SAFETY AND EFFECT OF CREATINE MONOHYDRATE SUPPLEMENTATION

by

FRANCÈ ROSSOUW

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SUPERVISOR: PROF PE KRÜGER

PRETORIA

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This thesis is dedicated to my loving and inspirational daughter Madya

CHAPTER 1

SCOPE AND INTENT

1.1 INTRODUCTION

Optimal exercise training and performance are crucially dependent on a well-designed diet, thus exemplifying the adage: *"You are what you eat"*. No wonder then that throughout the history of competitive sport athletes have attempted to improve their performance by ingesting a variety of substances as dietary supplements (Bishop, 2010).

Since its discovery in 1882 (Williams *et al.*, 1999) creatine (Cr) has become one of the most widely used non-doping performance-enhancing substances in sport (Kalinski, 2003; Tscholl *et al.*, 2010). It is a natural compound that is endogenously produced by the kidneys, pancreas and liver, and exogenously ingested by the consumption of meat and fish. Free Cr and its phosphorylated form, PCr, are found in a wide variety of excitable tissues. Both compounds can build up to high concentrations (150 - 160 mmol/kg dry mass) in most creatine kinase (CK)-containing cells and tissues (Harris *et al.*, 1992; Greenhaff, 1995; Wyss & Kaddurah-Daouk, 2000). The muscle (and other) cells' reservoir of PCr, Cr and a very small amount of ATP lies in the intervening space between the myofibril and the mitochondrion (Bessman, 1987), thereby allowing a high intracellular flux of high-energy phosphates. The supplementation regimens that have proved successful in significantly raising total muscular Cr (TCr) and PCr concentrations include: (i) the ingestion of 20 to 30 g/day of Cr for three to six successive days – the so-called *"loading dosage"* (Greenhaff *et al.*, 1994; Söderlund *et al.*, 1994; Balsom *et al.*, 1995; Febbraio *et al.*, 1995; Casey *et al.*, 1996; Green *et al.*, 1996; Hultman *et al.*, 1996; Vandenberghe *et al.*, 1996*a*; Vandenberghe *et al.*, 1996*b*; McKenna *et al.*, 1999; Volek *et* al., 1999; Bellinger *et al.*, 2000; Nelson *et al.*, 2001; Parise *et al.*, 2001; Preen *et al.*, 2001; Wiedermann *et al.*, 2001; Louis *et al.*, 2003*b*; Louis *et al.*, 2003*c*; Preen *et al.*, 2003; Tarnopolsky *et al.*, 2003*b*; Van Loon *et al.*, 2003; Rawson *et al.*, 2004; Deldicque *et al.*, 2005; Hadjicharalambous *et al.*, 2008); and (ii) prolonged low-dosage Cr ingestion (2 - 5 g/day) - the so-called *"maintenance dosage"* (Hultman *et al.*, 1996; Thompson *et al.*, 1996; Brose *et al.*, 2003; Tarnopolsky *et al.*, 2003*b*; Hickner *et al.*, 2010). Cr supplementation is also an efficient means of elevating and maintaining PCr and TCr content of blood and muscle when Cr loading is followed by longer-term

maintenance ingestion (Hultman *et al.*, 1996; Vandenberghe *et al.*, 1996*b*, Volek *et al.*, 1999; Burke *et al.*, 2003; Newman *et al.*, 2003; Tarnopolsky *et al.*, 2003*b*; Derave *et al.*, 2004).

Cr monohydrate is currently the most extensively studied form of Cr for use in dietary supplements, particularly since this form of Cr is regarded as the most clinically effective form of the substance in light of the muscle uptake and increase in high-intensity exercise capacity that can be achieved by using it for performance enhancing purposes (Buford *et al.*, 2007). In general, the ergogenic effects of Cr supplementation have been borne out by the studies conducted to determine the effect of such supplementation on performance in repeated bouts of high-intensity exercise (Greenhaff *et al.*, 1993*b*; Birch *et al.*, 1994; Earnest *et al.*, 1994; Casey *et al.*, 1996; Vandenberghe *et al.*, 1996*a*; Jones *et al.*, 1999; Rossouw *et al.*, 2000; Bennett *et al.*, 2001; Preen *et* al., 2001; Wiedermann *et al.*, 2001; Izquierdo *et al.*, 2002; Warber *et al.*, 2002; Ziegenfuss *et al.*, 2002; Van Loon *et al.*, 2003; Peyrebrune *et al.*, 2005; Jäger *et al.*, 2008; Rawson *et al.*, 2011). Maximal single-effort activities require an immediate, rapid supply of energy that is provided almost exclusively from the adenosine triphosphate (ATP) and PCr stored in the muscles. Therefore, it has been shown that performing activities of this nature improves as a direct result of Cr ingestion (Rossouw *et al.*, 2000; Ostojic, 2004; Gotshalk *et al.*, 2008; Saremi *et al.*, 2010).

The effect of Cr supplementation on aerobic endurance performance has received much less research attention. The increase in muscle free Cr as a result of Cr supplementation should potentially favour changes in oxidative phosphorylation in contracting muscle fibres because of an amplification of mitochondrial-CK activity and, consequently, the PCr shuttle (Rico-Sanz & Marco, 2000; McConell *et al.*, 2005). However, it seems that Cr supplementation has variable effects on this mode of exercise testing. While some studies report performance benefits for both sub-maximal (Ööpik *et al.*, 2002; Hadjicharalambous *et al.*, 2008; Graef *et al.*, 2009) and maximal (McConell *et al.*, 2005) aerobic exercise, the majority of results report no benefit to either sub-maximal (Van Loon *et al.*, 2003; Van Schuylenbergh *et al.*, 2003; McConell *et al.*, 2005; Stout *et al.*, 2006) or maximal (Jones *et al.*, 2002; Van Loon *et al.*, 2003; Reardon *et al.*, 2006; Graef *et al.*, 2009) aerobic performance. To date no studies have set out to test the benefits claimed for well-trained endurance athletes participating in ultradistance events. The nature of these events, i.e. extreme competition and training distances that tax all the body's energy systems, may lend it to benefit from Cr supplementation in terms of optimising energy stores for performance, post-exercise recovery and retention of muscle mass.

The vast majority of studies on Cr have been conducted to determine its performance-enhancing effects (Hathcock *et al.*, 2006). Very few studies in the literature have investigated the clinical safety of Cr supplementation (Van der Merwe *et al.*, 2009). The datasets on the safety and causally related adverse effects of non-essential nutrients, such as Cr, on humans are quite small and also vary in quality (Hathcock & Kriengsinyos, 2011). Supplementation at 5 g/day is currently considered safe for organ function (Hathcock *et al.*, 2006; Shao & Hathcock, 2006). However, most of the studies investigating the safety of Cr supplementation were relatively short-term, and incorporated small sample sizes and/or no control/placebo group (Hathcock & Kriengsinyos, 2011). Therefore, confidence in the existing datasets on the safety of Cr ingestion would be enhanced with longer-term data (Hathcock & Kriengsinyos, 2011).

The widespread media publicity surrounding the use and "resultant" success of reputable athletes following Cr supplementation has led to a change in the profile of the Cr user (Derman, 2000). The practice of Cr loading and long-term ingestion have spread from elite settings to the high-school locker rooms and health clubs (Derman, 2000). Findings in the literature indicate university students participating in sport and/or attending campus gymnasiums to be a prime target market for the sport-supplement industry (Jackson *et al.*, 2010), since they are amongst the greatest consumers of Cr globally (Sobolewski *et al.*, 2011). Thus, there is a need to investigate the safety of short-term and long-term Cr ingestion in a relatively homogeneous population of highly active university students.

Since the long-term safety and effects of Cr ingestion on well-trained ultradistance athletes and highly active male university students are still largely unknown, the present study will aim to shed light on effects in these population groups.

1.2 RESEARCH PROBLEM

The research problem is an area of concern in which there is a gap or a situation in need of solution, improvement or alteration, or where there is a discrepancy between the way things are and the way they ought to be (Burns & Grove, 2001; Brink *et al.*, 2006). A research problem can also be formulated from sources other than a problematic situation, such as a specific interest in a certain topic (Brink *et al.*, 2006). In the present study, the research problem stemmed from both these considerations namely:

- a public safety concern with regard to the short-term and long-term use of Cr in young male university athletes and the dearth of experimental research on the topic;

- a specific interest in the proposed cellular hydration effects of Cr supplementation and the shortfall in experimental research on the topic; and

- a specific interest in the benefits (if any) of Cr use in endurance athletes and the gap in experimental research on its specific usefulness as a means of enhancing the performance of ultradistance athletes.

The following research question was formulated to spell out the pivotal concern of the study at issue, namely to add to the extent and depth of the exact body of Cr research:

- **is short-term, moderate-term and long-term Cr monohydrate supplementation safe and ergogenic in well-trained male ultradistance runners and highly active male university students?**

1.3 RESEARCH HYPOTHESIS

A hypothesis is typically presented for quantitative research, provided sufficient relevant research has been conducted on the topic to lend reliable predictive capacity to the research at issue (Davis, 2008). The hypothesis is a prediction or tentative statement about the relationship between variables and gives direction to a study (Davis, 2008). The hypothesis is tested through scientific investigation and is provisionally deemed invalid if the findings of the study do not tally with the substantive content of the hypothesis, or alternatively deemed provisionally valid if the results of the study corroborate the assumptions embodied in the hypothesis. Never, strictly speaking, is the hypothesis conclusively declared to be true (or untrue) given the possibility of further evidence pointing either way at a future date (Nickerson, 2011).

The following research hypothesis was formulated in light of the research problem as stated above:

- **Cr supplementation (independent variable) will be safe for organ function (dependent variable) and will be ergogenic (dependent variable) in well-trained male ultradistance runners and highly active male university students.**

The following sub-hypotheses were derived:

- Cr supplementation (independent variable) will alter the pattern of substrate utilisation (dependent variable) during an incremental test for aerobic capacity in well-trained ultradistance runners;

- Cr supplementation (independent variable) will aid the maintenance of muscle mass (dependent variable) in well-trained ultradistance runners;

- Cr supplementation (independent variable) will increase total body water and intracellular water (dependent variables) in highly active male university students; and

- Cr supplementation (independent variable) will alter the mood state (dependent variable) of highly active male university students.

1.4 RESEARCH APPROACH

In the present study the **scientific method** of obtaining knowledge will be used. Research is a scientific process and refers to the exploration, discovery and careful study of unexplained phenomena (Brink *et al.*, 2006). It implies an orderly and logical engagement with the sum of what is known, commonly referred to as a body of knowledge (Macleod-Clark & Hockey, 1989), and indicates a diligent, systematic enquiry to validate and refine existing knowledge and create new knowledge (Burns & Grove, 2001). As a result, scientific knowledge is noticeable, objective, accurate, reliable and replicable (Ramos-Álvarez *et al.*, 2006). The research findings, and the methods used to acquire them, will be made known to the members of the research community (Brink *et al.*, 2006) to allow continuous review and replication (Ramos-Álvarez *et al.*, 2006).

The type of research to be conducted in the present instance is **applied research**. The major aim of the researcher will be to solve problems and make decisions for what are considered practical purposes (Brink *et al.*, 2006). A **quasi-experimental approach** will be used. The independent variable (creatine supplementation) will be manipulated in a controlled experimental situation (a placebo and a control group, respectively, will be included in the two experimental designs). **Quantitative** data will be collected in a controlled laboratory environment using structured procedures and formal instruments. Numerical information will be analysed through statistical procedures.

A critical literature review and two separate experimental protocols (Chapters 3 and 4) will be undertaken to investigate two primary areas of scientific debate regarding Cr use, namely the safety of long-term ingestion and its effects on endurance performance.

1.5 STUDY OBJECTIVES

1.5.1 Primary objectives

The primary objectives of (i) the study conducted with the aid of well-trained male ultradistance runners - who were participating in a sport-specific training programme for the duration of the study under review - were to determine whether or not both short-term Cr ingestion (6 g/day for 6 days) combined with moderate-term supplementation (3 g/day taken continuously for periods up to 3 months) would:

- lead to an improved training effect, and thus to enhanced performance during an incremental test for maximal aerobic capacity;
- change body mass, lean body mass (LBM) and/or other parameters of body composition;
- increase sub-maximal running economy; and
- prove safe for organ function (skeletal muscle, myocardium, blood, liver and kidney).

The primary objectives of (ii) the study on highly active male university students of Human Movement Sciences were to determine whether or not both short-term Cr loading (20 g/day for 6 days) together with long-term maintenance consumption (3 g/day taken continuously for periods up to 10 months) would:

- alter body water compartments, body weight and/or body composition; and
- be safe for organ function (skeletal muscle, myocardium, blood, liver and kidney).

1.5.2 Secondary objectives

The secondary objectives of (i) the study on well-trained ultradistance runners, who were participating in a sport-specific training programme, were to determine whether or not both short-term Cr ingestion (6 g/day for 6 days) combined with moderate-term maintenance supplementation (3 g/day taken continuously for periods up to 3 months) would:

- change the pattern of substrate utilisation during an incremental test for aerobic capacity; and

- decrease blood lactate accumulation during an incremental test for aerobic capacity.

The secondary objectives of (ii) the study on highly active male university students of Human Movement Sciences were to determine whether or not both short-term Cr loading (20 g/day for 6 days) and long-term maintenance consumption (3 g/day taken for periods up to 10 months) would:

- improve isokinetic strength; and

- alter mood state.

The scientific research process will be applied to elaborate a portrayal of a conceptual world (linked to theories and the research hypotheses) and an empirical world (linked to data) (Ramos-Álvarez *et al.*, 2006). The connection between these two worlds will be achieved by the method (linked to the hypotheses and able to obtain data that can contrast them) (Ramos-Álvarez *et al.*, 2006). Therefore, the study report submitted here as a doctoral thesis will be structured as follows:

- The work will begin with a literature review with particular reference to the basic characteristics and functions of the human energy systems for muscle contraction and exercise performance. Previous findings on the effects of Cr supplementation will also be reviewed. The chapter will conclude with an investigation of the literature dealing with the safety of Cr supplementation (chapter 2).

- The next section will cover the research methods employed in conducting the study at hand, the results of applying the methods concerned, followed by a discussion of the results and an account of the conclusions and recommendations following from the results and discussion thereof. The specific issues covered will be the following:
    - safety of creatine monohydrate supplementation and its effects on exercise performance and body composition in male ultradistance runners (chapter 3); and

- safety of long-term creatine monohydrate supplementation and its effects on isokinetic strength, body water compartments and mood in highly active male university students (chapter 4).

CHAPTER 2

LITERATURE REVIEW

Wyss (2004) warned against approaching the multifaceted fields of creatine (Cr) metabolism in general, and Cr supplementation in health and disease, without appropriate scientific care. It is with this warning in mind that the present author will review this seemingly simple energy pathway with complex kinetics as it applies to exercise physiology and safety of supplementation within the field of biokinetics.

1. HUMAN ENERGY: FUNDAMENTAL PRINCIPLES

An appreciation of the different energy systems is necessary to understand the role that Cr and its phosphorylated compound play in the provision of energy for muscle contraction.

1.1 ENERGY FOR RAPID MUSCLE CONTRACTION

1.1.1 Stored adenosine triphosphate

Adenosine triphosphate (ATP) is a complex molecule constructed with high-energy bonds that release energy very rapidly when split by enzyme action (Edwards, 1983; Powers & Howley, 2009). Although ATP is not the only energy-carrying molecule in the cell, it is the most important one, and without sufficient amounts of ATP most cells die quickly (Powers & Howley, 2009). Since the hydrolysis of ATP takes place whether oxygen is available or not the reaction is rapid, anaerobic and energy liberating (McArdle *et al.*, 2001).

$$H_2O + ATP \xrightarrow[\text{Actomyosin ATPase}]{} ADP + Pi + energy$$

(Lehninger, 1978)

The balance between ATP utilisation and ATP resynthesis is termed energy coupling. ADP, the end product of the actomyosin ATPase reaction, is maintained at low levels by activation of the

myokinase reaction (formation of ATP and adenosine monophosphate (AMP)) and subsequent deamination of AMP to inosine monophosphate (IMP). In itself, IMP can be further degraded to hypoxanthine, xanthine, and urate (Bellinger *et al.*, 2000).

$$ADP + H_2O \xrightarrow[\text{Myokinase}]{} AMP + Pi + energy$$

$$AMP + H_2O \rightarrow IMP + Pi + energy$$

(Lehninger, 1978)

ATP constitutes the only immediate energy source available for muscle contraction. Research results indicate that ATP decreases during the first two minutes of continuous work (Hultman, *et al.*, 1967; Giannesini *et al.*, 2002), and then remains unchanged (Hultman *et al.*, 1967; Giannesini *et al.*, 2002) or increases toward the basal value (Hultman *et al.*, 1967). At very heavy workloads with PCr levels down to zero the ATP concentration only decreases by 30 to 40% of the initial value (Hultman *et al.*, 1967; Giannesini *et al.*, 2002). Thus, at maximal consumption of energy during muscular work the ATP store remains largely intact.

Muscle stores of ATP are limited to ~24 mmol/kg (Maughan, 1995). It will supply energy for only four to six seconds of contraction and thus must be regenerated continuously if contraction is to continue (Marieb, 2004). To sustain muscle activity ATP is regenerated by the following three pathways:

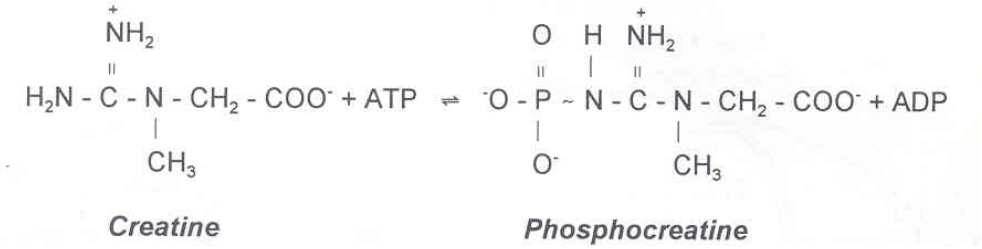
1.1.2 The ATP-PCr system

The creatine kinase-phosphocreatine system plays a key role in the control of ATP levels in tissues that have a high and fluctuating energy demand (Ponticos *et al.*, 1998; McLeish & Kenyon, 2005). Phosphocreatine (PCr) is a high-energy phosphate molecule used to store energy within muscle cells. Although it cannot be used as an immediate source of energy, the ATP molecule can be reconstructed by reducing PCr to Cr and phosphate (Pi), thereby providing energy for ATP production (Meyer *et al.*, 1984). Energy transfer from PCr is crucial during transitions from low to high energy demand, such as at the beginning of exercise (McArdle *et al.*, 2001). The sarcoplasmic enzyme creatine kinase (CK) catalyses the reversible transfer of Pi between PCr and ATP. This is a dead-end reaction in the sense that there is no other known reaction utilising PCr in cells (Meyer *et al.*, 1984).

Creatine kinase reaction:

$$ADP + PCr + H^+ \rightarrow ATP + Cr$$

ATP generated in the mitochondria during rest (from the oxidation of fats and carbohydrates), is utilised to resynthesise Cr released during muscle contraction back to PCr. This reaction is catalysed by the enzyme creatine phosphokinase (Mathews & Van Holde, 1990):



(Mathews & Van Holde, 1990)

Muscle cells store substantial amounts of PCr. In resting muscle the PCr concentration is approximately three to four times that of ATP (Maughan, 1995). At rest 60 to 90% of muscle Cr is in the form of PCr (Harris, 1993; Wyss & Kaddurah-Daouk, 2000), ensuring that ATP broken down during muscle contraction is rapidly (almost immediately) restored. Resting PCr concentrations are higher, and the rate of degradation greater, in type II (fast) muscle fibres compared with type I (slow) fibres (Casey *et al.*, 1996; Kraemer & Volek, 1999). Together, ATP and PCr provide energy fuelling maximum muscle power for about 15 to 20 seconds (Marieb, 2004). The initial store of PCr, and the rate at which it is resynthesised, must be optimal for the muscle to effectively maintain a high ATP content. During intense activity, however, the PCr support for ATP resynthesis will begin to fail. The subsequent build-up of adenosine diphosphate (ADP) is the starting point for further degradation of adenine nucleotide, with the formation of adenosine monophosphate (AMP) and membrane-damaging free radicals (Harris, 1993). This will challenge the normal functioning of the muscle cell and ultimately lead to fatigue.

A more detailed description of Cr biosynthesis and metabolism will be presented in 2.3.2 later in this chapter.

1.2 SUBSTRATE AVAILABILITY

The four energy systems discussed do not function in isolation, but under specific circumstances function simultaneously or complementary. The muscle copes with environmental demands (intensity and duration of exercise) by utilising a spectrum of substrates (Hultman & Sjöholm, 1983). To fully understand this, substrate availability - the availability of ATP, PCr, glycogen, glucose and lipids during exercise - needs to be examined.

1.2.1 Duration and time course of physical activity

Firstly, substrate choice is dependent on the duration and time course of physical activity. Although it is common to speak of aerobic versus anaerobic exercise, in reality the energy to perform most types of exercise comes from a combination of aerobic/anaerobic energy sources (Powers & Howley, 2009). Indications are that the duration of an event will determine the utilisation of energy sources. Events of five to ten seconds are mainly dependent on ATP and PCr. Whereas events of 40 to 60 seconds mainly depend on anaerobic glycolysis, events of two minutes require almost equal amounts of both anaerobic and aerobic energy, and events lasting more than two minutes depend increasingly on aerobic energy as the event continues (Thoden, 1991). Table 2-1 presents the relative contributions of the different energy systems during continuous activity of a specific duration. However, this table is not meant to apply directly to sports events that last two to three hours at a stretch, but are made up of five- to 20-second bursts at higher rates of energy release, interspersed with recovery periods of lower intensity (eg. tennis and rugby).

Furthermore, substrate availability depends on the time course of physical activity. The anaerobic pathways often kick in temporarily at an early stage in the exercise event as a result of the minuscule store of oxygen available to the muscle cells and a delay caused in the activation of mitochondrial respiration by ADP and inorganic phosphate. High-energy phosphates and glycogen are the immediately available substrates for energy production. They are stored in muscle tissue itself, and are therefore in close contact with the contractile units of the muscle cells. Endogenous hexose phosphates and free glucose inside muscle cells are additionally available for immediate anaerobic utilisation. The penetration by blood glucose of the plasma membrane is a slow process and will therefore only be available as substrate during prolonged exercise (Hultman & Sjöholm, 1983).

Table 2-1 Work time partitioned into aerobic and anaerobic contributions

Maximum-effort work time	ATP and PCr	Anaerobic glycolysis	Aerobic
5s	85	10	5
10s	50	35	15
30s	15	65	20
1 min	8	62	30
2 min	4	46	50
4 min	2	28	70
10 min	1	9	90
30 min	1	5	95
1 hour	1	2	98
2 hours	1	1	99

Note. Data are expressed as percentages. Adapted from Thoden (1991).

