burden on the fasted individuals. The only way to have a fair competition is to have competition timetable more than one week away from the end of Ramadan.

During one month of Ramadan fasting, reaction time as tested in the Stroop and Sternberg tests increased (reaction performance impaired) and did not recover one week after Ramadan.

Our findings demonstrate that cognitive function seems to be impaired by food and fluid deprivation showing the importance of food and fluid consumption in all sport tasks in particular in more concentration demanding sports and skills.

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