Aerobic mechanisms take over from the anaerobic mechanisms, unless the activity is so strenuous or prolonged that these mechanisms cannot keep pace (Marieb, 2004). The substrates available for aerobic utilisation are lipids and carbohydrates. The carbohydrates occur as muscle glycogen, liver glycogen and blood glucose, while lipids are stored primarily as triglycerides in the muscle and adipose cells (Williams, 2010). The availability of lipids in the form of blood-borne free fatty acids (FFA) is low during rest, but will increase during exercise. The peak level of FFA is, however, only reached after three to four hours of continuous exercise. According to Hultman and Sjöholm (1983), the time course of substrate utilisation is the following:

1. ATP → ADP + Pi + energy
2. ADP + PCr → ATP + Cr
3. ADP + glucose (glycogen) → ATP + lactate
4. ADP + O_2 + substrate → ATP + CO_2

In conclusion, the knowledge regarding the relationship between the utilisation of the different energy stores and the duration and time course of an activity can be applied to training for sporting success. The appropriate approach to exercise training includes analysis of the activity

in terms of its specific energy components, followed by the training of those systems to ensure optimal adaptations in physiologic and metabolic functions. An improved capacity for energy transfer usually translates into improved exercise performance (McArdle *et al.*, 2001).

1.2.2 Substrate choice and power output

Secondly, substrate choice is influenced by power output. According to Hultman and Sjöholm (1983) the levels of high-energy phosphates in human muscle are 24.0 ± 2.6 mmol ATP and 75.5 ± 7.6 mmol PCr per kilogram of dry muscle. The total creatine (TCr) content in human skeletal muscles ranges from a low of 100 mmol to a high of 160 mmol (Harris, 1993), with an average of 123 mmol (Harris *et al.*, 1974) to 125 mmol per kilogram dry muscle (Greenhaff, 1995). The glycogen content of muscle as measured during rest varies from 280.7 ± 51.4 mmol (Cheetham *et al.*, 1986) to 350 mmol per kilogram dry muscle (Harris *et al.*, 1974; Hultman & Sjöholm, 1983).

1.2.2.1 Phosphocreatine

All sports require utilisation of the high-energy phosphates, but many activities rely almost exclusively on this means for energy transfer. Exertions of short duration and high intensity such as the 100m dash and powerlifting require an immediate and rapid supply of energy (McArdle *et al.*, 2001). During the first seconds of intense muscular effort PCr is the primary source of energy. The role of PCr as an energy store is striking: one-half of the PCr is utilised in the first 10.5 seconds of intense muscular effort, and at the same time the ATP concentration is reduced by only 0.5% (Meyer *et al.*, 1984). Therefore, PCr has classically been considered to buffer changes in ATP and ADP levels.

The study by Hultman and Sjöholm (1983) on ATP turnover rate in the human quadriceps femoris muscle during electrical stimulation at a frequency of 50Hz (which gives a near maximum contraction force) demonstrated that 80% of ATP turnover during the 1.26-second period of stimulation was derived from the breakdown of PCr. According to Hultman *et al.* (1991) the PCr store in skeletal muscle declines by 50% during the first 10 seconds of exercise at maximum intensity.

1.2.2.1.1 Exercise at a constant work load

The PCr store in skeletal muscle can be utilised exhaustively during exercise, and the capacity is sufficient to maintain ATP production for about 30 seconds during exercise at 70% VO_2 max (Sahlin, 1986).

In their study on the breakdown and resynthesis of PCr Hultman *et al.* (1967) demonstrated a rapid breakdown of PCr during the first and second minutes of exercise at a constant workload of 900 kpm/min. After the initial decrease, the fall in PCr concentration slowed down considerably, until after five minutes of exercise no further reduction in PCr was observed. In the same publication, when workloads were altered every five minutes with a rest period of 10-15 minutes in between to allow resynthesis of PCr and ATP, an inverse correlation between workload and PCr concentration (a high load corresponds to a low PCr concentration, and *vice versa*) was shown to exist. They further demonstrated that muscle PCr concentration remained at a fairly constant level during endurance exercise lasting 15 to 60 minutes.

1.2.2.1.2 Single and repeated bouts of maximal sprinting

In a study conducted by Cheetham *et al.* (1986) post-exercise muscle metabolites demonstrated a 64% fall in muscle PCr concentration, and a 37% fall in ATP during a single 30-second maximal sprint on a non-motorised treadmill. It was calculated that approximately 36% of the ATP used during the sprint came from PCr. However, the substrate profile changes during repeated maximal sprinting in bouts of 30 seconds in duration. Bogdanis *et al.* (1996) showed that PCr content in the muscle fell to 1.4% of the resting value after sprint 1 of a 30-second Wingate Test protocol on a bicycle ergometer. During the 4 minute passive recovery period, PCr was resynthesised to 3.3% of the resting value. During the first 10 seconds of sprint 2 the PCr level dropped rapidly to the post-sprint 1 level with no significant decrease thereafter, demonstrating that PCr is utilised exhaustively during the first 10 seconds of sprint 2. Results obtained in a study conducted by Trump *et al.* (1996) suggest that PCr contributes on average 15% of the total ATP provision during the third 30-second bout of maximal isokinetic cycling. According to Bogdanis *et al.* (1996) the amount of PCr available before a repeated sprint may be related to the ability to generate high power during the initial seconds of the sprint.

1.2.2.1.3 Recovery

PCr resynthesis occurs rapidly after strenuous exercise and reaches completion within 15 minutes - 90% being resynthesised within the first two minutes of recovery (Hultman *et al.*,

1967). Resynthesis of PCr during recovery is mediated by mitochondrial-membrane-bound CK, thereby linking oxidative ATP production to cytoplasmic PCr resynthesis (Meyer *et al.*, 1984). Thus, with the aerobic rephosphorylation of ATP, the synthesis of PCr will occur simultaneously thereby allowing long-distance athletes to produce bursts of speed despite having run for periods exceeding two hours.

As explained in the previous paragraph, net PCr resynthesis is usually observed during post-exercise recovery, that is as soon as the muscle ceases to produce any mechanical work. Surprisingly, Giannesini *et al*. (2002) demonstrated a net PCr resynthesis in rat gastrocnemius muscle during exhaustive muscle contraction. Thus, muscle ATP was used to promote PCr resynthesis, instead of being used to produce force. This paradoxical phenomenon was explained as follows: *"Rat gastrocnemius muscle is a mixed muscle composed of slow-twitch oxidative muscle fibres (SO), fast-twitch oxidative glycolytic fibres (FOG), and fast-twitch glycolytic fibres (FG). By the end of the exhaustive exercise, SO fibres (fatigue resistant) could continue to produce force, whereas FG and FOG fibres (fast fatigable) were partially or totally inactive. This alteration in the pattern of fibre recruitment through fatigue development could, thus, allow PCr resynthesis to occur in inactivated fibres (FG and FOG) toward the end of exhaustive exercise (as energy production becomes more aerobic)*" (Giannesini *et al*. (2002 : 228)

Bogdanis *et al.* (1996) further demonstrated that the resynthesis of PCr and the recovery of power output were related to endurance fitness (%VO_2 max at 4 mmol/ℓ blood lactate), thus supporting the notion that endurance-trained individuals will have a faster rate of PCr resynthesis.

1.2.2.2 Summary of substrate utilisation during exercise at different intensities

Substrate utilisation during exercise at different intensities has three important breakpoints (Figure 2-1) where the metabolism is changed. The carbohydrate (CHO) threshold occurs when the energy requirement exceeds the maximum power for FFA oxidation, which therefore must be complemented by CHO oxidation. If available, CHO will also be oxidised when the exercise intensity is below the CHO threshold. The proportion of fat and CHO oxidation will be dependent on the muscle glycogen level and the concentration of FFA in the blood (Sahlin, 1986). It must be noted though, that the mechanism regulating the relative contribution of CHO and fats to energy production during exercise is not well understood. The results of a study by Sidossis and

Wolfe (1997) suggest that besides changes in plasma FFA availability, fatty-acid oxidation during exercise is directly regulated by glycolytic flux (the rate at which glucose is metabolised to pyruvate). When glycolytic flux is high the activity of the key enzyme carnitine palmitoyltransferase I (CPT I) is inhibited, blocking fatty acid entry into the mitochondria for oxidation. On the other hand, a slow glycolytic flux would disinhibit CPT I activity, allowing fatty acids to flow into the mitochondria for oxidation (Sidossis & Wolfe, 1997).

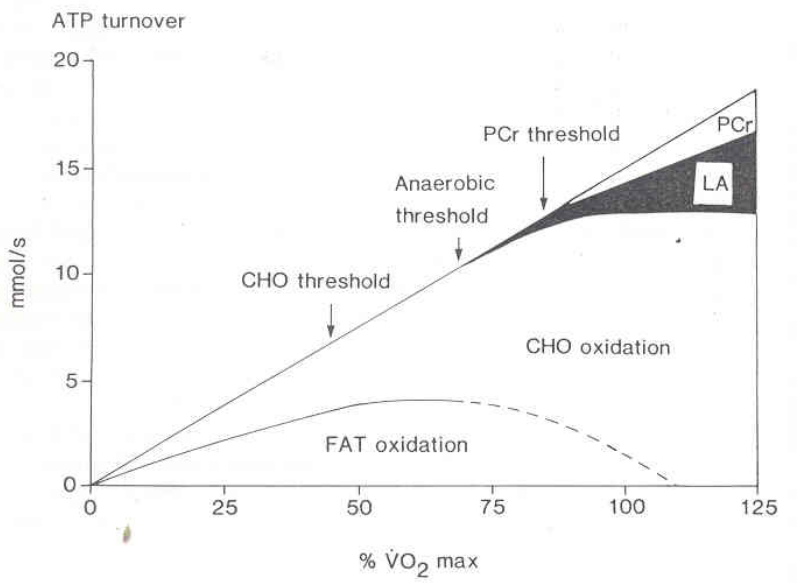


Figure 2-1 **Estimated rate of ATP production from different energy sources in the working legs (Sahlin, 1986)**
The CHO threshold denotes the workload where the maximal power of fat oxidation is sufficient to produce all of the required ATP. The anaerobic threshold denotes the workload, when blood lactate exceeds 2 mmol/ℓ. The PCr threshold denotes the workload where the whole PCr store is depleted in the working muscle.

The second breakpoint (the anaerobic threshold) occurs when anaerobic energy utilisation is initiated (Figure 2-1), with the result that rapid depletion of the local glycogen store will reduce work time (Sahlin, 1986; Powers & Howley, 2009).

The third breakpoint (the PCr threshold) occurs when the formation of lactate exceeds internal removal (Figure 2-1). At this high exercise intensity (80 - 95% VO_2 max), lactate and hydrogen ions (H^+) will continuously accumulate in the muscle and PCr will decrease until a level is reached where anaerobic energy production is too low to meet the demand and muscle

contraction ceases (Sahlin, 1986). However, the position of the three breakpoints is highly dependent upon the training status of an individual (Sahlin, 1986).

1.2.3 Respiratory exchange ratio (RER)

The respiratory exchange ratio (RER) is a variable that indicates substrate utilisation during incremental exercise. RER is the ratio between the amount of carbon dioxide (CO_2) produced and the amount of oxygen (O_2) consumed. The RER value provides information regarding the proportion of energy derived from various nutrients at rest and during steady-state sub-maximal exercise. It also indicates the attainment of exhaustion. RER values of less than 1.0 at peak exercise generally signify inadequate effort or poor motivation on the part of the participant (Franklin *et al.*, 1989), while values exceeding 1.0 indicate metabolism beginning to rely mainly on anaerobic processes (McArdle *et al.*, 2001). When the RER value exceeds 1.0, as when the individual approaches exhaustion, an accurate estimate of the fuel type being used is no longer possible. Elevations of 1.1 or higher during recovery are associated with an unloading of the excess CO_2 that accumulated in the blood during exercise (Wilmore & Costill, 2008). In general, the amount of carbon within a molecule of carbohydrate or fat is proportional to the amount of oxygen needed to oxidise the fuel completely.

Although the body derives more energy when it metabolises a given amount of fat than when it metabolises the same amount of carbohydrate, it takes proportionally more oxygen to oxidise fat (Marieb, 2004). Wilmore and Costill (2008) observe that it is impossible to calculate the use of protein from the RER since protein oxidises less completely than carbohydrate and fat. But, according to Franklin *et al.* (1989), the RER value for protein metabolism is approximately 0.8. Although, protein has been thought to contribute little to energy used in short duration activities, it may well contribute eight to nine percent of the total energy used in bouts of exercise lasting several hours (Wilmore & Costill, 2008; Williams, 2010). Indeed, Janssen (1987) speculated that for endurance sport, five to 15 percent of the energy supplied is derived from proteins (Janssen, 1987). This percentage may even rise when some very strenuous workouts are done successively, or when the duration of the exertion increases further (Williams, 2010). Table 2-2 shows that the RER will vary with the substrates being used for energy.

Table 2-2 The Respiratory Exchange Ratio (RER) and fraction (%) of energy derived from the oxidation of carbohydrates and fat

Respiratory exchange ratio	% of Calories from	
	Carbohydrates	Fat
0.71	0	100.0
0.75	15.6	84.4
0.80	33.4	66.6
0.85	50.7	49.3
0.90	67.5	32.5
0.95	84.0	16.0
1.00	100.0	0

Adapted from Wilmore and Costill (2008).

2. CREATINE: SOURCES, METABOLISM AND EFFECTS

2.1 A CONCISE HISTORY OF CREATINE

In 1832 a French scientist, Michel Eugene Chevreul, extracted from animal flesh a new organic compound (Williams *et al.*, 1999). He called it creatine (from the Greek *kreas*, flesh). In 1847 his finding was confirmed by Liebig, who also observed that the meat of wild foxes killed in the chase contained 10 times more Cr than that of their captive counterparts. Liebig thus concluded that muscle work results in the accumulation of Cr (Demant & Rhodes, 1999; Williams *et al.*, 1999). Even in these early days, the knowledge of Cr's fairly specific distribution and its absence from normal urine led to the realisation that it was not merely a waste product of metabolism. This realisation was confirmed when it was observed by Chanutin in 1926 that if Cr was administered a major portion of the compound was retained by the body (Greenhaff, 1995). Due to the unstable nature of PCr, and therefore difficulties in its isolation from muscle tissue, almost a hundred years elapsed from the first discovery of Cr to the discovery of its phosphorylated form. In 1927 Fiske and Subbarow, as well as and Eggleton and Eggleton, isolated PCr. They named it "phosphagen" and observed that it was involved in exercise energy expenditure (Demant & Rhodes, 1999; Williams *et al.*, 1999). Nowadays the term *"phosphagen"* is used generally for all phosphorylated guanidine compounds that may serve to generate ATP (Wyss & Kaddurah-Daouk, 2000). CK, the enzyme that catalyses the reversible transfer of the γ-phosphate group of ATP to Cr to yield ADP and PCr, was discovered in 1934 by Lohman (Williams *et al.*, 1999; Wyss & Schulze, 2002).

In England and the United States of America (USA), Cr supplementation has a relatively short history with the first anecdotal reports of Cr benefiting sports performance only surfacing after the 1992 Barcelona Summer Olympic Games. Two Olympic champions, Linford Christie in the men's 100m dash and Sally Gunnell in the women's 400m hurdles, reportedly used creatine supplements (Williams *et al.*, 1999). Silber (1999) speculated that Cr supplementation for the enhancement of sport performance had a much longer history in the Soviet and East Block countries. This statement is supported by a publication (Kalinski, 2003) tracing back research on the ergogenic effect of Cr to the 1970s. Reportedly (Kalinski, 2003), the Central Institute of Physical Culture in Moscow initiated a long-term research programme to specify the role of Cr in muscle performance and its use to enhance muscle function. Soviet scientists thus redirected their research from academic studies on Cr metabolism in animals to applied studies on the effects of Cr supplements on human physical performance. The pioneering studies were undertaken by Olexander Palladin, a leading Soviet biochemist. Information on the results from the studies was embargoed, and thus not published in Western journals. Kalinski (2003) reports that Cr supplementation at 0.1g/kg body weight per day was shown to significantly enhance biochemical and physiological measures of both aerobic and anaerobic metabolism in members of the Union of Soviet Socialist Republics (USSR) national track and field team. As a result, the Central Institute of Physical Culture officially recommended the use of Cr supplements to enhance physical capacity - as regards athletic performance - and the efficacy of exercise training. Thus, the USSR national track and field team were routinely given Cr supplements (Kalinski, 2003).

In the early 1990s Western studies on the ergogenic effect of Cr grew rapidly, and a number of publications reported delayed muscle fatigue (Balsom *et al.*, 1993*a*; Greenhaff *et al.*, 1993*b*; Söderlund *et al.*, 1994), enhanced muscle energy recovery (Greenhaff *et al.*, 1992; Greenhaff *et al.*, 1994; Bogdanis *et al.*, 1996; Vandenberghe *et al.*, 1996*a*), and enhanced exercise performance (Balsom *et al.* 1993*a*; Greenhaff *et al.*, 1993*b*; Birch *et al.*, 1994; Earnest *et al.*, 1994; Söderlund *et al.*, 1994). Recent publications on the benefits of Cr supplementation for athletes in general support the findings of the early 1990s (Rossouw *et al.*, 2000*a*; Bemben *et al.*, 2001; Kambis & Pizzedaz, 2003; Van Loon *et al.*, 2003; Ostojic, 2004; Peyrebrune *et al.*, 2005; Gotshalk *et al.*, 2008; Jäger *et al.*, 2008; Saremi *et al.*, 2010; Rawson *et al.*, 2011). Cr has become one of the most widely used non-doping exercise-enhancing supplements in sport (Kalinski, 2003; Tscholl *et al.*, 2010).

It seems that studies on Cr metabolism in general were scarce in the 1990s, with most research effort being directed towards its ergogenic properties. However, it seems that the focus of research has since shifted. The safety of supplementation has received some attention (Poortmans & Francaux, 2000; Potteiger *et al.*, 2001; Mayhew, *et al.*, 2002; Crowe, *et al.*, 2003; Tarnopolsky *et al.*, 2003*a*; Archer, 2004; Taes & De Vriese, 2005), but conclusive long-term research results are still lacking. However, fascinating biochemical and physiological discoveries have been made of late. First of all, Cr analogs have proved to be potent anticancer agents (Wyss & Kaddurah-Daouk, 2000); and secondly, oral Cr supplementation protect muscle (Braegger *et al.*, 2003; Louis *et al.*, 2003*a*; Aoki *et al.*, 2004; Chetlin *et al.*, 2004; Tarnopolsky, 2011) and neural (Stout *et al.*, 2001; Wyss & Schulze, 2002; Lambert *et al.*, 2003; Berger *et al.*, 2004; Smith *et al.*, 2006) tissue from deteriorating as a result of disorders, and the cloning of many of the enzymes involved in Cr metabolism has opened the door to a wide variety of clinical investigations and applications (Wyss & Kaddurah-Daouk, 2000).

2.2 STRUCTURE

2.2.1 Creatine, phosphocreatine and creatinine

Cr, or β-methylguanidine-acetic acid, is the most abundant low molecular weight compound found in human muscle. It is not an amino acid, but a nitrogen-containing compound known as an amine (Williams, 2010). This is found together with its phosphorylated form, PCr, in a wide variety of excitable tissues (Figure 2-2). In the guanidine family of compounds, PCr is unique that it is the only natural phosphagen with a methyl group attached to the guanidine moiety of the molecule. PCr and Cr are smaller and less negatively charged molecules compared to ATP and ADP, with the result that it can build up to much higher concentrations in most CK-containing cells and tissues, thereby enabling a higher intracellular flux of high-energy phosphates (Wyss & Kaddurah-Daouk, 2000). The muscle (and other) cells' reservoir of PCr, Cr and a very small amount of ATP, lies in the intervening space between the myofibril and the mitochondrion (Bessman, 1987). This "*reservoir*" is in reality the fraction of free or phosphorylated Cr in transit between the mitochondrion and myofibril (Bessman, 1987). Any reactions occurring in the CK-enzyme compartments – also called the Cr compartments (Speer *et al.*, 2004) - of the mitochondria and the myofibrils are separate from this equilibrium pool (Bessman, 1987).

Muscle Cr is slowly converted non-enzymatically to creatinine (Crn) by the removal of water and the formation of a ring structure (Purchas *et al.*, 2004).

2.2.2 Endogenous Cr synthesis

The total creatine (TCr) pool in the body is derived from dietary Cr present in meat and fish products, and from biosynthesis occurring in the kidneys, liver and pancreas. Cr is not considered an essential nutrient in the diet because the body has all the enzymes required for Cr biosynthesis (Harris, 1993; Kraemer & Volek, 1999).

Endogenous Cr synthesis requires the amino acid arginine (Arg), with further additions from glycine (Gly) and methionine (Met) (Harris, 1993). The entire Gly molecule is incorporated into Cr, whereas Arg furnishes only its amidino group and Met its methyl group (Walker, 1979). The biosynthesis of Cr occurs in two steps and involves two different enzymes (Figure 2-2). The first step occurs mostly in the kidneys (Silber, 1999; Brudnak, 2004), and begins with a process of transamidination. In a reversible reaction catalysed by the enzyme arginine:glycine amidinotransferase (AGAT), the amidino group of Arg is transferred to Gly to yield ornithine and guanidinoacetate (GAA) (Demant & Rhodes, 1999; Wyss & Kaddurah-Daouk, 2000). It is common cause that the GAA then enters the blood and is transported to the liver where the second step in biosynthesis occurs (Wyss & Kaddurah-Daouk, 2000). The second (and last) step in Cr biosynthesis is an irreversible reaction known as transmethylation. The action of the enzyme guanidinoacetate methyltransferase (GAMT) adds a methyl group from *S*-adenosylmethionine to GAA, thereby yielding Cr and *S*-adenosylhomocysteine (Wyss & Kaddurah-Daouk, 2000; Wyss & Schulze, 2002). Cr is then transported via the blood to be concentrated into the skeletal and heart muscles and other tissues requiring high and fluctuating energy levels (Ponticos *et al.*, 1998; McLeish & Kenyon, 2005). These tissues include the brain, testes and retina (Walker, 1979; Harris, 1993).

2.2.3 Exogenous Cr production

Cr supplements are available to the public mainly as creatine monohydrate (CrM). However, other forms such as creatine citrate (CrC), creatine phosphate, creatine pyruvate, creatine malate, Kre-Alkalyn® (CrA) (a special patented form of CrM buffered at basic pH) and creatine ethyl ester are also available in both their pure form or in formulation with other ingredients (Moret *et al.*, 2011). Each gram of pure CrM provides 0.879g of Cr (Hathcock *et al.*, 2006; Shao & Hathcock, 2006). In 2007 the International Society for Sports Nutrition declared as a myth the belief that the newer Cr formulations are more beneficial than CrM and caused fewer side-effects (Buford *et al.*, 2007). This issue will be addressed later in this review.

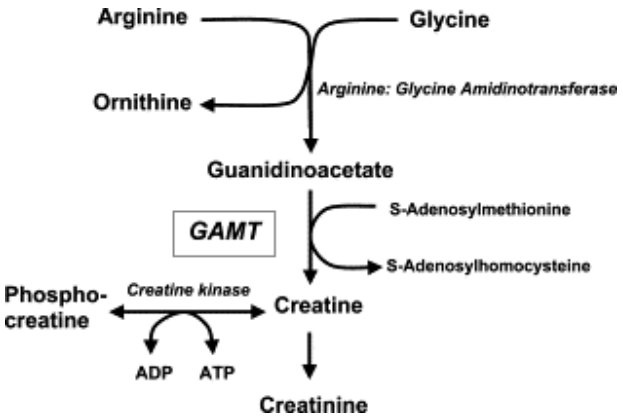


Figure 2-2 Creatine biosynthesis (Feldman, 1999)

The exogenous commercial production of Cr (Figure 2-3) entails the reaction over time of cyanamide and either sodium or potassium sarcosinate in the presence of heat. Crystals precipitate out and are isolated and dried (Brudnak, 2004; Moret *et al.*, 2011). The alternative low-cost route of preparation uses sarcosine (and/or potassium or sodium sarcosinate) and S-methylisothiourea (and/or methylisothiourea sulphate) as starting materials (Moret *et al.*, 2011).

2.2.4 Exogenous Cr intake

Cr is present in meat and fish and is thus ingested by following a healthy, balanced, omnivorous diet. Harris *et al.* (1997) report that uncooked chicken, beef and rabbit meat contain approximately 30 mmol/kg of Cr. Ox-heart and ox-liver were found to have concentrations of 22 and 2 mmol/kg respectively. Unfortunately for South Africans who love biltong and consider it a source of additional Cr intake, Harris *et al.* (1997) report that in the dried meat samples of their study, Cr had degraded variably to Crn.

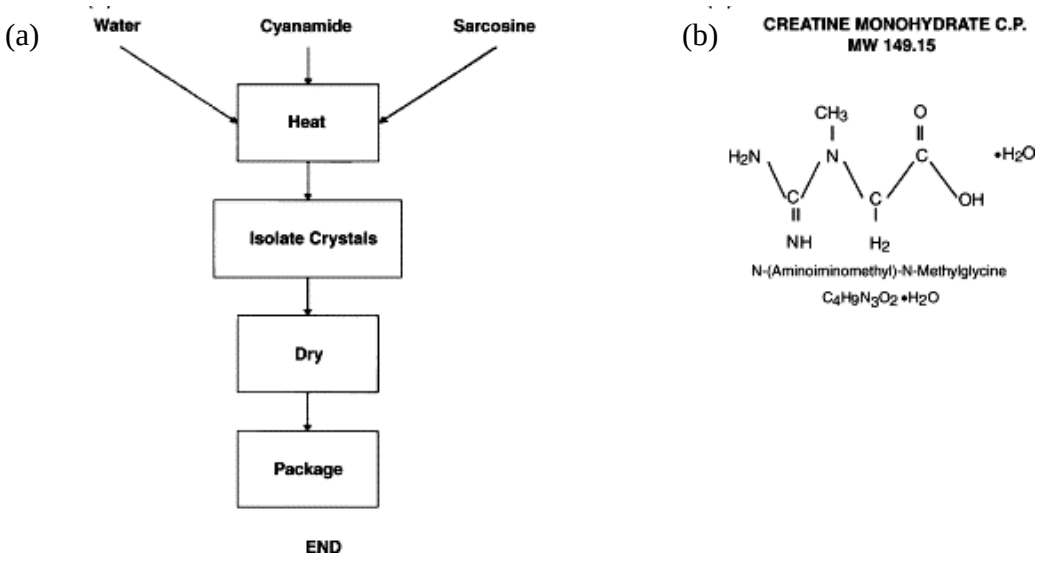


Figure 2-3: (a) Commercial production of creatine. (b) Structural formula for commercially produced creatine monohydrate (Brudnak, 2004)

2.3 METABOLISM

2.3.1 Tissue uptake of Cr

Little Cr is found in the major sites of synthesis (Greenhaff, 1997; Persky & Brazeau, 2001). It follows therefore, that Cr is transported from sites of synthesis (liver, kidneys, and pancreas) to sites of storage and utilisation (skeletal muscle, heart, nervous system and brain, macrophages, and spermatozoa) (Silber, 1999; Persky & Brazeau, 2001). It has been suggested that the significant presence of Cr in skeletal muscle is attributed to special mechanisms for Cr entry and intracellular trapping (Fitch & Shields, 1966).

Cr, whether from exogenous or endogenous sources, travels in the blood stream. Once it arrives at the muscle and nerve cells its uptake occurs *via* a specific Cr transporter system at a 200 : 1 concentration gradient (Feldman, 1999). A specific, saturable sodium and chloride dependent Cr transporter (Crea T) responsible for Cr uptake across the plasma membrane has been described for a variety of cells. Crea Ts are integral membrane proteins (glycoproteins) and are highly substrate-specific (Guimbal & Kilimann, 1993; Peral *et al.*, 2002; Zhao *et al.*, 2002; Speer *et al.*, 2004). They are most closely related to the γ-aminobutyric acid, taurine/betaine transporter sub-family (Walzel *et al.*, 2002). Cr uptake into skeletal muscle cells may thus be influenced by the expression and activity of the Crea T. Furthermore, changes in the extra- and/or intracellular Cr content may alter the activity of the Crea T (Speer *et al.*, 2004).

It is currently believed that the intracellular trapping of Cr occurs through the compound's phosphorylation in the cytosol *via* the CK-reaction (Geenhaff, 1997). PCr (the form in which 60 - 70% of muscle TCr exists) is unable to escape from the cell due to its polarity (Greenhaff, 1997) and the specificity of the plasma membrane Crea T to Cr (Speer *et al.*, 2004). Another mechanism of intracellular trapping of Cr consists in Cr binding to intracellular components (Greenhaff, 1997). An inner mitochondrial membrane Crea T that has been identified provides strong evidence that mitochondria can accumulate Cr (Walzel *et al.*, 2002; Tarnopolsky *et al.*, 2003*b*; Speer *et al.*, 2004). It is still uncertain whether the Cr transported into mitochondria is immediately recharged *via* mitochondrial CK to PCr, or whether it could fulfil some protective role as an osmolyte to guarantee mitochondrial integrity under conditions of stress (Walzel *et al.*, 2002; Sobolewski *et al.*, 2011).

At a cellular level in heart and skeletal muscle, the site of ATP production (eg. the mitochondrial intermembrane space) is separate from the sites of ATP consumption (eg. the myofibrillar M-line, sarcoplasmic reticulum, or the cell membrane) (Wyss & Kaddurah-Daouk, 2000; McLeish & Kenyon, 2005). According to the "transport" *("shuttle")* hypothesis concerning the CK system the transport of high-energy phosphates between the sites of ATP production and ATP consumption is achieved mainly by PCr, Cr and distinct CK iso-enzymes (Bessman, 1987; Harris, 1993; Wyss & Kaddurah-Daouk, 2000; Echegaray & Rivera, 2001; McLeish & Kenyon, 2005). This system of energy transport in muscle has been described as the creatine phosphate shuttle (Figure 2-4) because the phosphate-"laden" Cr moves vectorially to where it is picked up by the myofibril, and the product, free Cr, returns to the mitochondrion (Bessman, 1987).

The shuttle proposal states that the enzyme CK is intimately linked to the sites of ATP production and utilisation. It can be explained as follows (Wyss & Kaddurah-Daouk (2000 : 1109): "*The γ-phosphate group of ATP, synthesized within the mitochondrial matrix, is transferred by mitochondrial CK (Mi-CK) in the mitochondrial inter-membrane space to Cr to yield ADP plus PCr. ADP liberated by the Mi-CK reaction may be transported back directly to the matrix where it is rephosphorylated to ATP. PCr leaves the mitochondria and diffuses through the cytosol to the sites of ATP consumption (i.e. the myofibrils) where cytosolic CK isoenzymes regenerate ATP locally, thus conducing to a high phosphorylation potential in the immediate vicinity of the respective ATPases. Cr thus liberated diffuses back to the mitochondria, thereby closing the cycle".* PCr and Cr thus fulfil necessary mechanistic roles as carriers of high-energy phosphates, and as regulatory signals linking the sites of ATP production

and consumption during muscle contraction over long periods (Meyer *et al.*, 1984; Echegaray & Rivera, 2001; Speer *et al.*, 2004). The CK system, however, displays a high degree of flexibility and is able to adapt to the peculiar physiological requirements of a given tissue (Bessman & Savabi, 1988; Wyss & Kaddurah-Daouk, 2000; McLeish & Kenyon, 2005). The question whether the Cr-PCr shuttle hypothesis accurately describes the function of the CK system in endurance-type tissues remains unsettled.

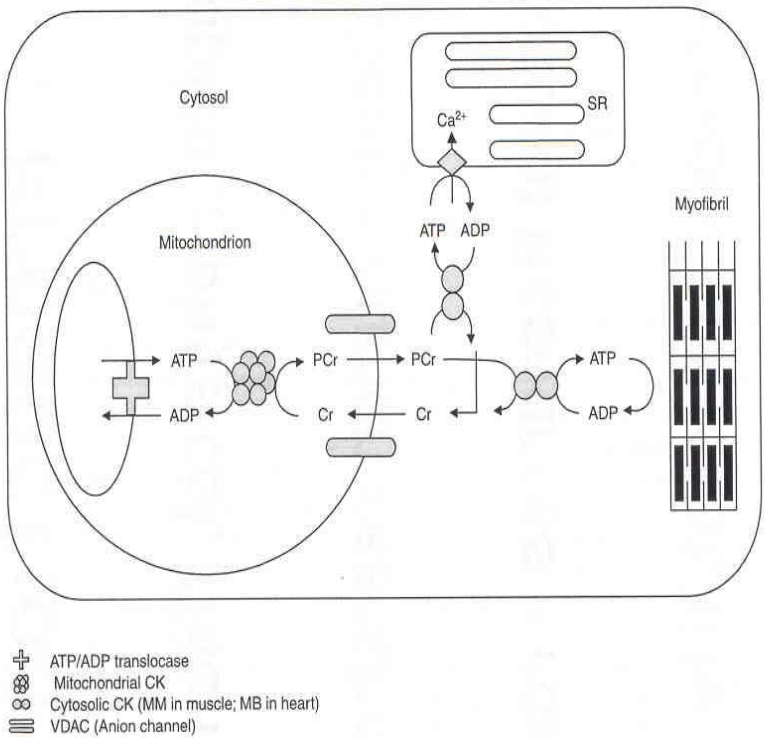


Figure 2-4 Schematic presentation of the CK-PCr system in muscle (Echegaray & Rivera, 2001)

2.3.2 Regulation of Cr homeostasis in humans

The total cellular Cr content depends on the balance of uptake, retention and efflux of Cr. The control mechanisms may vary in importance and effectiveness depending in part on amino acid requirements, and whether or not Cr is a common component of the relevant diet (Walker, 1979).

2.3.2.1 Cr distribution in the body

Cr is located in three compartments, namely blood serum, cytosol and mitochondria (Speer *et al.*, 2004). These pools are interconnected *via* two different Cr uptake mechanisms: the high-

affinity, high-efficiency plasma membrane Crea T and the low-affinity mitochondrial Crea T (Speer *et al.*, 2004).

Cr and its three other sources, PCr, creatinine (Crn), and phosphocreatinine (CnP), follow a specific equilibrium in different organs and body fluids, under different physiological and pathological conditions (Silber, 1999). If skeletal muscle is at rest and pH and body temperature are normal, the equilibrium among them is shifted toward PCr, CnP, and Cr (Silber, 1999). The bulk of the body's TCr pool (95%) is located in skeletal muscle at an average concentration of 125 mmol/kg of dry mass (Hultman *et al.*, 1996), but individual variance may range from 90 to 160 mmol/kg (Greenhaff, 1995). Of the total Cr content of muscle, approximately 60% exists in the form of PCr at rest (Harris *et al.*, 1992; Greenhaff, 1995). The estimated total body pool of Cr in man weighing 70 kg is approximately 120 g (Walker, 1979; Harris *et al.*, 1992). There appears to be no difference between elderly and young persons where total Cr content is concerned (Moeller *et al.*, 1980). Tarnopolsky *et al*. (2003*b*) demonstrate similar baseline TCr concentrations and similar increases in muscle TCr in response to acute and moderate-term Cr supplementation in men and women. Thus, no gender differences in total body Cr is observed.

2.3.2.2 Regulation of Cr biosynthesis

The biosynthesis of Cr requires three amino acids, Arg, Gly, and Met. It follows, therefore, that regulatory mechanisms should exist to prevent excessive conversion of these amino acids from Cr biosynthesis. As explained earlier (section 2.2.2), the first step in Cr biosynthesis is the formation of GAA in a reaction catalysed by the enzyme AGAT. This is normally the rate-limiting step of Cr biosynthesis (Walker, 1979). The expression of AGAT is repressed by increased Cr levels, regardless of whether the metabolic origin of the Cr is endogenous or exogenous (Walker, 1979; Derave *et al.*, 2004). This most probably serves to conserve the dietary essential amino acids Arg and Met (Wyss & Kaddurah-Daouk, 2000; Derave *et al.*, 2004) and energy (Derave *et al.*, 2004) in the human body.

In addition to Cr, the expression of AGAT may be modulated by dietary and hormonal factors. In animal studies, fasting and inadequate diets differentially lower AGAT levels. According to Walker (1979) this makes sense physiologically since Cr biosynthesis is unnecessary during the muscle atrophy associated with fasting. Cr biosynthesis is optimal under conditions of good food supply and optimal blood concentrations of insulin, somatotropin, thyroid hormone, and testosterone (Walker, 1979).

There is a good reason to expect that Cr supplementation, especially large quantities, would markedly reduce normal Cr synthesis in the body (Silber, 1999; Terjung *et al.*, 2000). This surmise has been borne out where human subjects are concerned. Derave *et al.* (2004) demonstrated a significant down-regulation of endogenous Cr synthesis during both the loading and maintenance stages of supplementation (20 g/day Cr for 5 days followed by 5 g/day for 19 weeks). The mechanism of down-regulation of endogenous Cr synthesis provides a valuable way to save amino acids and energy in the human body (Derave *et al.*, 2004). However, it is reasonable to expect that shortly after the cessation of Cr supplementation Cr synthesis within the body would revert to its pre-existing rate. The long half-life of Cr in muscle probably serves as a buffer that would allow for the recovery of any potential down-regulation in either synthesis or transport (Terjung *et al.*, 2000).

2.3.2.3 Regulation of Cr uptake and retention

The question of how Cr uptake and retention are regulated must be addressed in detail to ensure that the processes of Cr metabolism in health and disease are properly understood.

Cellular Cr uptake across membranes is regulated through control of Crea T expression and activity. As explained earlier, Cr uptake into skeletal muscle, heart, brain, or kidney is effected by a specific sodium- and chloride-dependent Crea T (Walker, 1979; Guerrero-Ontiveros & Walliman, 1998; Wyss & Kaddurah-Daouk, 2000; Tarnopolsky *et al.*, 2003*b*; Speer *et al.*, 2004). The Crea T is saturable, and its expression and/or specific activity seem to be influenced by dietary and hormonal factors. Odoom *et al.* (1996) identified a series of hormones that influence net Cr uptake into a cultured mouse myoblast cell line. They have shown that catecholamines (noradrenalin, isoproterenol and clenbuterol) can stimulate net Cr uptake preferentially through β_2-adrenergic receptors. It was also demonstrated (Odoom *et al.*, 1996) that insulin at supra-physiological concentrations, and insulin-like growth factors (Odoom *et al.*, 1996; Louis *et al.*, 2004) may also stimulate net Cr uptake. Results from various studies (Green *et al.*, 1996; Steenge *et al.*, 2000; Preen *et al.*, 2003) and discussions (Terjung *et al.*, 2000; Buford *et al.*, 2007) indicate that combining Cr with either a carbohydrate or a carbohydrate-and-protein source produces optimal cellular uptake and retention. This effect has been attributed to increased insulin levels and/or improved insulin sensitivity (Steenge *et al.*, 2000; Buford *et al.*, 2007).

According to Silber (1999), experimental data and calculation show a leakage of Cr from the muscle cell into the blood plasma during maximal muscle contractions, with the result that plasma levels of Cr may reach 115 to 150 μmol/ℓ. However, this efflux of Cr from the muscle cell has the tendancy to slow down along with the ongoing adaptation of skeletal muscle to extreme exercise.

Peral *et al.* (2002) measured the expression of the Crea T in the human small intestine to determine the mechanism of absorption of orally ingested Cr. Results indicate that enterocytes accumulate Cr against a concentration gradient. This accumulation is electrogenic, sodium and chloride dependent, with a probable ratio of 2 Na^+ : 1 Cl^- : 1 Cr. The Crea T is located in the brush-border membrane in the cells lining the intestinal villus, and expresses high substrate specificity (Peral *et al.*, 2002).

Dietary Cr supplementation could potentially influence the Crea T through an increase in extracellular Cr, and/or an increase in intracellular Cr (Speer *et al.*, 2004). In a study by Guerrero-Ontiveros and Walliman (1998) on rats, Cr supplementation of the diet decreased Crea T expression in cell cultures. The experiments concluded that the effect of extracellular Cr on the transporter protein is to control, by a negative feedback repression mechanism, the synthesis of the Crea T itself. According to Guerrero-Ontiveros and Walliman (1998) extrapolation of this result to athletes who chronically ingest Cr seems highly likely. However, this presupposition was disproved by Tarnopolsky *et al.* (2003*b*) who showed that no down-regulation occurred in plasma membrane Crea T in response to Cr loading and moderate-term Cr maintenance dosages in both young and elderly human subjects. This finding is probably attributable to the supra-physiological Cr dosages of five to 10 times those normally used in human studies compared to those used in the rat study (Tarnopolsky *et al.*, 2003*b*). Also, dietary supplementation of Cr in human subjects despite a three- to 20-fold increase in the serum concentration of Cr, results in an increase in the muscle levels of Cr and PCr of only 10% to 25% (Harris *et al.*, 1992; Francaux *et al.*, 2000; Wiedermann *et al.*, 2001; Burke *et al.*, 2003; Rawson *et al.*, 2004). It follows, according to Wyss and Kaddurah-Daouk (2000) that it is difficult to envisage that intracellular Cr concentration would be a key regulator of Cr uptake in healthy human subjects, and the mechanism whereby the extracellular Cr concentration is transformed into an intracellular signal has yet to be explained.

It was found that bioavailability of Cr in the digestive tract at 2 g/day supplementation was not affected by the form in which Cr was ingested. Deldicque *et al*. (2008) found no difference in the extent of Cr absorption resulting from ingestion of an aqueous solution of Cr powder, a beta glucan-rich Cr-containing food bar and a protein-rich Cr-containing food bar, but the retention of whole-body Cr was enhanced following the beta glucan-rich bar. The authors (Deldicque *et al*., 2008) attributed this result to the structural polysaccharides (dietary fibres) increasing the viscosity of the intestinal content, thereby slowing down the rate of Cr absorption and consequently favouring its retention by the body.

2.3.2.4 Regulation of Cr degradation and reabsorption

Cr is essentially stable at neutral to basic pH values, but rapidly converts to Crn in acidic environments (Brudnak, 2004). Once ingested, Cr has potentially various fates. Due to the low pH in the stomach (pH ~ 2), some of it is transformed into Crn (Deldicque *et al*., 2008). The rate of Cr degradation is in the same range in the small intestine (pH = 6 - 7) and amounts to only 1% within the first hour. The stable plasma Crn concentration following the ingestion of two grams of creatine, in addition to its undetectable amount in faeces, indicates that the conversion of Cr to Crn remains negligible in the gastrointestinal tract (Deldicque *et al*., 2008). Thus, oral Cr is well absorbed and does not undergo significant deterioration in the gastrointestinal tract.

Under conditions that exist in the stomach it is possible in theory for Cr to be degraded and give rise to toxic intestinal methylamine and formaldehyde. However, according to evidence gathered by Deldicque *et al*. (2008) such transformations are quantitatively negligible and the bulk of Cr ingested remains available for absorption.

The degradation of Cr and PCr in humans is, for the most part, a spontaneous, non-enzymatic process (Wyss & Kaddurah-Daouk, 2000). *In vivo*, once this irreversible conversion (Greenhaff, 1995) happens, Crn passively diffuses out of the body cells and is excreted by the kidneys into the urine (Wyss & Schulze, 2002; Brudnak, 2004). An almost constant fraction of the body's Cr (1.1% per day) and PCr (2.6% per day) is converted into Crn. Consequently, in a 70-kg man containing ~120g of TCr, roughly 2 g/day is converted into Crn and has to be replaced by Cr from the diet or from biosynthesis (Walker, 1979; Wyss & Kaddurah-Daouk, 2000). According to Wyss and Kaddurah-Daouk (2000) this loss can be made up by ingesting 500 g of raw meat per day.

With continued daily supplementation a large portion (up to 90%) of ingested Cr is excreted as Cr in the urine (Terjung *et al.*, 2000). It seems that when the capacity of the muscle to extract Cr from the blood is exceeded (i.e. during the first days of high-dosage supplementation) (Terjung *et al.*, 2000; Hadjicharalambous *et al.*, 2008), the excess Cr is simply excreted into the urine with the result that continued ingestion of large doses of Cr simply produces Cr-enriched urine (Terjung *et al.*, 2000). Recent evidence (Deldicque *et al.*, 2008) indicates that the high rate of excretion can be reduced to about 15% by reducing the daily dosage to 2 g.

The kidney effectively salvages Cr from the urine through simple diffusion: notable quantities are only excreted (creatinuria) in certain diseases, under muscle-mass reducing conditions, and also fasting or high dietary intake of Cr (Silber, 1999).

To conclude, the most critical determinant for the regulation of Cr metabolism seems to be the serum concentration of Cr. An elevation of serum Cr over an extended period of time would point to excess biosynthesis or dietary intake of Cr, and, in addition, would indicate that the tissue pools of Cr and PCr are replenished. This could lead to the down-regulation of the activity and/or expression of AGAT and possibly also the Crea T. Net Cr uptake into tissues may be enhanced by the simultaneous ingestion of anabolic agents, simple carbohydrates and/or carbohydrate/protein combinations. Normal and steady levels of Cr and PCr are maintained in CK-containing tissues. The bioavailability of pure Cr monohydrate is not affected by the form (aqueous solution or solid food) in which it is ingested. However, whole-body Cr retention may be enhanced by the simultaneous ingestion of beta glucans. It is also recommended that Cr be ingested at low dosages to increase whole-body retention and decrease excretion in the urine. This practice can also be expected to decrease the load placed on the kidneys.

2.4 INHERENT FUNCTIONS IN THE BODY

The availability of Cr and PCr is essential to muscle function during fatiguing maximal exercise of short duration, because both play several key roles in muscular activity.

2.4.1 Energy buffer

ATP concentrations maintain physiological processes and protect tissue from hypoxia-induced damage (Persky & Brazeau, 2001). Meyer *et al.* (1984) suggest the term "*temporal buffer*" to describe the familiar role of PCr in the maintenance of ATP levels during periods of muscle contraction. Since then, the term has been commonly used (Juhn & Tarnopolsky, 1998;

Kraemer & Volek, 1999; Persky & Brazeau, 2001; Wiedermann *et al.*, 2001; Brudnak, 2004) to indicate the role of PCr as a reservoir of potential ATP-synthesis. The phosphate bonds of PCr act as an energy source for the first few seconds of any activity. During the initial seconds of intense muscle contraction PCr acts as a buffer to the delay in energy provision from glycolysis. The CK reaction is very rapid and, therefore, the regeneration of ATP at a rate close to that of ATP hydrolysis is achieved and fatigue delayed (Hultman *et al.*, 1991; Powers & Howley, 2009). It is important to remember that the synthesis of ATP from PCr is anaerobic in nature, while the resynthesis of PCr during recovery takes place in the mitochondria, and is thus aerobic (Persky & Brazeau, 2001).

2.4.2 Energy transport/distribution

CK has two isoforms in skeletal muscle, namely cytosolic and mitochondrial CK. The formation of the polar PCr "*locks*" Cr in the muscle and maintains the retention of Cr because the charge prevents partitioning through biological membranes (Greenhaff, 1997; Persky & Brazeau, 2001). Since these spatially separate isoforms catalyse the reversible reaction PCr + ADP ↔ ATP + Cr in their respective compartments, Meyer *et al.* (1984) suggest that PCr acts as a "*spatial energy buffer*". As explained earlier, PCr may facilitate the transfer of high-energy phosphate, formed as ATP in the mitochondria, to the cytoplasm (Thompson, 1996; Wyss & Kaddurah-Daouk, 2000). It may therefore act as an energy transport shuttle from mitochondria to the contractile sites of the muscle (myofibrils), the so called Cr-PCr shuttle as demonstrated in Figure 2-1. Thus, the shuttle system provides more efficient energy transport between sites of ATP synthesis and utilisation. The shuttle system may also be important during aerobic exercise and/or in the facilitation of recovery after exercise (Juhn & Tarnopolsky, 1998; Echegaray & Rivera, 2001). Whether or not the spatial buffering effect is physiologically important is uncertain, although calculations suggest that it would be most important in large cells with nonuniform mitochondrial distributions in which diffusion distances are greatest e.g. neuronal axons (Meyer *et al.*, 1984).

2.4.3 Maintenance of inorganic phosphate levels

An increase in the pre-exercise PCr stores provides an enhanced buffer to ATP during exercise, resulting in less degradation of the total adenine nucleotide pool (Balsom *et al.*, 1993*a*; Bellinger *et al.*, 2000). The plasma concentrations of other products of adenine nucleotide degradation (e.g. ammonia, hypoxanthine, and urate) are also decreased (Bellinger *et al.*, 2000). The CK reaction thus prevents a build-up of ADP with less activation of the myokinase reaction and less

subsequent catabolism of AMP. The intracellular environment will therefore remain stable, enabling normal muscular function to continue.

2.4.4 Hydrogen ion (H^+) buffer

As mentioned previously, the breakdown of PCr and anaerobic glycolysis are the only mechanisms capable of rapidly replenishing ATP during intense activity. Anaerobic glycolysis will, however, result in an accumulation of lactate within the muscle. When this lactate dissociates, it floods the cellular environment with hydrogen ions (H^+), causing the muscle pH to fall. The fall in pH has been implicated in the fatigue process (Sahlin, 1986; Booth & Thomason, 1991; Fitts, 1994; Chaudhuri & Behan, 2004). The breakdown of PCr is one of several buffers in the cell that resist changes in pH (Persky & Brazeau, 2001). The CK reaction can be rewritten (Maughan, 1995; Juhn & Tarnopolsky, 1998; Wyss & Kaddurah-Daouk, 2000) to account for the changes involved:

$$PCr^{2-} + ADP^{3-} + H^+ \rightarrow ATP^{4-} + Cr$$

Increased availability of PCr for breakdown therefore has the potential to increase the intramuscular buffering capacity, delaying the point at which pH reaches a critically low level (Maughan, 1995; Persky & Brazeau, 2001).

2.4.5 Modulation of glycolysis

Phosphofructokinase (PFK), the key enzyme of anaerobic glycolysis (Powers & Howley, 2009), is at least partially inhibited by PCr (Demant & Rhodes, 1999; Wyss & Kaddurah-Daouk, 2000). Therefore, during intense physical activity requiring rapid ATP resynthesis, PCr levels decline. As a result, PFK becomes less inhibited and the rate of glycolysis increases.

In conclusion, in skeletal muscle with many mitochondria, the classic energy storage function, the potential role in the activation of glycolysis and lactate buffering are proposed as the most reasonable functions of PCr. In the neuron, PCr can accumulate toward the synapse and assist with relatively high and stable synaptic potential (Brudnak, 2004). The transport of high-energy phosphates between the sites of ATP synthesis (mitochondria) and utilisation (synapses) may thus play a larger role in cells of the nervous system where greater diffusion distances are concerned.

2.5 COMMON DOSAGES AND FORMS OF SUPPLEMENTATION

The Cr requirement of a 70 kg male is approximately two grams per day, but Western man eating a typical modern diet may consume just 0.5 to 1g Cr per day (Walker, 1979; Harris, 1993). The Cr content of fresh, top quality, uncooked meat is approximately four to a maximum of 6 g/kg (Harris, 1993; Purchas *et al.*, 2004), varying between muscles, individual animals, and with cooking. Slow cooking for 90 minutes at 70 °C would result in at least half the Cr being lost, most probably in the cooking juices (Purchas *et al.*, 2004). Normal levels of cooked meat consumption (approximately 100 – 200 g/day) will lead to appreciably lower intakes than those required to achieve and maintain optimal daily muscle TCr levels. It seems logical, therefore, to supplement an athlete's diet with Cr. But for Cr supplementation to benefit exercise performance the supplementation regimen has to be effective at increasing the muscle PCr concentration, and/or the TCr pool (sum of PCr + free Cr) of the muscles.

Varying amounts of Cr can be consumed depending on the recommendations made by manufacturers of Cr products, and what the individual is trying to accomplish (eg. muscle hypertrophy or improved aerobic power). Typically, supplementation at a rate of 20 – 30 g/day over five to seven days (usually as Cr monohydrate) is utilised in studies (Harris *et al.*, 1992; Casey *et al.*, 1996; Schedel, *et al.*, 2000; Steenge *et al.*, 2000; Warber *et al.*, 2002; Louis *et al.*, 2003*c*; Mendes *et al.*, 2004; Santos *et al.*, 2004; Hadjicharalambous *et al.*, 2008; Rahimi *et al.*, 2010). This is referred to as the "*loading dosage*", and is followed in many studies by a "*maintenance dosage*" of 2 – 5 g/day (Becque *et al.*, 2000; Huso *et al.*, 2002; Burke *et al.*, 2003; Kutz & Gunter, 2003; Preen *et al.*, 2003; Olsen *et al.*, 2006; Stout *et al.*, 2006; Van der Merwe *et al.*, 2009). However, without a loading phase a dosage of 3 g/day would achieve similar increases in TCr and PCr levels after 28 days (Hultman *et al.*, 1996; Hickner *et al.*, 2010).

If dosing by body weight, a loading regimen of 0.3 g/kg/day for five days, followed by a maintenance regimen of 0.03 g/kg/day is recommended (Hultman *et al.*, 1996). Studies incorporating Cr supplementation according to body weight are relatively scarce (Kirksey *et al.*, 1999; Kambis & Pizzedaz, 2003; Gotshalk *et al.*, 2008; Saremi *et al.*, 2010). This is probably due to practical complications in the dosing of individual participants. According to Rawson *et al.* (2011) the lowest effective dosage (ie. enhancing exercise performance) of Cr is approximately 2.3 g/day (0.03 g/kg/d), if the supplementation period is extended beyond five days.

Some authors recommend the loading dosage not be repeated within three months, and low-dosage supplementation not continued for periods longer than three months, due to organ safety concerns and/or fear of Crea T down-regulation (Guerrero-Ontiveros & Walliman, 1998). For these reasons, some consumers employ cycling protocols involving the consumption of Cr "*loading*" doses only, for 3 - 5 days every 3 to 4 weeks. According to Buford *et al*. (2007) these protocols are effective in increasing and maintaining muscle Cr content. However, if product purity has been established, concerns for organ safety are probably unfounded. Extensive risk analyses (Hathcock *et al.*, 2006; Shao & Hathcock, 2006) have established the safe dosage of Cr to be 5 g/day, with no limitation on the time period of the ingestion routine. Also, Tarnopolsky *et al.* (2003*b*) reported no down-regulation of the plasma membrane Crea T in response to Cr loading and moderate-term Cr maintenance dosages in both young and elderly humans.

The effect of oral Cr supplementation on muscle Cr content varies considerably between people. The effect of supplementation is the greatest in people at the lowest end of initial Cr content and the least effect occurs where the initial content is close to the upper end of the normal range (Harris *et al.*, 1992; Greenhaff *et al.*, 1993*b*; Francaux *et al.*, 2000; Lukaszuk *et al.*, 2002; Burke *et al.*, 2003). Some subjects retain more than twice as much Cr as others (Rossiter *et al.*, 1996). Studies (Greenhaff *et al.*, 1996; Van Loon, 2003; Hadjicharalambous *et al.*, 2008) have revealed that 20 - 30% of a given population "*do not respond*" to Cr supplementation. The current explanations (Harris, 1993) for this phenomenon are that it may reflect differences in individuals' capacity to synthesise Cr, their ability to concentrate it into tissues, or differences in the amount taken in with the diet.

Several studies have examined the effect of simultaneous supplementation of Cr in combination with other compounds. The ingestion/addition of CHO in the form of glucose or simple sugars augments Cr retention in muscle (Green *et al.*, 1996; Odoom *et al.*, 1996; Preen *et al.*, 2003; Buford *et al.*, 2007; Hadjicharalambous *et al.*, 2008). Indeed, Preen *et al.* (2003) concluded that Cr + glucose is potentially the most effective means of elevating TCr accumulation in human skeletal muscle. The quantity of glucose required is at least one gram per kilogram of body weight taken twice daily in conjunction with Cr (Preen *et al.*, 2003). Although simultaneous ingestion of caffeine with Cr has been shown to have no negative effect on Cr uptake into blood plasma (Vanakosi *et al.*, 1998) or muscle (Vandenberghe *et al.*, 1996*a*), most users are still wary because the ergogenic effect of Cr has been shown to be compromised by this mixture (Vandenberghe *et al.*, 1996*a*) and there are concerns about the dehydration effects of caffeine

(Hadjicharalambous *et al.*, 2008). The simultaneous ingestion of Cr and solid food (specifically the addition of beta glucans) may slow down the absorption rate of Cr and subsequently improve its whole-body retention (Deldicque *et al.*, 2008). Although the Cr content of combination products may be considered too low to be effective (eg. 1.5 g/day or less), it is speculated that low doses of several ingredients (eg. caffeine, Cr, branched-chain amino acids, whey protein, and others) may collectively amount to increases in exercise performance, training volume and the maintenance of lean body mass (Smith *et al.*, 2010).

2.6 EFFECTS OF CREATINE SUPPLEMENTATION

2.6.1 Effects of Cr supplementation on Cr, PCr, and TCr content of blood and muscle

The first study to systematically investigate the effect of Cr supplementation in humans was that of Harris *et al.* (1992). Seventeen subjects who varied considerably in fitness levels volunteered for the study. Results showed that ingestion of low doses of Cr (1 g or less) had a negligible effect on the plasma Cr concentration, whereas 5 g resulted in an approximately tenfold increase one hour after administration. Repeated feeding of 20 g Cr per day over a period of four to five days resulted in a 20% increase in the TCr pool of the quadriceps femoris muscle, raising the mean TCr content to 148.6 mmol/kg dry mass. Through the years other researchers also proved that the Cr supplementation regimen of 20 - 30 g/day at a stetch for three to six days significantly raises muscle TCr content and PCr concentration (Greenhaff *et al.*, 1994; Söderlund *et al.*, 1994; Balsom *et al.*, 1995; Febbraio *et al.*, 1995; Casey *et al.*, 1996; Green *et al.*, 1996; Hultman *et al.*, 1996; Vandenberghe *et al.*, 1996*a*; Vandenberghe *et al.*, 1996*b*; McKenna *et al.*, 1999; Volek *et* al., 1999; Bellinger *et al.*, 2000; Nelson *et al.*, 2001; Parise *et al.*, 2001; Preen *et al.*, 2001; Wiedermann *et al.*, 2001; Louis *et al.*, 2003*b*; Louis *et al.*, 2003*c*; Preen *et al.*, 2003; Tarnopolsky *et al.*, 2003*b*; Van Loon *et al.*, 2003; Rawson *et al.*, 2004; Deldicque *et al.*, 2005; Hadjicharalambous *et al.*, 2008). This increase in the muscle's potential to generate energy quickly, seem to translate to exercise performance (the ergogenic benefits of Cr will be further addressed in section 2.6.4).

Greenhaff (1995) found that one hour of hard one-legged bicycle ergometer exercise per day, accompanied by the mentioned supplementation regimen, augmented the increase in TCr content in the exercised leg but had no effect on the collateral. The author proposed that the increase in Cr uptake resulted from the increase in total blood flow to the exercised muscle, or a change in the transport kinetics of Cr across the fibre membranes. It is therefore recommended that Cr supplementation be accompanied by physical training to be optimally effective.

It was also found that low dosage supplementation at 3 g/day for 28 days is just as effective at elevating muscle Cr levels as the loading dosage (Hultman *et al.*, 1996). Other low-dosage-only studies demonstrated that supplementation at 2 g/day (Thompson *et al.*, 1996), 3 g/day (Hickner *et al.*, 2010) and 5 g/day (Brose *et al.*, 2003; Tarnopolsky *et al.*, 2003*b*) were effective (ie. each regimen separately) at raising muscle metabolite ratios. Hickner *et al.* (2010) reported that the increases in muscle TCr and PCr were of similar magnitude (respectively in the order of 10 and 20 mmol/kg) to those demonstrated by Hultman *et al.* (1996) after Cr loading. It was, however, suggested that a dosage of 2 g/day might be too low for some individuals (Van Loon *et al.*, 2003); hence it is generally recommended that continuous low dosage supplementation be ingested at 3 g/day or at 0.03 g/kg/day (Hultman *et al.*, 1996; Buford *et al.*, 2007).

Cr supplementation is also successful at elevating and maintaining Cr, PCr, and TCr content of blood and muscle when high dosage Cr supplementation *("loading")* is followed by longer-term low dosage *("maintenance")* feeding (Hultman *et al.*, 1996; Vandenberghe *et al.*, 1996*b*, Volek *et al.*, 1999; Burke *et al.*, 2003; Newman *et al.*, 2003; Tarnopolsky *et al.*, 2003*b*; Derave *et al.*, 2004). This mode of supplementation has been refined due to Hultman *et al.* (1996) indicating that a more rapid way to increase the muscle Cr store, was to ingest a dosage close to 0.3 g/kg body mass for six days. This high tissue level could be maintained by ingesting Cr in doses close to 0.03 g/kg body mass per day.

Persuasive evidence has been reported suggesting that there are definite limits to the possible benefits from Cr supplementation (Kinugasa *et al.*, 2004). The limit is apparently set by a maximal TCr concentration approaching 150 – 160 mmol/kg (Harris *et al.*, 1992; Greenhaff, 1995).

Lastly, Lukaszuk *et al.* (2002) found no significant difference in the increase in muscle TCr, Cr and PCr concentrations, compared to a placebo group, between lacto-ovo-vegetarians (n = 12) and omnivores (n = 14) after five days of Cr loading at 0.3 g/kg/day.

Burke *et al.* (2003) compared the change in muscle TCr content and exercise performance between vegetarians and non-vegetarians after eight weeks of Cr supplementation combined with resistance training. Forty-two recreational athletes (18 vegetarians, 24 non-vegetarians) were randomly and in a double-blind fashion assigned to four groups: vegetarian + Cr (n = 10), vegetarian + placebo (n = 8), non-vegetarian + Cr (n = 12), and non-vegetarian + placebo

(n = 12). Supplement dosage was based on lean tissue mass (0.25 g/kg/day for seven days, then 0.06 g/kg/day for 49 days). The average absolute daily supplementation dosages for subjects during loading and maintenance were respectively in the order of 17 g/day and 4 g/day. Muscle biopsy samples indicated that TCr levels were significantly lower in vegetarian compared with non-vegetarian subjects at baseline. After the 8-week supplementation period, subjects in the Cr groups were found to have gained more muscle TCr and PCr than subjects exposed to the placebo. Vegetarians who had taken Cr had a greater increase in TCr, PCr, and total work performance than non-vegetarians who had taken Cr. These findings confirm that subjects with initially low levels of intramuscular Cr (eg. vegetarians) are more responsive to supplementation than non-vegetarians.

Carbohydrate ingestion in conjunction with Cr loading conclusively augments Cr retention in muscle. The first study to prove this statement was done by Green *et al.* (1996). Six male volunteers consumed five grams of Cr monohydrate dissolved in 250 ml hot, sugar-free orange juice, followed by 93 g of simple carbohydrate in solution (500 ml Lucozade) for three consecutive days. Cr retention was not enhanced further by one hour of cycling exercise at 70% VO_2 max immediately before Cr and Lucozade ingestion. More recently Preen *et al.* (2003) demonstrated that 4 x 5 g/day Cr together with glucose ingestion at 1 g/kg twice daily (with the second and fourth Cr doses) produced a significantly greater elevation in muscle TCr concentration (25%) than Cr alone (16%).

Muscle Cr retention can also be enhanced by ingesting protein when supplementing with Cr. Steenge *et al.* (2000) found that the ingestion of Cr conjoined to 50 g of protein + 47 g of CHO was as effective at potentiating insulin release and Cr retention as ingesting Cr in combination with almost 100 g of CHO.

In a placebo-controlled study (Vandenberghe *et al.*, 1996*a*) it was reported that caffeine ingestion (5 mg/kg/day) in conjunction with Cr loading (0.5 g/kg/day for 6 days) increased increase muscle PCr levels by 6%. The ingestion of Cr alone increased muscle PCr by 4%. The authors concluded that there was no difference in the effects of either Cr or Cr + caffeine on muscle PCr concentration. However, the improvement in intermittent knee extension torque seen after Cr supplementation, was completely absent after Cr + caffeine. Therefore, some researchers still recommend that research participants abstain from the ingestion of caffeine

and caffeine-containing beverages throughout the experimental period (Hoffman *et al.*, 2005; Kilduff *et al.*, 2007; Hadjicharalambous *et al.*, 2008).

In conclusion, Cr supplementation can be expected to significantly raise muscle TCr and PCr levels. However, the lowest effective dosage seems to be at 3 g/day or 0.03 g/kg/day. The simultaneous ingestion of simple CHO (90 - 100g) or a protein-CHO combination (50 g protein + 50 g CHO) may improve muscle Cr uptake and retention via insulin action and is therefore recommended. Some concerns remain about caffeine ingestion inhibiting the ergogenic effect of Cr. However, a supplement recently formulated and tested for its ergogenic potential incorporated both Cr and caffeine (Smith *et al.*, 2010). The authors found an ergogenic effect, but were unsure which of the supplement components (Cr, caffeine or amino acids) to credit. They concluded that the unique low-dosage combination of ingredients might have been responsible for the improved aerobic performance demonstrated (Smith *et al.*, 2010). The potential effects of poly-supplementation will be discussed later in this thesis (section 3.1.3).

2.6.2 Effect of Cr supplementation on muscle ATP levels

Harris *et al.* (1992) found the ATP concentration in resting skeletal muscle to be unaffected by high-dosage Cr supplementation. This is to be expected, since the cellular ATP concentration is one of the most tightly regulated biochemical entities (Wiedermann *et al.*, 2001). More recent studies have shown no change in muscle ATP concentration after Cr supplementation (Hultman *et al.*, 1996; Wiedermann *et al.*, 2001; Louis *et al.*, 2003*c*; Newman *et al.*, 2003; Van Loon *et al.*, 2003). Thus, Cr supplementation does not influence resting ATP concentrations in skeletal muscle.

2.6.3 Effect of Cr supplementation on PCr resynthesis after exercise

Fatigue associated with intense muscle contraction, especially during repeated bouts of maximal exercise, is related to the availability of pre-exercise PCr stores (Wiedermann *et al.*, 2001). The depletion of these stores will limit the rate of ADP re-phosphorylation. Several authors have demonstrated the enhanced resynthesis of PCr during recovery to be one of the key factors underlying the ergogenic potential of Cr supplementation (Greenhaff *et al.*, 1992; Greenhaff *et al.*, 1993*a*; Greenhaff *et al.*, 1994; Bogdanis *et al.*, 1996; Smith *et al.*, 1998; Preen *et al.*, 2001). The larger PCr store available before the onset of a consecutive exercise bout may enhance ATP resynthesis during exercise, thereby improving performance and delaying fatigue during repeated bouts of high-intensity exercise. Some studies (Thompson *et al.*, 1996;

Vandenberghe *et al.*, 1999; Francaux *et al.*, 2000; Wiedermann *et al.*, 2001; Kinugasa *et al.*, 2004; Jäger *et al.*, 2008) have failed to detect changes in PCr resynthesis kinetics during recovery between exercise bouts. These contrasting reports may be due to differences in experimental set-up and low sample sizes. However, more studies are needed to clarify whether or not this proposed theory of the ergogenic action of Cr has merit.

The timing of Cr ingestion may influence its effect. Exercise is known to enhance Cr uptake into muscle cells (Greenhaff, 1995). Also, Chilibeck *et al.* (2004) found that Cr ingestion immediately after unilateral training of the arm and thigh, respectively, results in a greater increase in muscle thickness of the exercised arm but not the quadriceps. This finding might suggest that Cr should be ingested after exercise when blood supply to the exercised limbs has increased, and activation of the sodium-potassium pump which cotransports Cr across the sarcolemma has taken place (Greenhaff, 1995; Chilibeck *et al.*, 2004). Refinements to the Cr supplementation protocol have been made accordingly, hence many athletes now consume only one 5 g dosage approximately 60 min prior to, or immediately after training. High-dosage ingestion of Cr during exercise is not recommended and has been found to cause post-exercise distress and even syncope (Vandebuerie *et al.*, 1998).

PCr is an unstable compound. It does not remain in the muscle for long and breaks down to Crn if it is not utilised, and is excreted in the urine at a rate of approximately 20 mg/kg/day (Harris, 1993). Earlier studies (Febbraio *et al.*, 1995; Hultman *et al.*, 1996; Vandenberghe *et al.*, 1996*b*) found a 28-day washout period sufficient to return muscle TCr and PCr concentrations to baseline values. However, more recent publications (Juhn & Tarnopolsky, 1998; Preen *et al.*, 2003; Rawson *et al.*, 2004; Deldicque *et al.*, 2008) found a 30-day washout period insufficient. A washout time for crossover research studies of at least five weeks (Juhn & Tarnopolsky, 1998) or longer than 40 days (Deldicque *et al.*, 2008) is therefore recommended.

2.6.4 Effects of Cr supplementation on physical performance

The body of evidence supporting a beneficial effect of Cr supplementation on performance outcomes in both humans and animals continues to grow (Shao & Hathcock, 2006). At present the most extensively studied and clinically effective form of Cr for use in nutritional supplements in terms of muscle uptake and ability to increase high-intensity exercise capacity is Cr monohydrate (Buford *et al.*, 2007). Human data that support this observation are primarily derived from three types of studies: (i) acute studies involving supplementation at high dosage

rates (20 g/day) for short stretches of time (up to 6 weeks), (ii) chronic studies involving lower dosages (3 - 5 g/day) for longer durations (up to 1 year, or longer), or (iii) a combination of high-dosage supplementation followed by a maintenance dosage regimen (Shao & Hathcock, 2006).

2.6.4.1 Repeated bouts of high-intensity exercise

As explained in section 1.1, the ATP-PCr energy system is the predominant energy supplier for muscular work during the first few seconds of high-intensity exercise. The pre-exercise availability of Cr and PCr directly influences the muscle's ability to replenish ATP during subsequent maximal exertions. Cr thus plays a pivotal role in maintaining high work and power levels during repeated bouts of maximal exercise (Kirksey *et al.*, 1999).

In general, results of studies conducted to determine the effect of Cr supplementation on exercise performance in repeated bouts of high-intensity exercise (performance testing done pre- to end-2000) support its ergogenic effects. The benefits seemed to extend specifically, but not exclusively, to performance parameters for isokinetic strength (Greenhaff *et al.*, 1993*b*; Vandenberghe *et al.*, 1996*a*; Rossouw *et al.*, 2000), cycle ergometry (Birch *et al.*, 1994; Earnest *et al.*, 1994; Casey *et al.*, 1996; Jones *et al.*, 1999) and jump squats (Volek *et al.*, 1997; Volek *et al.*, 1999). Studies to determine the effect of Cr supplementation on isometric performance generally showed no benefit (Gilliam *et al.*, 2000; Jakobi *et al.*, 2000; Rawson & Clarkson, 2000). High-dosage short-term Cr supplementation (20 - 30 g/day for 5 - 7 days) (Greenhaff *et al.*, 1993*b*; Birch *et al.*, 1994; Earnest *et al.*, 1994; Vandenberghe *et al.*, 1996*a*; Volek *et al.*, 1997), moderate-dosage, short-term supplementation (Kreider *et al.*, 1998; Peyrebrune *et al.*, 1998; Rossouw & Rossouw 2000*b*) and high-dosage followed by prolonged maintenance Cr feeding at 2 - 5 g/day seemed to be effective (Jones *et al.*, 1999; Volek *et al.*, 1999). The effectiveness of low-dosage supplementation seemed to improve with longer supplementation periods (Rossouw & Rossouw, 2000*a*).

The interpretation of the available research results (pre- to end-2000) on Cr supplementation was, however, not quite as simple as it might appear from the previous paragraph. Large variations existed between studies with regard to subject characteristics and research protocol. For example, two studies (Dempsey *et al.*, 2002; Branch, 2003) attempted to quantify the effects of Cr supplementation. To identify possible research for inclusion in their analysis, both groups searched the MEDLINE electronic database (1966 – December 2000). One hundred English-language, peer-reviewed studies that included randomised group formation, a placebo control,

and human subjects who were blinded to treatments, were identified and analysed. Quantitative results of measuring performance in intermittent laboratory-based exercise bouts lasting less than 30 seconds each (eg. isokinetic and isotonic exercise), and upper-body exercise, revealed a significantly greater effect of Cr supplementation compared to placebo. Cr supplementation did not appear to be effective in improving intermittent swimming, running, or isometric performance. Also, no evidence was found that gender or training status affected performance after Cr supplementation.

Studies conducted since 2000 showed either a benefit (Bennett *et al.*, 2001; Preen *et* al., 2001; Wiedermann *et al.*, 2001; Izquierdo *et al.*, 2002; Warber *et al.*, 2002; Ziegenfuss *et al.*, 2002; Van Loon *et al.*, 2003; Peyrebrune *et al.*, 2005; Jäger *et al.*, 2008; Rawson *et al.*, 2011) or no effect (Bennett *et al.*, 2001; Jakobi *et al.*, 2001; Warber *et al.*, 2002; Van Schuylenbergh *et al.*, 2003; Ahmun *et al.*, 2005; Glaister *et al.*, 2006; Pluim *et al.*, 2006) on performance achieved during repeated bouts of maximal exercise. The subjects involved in all of these studies were physically active or well-trained (eg. soldiers, students enrolled for sports science programmes and active participants in sports), with the exception of Van Loon *et al.* (2003) and Jäger *et al.* (2008) who utilised sedentary males. The possible reasons for discrepancies in results will be discussed at the end of this section.

Several potential mechanisms could be responsible for enhanced strength-power performance during multiple bouts of high-intensity short-duration exercise following Cr supplementation. Theoretically, the increased muscle stores of both Cr and PCr make it possible to maintain the rate of ATP resynthesis for longer during the first bout of exercise (Balsom *et al.*, 1993*a*; Greenhaff *et al.*, 1993*b*). It also shortens the recovery time between the first two exercise bouts due to an enhanced ability to regenerate ATP and PCr (Harris *et al.*, 1992; Green *et al.*, 1996; Francaux *et al.*, 2000; Lemon, 2002; Jäger *et al.*, 2008). The increase in performance level at the beginning of intermittent exercise (ie. the first interval) is thus related to an increased muscle contraction speed enabled by reduced ADP and Pi accumulation after the first bout of exercise and increased ATP resynthesis before the second bout of exercise (Jäger *et al.*, 2008).

Studies have also demonstrated a resistance to fatigue during the latter bouts of intermittent exercise after Cr supplementation (Balsom *et al.*, 1993*a*; Greenhaff *et al.*, 1993*b*; Mujika *et al.*, 2000; Rossouw & Rossouw, 2000*a*; Izquierdo *et al.*, 2002; Jäger *et al.*, 2008; Rawson *et al.*, 2011). In theory this effect is attributable to improvements in the function of the PCr shuttle as

explained in sections 2.2.4 and 2.4.2 above. It is thus postulated that Cr supplementation facilitates recovery from fatigue during rest, rather than delaying the process of fatigue during repeated bouts of high-intensity exercise (Vandenberghe *et al.*, 1996*a*). After Cr supplementation, the rate of PCr resynthesis from mitochondrial ATP is improved due to increased Cr availability and a faster CK reaction (Ahmun *et al.*, 2005) as first postulated by Greenhaff (1997). PCr resynthesis and its shuttling to the myofibrils are therefore enhanced between exercise bouts. This enables the subject to start the next bout with an improved capacity to regenerate ATP (Greenhaff *et al.*, 1992; Greenhaff *et al.*, 1993*a*; Greenhaff *et al.*, 1993*b*; Greenhaff *et al.*, 1994; Bogdanis *et al.*, 1996; Green *et al.*, 1996; Smith *et al.*, 1998*a*; Preen *et al.*, 2001; Ahmun *et al.*, 2005). The result is that strength and power output can be better maintained during the latter bouts of high-intensity exercise. However, as indicated in section 2.6.3, several investigations have failed to demonstrate any improvement in post-exercise PCr resynthesis after Cr supplementation (Thompson *et al.*, 1996; Vandenberghe *et al.*, 1999; Francaux *et al.*, 2000; Wiedermann *et al.*, 2001; Jäger *et al.*, 2008). These inconsistencies may be caused by a number of factors such as small sample sizes, differences in exercise testing modalities, and/or differences in recovery periods between consecutive exercise bouts. Also, during repetitive exercise sequences a significant proportion of energy is derived from aerobic metabolism, hence fatigue during the latter bouts of exercise to exhaustion may be unrelated to PCr availability during these protocols.

Accelerated PCr recovery kinetics following Cr supplementation has been suggested to reduce acidosis during repeated bouts of exercise (Glaister *et al.*, 2006). During the latter bouts of intermittent exercise, increased reliance on anaerobic glycolysis may result in an accumulation of lactate within the muscle. When this lactate dissociates it may flood the cellular environment with H^+, causing the muscle pH to fall. The fall in pH has been shown to decrease PCr resynthesis and has been implicated in the fatigue process (Janssen, 1987; Sahlin, 1986; Booth & Thomason, 1991; Fitts, 1994; Chaudhuri & Behan, 2004). Thus, the enhanced availability of PCr at the beginning of each consecutive exercise bout could increase the muscle's hydrogen ion buffering capacity, thereby facilitating PCr re-synthesis between exercise bouts (Parkhouse & McKenzie, 1984; Lemon, 2002; Glaister *et al.*, 2006) and/or reducing the glycolytic flux (Peyrebrune *et al.*, 2005; Glaister *et al.*, 2006). Greenhaff (1995) calculated that the average increase in muscle PCr concentration after Cr feeding at the loading dosage would raise muscle buffering capacity by about 7%. This should allow the muscle to accumulate more lactate before reaching a limiting muscle pH, thus allowing increased high-intensity exercise (Parkhouse &

McKenzie, 1984; Greenhaff, 1995). According to Peyrebrune *et al.* (2005) the resynthesis of ATP from ADP and PCr, which consumes a H^+ in the process of reaction, thus maintaining blood pH, has a greater impact on exercise performance than any benefits gained from training.

Studies conducted under conditions of high (Greenhaff *et al.*, 1993*b*; Söderlund *et al.*, 1994) and low (Rossouw & Rossouw, 2000) rate of Cr supplementation demonstrated either a significant reduction in blood lactate accumulation in spite of a higher work output (Söderlund *et al.*, 1994; Rossouw & Rossouw, 2000), or an increased work output with no change in blood lactate levels (Peyrebrune *et al.*, 1998; Greenhaff *et al.*, 1993*b*). All of these studies incorporated repeated bouts of maximal exercise. The effects could be attributable to a change in the source driving energy metabolism. More specifically, the reason could be that the increased availability of muscle PCr delayed anaerobic glycolysis during the exercise bouts, thereby leading to a decrease in lactate production (Balsom *et al.*, 1993*a*; Rossouw & Rossouw, 2000*a*).

2.6.4.2 Single-effort exercise lasting 30 seconds or less

The body's demand for energy increases significantly during exercise. Short-duration, high-intensity activities (maximal single-effort activities) require an immediate, rapid supply of energy that is provided almost exclusively from the ATP and PCr stored in the muscles. For example, during sprint running and swimming, energy output can rise to a level of 120 times higher than that maintained during rest (Feldman, 1999). Dempsey *et al.* (2002) and Branch (2003) performed meta-analyses of literature up to December 2000 (as stated previously) with a view to quantifying the effect of Cr supplementation on these events. They found that different researchers may define strength differently because muscle strength is related to muscle endurance. Also, there is no obvious point at which a single-effort exercise becomes a test of endurance and not of strength alone. The meta-analyses of these investigators were therefore limited to "*pure strength*" or "*pure power*" measurements (eg. 1RM strength) to enable comparisons between similar outcomes. Results (Branch, 2003) revealed that the effect of repetitive bouts of exercise following Cr supplementation was greater than that for single-bout or first-bout exercise.

More studies on the effect of Cr supplementation on performance in single-bout strength feats have become available since 2000. These show Cr to either benefit (Rossouw *et al.*, 2000; Ostojic, 2004; Gotshalk *et al.*, 2008; Saremi *et al.*, 2010) or have no effect on (Izquierdo *et al.*,

2002) strength/power output achieved during a single event. Thus, results from the majority of studies lend support to the finding (Branch, 2003) that the effects of Cr supplementation on exercise performance are most pronounced in repetitive tasks lasting 30 seconds or less.

As explained previously - and as might be expected after Cr supplementation - the increased muscle stores of both Cr and PCr should make it possible to maintain the rate of ATP resynthesis for longer periods during maximal exercise and thereby improve muscle contraction speed and explosive strength (Balsom *et al.*, 1993*a*; Greenhaff *et al.*, 1993*b*).

2.6.4.3 Exercise performance after participation in a resistance training programme

Research findings indicate that the effects of Cr supplementation on exercise performance in relatively simple single-effort movements such as 1RM bench press, leg press, biceps curl, squat, sprint running and sprint cycling are most pronounced when individuals take part in a concomitant structured resistance training programme (Peeters *et al.*, 1999; Stout *et al.*, 1999; Volek *et al.*, 1999; Arciero *et al.*, 2001; Bemben *et al.*, 2001; Huso *et al.*, 2002; Brilla *et al.*, 2003; Brose *et al.*, 2003; Burke *et al.*, 2003; Ostojic, 2004; Olsen *et al.*, 2006; Law *et al.*, 2009).

The mechanisms of Cr supplementation combined with resistance (or other) training may increase performance in two ways. Firstly, the ergogenic effects of prolonged Cr supplementation may improve training quality, thus enabling a "*super-training"* effect (Lemon, 2002). As indicated in Figure 2-5, the increase in muscle Cr levels after Cr loading may increase both pre-exercise PCr availability and post-exercise PCr resynthesis. These two factors may then improve training intensity and augment physiological adaptations to training, thereby improving exercise-test performance after a period of supplementation (Kraemer & Volek, 1999; Lemon, 2002; Souza-Junior *et al.*, 2011). Secondly, as indicated in Figure 2-5, Cr supplementation may indirectly increase muscle cross-sectional area and lean body weight (LBW). These adaptations are associated with increased strength and/or power output (Olsen *et al.,* 2006; Peeters *et al.* 1999; Law *et al.*, 2009; Saremi *et al.*, 2010; Souza-Junior *et al.*, 2011) that may improve the exercise stimulus and result in an enhanced training effect.

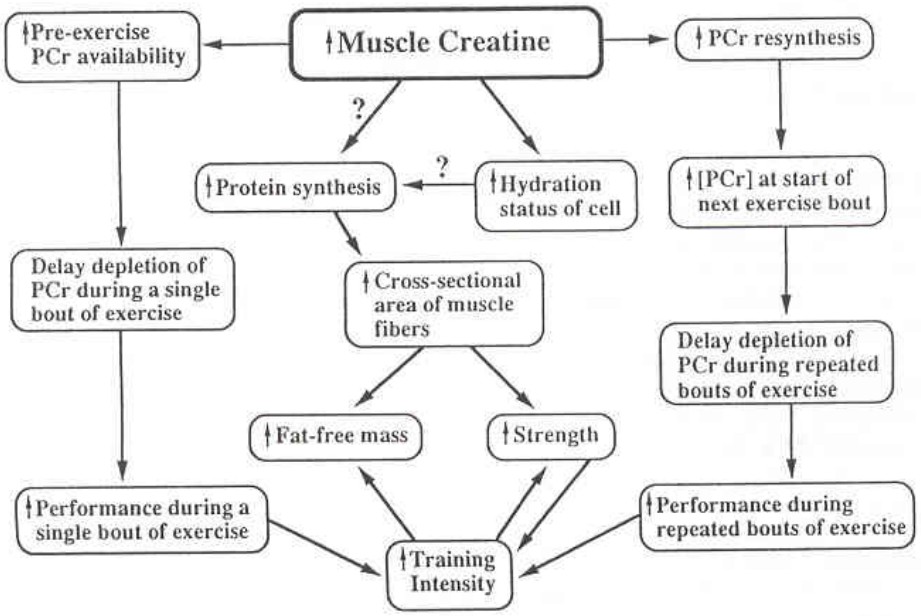


Figure 2-5 **Proposed theoretical mechanisms of action by which Cr supplementation may lead to enhancement in the exercise stimulus and augment physiological adaptations to physical exercise (Kraemer & Volek, 1999)**

2.6.4.4 Prolonged anaerobic exercise incorporating the glycolytic system

It is logical to expect that any ergogenic effect of Cr supplementation would be best observed in high-intensity, short-duration tasks, since ATP for the performance of such tasks comes from the ATP-PCr energy system (Branch, 2003). However, it has been suggested (Williams *et al.*, 1999) that it may improve maximal anaerobic performance over longer periods due to H^+ buffering and less reliance on anaerobic glycolysis. In his quantitative analysis, Branch (2003) found that the overall effect required to support the hypothesis that Cr may also improve performance in tasks such as push-ups performed repeatedly for 1 - 2 min, isotonic lifting performed repeatedly, isometric work (Nm), and leg ergometer power (W) and work (J) that rely primarily on anaerobic glycolysis for energy. Recent findings both support (Kambis & Pizzedaz, 2003) and refute (O'Connor & Crowe, 2003; Armentano *et al.*, 2007) this claim. Again, the reasons for these inconsistent findings will be discussed in section 2.6.4.7.

2.6.4.5 Endurance (aerobic) exercise performance

Researchers have paid little attention to the effect of Cr supplementation on aerobic endurance performance. However, the performance graph of endurance events usually describes an undulating path. This will cause a shift to anaerobic energy provision when athletes encounter a

hill and/or increase their pace (Stroud *et al.*, 1994; Rico-Sanz & Marco, 2000). Furthermore, research indicates that substrate utilisation during aerobic activity (Stroud *et al.*, 1994) and during rest (Huso *et al.*, 2002) may be modified by Cr use. These arguments lend support to the potential benefit of supplementing Cr to enhance endurance performance (> 150 s).

An increase in muscle free AMP accumulation becomes manifest during sustained exercise, when the rate of muscle ATP resynthesis fails to meet the rate of muscle ATP demand (McConell *et al.*, 2005), with the result that inosine monophosphate (IMP) production increases and indicates an energy imbalance in the contracting skeletal muscle (McConell *et al.*, 2005). Increases in levels of muscle-energy metabolites such as ADP, AMP, IMP, ammonia and lactate are thus likely to provide an accurate indication of muscle energy balance (McConell *et al.*, 2005).

Rico-Sanz and Marco (2000) suggest that increasing muscle Cr and PCr stores through Cr supplementation may lower PCr degradation (resulting in lower AMP and IMP levels) and pH drop (due to less reliance on anaerobic glycolysis), thereby enhancing oxidative phosphorylation during an endurance event. The increase in muscle free Cr should potentially favour changes in oxidative phosphorylation in contracting muscle fibres because of an amplification of mitochondrial-CK activity and, consequently, the PCr shuttle (Rico-Sanz & Marco, 2000; McConell *et al.*, 2005). Energy is shuttled out of the mitochondria by the transporter PCr, and is then transferred back to ADP in the cytosol, thereby making ATP available at the actin-myosin cross-bridge interface (Rico-Sanz & Marco, 2000; McConell *et al.*, 2005).

In summary, Cr supplementation may benefit endurance performance by: (i) enabling slow-twitch muscle fibres to rely for longer on oxidative metabolism, as would be evident from an increase in oxygen uptake during exercise testing; (ii) increasing muscle TCr and PCr content, and consequently buffering larger amounts of ADP at myofibrillar sites in both muscle fibre types I and II (ie. facilitating the PCr energy shuttle), as would be evident from an increase in work output and/or a delayed onset of fatigue during exercise testing (Rico-Sanz & Marco, 2000); (iii) reducing intramuscular lactate accumulation by decreasing the reliance on anaerobic glycolysis during periods of increased exercise intensity/pace (Stout *et al.*, 2006) and/or buffering H^+ accumulation during incremental aerobic exercise to fatigue (Stout *et al.*, 2006).

The earlier quantitative analysis done by Branch (2003) reports bicycle ergometry to be the only mode of aerobic exercise to show any benefit from Cr supplementation. In the first study conducted to determine the effect of Cr supplementation on endurance performance, Balsom *et al.* (1993*b*) reported neither an enhancement of performance, nor an increase in peak oxygen uptake in a supramaximal treadmill run to exhaustion (~4 min).

A small number of recent findings (post-2000) report performance benefits for both sub-maximal (Ööpik *et al.*, 2002; Hadjicharalambous *et al.*, 2008; Graef *et al.*, 2009) and maximal (McConell *et al.*, 2005) aerobic cycling exercise. However, the majority of results report no benefit to either sub-maximal (Van Loon *et al.*, 2003; Van Schuylenbergh *et al.*, 2003; McConell *et al.*, 2005; Stout *et al.*, 2006) or maximal (Jones *et al.*, 2002; Van Loon *et al.*, 2003; Reardon *et al.*, 2006; Graef *et al.*, 2009) aerobic cycling performance. It thus seems that Cr supplementation has variable effects on this mode of exercise testing. This may be attributable to person-by-treatment differences in physiological responses to Cr supplementation (Hadjicharalambous *et al.*, 2008), differences in the frequency of oxygen consumption measurements (Rico-Sanz & Marco, 2000) and/or other variations in study methodology.

As explained in section 1.2.3, the respiratory exchange ratio (RER) is a measure that provides information regarding the proportion of energy derived from various nutrients at rest and during steady-state sub-maximal exercise. It has been hypothesised that Cr supplementation might increase the RER during rest (Huso *et al.*, 2002) and endurance exercise (Jones *et al.*, 2002; Van Loon *et al.*, 2003; McConell *et al.*, 2005; Hadjicharalambous *et al.*, 2008), thereby indicating a shift towards greater CHO oxidation and less fat oxidation. As it is suspected that Cr supplementation increases cell hydration and thus cell volume, it seems justifiable to expect a concomitant increase in muscle glycogen (Huso *et al.*, 2002). To date, none of the studies measuring RER at rest (Huso *et al.*, 2002) or during endurance exercise of varying intensities (Jones *et al.*, 2002; Van Loon *et al.*, 2003; McConell *et al.*, 2005; Reardon *et al.*, 2006; Hadjicharalambous *et al.*, 2008; Hickner *et al.*, 2010; Beis *et al.*, 2011) have demonstrated a significant change in RER, and thus CHO oxidation, in response to Cr supplementation. Also, no significant changes in VO_2 max (Balsom *et al.*, 1993*b*; Van Loon *et al.*, 2003) or VO_2 peak (Graef *et al.*, 2009; Hickner *et al.*, 2010) have been reported.

Blood lactate accumulation during endurance rowing (Chwalbinska-Moneta, 2003) and sub-maximal cycling (Jones *et al.*, 2002) to exhaustion have been shown to benefit from Cr

supplementation. As explained earlier, it is logical to expect that the increased availability of muscle PCr would delay anaerobic glycolysis during periods of increased exercise intensity, thereby leading to a decrease in blood lactate accumulation (Balsom *et al.*, 1993*b*; Rossouw & Rossouw, 2000*a*). However, no benefits were reported during a 6 km terrain run (Balsom *et al*., 1993*b*), 10 mile forced march and a 5 mile run (Bennett *et al.*, 2001), a test for maximum aerobic capacity on a cycle ergometer (Van Loon *et al.*, 2003), a 1 h cycling time trial (Van Schuylenbergh *et al.*, 2003), sub-maximal alternating-intensity cycling to exhaustion (Rico-Sanz & Marco, 2000; Van Loon *et al.*, 2003), constant-load cycling to fatigue in a hot environment (Hadjicharalambous *et al.*, 2008) and sub-maximal running in hot and cool conditions (Beis *et al.*, 2011). Thus, Cr supplementation does not appear to significantly alter blood lactate accumulation during endurance exercise to fatigue.

Very few studies have reported on changes in the heart rate (HR) response during aerobic exercise after Cr supplementation. Hadjicharalambous *et al.* (2008) reported that Cr supplementation decreased the HR-response to constant-load cycling to fatigue in a hot environment, while Beis *et al.* (2011) reported the same effect during sub-maximal running in the heat after Cr combined with glycerol ingestion. McConell *et al.* (2005) reported that HR showed a significant trial-by-time interaction. The HR of participants on Cr supplementation was on average 2 - 4 bpm higher in the early stages of cycling exercise, but 2 - 5 bpm lower during the final sprint of the time-trial stage (McConell *et al.*, 2005). None of these authors explained their results. However, the decrease in HR might indicate less cardiovascular strain during exercise, and thus, less effort in maintaining the desired work output.

There are numerous reasons for the varying responses in endurance performance after Cr supplementation. No treatment response would have been apparent if the supplementation protocol had not been effective at raising muscle TCr and PCr levels. This was the explanation given by both Van Schuylenbergh *et al*. (2003) and Stout *et al.* (2006) as to why Cr supplementation did not increase endurance performance. Person-by-treatment differences in physiological response to Cr supplementation – the *"non-responders"* – are also given as a reason why differences in performance and other parameters measured did not reach statistical significance (Van Loon *et al*., 2003; Hadjicharalambous *et al.*, 2008). Furthermore, factors other than muscle energy balance may determine exercise capacity during endurance performance (McConell *et al.* 2005; Hadjicharalambous *et al.*, 2008). Jones *et al.* (2002) suggested that the effect of Cr supplementation on sub-maximal endurance performance might be related to

changes in individuals' motor-unit recruitment patterns or the volume of muscle activated, and that Cr loading might have increased recruitment of type II muscle fibres during intense sub-maximal endurance exercise, and/or might have caused a reduction in the total amount of muscle mass recruited to perform this type of exercise. Finally, the fact that Cr supplementation had no discernible effect on endurance performance can be attributed to a combination of acute rather than long-term supplementation protocols utilised by most studies, and to the absence of an adequate exercise stimulus during the supplementation period (Reardon *et al.*, 2006). Also, a type II statistical error may have been induced by the small sample sizes that typified the studies (Reardon *et al.*, 2006). Thus, there is currently no consensus as to the effect of Cr supplementation on endurance performance achieved during exercise at a steady, or at a variable level of intensity (Hickner *et al.*, 2010).

2.6.4.6 Sports performance

Exercise testing in a laboratory setting is often preferred by researchers since it offers a way to control environmental influences. This form of evaluation has therefore been employed in most of the research done on Cr. However, from early on researchers became interested in whether or not the performance benefits demonstrated in controlled environments (Balsom *et al.*, 1993*a*; Greenhaff *et al.*, 1993*a*; Greenhaff *et al.*, 1993*b*; Harris, 1993; Harris *et al.*, 1993*a*) would transfer to actual sports performance (uncontrolled environments). Due to the difficulty in measuring complex movements requiring strength, speed and the simultaneous coordination of multiple muscle groups (ie. sports performance) in a field setting during competition, researchers have attempted to simulate these events by testing well-trained athletes in laboratory environments (Balsom *et al.*, 1993*b*; Harris *et al.*, 1993*b*; Rossiter *et al.*, 1996; Ööpik *et al.*, 1998; Hamilton *et al.*, 2000; Dempsey *et al.*, 2002; Ööpik *et al.*, 2002; Van Schuylenbergh *et al.*, 2003; Hickner *et al.*, 2010).

The studies have yielded mixed results. Ööpik *et al.* (1998; 2002) found high-dosage Cr supplementation effective in restoring sport-specific strength and muscle endurance after a period of rapid weight loss in karate athletes and wrestlers. After Cr loading, Harris *et al.* (1993*b*) reported improved 300 m and 1000 m running times in middle-distance runners while Rossiter *et al.* (1996) found that competitive rowers had significantly improved their 1000 m rowing time. Hamilton *et al.* (2000), however, found the increase in upper extremity work capacity in females taking part in overhand sports after Cr loading not to extend to the muscles responsible for performance in these sports.

According to results achieved in the two most recent studies no beneficial impact became evident from either short-term (7 g/d for 7days) or prolonged (3 g/d for 28 days) low-dosage Cr supplementation (Van Schuylenbergh *et al.*, 2003 and Hickner *et al.*, 2010, respectively) in the course of either a 1 h time-trial performance (Van Schuylenbergh *et al.*, 2003) or total power output during a 2 h bout of cycling to fatigue (Hickner *et al.*, 2010). As can be seen, the earlier studies incorporated Cr loading while the last two incorporated low-dosage supplementation. It is thus possible, as Van Schuylenbergh *et al.* (2003) suggested, that the dosage/product (Cr-Pyr) was below the level required to raise muscle Cr content. This explanation cannot be true for the last study, however. Hickner *et al.* (2010) reported a significant increase in muscle TCr and PCr concentrations after their supplementation protocol. Thus, the reason for the failure of Cr to enhance endurance cycling performance in the study under review (Hickner *et al.*, 2010) may be attributable to either a type II statistical error, or to other reasons discussed in section 2.6.4.7 below.

There is a shortage of scientific data concerning the possible effects of Cr supplementation on specific performance in sports (Ostojic, 2004). In one of the first studies to measure actual sports performance, Balsom *et al.* (1993*b*), reported a decline in performance level during a 6 km terrain run in the Cr-supplementation group (20 g/d for 6 days). As this group's body weight increased significantly (~2kg) after Cr loading, the decrease in endurance performance may be attributable to a decrease in running economy (greater mechanical effort in supporting the larger body mass) during the time trial.

The effects of Cr supplementation on swimming performance have been studied quite extensively. The majority of studies demonstrate no benefit to sport-specific sprinting after either Cr loading (Burke *et al.,* 1996; Thompson *et al.*, 1996; Peyrebrune *et al.*, 1998; Mendes *et al.*, 2004; Peyrebrune *et al.*, 2005) or long-term supplementation (Peyrebrune *et al.*, 2005). Good hydrodynamics increases the ability of a body to float and helps the swimmer to maintain floating and swimming position by reducing body area and water resistance (Mendes *et al.*, 2004). According to Mendes *et al.* (2004) the gain in body weight associated with Cr loading can be considered deleterious to swimming performance as it may change the mechanics of swimming styles and result in greater energy expenditure during movement. This statement was challenged, however, by results from Silva *et al.* (2007), as Cr supplementation administered to female athletes at 20 g/d for 21 days significantly improved gross and propelling efficiency during swimming. This study (Silva *et al.*, 2007) incorporated high-dosage supplementation over

a longer period. The high Cr dosage may have led to enhanced training quality (recovery ability) during the supplementation period, thereby significantly improving performance. The most recent study (Vatani *et al.*, 2011) is the first to report improved 50 m sprint performance in male swimmers after Cr loading.

Tennis can be characterised as a sport with intermittent activity (Smekal *et al.*, 2001). The demands imposed on the athletes during competition cannot be simulated in a controlled laboratory setting and instead have to be determined during real matchplay (Smekal *et al.*, 2001). Despite its stop-start nature, tennis has a high aerobic component because high-energy phosphates used for immediate energy requirements of muscles (ie. PCr) are mainly resynthesised by oxidation during recovery periods (Smekal *et al.*, 2001).

Pluim *et al.* (2006) hypothesised that Cr supplementation would be effective in improving selected factors of tennis-specific performance (ie. forehand velocity, backhand velocity and serving velocity) in a situation where a high stroke velocity is repeatedly required (a ball machine for ground-stroke drill, repeated serving and intermittent sprinting). Neither six days nor five weeks of Cr supplementation had a significant effect on these parameters. The authors therefore concluded that Cr supplementation should not be recommended for tennis players.

However, sport-specific skill performance in another sport characterized by intermittent sprinting, namely soccer (specific dribble test times and sprint-power test times), have reportedly benefited from Cr supplementation (Ostojic, 2004). Thus further research on tennis should be considered. As stated previously, tennis performance should ideally be measured during real matchplay. Therefore, future research might focus on the factors that significantly influence the energy demands of tennis matchplay: (i) the frequency of shots performed in the rallies, (ii) the accuracy of these shots, (iii) the duration of rallies, and (iv) the effective playing time (Smekal *et al.*, 2001). Also, further studies are needed to determine whether Cr supplementation may be beneficial for tennis players in combination with a strength training programme (Pluim *et al.*, 2006).

Team sports have a ubiquitous nature (Bishop, 2010). This is partly because the exact physical demands will differ between sports (and also between matches), but mostly because team sports are ultimately decided by points/goals scored rather than the speed, strength or endurance of individual players (Bishop, 2010). As in tennis, the metabolic demands typically

alternate between aerobic and anaerobic metabolism (ie. soccer, basketball, handball and hockey) (Rico-Sanz & Marco, 2000). Since the important physical determinants of team-sport performance include speed, strength and power, repeated- and intermittent-sprint ability and aerobic endurance (to hasten recovery between sprints), Cr supplementation can be expected to enhance performance in these events (Bishop, 2010). However, there is no guarantee that the effectiveness of a dietary supplement such as Cr, which improves isolated performance (ie. single-sprint or jump performance), will remain effective in the context of a team sport match (Bishop, 2010). While Cr supplementation could potentially improve some aspects of team sport performance, its true sport-specific effects remain untested.

Finally, Rahimi *et al.* (2010) investigated the effect of Cr supplementation (20 g/d for 7 days) on the quality of resistance training sessions. Results indicate that Cr supplementation, with no physical training, increased the number of repetitions performed and training volume attained during a training session (Rahimi *et al.*, 2010). The authors attributed these improvements to an increased availability of PCr for ATP resynthesis during contraction, increased availability of free Cr for PCr resynthesis during recovery, and improved muscle H^+ buffering capacity. In a sport-specific strength feat, well-trained power-lifters significantly improved their deadlift lifting volume after Cr loading (Rossouw *et al.*, 2000). These results (Rossouw *et al.*, 2000; Rahimi *et al.*, 2010) look promising for trained individuals who want to improve the quality of their respective resistance training sessions.

In conclusion, Cr seems to provide the most beneficial effects for sport performance when the activity involves repeated short bouts of high-intensity exercise. It appears that athletes who perform in sports that are most similar to this type of activity derive the greatest benefit from Cr supplementation (Bemben & Lamont, 2005). More research needs to be conducted in the areas of tennis, sport-specific endurance and team-sport performance.

2.6.4.7 Possible reasons for inconsistent research findings

As an ergogenic aid, Cr has a widespread following among athletes (Froiland *et al.*, 2004; Dascombe *et al.*, 2010; Tscholl *et al.*, 2010). As indicated in this section, many studies support the benefits of Cr supplementation. However, a fairly large body of well-controlled research studies demonstrate either no benefit (Cooke & Barnes, 1997; McKenna *et al.*, 1999; Bennett *et al.*, 2001; Jakobi *et al.*, 2001; Jones *et al.*, 2002; Warber *et al.*, 2002; Van Schuylenbergh *et al.*, 2003; Mendes *et al.*, 2004; Ahmun *et al.*, 2005; Peyrebrune *et al.*, 2005; Glaister *et al.*, 2006;

Reardon *et al.*, 2006; Stout *et al.*, 2006; Hickner *et al.*, 2010; Pluim *et al.*, 2006) or variable benefit (Peyrebrune *et al.*, 1998; Graef *et al.*, 2009) in terms of exercise performance achieved with Cr supplementation. The following are reasonable explanations that might account for the inconsistency of research results (adapted from Lemon, 2002):

- Low statistical power can explain some (perhaps most) of the inconsistency in the Cr literature. Typically, small sample sizes are studied, and adequate control of important factors such as habitual diet and exercise is difficult. Furthermore, not all individuals respond in the same manner to Cr supplementation. The small sample sizes and variable responses to supplementation thus make the documentation of real statistical effect difficult. This may give rise to a type I statistical error (ie. inability to observe a significant improvement in the Cr group, when there is one). Sample sizes of ≥15 per group may be necessary to document a real effect.

- It is possible that in some studies the muscles of placebo and/or experimental subjects (ie. the *"non-responders"*) may have been loaded with Cr at baseline testing due to a chronic high meat intake, thereby invalidating any experimental comparison/effect (Van Loon *et al.*, 2003; Ahmun *et al.*, 2005; Stout *et al.*, 2006; Hadjicharalambous *et al.*, 2008), and possibly giving rise to a type II statistical error (ie. inability to observe a real treatment effect due to response variability). The quantification of initial muscle Cr concentration prior to any treatment might therefore be critical. However, in many published studies quantification remained outstanding due to the expense/invasiveness of the measurement techniques (magnetic resonance spectroscopy/needle biopsy).

- The type of exercise test employed differs greatly between studies, making comparison of results difficult. Also, the recovery periods allowed between bouts of repeated high-intensity exercise differ between studies. Greenhaff *et al.* (1994) suggest that the additional muscle Cr supply following supplementation might have little effect on PCr resynthesis or performance unless sufficient recovery time was available. It is likely that a recovery time of 60 - 120 s between successive bouts of exercise is necessary.

- Most studies have investigated the response in male subjects, but some have combined both genders in the same experiment. The latter approach may have confounded the

data because it is still unclear whether women and men respond similarly to Cr supplementation.

- Most published experiments have concentrated on body-mass supported activities (eg. cycle ergometer exercise) which would tend to minimise any adverse performance effect of body weight gain typically associated with Cr loading. More systematic study is needed to document fully the significance of any differences between mass-dependent and mass-supported exercise tasks following Cr supplementation.

The form in which Cr is ingested may also limit its effect. Van Schuylenbergh *et al.* (2003) demonstrated no benefit of low-dosage (7 g/day) creatine-pyruvate (Cr-Pyr) ingestion on either endurance capacity or intermittent sprint performance achieved by cyclists. The authors explained that, in the acid environment of the stomach, Cr-Pyr rapidly splits into its Cr and pyruvate moieties to be separately absorbed into the intestinal tract. The supplement failed to increase pyruvate levels in the blood (Van Schuylenbergh *et al.*, 2003), indicating that it (pyruvate) was poorly absorbed from the intestinal tract. The Cr subsequently absorbed from the intestinal tract and distributed to the muscles might have been too low to elicit an effect. Thus, short-term Cr-Pyr supplementation at the dosage advocated by sports nutrition dealers did not prove to be ergogenic (Van Schuylenbergh *et al.*, 2003). A more recent study incorporating a longer period of Cr-Pyr supplementation (5 g/day for 28 days) did, however, delay muscle fatigue during maximal intermittent handgrip exercise (Jäger *et al.*, 2008). Thus, a longer period of Cr-Pyr supplementation might benefit exercise performance, but a conclusion to that effect cannot be sustained on the grounds of just two published studies.
Finally, Butterly *et al.* (2006) found that the potentiating effect of Cr supplementation might be under-estimated in the Wingate Anaerobic Test (WAnT), if the inertial effects of the flywheel are not considered in the power output determination.

Thus, it is clear that more research is needed to document the potential benefits of Cr supplementation for exercise performance and to determine the exact reasons for the contradictory results observed to date (Lemon, 2002).

2.6.4.8 CONCLUSION

Attempting to draw clear conclusions on the effect of Cr supplementation on exercise performance in general, and on sport performance in particular, is a daunting task. Up to

approximately 1997, limited studies were available and most reported clear benefits. Conclusions seemed to favour Cr as an ergogenic aid in a controlled environment, especially in the context of repeated bouts of high-intensity exercise and single-effort strength-power feats. Many studies have supported these claims since then, but at the same time it must be noted that a significant number have demonstrated no benefit. Salespeople, and others intent on using Cr, tend to focus on the former. The benefit of Cr to performance in sports events still needs to be established beyond reasonable doubt.

The ergogenic benefit of Cr supplementation to exercise performance has been established in males (Preen *et al.*, 2001; Warber *et al.*, 2002; Souza-Junior *et al.*, 2011; Vatani *et al.*, 2011) and females (Vandenberghe *et al.*, 1996*b*; Ziegenfuss *et al.*, 2002; Kambis & Pizzedaz, 2003; Gotshalk *et al.*, 2008), as well as the young (Kreider *et al.*, 1998; Volek *et al.*, 1999; Ostojik, 2004; Olsen *et al.*, 2006) and the old (Rawson & Clarkson, 2000; Brose *et al.*, 2003; Gotshalk *et al.*, 2008). The mechanisms whereby Cr exerts this effect include: (i) an increased ability to maintain the rate of ATP resynthesis for longer during exercise (Balsom *et al.*, 1993*a*; Greenhaff *et al.*, 1993*b*), (ii) an enhanced ability to regenerate ATP and PCr during recovery (Harris *et al.*, 1992; Green *et al.*, 1996; Francaux *et al.*, 2000; Lemon, 2002; Jäger *et al.*, 2008; Rahimi *et al.*, 2010), (iii) reduced ADP, Pi, AMP and IMP accumulation during consecutive bouts of exercise (Rico-Sanz & Marco, 2000; McConell *et al.*, 2005), (iv) a "*super-training*" effect due to enhanced post-exercise recovery rate (Kraemer & Volek, 1999; Lemon, 2002; Rahimi *et al.*, 2010; Souza-Junior *et al.*, 2011), (v) enhanced lactate/H^+ buffering capacity (Söderlund *et al.*, 1994; Rossouw & Rossouw, 2000*a*; Peyrebrune *et al.*, 2005; Glaister *et al.*, 2006; Stout *et al.*, 2006; Rahimi *et al.*, 2010); and (vi) an indirect anabolic effect on muscle tissue (Francaux & Poortmans, 1999; Jówko *et al.*, 2000; Arciero *et al* 2001; Bemben *et al* 2001; Olsen *et al.*, 2006; Saremi *et al.*, 2010).

Gualano *et al.* (2011) argue that since Cr is not a drug, ''Cr loading'' is an effective dietary practice much like ''carboloading''. This author is in agreement as far as the dietary practice goes; however the safety of the product, the intentions of the salespeople who promote it, and the intentions of the consumers who use it, also need to be considered.

2.6.4.9 RECOMMENDATIONS

- Before commencing a study research participants should be screened for potential "non-responders" to Cr treatment in order to decrease the likelihood of falling into a type II statistical error (Hadjicharalambous *et al.*, 2008).

- More research is required to investigate the effects of Cr supplementation on simulated or actual sport performance (Bishop, 2010) and endurance events.

2.6.5 Effects of creatine supplementation on body composition

2.6.5.1 Tabulated summary of literature

Table 2-3 Effect of Cr ingestion on body weight, fat-free weight and fat% – a summary of the literature

Authors	Year	Dosage	♂/♀	Description	Method	Mean change: Body weight (kg)		Mean change: Fat free weight (kg)		Mean change: Fat%	
						Cr	Placebo	Cr	Placebo	Cr	Placebo
Ahmun *et al.*	2005	20g/day 5 days	♂	Highly-trained rugby players	4-site skinfold,	+ 0.4	+ 0.2	NR	NR	+ 1.1	+ 1.6
Armentano *et al.*	2007	20g/day 7 days	♂, ♀	Active duty military personnel	Biodynamics BIA	+ 0.8	- 0.1	+ 0.6	- 1.0	+ 0.1	+ 1.0
Balsom *et al.*	1993 (a)	25g/day 6 days	♂	Physical Education students	Scale	+ 1.1*	NC	NR	NR	NR	NR
Balsom *et al.*	1993 (b)	20g/day 6 days	♂	Active to well-trained	Scale	+ 0.9*	- 0.1	NR	NR	NR	NR
Balsom *et al.*	1995	20g/day 6 days	♂	Physically active	Scale	+ 1.1*	No placebo group	NR	NR	NR	NR
Francaux *et al.*	2000	21g/day 14 days	♂	Physically active	BodyStat BIA	+ 0.4	+ 0.4	NR	NR	NR	NR
Glaister *et al.*	2006	20 g/day 5 days	♂	Physically active sport science students	NR	+ 0.7	NR	NR	NR	- 0.4	NR
Gotshalk *et al.*	2008	0.3g/day 7 days	Older ♀	Healthy, active,	Scale, skinfold calliper	+ 0.6*	+ 0.01	+ 0.5*	+ 0.1	- 0.2	+ 0.01
Green *et al.*	1996	20g/day 3 days	♂	Healthy	Scale	Cr: + 0.6 Cr-CHO: + 2.1*	NC	NR	NR	NR	NR
Greenhaff *et al.*	1994	20g/day 5 days	♂	Recreationally active	Scale	+ 1.6*	No placebo group	NR	NR	NR	NR

Authors	Year	Dosage	♂/♀	Description	Method	Mean change: Body weight (kg)		Mean change: Fat free weight (kg)		Mean change: Fat%	
						Cr	Placebo	Cr	Placebo	Cr	Placebo
Hadjicharalambous *et al.*	2008	20g/day 7 days	♂	Endurance-trained	BIA	+ 0.7*	+ 0.2	NR	NR	NR	NR
Hamilton *et al.*	2000	25g/day 7 days	♀	Resistance-trained, participating in overhand sports	7-site skinfold, circumferences	+ 0.6	+ 0.3	NR	NR	NC	NC
Jakobi *et al.*	2000	20g/day 5 days	♂	Moderately active	7-site skinfold, circumferences	+ 1.0*	NC	NR	NR	NR	NR
Jakobi *et al.*	2001	20g/day 5 days	Older ♂	Moderately active	7-site skinfold, circumferences	+ 1.0*	- 0.3	NR	NR	NR	NR
Kambis & Pizzedaz	2003	0.5g/kg/day 5 days	♀	College students	3-site skinfold, circumferences	NC	NC	+ 0.1	- 0.1	NC	+ 0.2
Kilduff *et al.*	2007	20g/day 7 days	♂	Athletes	Hydrostatic weighing, Bodystat Quadscan BIA, 7-site skinfold	+1.0*	No placebo group	+0.9*	No placebo group	NR	NR
Kinugasa *et al.*	2004	20g/day 5 days	♂	Healthy	Scale	+1.0*	NC	NR	NR	NR	NR
Kirksey *et al.*	1999	0.3g/kg/day 6 weeks	♂, ♀	Collegiate track and field athletes	7-site skinfold, underwater weighing	+ 2.1*	+ 1.3*	+ 2.6*	+ 1.0	- 0.8	+ 0.1
Koçak & Karli	2003	20g/day 5 days	♂	Elite wrestlers	Scale	+ 1.0*	+ 0.9	NR	NR	NR	NR
Louis *et al.*	2003 (b)	21g/day 5 days	♂	Physical education students, not highly trained	Scale	NC	NC	NR	NR	NR	NR

Authors	Year	Dosage	♂/♀	Description	Method	Mean change: Body weight (kg)		Mean change: Fat free weight (kg)		Mean change: Fat%	
						Cr	Placebo	Cr	Placebo	Cr	Placebo
Louis *et al.*	2003 (c)	21g/day 5 days	♂	Physical education students, not highly trained	Scale	NC	NC	NR	NR	NR	NR
McNaughton *et al.*	1998	20g/day 5 days	♂	Elite kayak paddlers	Sum of 8 skinfolds	NC	NC	NR	NR	NR	NR
Mendes *et al.*	2004	20g/day 8 days	♂, ♀	Competitive swimmers	Skinfolds, BIA	+ 1.3*	- 0.1	+ 1.5*	- 0.7	NR	NR
Mihic *et al.*	2000	20g/day 5 days	♂, ♀	Physically active	DEXA scan: Hologic QDR 1000	+ 1.04* ♂ + 1.6* ♀ + 0.4	- 0.1 ♂ - 0.3 ♀ + 0.1	+ 0.9* ♂ + 1.4* ♀ + 0.4	NC ♂ - 0.2 ♀ + 0.1	NC ♂ NC ♀ NC	NC ♂ NC ♀ NC
Mujika *et al.*	1996	20g/day 7 days	♂, ♀	Elite swimmers	Scale	+ 0.7*	- 0.3	NR	NR	NR	NR
Op 'T Eijnde & Hespel	2001	20g/day 5 days	♂	Healthy, physical education students	Scale	+ 0.4	+ 0.2	NR	NR	NR	NR
Preen *et al.*	2001	20g/day 5 days	♂	Healthy	Scale	+ 0.9*	NC	NR	NR	NR	NR
Rahimi *et al.*	2010	20g/day 7 days	♂	Resistance-trained	Skinfold, body density	+ 0.7*	+ 0.1	+ 0.6*	+ 0.1	- 0.2	- 0.1
Rawson & Clarkson	2000	20g/day 5 days	Older ♂	Healthy	3-site skinfold, circumferences, DEXA scan: Xitron	+ 0.5*	NC	+ 0.6	+ 0.7	- 0.2	+ 0.1
Redondo *et al.*	1996	25g/day 7 days	♂, ♀	Well-trained athletes	Scale	- 0.8	NC	NR	NR	NR	NR
Volek *et al.*	1997	25g/day 6 days	♂	Resistance-trained	7-site skinfold, electronic scale	+ 1.4*	NC	NR	NR		
Volek *et al.*	2001	0.3g/kg/day 7 days	♂	Healthy	7-site skinfold, Tanita BIA	+ 0.6*	NC	NR	NR	NR	NR

Authors	Year	Dosage	♂/♀	Description	Method	Mean change: Body weight (kg)		Mean change: Fat free weight (kg)		Mean change: Fat%	
						Cr	Placebo	Cr	Placebo	Cr	Placebo
Warber *et al.*	2002	24g/day 5 days	♂	Soldiers	DEXA scan	+ 1.4*	NC	NR	NR	- 2.3*	- 1.3
Ziegenfuss *et al*	2002	0.35g/kg/day 3 days	♂, ♀	Division I national athletes	Magnetic resonance images	+ 0.9*	NC	NR	NR	NR	NR
Graef *et al.*	2009	10g/day 30 days	♂	Recreationally active	Scale	+ 0.4	+ 0.3	NR	NR	NR	NR
Kreider *et al.*	1998	15.75g/day 28 days	♂	NCAA Division IA football team	DEXA scan: QDR 2000; Valhalla BIA	+ 2.3*	+ 0.7	+ 2.4	+ 1.3	- 0.2	- 0.7
Rossouw *et al.*	2000	9g/day 6 days	♂, ♀	Well trained power-lifters	7-site skinfold, circumferences	+ 0.2	+ 0.9	+ 0.1	- 0.2	NC	- 0.1
Arciero *et al.*	2001	20g/day, 6 days; 10g/day, 22days	♂, ♀	Active, not resistance-trained	DEXA scan: Xitron	Cr: + 1.7* Cr-RT: + 2.0*	P: NC P-RT: + 0.2	Cr: + 0.9 Cr-RT: + 1.7*	P: NC P-RT: + 0.2	Cr: + 0.3 Cr-RT: NC	P: NC P-RT: + 0.1
Becque *et al.*	2000	20g/day, 5days; 2g/day, 5weeks	♂	Recreational weight lifters	Underwater weighing	Cr-RT: + 2.0*	P-RT: + 0.4	Cr-RT: + 1.6*	P-RT: - 0.1	Cr-RT: -0.1	P-RT: + 0.5
Bemben *et al.*	2001	20g/day, 5days; 5g/day, 8weeks	♂	NCAA Division I football team	Underwater weighing	Cr-RT: + 3.1*	P-RT: + 0.6	Cr-RT: + 2.9*	P-RT: - 0.2	Cr-RT: - 3.2	P-RT: + 7.2
Bennett *et al.*	2001	20g/day, 6days; 6g/day, 4weeks	♂	Trained military personnel	Akern BIA	Cr-MT: + 2.9*	P-MT: - 0.2	Cr-MT: + 0.3	P-MT: - 0.7	Cr-MT: +0.6	P-MT: - 0.2
Bermon *et al.*	1998	20g/day, 5days; 3g/day, 7weeks	Older ♂, ♀	Sedentary to moderately active	Skinfold	Cr: + 0.1 Cr-RT: + 0.5	P: + 0.2 P-RT: - 0.3	NR	NR	Cr: - 0.7 Cr-RT: - 0.4	P: - 0.5 P-RT: - 0.3

Authors	Year	Dosage	♂/♀	Description	Method	Mean change: Body weight (kg)		Mean change: Fat free weight (kg)		Mean change: Fat%	
						Cr	Placebo	Cr	Placebo	Cr	Placebo
Burke *et al.*	2003	17g/day, 7days; 4g/day, 49days	♂, ♀	Recreationally active	DEXA scan: Hologic QDR-2000	NR	NR	Cr-Vg: + 2.4* Cr-NVg: + 1.9*	P-NVg: NC	NR	NR
Francaux & Poortmans	1999	21g/day, 5days 3g/day, 58days	♂	Active, not resistance-trained	BodyStat BIA	Cr-RT: + 2.0*	P-RT: + 0.5	NR		NR	NR
Huso *et al.*	2002	20g/day, 4days; 2g/day, 17days	♂	Recreationally active	Bod Pod whole body densitometry	Cr-RT: + 1.6*	P-RT: - 0.4	Cr-RT: + 1.9*	P-RT: + 2.2*	Cr-RT: - 0.7	P-RT: - 3.2*
Jówko *et al.*	2001	20g/day, 7days; 10g/day, 14days	♂	Active, not resistance-trained	Akern BIA	Cr: + 2.0* Cr-HMB: + 3.4*	P: + 1.0 HMB: + 1.3*	Cr: + 1.7* Cr-HMB: + 2.3*	P: + 0.8 HMB: + 1.2	NR	NR
Kohler	2001	20g/day, 5days; 5g/day, 59days	♂	Club rugby players	Scale	Cr-RT: - 2.2	P-RT: - 0.8	NR	NR	NR	NR
Kutz & Gunter	2003	30g/day, 2weeks; 15g/day, 2weeks	♂	Active	Underwater weighing; BIA	+ 1.7*	+ 0.7	NR	NR	- 0.4	- 0.3
Parise *et al.*	2001	20g/day, 5days; 5g/day, 4days	♂, ♀	Physically active	DEXA scan: Hologic QDR-1000	♂: + 0.7 ♀: + 0.2	♂: + 0.5 ♀: + 0.8	♂: + 1.0 ♀: + 0.5	♂: + 0.8 ♀: + 0.5	NR	NR

Authors	Year	Dosage	♂/♀	Description	Method	Mean change: Body weight (kg)		Mean change: Fat free weight (kg)		Mean change: Fat%	
						Cr	Placebo	Cr	Placebo	Cr	Placebo
Peeters *et al.*	1999	20g/day, 3days; 10g/day, 6weeks	♂	Resistance-trained	7-site skinfold	Cr-RT: + 1.8*	P-RT: - 0.1	Cr-RT: + 1.6*	P-RT: - 0.1	Cr-RT: - 0.9	P-RT: - 0.2
Peyrebrune *et al.*	2005	20g/day, 5days; 3g/day, 26weeks	NR	Physically active	BIA	Cr + ET: NC	P + ET: - 0.5	Cr + ET: NC	P + ET: NC	Cr + ET: + 0.2	P + ET: + 0.3
Reardon *et al.*	2006	20g/day, 7days; 5g/day, 3weeks	♂, ♀	Elite swimmers	Scale	+ 0.6	- 0.4	NR	NR	NR	NR
Safdar *et al.*	2008	20g/day, 3days; 5g/day, 7days	♂	Healthy	DEXA scan, BIA	+ 2.0*	NR	+1.0*	NR	NC	NR
Saremi *et al.*	2010	0.3g/kg/day, 7days; 0.05g/kg/day, 7weeks	♂	Healthy, not resistance-trained university students	Lunar DPX-L dual energy X-ray absorptiometer	Cr-RT: +2.1	P-RT: +1.6	Cr-RT: +2.6*	P-RT: +2.0*	Cr-RT: -1.3	P-RT: -1.0
Stout *et al.*	1999	21g/day, 5days; 10g/day, 7weeks	♂	NCAA Division II football team	DEXA scan: Hologic QDR 2000+	NR	NR	Cr: + 2.8* Cr-CHO: + 3.2*	P: - 0.3	NR	NR
Van der Merwe *et al.*	2009	25g/day, 7days; 5g/day, 14days	♂	Rugby players from a Rugby Institute	6-skilfold	+0.8	+0.5	+0.8	NC	-0.1	-0.1

Authors	Year	Dosage	♂/♀	Description	Method	Mean change: Body weight (kg)		Mean change: Fat free weight (kg)		Mean change: Fat%	
						Cr	Placebo	Cr	Placebo	Cr	Placebo
Van Loon *et al.*	2003	20g/day, 5days; 2g/day, 37days	♂	Sedentary	Underwater weighing	+ 1.1*	- 0.1	+ 1.0	- 0.2	- 0.2	+ 1.0
Volek *et al.*	1999	25g/day, 7days; 5g/day, 11weeks	♂	Resistance-trained	Underwater weighing	Cr-RT: + 5.2*	P: + 3.0*	Cr-RT: + 4.3*	P: + 2.1*	NC	NC
Brilla *et al.*	2003	5g/day 14 days	♂, ♀	Recreationally active	Akern BIA	Cr-MgO: + 0.75* Cr-MgC: + 0.4	P: + 0.04	NR	NR	NR	NR
Brose *et al.*	2003	5g/day 14 weeks	Older ♂, ♀	Healthy, not resistance-trained	DEXA scan: Hologic QDR 4500 A	♂ Cr-RT: + 1.4* ♀ Cr-RT: + 1.1*	♂ P-RT: - 0.4 ♀ P-RT: NC	♂ Cr-RT: + 1.4* ♀ Cr-RT: + 2.0*	♂ P-RT: NC ♀ P-RT: + 0.6	♂ Cr-RT: - 0.2 ♀ Cr-RT: - 2.0	♂ P-RT: - 0.2 ♀ P-RT: - 0.9
Hickner *et al.*	2010	3g/day 28 days	♂	Endurance-trained cyclists	Hydrostatic weighing	+ 2.0*	+ 0.7	NR	NR	+ 0.3	-0.1
Hoffman *et al*,	2005	6g/day 6 days	♂	Physically active	Scale	+ 0.2	NC	NR	NR	NR	NR
Jäger *et al.*	2008	5g/day 28 days	♂	Healthy	Bioelectrical impedance; forearm circumference	Cr-Pyr: + 1.5* Cr-Cit: + 1.4*	+ 0.1	NR	NR	Cr-Pyr: -0.1 Cr-Cit: - 0.3	- 0.2
Rawson *et al.*	2011	0.03g/kg/day 6 weeks	♂, ♀	Healthy	Tanita BIA	- 0.4	+ 0.1	- 0.5	- 0.1	+ 0.4	- 2.0
Willoughby & Rosene	2001	6g/day 12 weeks	♂	Untrained	7-site skinfold	Cr-RT: + 5.0*	P-RT: + 1.0*	Cr-RT: + 3.2*	P-RT: + 0.9*	Cr-RT: + 0.7	P-RT: + 1.1

* $p \leq 0.05$

High-dosage supplementation
Moderate-dosage supplementation
DEXA duel energy x-ray absorptiometry
NC no change
High-dosage, followed by low-dosage, supplementation
Low-dosage Cr supplementation
BIA bioelectrical impedance analyzer
NR not reported

Abbreviations: Cr, creatine only; Cr-CHO, creatine and carbohydrate ingestion; Cr-Cit, creatine and citrate ingestion; Cr-HMB, creatine and HMB ingestion; Cr-MgO, Cr and Mg oxide ingestion; Cr-MgC, Mg-creatine chelate; Cr-MT, creatine and military training; Cr-NVg, non-vegetarians consuming Cr; Cr-Pyr, creatine and pyruvate ingestion; Cr-RT, creatine and resistance training; Cr-Vg, vegetarians consuming Cr; ET, endurance training; P, placebo alone; P-MT, placebo and military training; P-RT, placebo and resistance training; DEXA scan, dual-energy x-ray absorptiometry; P-NVg, non-vegetarians consuming placebo; P-Vg, vegetarians consuming placebo.

Table 2-4 Effect of Cr ingestion on body water compartments - a summary of the literature

Authors	Year	Dosage	♂/♀	Description	Method	Total Body Water (TBW) (ℓ)		Intra-cellular Water (ICW) (ℓ)		Extra-cellular Water (ECW) (ℓ)	
Armentano *et al.*	2007	20g/day 7 days	♂, ♀	Active duty military personnel	Biodynamics BIA	- 1.6	- 1.0	NR	NR	NR	NR
Francaux *et al.*	2000	21g/day 14 days	♂	Physically active	BodyStat BIA	- 0.4	NC	+ 0.1	- 0.2	- 0.5	+ 0.2
Hadjicharalambous *et al.*	2008	20g/day 7 days	♂	Endurance-trained	BIA	+ 0.6*	+ 0.1	+ 0.5	NC	+ 0.1	NC
Rawson & Clarkson	2000	20g/day 5 days	Older ♂	Healthy	DEXA scan: Xitron	+ 0.7	- 0.5	+ 0.8	- 0.2	- 0.1	- 0.2
Volek *et al.*	2001	0.3g/kg/day 7 days	♂	Healthy	7-site skinfold, Tanita BIA	+ 0.4*	- 0.1	NR	NR	NR	NR
Arciero *et al.*	2001	20g/day, 6 days; 10g/day, 22days	♂, ♀	Active, not resistance-trained	DEXA scan: Xitron	Cr: + 2.2* Cr-RT: + 1.7*	P: NC P-RT: + 0.1	NR	NR	NR	NR
Bemben *et al.*	2001	20g/day, 5days; 5g/day, 8weeks	♂	NCAA Division I football team	DEXA scan: Xitron	Cr-RT: + 3.2*	P-RT: - 0.5	Cr-RT: + 3.3*	P-RT: - 0.3	NC	NC

Authors	Year	Dosage	♂/♀	Description	Method	Total Body Water (TBW) (ℓ)		Intra-cellular Water (ICW) (ℓ)		Extra-cellular Water (ECW) (ℓ)	
Bennett *et al.*	2001	20g/day, 6days; 6g/day, 4weeks	♂	Trained military personnel	Akern BIA	Cr-MT: - 0.1	P-MT: - 2.2	NR	NR	NR	NR
Francaux & Poortmans	1999	21g/day, 5days; 3g/day, 58days	♂	Active, not resistance-trained	BodyStat BIA	Cr-RT: + 1.1*	P-RT: + 0.5	Cr-RT: + 0.6*	P-RT: + 0.1	Cr-RT: + 0.5	P-RT: + 0.4
Jówko *et al.*	2001	20g/day, 7days; 10g/day, 14days	♂	Active, not resistance-trained	Akern BIA	Cr: + 1.6* Cr-HMB: + 2.1*	P: + 0.7 HMB: + 1.1	Cr: + 1.1 Cr-HMB: + 1.5	P: + 0.4 HMB: + 0.7	NR	NR
Kutz & Gunter	2003	30g/day, 2weeks; 15g/day, 2weeks	♂	Active	BIA	+ 3.4*	+ 0.3	NR	NR	NR	NR
Safdar *et al.*	2008	20g/day, 3days 5g/day, 7days	♂	Healthy	BIA	+ 1.0*	NR	NR	NR	NR	NR
Brilla *et al.*	2003	5g/day 14 days	♂, ♀	Recreationally active	Akern BIA	Cr-MgO: + 0.5 Cr-MgC: + 0.9	P: + 0.4	Cr-MgO: + 0.5 Cr-MgC: + 1.7*	P: + 1.3	Cr-MgO: - 0.02 Cr-MgC: - 0.8*	P: - 0.7
Rawson *et al.*	2011	0.03g/kg/day 6 weeks	♂, ♀	Healthy	Tanita BIA	- 0.4	+ 0.4	NR	NR	NR	NR

* $p \leq 0.05$

High-dosage supplementation

High-dosage, followed by low-dosage, supplementation

Low-dosage Cr supplementation

DEXA duel energy x-ray absorptiometry

BIA bioelectrical impedance analyser

NC no change

NR not reported

Abbreviations: Cr, creatine only; Cr-CHO, creatine and carbohydrate ingestion; Cr-RT, creatine and resistance training; P, placebo alone; P-RT, placebo and resistance training; Cr-MT, creatine and military training; P-MT, placebo and military training; Cr-HMB, creatine and ß-Hydroxy-ß-Methylbuterate (HMB) ingestion; Cr-MgO, Cr and Mg oxide ingestion; Cr-MgC, Mg-creatine chelate; DEXA scan, dual-energy x-ray absorptiometry; Cr-Vg, vegetarians consuming Cr; Cr-NVg, non-vegetarians consuming Cr; P-Vg, vegetarians consuming placeb0; P-NVg, non-vegetarians consuming placebo;

Table 2-5 Effect of Cr supplementation on muscle morphology – a summary of the literature

Authors	Year	Dosage	♂/♀	Description	Method	Results
Dangott *et al.*	2000	0.9g/kg/day 4weeks	♂	Animal study: rats	Surgery to hind-limb to induce compensatory hypertrophy in plantaris muscle	* Increased satellite-cell mitotic activity with Cr supplementation only in combination with increased functional activation of muscle (ie. exercise). Myotube diameter and muscle mass – no change. Thus, Cr supplementation alone is not sufficient to supply new DNA (*via* the proliferation of satellite cells) for enlarging muscle fibres. Supplementation needs to be combined with functional muscle activity.
Deldicque *et al.*	2005	21g/day 5 days	♂	Physically active, crossover design	Muscle biopsy – vastus lateralis	*Both resistance exercise and Cr supplementation increased the expression of growth factors (IGF-I and IGF-II), but these effects were not cumulative. Cr did not potentiate the muscle phosphorylation state. Thus, Cr supplementation did not directly stimulate muscle growth, but could enhance the anabolic status of the cell by a late-response involving IGF.
Kinugasa *et al.*	2004	20g/day 5 days	♂	Healthy	mfMRI	No change in muscle volume of the mid-thigh. Thus, no fluid shift or protein synthesis with Cr supplementation.
Louis *et al.*	2003*b*	21g/day 5 days	♂	Physical education students, not highly trained	Muscle biopsy – vastus lateralis, blood analyses	No increase in myofibrillar protein synthesis. Thus, short-term Cr supplementation accompanied by acute resistance exercise was without any anabolic effect.
Louis *et al.*	2003*c*	21g/day 5 days	♂	Physical education students, not highly trained	Muscle biopsy – vastus lateralis, blood analyses	Increased myofibrillar protein synthesis, decreased muscle protein breakdown, but no net gain in muscle protein content. Thus, to increase muscle mass Cr supplementation should probably be accompanied by increased physical activity.
Louis *et al.*	2004	5 mM 2 days	n.a.	Cell cultures	C_2C12 myoblast cell lines in very high level of Cr	* Increased expression of IGF-I. * Increased diameter of myotubes. Thus, Cr stimulated growth (differentiation) and protein accumulation in myogenic cells in culture.

Authors	Year	Dosage	♂/♀	Description	Method	Results
Ziegenfuss *et al.*	2002	0.35g/kg/day 3 days	♂, ♀	Division I national athletes	MRI	* Increased mid-thigh muscle volume.
Chilibeck *et al.*	2004	0.2g/kg after training, 6weeks	♂, ♀	Resistance-trained	Ultrasound	*Increased muscle thickness and lean tissue mass of arm flexors with acute Cr supplementation; effect significantly greater in ♂ than in ♀. Muscle thickness and lean tissue mass of knee extensors - no differences between Cr and placebo. *Increased arm and leg values for muscle thicknesses combined in Cr group. Arm and leg values for lean tissue mass combined – no significant difference between Cr and placebo.
Burke *et al.*	2003	17g/day, 7days; 4g/day, 49days	♂, ♀	Recreationally active	Muscle biopsy – vastus lateralis	*Cr supplementation combined with resistance training increased Type II muscle fibre area in both vegetarians and non-vegetarians. Thus, Cr supplementation resulted in selected hypertrophy of Type II muscle fibres.
Olsen *et al.*	2006	24g/day, 7days; 6g/day, 15weeks	♂	Healthy	Muscle biopsy – vastus lateralis Immunochemsitry	*Cr supplementation combined with resistance training amplified the training-induced increase in the number of satellite cells and myonuclei, and increased the number of myonuclei per muscle fibre. *An amplified hypertrophy response was indicated by increased muscle fibre area.
Parise *et al.*	2001	20g/day, 5days; 5g/day, 4days	♂, ♀	Physically active	Muscle biopsy – vastus lateralis, blood and urine analyses	*Reduced rate of leucine oxidation and protein breakdown in men but not in women. No increase in the rate of mixed-muscle protein synthesis. Thus, Cr supplementation may provide an anti-catabolic effect in men. Not clear why this effect was not observed in women but does not appear to be related to muscle TCr and PCr.
Souza-Junior *et al.*	2011	20g/day, 7days; 5g/day, 7weeks	♂	Recreationally active	Magnetic resonance imaging	*Cr supplementation combined with resistance training increased muscle cross-sectional area of the arm and thigh. No control/placebo group.

Authors	Year	Dosage	♂/♀	Description	Method	Results
Volek *et al.*	1999	25g/day, 7days; 5g/day, 11weeks	♂	Resistance-trained	Muscle biopsy – vastus lateralis	* Cr supplementation combined with resistance training increased muscle fibre cross-sectional areas in Types I, IIA, and IIB fibres (35%, 36%, and 29%, respectively). Cr subjects possessed smaller muscle fibre areas before the training and supplementation programme compared with placebo subjects.
Brose *et al.*	2003	5g/day 14weeks	Older ♂, ♀	Healthy, not resistance-trained	Muscle biopsy – vastus lateralis	* Resistance training increased mean fibre area for Type I and IIB muscle fibres in both Cr and placebo groups. No effect of Cr supplementation on enhancing muscle fibre area increases.
Jäger *et al.*	2008	5g/day 28days	♂	Healthy	Forearm circumference	*Forearm circumference increased significantly with both Cr + pyruvate and Cr + citrate ingestion.
Sipilä *et al.*	1981	1.5g/day 12months	♂	Patients with gyrate atrophy	Muscle biopsy – vastus lateralis	*Selected hypertrophy of Type II muscle fibres.
Willoughby & Rosene	2001	6g/day 12weeks	♂	Untrained	Muscle biopsy – vastus lateralis	*Increased myofibrillar protein content (expressed as myosin heavy chain (MHC) isoforms).
Willoughby & Rosene	2003	6g/day 12weeks	♂	Untrained	Muscle biopsy – vastus lateralis	*Cr supplementation combined with resistance training increased the expression of myogenic regulatory factors (MRF's).

* $p \leq 0.05$

High-dosage supplementation

Moderate-dosage supplementation

IGF insulin-like growth factor

DEXA dual energy x-ray absorptiometry

NC no change

High-dosage, followed by low-dosage, supplementation

Low-dosage Cr supplementation

NR not reported

mfMRI muscle-functional magnetic resonance imaging

2.6.5.2 Effect of Cr on body weight

The largest increases in body weight seem to be reported when longer-term Cr supplementation is combined with resistance training (Francaux & Poortmans, 1999; Peeters *et al.*, 1999; Volek *et al.*, 1999; Becque *et al.*, 2000; Arciero *et al.*, 2001; Bembem *et al.*, 2001; Bennett *et al.*, 2001; Kohler, 2001; Willoughby & Rosene, 2001; Huso *et al.*, 2002; Brose *et al.*, 2003; Saremi *et al.*, 2010) or HMB (Jówko *et al.*, 2001). Most researchers attribute these increases to water retention (Mendes *et al.*, 2004; Ahmun *et al.*, 2005; Gotshalk *et al.*, 2008; Safdar *et al.*, 2008; Rahimi *et al.*, 2010; Vatani *et al.*, 2011), and not to direct anabolic endocrine influences. However, this theory has been challenged (Kraemer & Volek, 1999; Francaux *et al.*, 2000; Rawson & Clarkson, 2000; Paddon-Jones *et al.*, 2004) as will be explained under point 2.6.5.3.

Van der Merwe *et al.* (2009) attributed the fact that well-trained rugby players did not undergo a significant increase in body weight in response to short-term Cr ingestion, to their being recruited during their competitive season, after their initial pre-season strength-orientated training and concomitant increase in body weight. However, other short-term studies incorporating well-trained strength/power athletes did report significant changes in body weight and/or composition (Kirksey *et al.*, 1999; Ziegenfuss *et al.*, 2002; Koçak & Karli, 2003; Mendes *et al.*, 2004). These discrepancies in results are probably attributable to differences in the comprehension of what is meant by reference to a "*well-trained*" athlete, different athletic codes, and variations in methods used to determine body composition. Longer supplementation periods altered body composition even in these subjects whose training would not have changed appreciably during the course of the research studies (Kreider *et al*, 1998; Stout *et al.*, 1999).

In conclusion, increased body weight is reported as a common side-effect of Cr ingestion (Table 2-3). Since oral Cr supplementation has been found to increase skeletal muscle Cr content by as much as 20% (Harris *et al.*, 1992; Greenhaff, 1995; Francaux *et al.*, 2000; Rawson *et al.*, 2004; Deldicque *et al.*, 2005; Hadjicharalambous *et al.*, 2008; Safdar *et al.*, 2008), and Cr is an osmotically active substance, increases in body weight are usually attributed to water retention (Rawson & Clarkson, 2000; Persky & Brazeau, 2001; Mendes *et al.*, 2004; Ahmun *et al.*, 2005; Gotshalk *et al.*, 2008; Safdar *et al.*, 2008; Rahimi *et al.*, 2010; Vatani *et al.*, 2011). Because the increase in body weight could not always be accounted for by fat free weight (or water), some researchers have also examined changes in the fat% (Table 2-3) and/or muscle morphology (Table 2-5). However, increases in body weight may also result from supplemented subjects

being able to perform more total work during resistance training and recover faster between sets compared to non-supplemented controls, thereby facilitating muscle protein synthesis (Paddon-Jones *et al.*, 2004; Saremi *et al.*, 2010). This may account for the concomitant increase in FFW reported by most longer-term studies (Kreider *et al.*, 1998; Peeters *et al.*, 1999; Volek *et al.*, 1999; Becque *et al.*, 2000; Arciero *et al.*, 2001; Bembem *et al.*, 2001; Bennett *et al.*, 2001; Willoughby & Rosene, 2001; Huso *et al.*, 2002; Brose *et al.*, 2003; Saremi *et al.*, 2010). The beneficial effects of Cr on muscle strength and body composition probably occur in the following sequence: increased muscle Cr, increased training intensity, greater training stimulus, and enhanced physiological adaptations to training (Paddon-Jones *et al.*, 2004). To date, Cr supplementation seems to elicit no significant change in body fat% (Table 2-3).

2.6.5.3 Effect of Cr on body water and protein turnover

Depending on the stage of life and adiposity of a human, total body water (TBW) may comprise 50 to 65% of the body weight and 70 to 80% of the fat-free body (Schoeller, 2005; Horswill & Janas, 2011). TBW can be compartmentalised into intracellular fluid ICF) and extracellular fluid (ECF), with ECF further divided into interstitial and plasma volumes. The ICF represents approximately 60% and the ECF 40% of the TBW. The plasma volume comprises 7% of TBW (Schoeller, 2005; Horswill & Janas, 2011).

As mentioned before, Cr supplementation protocols often result in significant increases in body weight, which may be attributable to increases in TBW. It is hypothesised (Francaux *et al.*, 2000; Bemben *et al.*, 2001; Jówko *et al.*, 2001; Brilla *et al.*, 2003; Safdar *et al.*, 2008; Saremi *et al.*, 2010) that the rise in muscle Cr concentration associated with supplementation could increase the osmolarity of the intracellular environment, despite the large number of mechanisms allowing the cell to regulate its own osmolarity, thereby drawing water into the cell. Cellular hydration (swelling) can be considered an anabolic proliferative signal (Häussinger *et al.*, 1993; Schliess & Häussinger, 2002; Baechle & Earle, 2008). It favours the synthesis and inhibits the degradation of muscle protein (Häussinger *et al.*, 1993; Schliess & Häussinger, 2002; Brilla *et al.*, 2003). Factors such as hormones, substrates (ie. Cr) and oxidative stress can change the cellular hydration status within minutes, thereby affecting protein turnover (Häussinger *et al.*, 1993; Schliess & Häussinger, 2002; Brilla *et al.*, 2003). It is also postulated that short-term alterations of cell volume - induced by hormones (ie. insulin), cumulative substrate uptake (eg. Cr) and/or oxidative stress - may activate independent, volume-sensitive

signalling cascades which contribute to the regulation of metabolic cell function and gene expression (Häussinger *et al.*, 1993; Schliess & Häussinger, 2002; Safdar *et al.*, 2008).

If the water retention hypothesis is correct the increase in body weight observed together with Cr supplementation could be attributable in the short-term to water retention whereas protein synthesis could be a longer-term phenomenon (Francaux *et al.*, 2000; Saremi *et al.*, 2010). Unfortunately not many studies have included body water analyses in their study designs.

Three of the five studies that reported on the effect of Cr loading on the body water compartments (Table 2-4) demonstrated no changes (Francaux *et al.*, 2000; Rawson & Clarkson, 2000; Armentano *et al.*, 2007). Surprisingly, Francaux *et al.* (2000) found no evidence of an increase in body weight despite reporting an increase of 20% in muscle PCr. They argued that the negative results could not be explained by a lack of sensitivity of the bioelectrical impedance spectroscopy technique, since they previously observed an increase in both TBW and ICW after respectively six and nine weeks of Cr supplementation (Francaux & Poortmans, 1999). Although Rawson and Clarkson (2000) report a significant increase in body weight, no significant changes in the body water compartments were found. However, Volek *et al.* (2001), and Hadjicharalambous *et al.* (2008) reported significant increases in both body weight and TBW. Thus, the effect of Cr loading on the body water compartments seems variable. It seems that the hydration effect of Cr might not be a short-term phenomenon, and that a supplementation period of more than two weeks is needed for significant changes to occur. It is interesting that none of the studies reported any change in intra-cellular water. These findings lend support to the remark by Kraemer and Volek (1999) that the impact of Cr supplementation on body composition seems to be mediated more by the long-term use of higher intensities in weight-training, rather than the acute effects of short-term water influx to maintain proper solute to hydration relationships in the muscle cell.

This possibility is supported by six of the seven studies that incorporated Cr loading followed by long-term maintenance feeding (Table 2-3). Significant mean increases in body weight ranging between 1.7 and 3.4 kg with TBW increasing between 1.0 to 3.2 ℓ, and FFW between 1.0 to 2.9 kg were reported (Francaux & Poortmans, 1999; Arciero *et al*, 2001; Bemben *et al*, 2001; Jówko *et al.*, 2001; Kutz & Gunter, 2003; Safdar *et al.*, 2008). Thus, the increase in TBW associated with long-term Cr use (loading followed by maintenance feeding) combined with resistance training, may be due to increased cell hydration (ICW) and/or an associated increase in FFW. It

is unfortunate, however, that not all studies reported on the state of the ICW compartment. Thus, as suggested previously by Volek and Rawson (2004), a determination beyond doubt cannot be made as to whether or not increases in ICW with Cr supplementation are of sufficient magnitude to influence measures of protein synthesis or breakdown.

Longer-term (14 days) low-dosage (5 g/day) Cr supplementation did increase body weight, TBW, ICW and extracellular (ECW) water in recreationally active men and women (Brilla *et al.*, 2003). Thus, this regimen of Cr supplementation may have a hydrating effect on the body with concomitant increased FFW (although the authors did not report on FFW).

In conclusion, reports about the effect of Cr supplementation on body water compartments remain both scarce and varied, with the most effective supplementation regimen seeming to be Cr loading followed by maintenance feeding.

2.6.5.4 Effect of Cr on muscle hypertrophy

Increases in muscle fibre hypertrophy and muscle protein content have been observed with Cr supplementation, especially when combined with resistance training (Table 2-5). However, the supplementation regimen seems to play an important role in facilitating these outcomes. Short-term Cr loading in humans did not result in net gains in muscle protein synthesis or hypertrophy (Louis *et al.*, 2003*b*; Louis *et al.*, 2003*c*; Kinugasa *et al.*, 2004; Deldicque *et al.*, 2005). It was found that Cr loading followed by an extended maintenance period (6 - 15 weeks) induced muscle hypertrophy (Volek *et al.*, 1999; Burke *et al.*, 2003; Chilibeck *et al.*, 2004; Souza-Junior *et al.*, 2011), as well as an amplified muscle hypertrophy response (Olsen *et al.*, 2006) in subjects who participated in an associated resistance training programme. Prolonged low-dosage Cr supplementation also proved effective in supporting muscle hypertrophy in that it increased myofibrillar protein content (Sipilä *et al.*, 1981; Willoughby & Rosene, 2001; Jäger *et al.*, 2008) and the expression of myoregulatory factors (Willoughby & Rosene, 2003).

Some of the studies that reported gains in muscle protein also reported gains in muscle strength (Volek *et al.*, 1999; Willoughby & Rosene, 2001; Brose *et al.*, 2003; Burke *et al.*, 2003; Chilibeck *et al.*, 2004; Olsen *et al.*, 2006; Souza-Junior *et al.*, 2011). However, the underlying physiological mechanism(s) to explain this ergogenic effect remain unclear. In essence, a Cr-induced enhancement of strength and particularly muscle fibre area must depend on interaction in some manner between Cr and known or postulated mechanisms of muscle-fibre hypertrophy

(Volek & Rawson, 2004). As explained in the previous paragraphs, one of the explanatory mechanisms applied to Cr, namely cellular hydration and swelling, has been difficult to validate.

Another theory to consider here is that Cr supplementation combined with resistance training may influence the expression of certain myogenic regulatory factors (MRF), which may increase myosin heavy chain (MHC) synthesis. This theory is supported by study results achieved by Willoughby and Rosene (2001; 2003), Louis *et al.* (2004), Deldicque *et al.* (2005) and Saremi *et al.* (2010). However, further research is needed to clarify whether oral Cr supplementation has a direct effect on the expression of MRF and MHC, or whether the effect is indirectly mediated through a greater training volume resulting in increased stimulation of muscle hypertrophy (Burke *et al.*, 2003; Louis *et al.*, 2003*b*; Chilibeck *et al.*, 2004; Volek & Rawson, 2004; Rahimi *et al.*, 2010; Souza-Junior *et al.*, 2011), in which case it may either be that acute exercise unmasks some anabolic effect of Cr not seen at rest (Schedel *et al.*, 2000), or that, because Cr increases force development through increases in muscle PCr stores, work output during training can be increased during Cr supplementation with a benefit to muscle accretion (Louis *et al.*, 2003*c*; Souza-Junior *et al.*, 2011).

An increase in myonucleus number is not a permissive factor to achieve muscle fibre hypertrophy, but it may set the limit for muscle hypertrophy by regulating the nuclear domain of the muscle cell (Olsen *et al.*, 2006). It has been found that Cr supplementation combined with resistance training elevates myonucleus number respectively by 14% and 17% at weeks 4 and 16 (Olsen *et al.*, 2006). The authors attributed the accelerated time course and more marked muscle-fibre hypertrophy of the Cr group to this phenomenon. The study (Olsen *et al.*, 2006) also reported increased satellite cell numbers of myofibres. This effect may have been mediated, at least in part, *via* Cr-induced facilitation of MRF pathways. The results of the study therefore support a role for Cr in activating myogenic satellite cells (Olsen *et al.*, 2006). These activated cells then donate their nuclei to muscle fibres, thereby augmenting repair and recovery of muscle fibres and the training-induced accretion of muscle mass, especially in the early part of the training bout (Olsen *et al.*, 2006).

In another study that supports the possible anabolic effects of Cr combined with resistance training, Saremi *et al.* (2010) demonstrated that Cr supplementation added to the effects of resistance training by decreasing serum levels of myostatin and elevating serum growth and differentiation-factor associated serum protein-1 (GASP-1). Myostatin is a secreted protein that

negatively regulates human skeletal muscle mass by determining both muscle fibre number and size (Tobin & Celeste, 2005). GASP-1 is a protein that interacts with myostatin and down-regulates its actions, thereby leading to increased muscle mass (Ratkevicius *et al.*, 2011). Myostatin acts, in part, by inhibiting the activation of muscle satellite cells (Tobin & Celeste, 2005). Satellite cells are mononucleated cells found between the basement membrane and the sarcolema of muscle fibers, and are thought to represent the stem-cell population in muscle (Tobin & Celeste, 2005). Satellite cells are normally quiescent but can enter the cell cycle and fuse with existing fibres during muscle growth (Tobin & Celeste, 2005; Baechle & Earle, 2008; Karagounis & Hawley, 2010). They do not create more fibres but increase the size and number of the contractile proteins, namely actin and myosin (Karagounis & Hawley, 2010). The inhibition of myostatin activity – which cancels the inhibition of satellite-cell activation and self-renewal (Tobin & Celeste, 2005) - can lead to increased skeletal muscle mass in adult humans *via* muscle fibre hypertrophy, but not hyperplasia (Tobin & Celeste, 2005). Saremi *et al.* (2010) concluded that the increase in serum GASP-1 that accompanied the decrease in serum myostatin during Cr supplementation combined with resistance training, may have served to inhibit myostatin signaling and muscle catabolism that could conceivably have accompanied heavy resistance exercise, thereby augmenting the training-induced accretion of muscle mass and strength.

A third theoretical mechanism for Cr-induced muscle anabolism is through stimulation of transcriptional changes in muscle gene expression. This late-response involves insulin-like growth factors I and II (IGF-I and IGF-II). IGFs activate several cellular pathways regulating muscle atrophy and hypertrophy. Muscle protein synthesis is known to be increased by IGF-I, while IGF-II is involved in muscle differentiation and regeneration (Deldicque *et al.*, 2005). Previously, this response to Cr supplementation was only evident in cell cultures (Louis *et al.*, 2004), but more recently Deldicque *et al.* (2005) demonstrated increased expression of IGF-I and IGF-II in Cr-supplemented males participating in a heavy resistance training programme.

Safdar *et al.* (2008) demonstrated in the only study of its nature to date, that short-term Cr supplementation, independent of confounding factors such as exercise or disuse atrophy, may exert a significant direct effect on skeletal muscle cell metabolism. The researchers found that the increase in body weight and fat-free weight in response to Cr loading was attributable to a rapid and coordinated increase in gene activation that constitutes the cellular signal transduction network. More specifically, that the increase in cell swelling/hydration, activated volume-

sensitive signaling cascades in skeletal muscle cells that, in turn, activated genes that up-regulate cytoskeleton remodelling, protein synthesis, satellite cell proliferation and differentiation, RNA transcription, and DNA replication and repair (Safdar *et al.*, 2008).

In summary, results suggest that the effect of Cr on muscle hypertrophy occur in two stages, and that concomitant resistance training participation might be a prerequisite for this effect. The early stage of hypertrophy in the time duration of training seems to be related to the interaction of (i) signaling cascades that up-regulate anabolic gene expression, thereby increasing protein repair and synthesis in skeletal muscle and/or (ii) MRF (ie. myostatin and GASP-1, among others) affecting the activation state of satellite cells, thereby augmenting the repair and anabolic state of the muscle cell. The late stage may be mediated through the enhanced ability to tolerate a greater training volume and/or the actions of insulin-like growth factors.

2.6.5.5 Effect of Cr on hormone levels

Two main approaches have been adopted to investigate mechanisms by which Cr may influence skeletal muscle. One entails the investigation of skeletal muscle biopsies, as explained in the previous section, the other investigation of possible effects of Cr supplementation on the humoral endocrine response to exercise (Van der Merwe *et al.*, 2009).

Some *in vitro* studies of muscle cell cultures have suggested that Cr may directly stimulate muscle protein synthesis (Louis *et al.*, 2004). The idea that Cr supplementation can exert an anabolic effect on muscle cells *via* endocrine mechanisms may have gained credence from this suggestion. Of the four studies conducted on healthy human subjects, three reported no significant alterations either in resting circulating anabolic hormones following Cr loading (Rahimi *et al.* 2010; Vatani *et al.*, 2011), or after Cr loading with subsequent maintenance feeding (Van der Merwe *et al.*, 2009). These results suggest that Cr supplementation does not promote muscle hypertrophy through endocrine mechanisms. However, Schedel *et al.* (2000) showed a major (83%) increase in serum growth hormone (GH) levels after Cr ingestion. It is important to note that participants in the study demonstrated large inter-individual variations in the GH response. The authors concluded that in resting conditions, and at high-dosage Cr ingestion, GH secretion was stimulated, thereby mimicking the body's anabolic response to high-intensity exercise and exerting an indirect anabolic effect on skeletal muscle tissue (Schedel *et al.*, 2000). It is difficult to explain why the results from one study (Schedel *et al.*, 2000) differ so clearly from the others (Van der Merwe *et al.*, 2009; Rahimi *et al.* 2010; Vatani *et*

al., 2011). Possible reasons include the difference in subjects recruited: Schedel *et al.* (2000) recruited healthy, untrained males while the other investigators (Van der Merwe *et al.*, 2009; Rahimi *et al.* 2010; Vatani *et al.*, 2011) recruited well-trained individuals, including rugby players, resistance-trained men and male swimmers. The inconsistent results may further be attributable to differences in supplementation regimens: Schedel *et al.* (2000) reported the hormonal effects elicited by a once-off high dosage of Cr, while the others (Van der Merwe *et al.*, 2009; Rahimi *et al.* 2010; Vatani *et al.*, 2011) reported the effects of Cr ingestion over longer periods (6 - 14 days). These studies suggest that the proposed indirect anabolic effect of Cr supplementation *via* hormonal mechanisms either does not exist, or is blunted in well-trained athletes and in response to repeated Cr ingestion. However, the physiological mechanisms responsible for these effects remain elusive.

It is well known that progressive high-intensity high-volume resistance exercise facilitates an anabolic response in skeletal muscle tissue (Baechle & Earle, 2008). It seems reasonable to argue in theory that Cr supplementation may enhance the long-term hypertrophic response to resistance training by shifting the hormonal signals impinging on the muscle cells to the "anabolic" pole (Op't Eijnde & Hespel, 2001). It is well documented that signaling by growth hormone, testosterone and cortisol play a pivotal role in stimulating muscle protein synthesis in response to heavy resistance training (Op't Eijnde & Hespel, 2001; Baechle & Earle, 2008; Rahimi *et al.*, 2010). Thus, enhanced stimulation of GH and testosterone secretion (anabolic hormones) versus decreased secretion of cortisol (catabolic hormone) after bouts of heavy resistance training, could be supportive of muscle hypertrophy (Op't Eijnde & Hespel, 2001; Baechle & Earle, 2008). A more pronounced anabolic hormone response could also be significantly implicated in the repair and recovery of skeletal muscle after resistance exercise sessions and may subsequently play a vital role in muscle remodeling (Rahimi *et al.*, 2010).

On investigating their hypothesis that in response to resistance training Cr induces muscle hypertrophy through endocrine mechanisms, Rahimi *et al.* (2010) reported that Cr supplementation (20 g/day for 7 days) appeared to provide an enhanced exercise response that was evident from an increase in training volume and the number of repetitions performed. These changes appeared to result in greater increases in GH and testosterone levels in the Cr, compared to the placebo group during recovery after exercise (15 min post-exercise), reflecting an augmented anabolic hormone response. However, a study on the effect of a longer period of

training combined with Cr supplementation (22 - 27 weeks) demonstrated no benefit to integrated 24 h GH secretion in elite athletes (Peyrebrune *et al.*, 2005).

Androgens stimulate myogenesis, thereby causing hypertrophy of both Type I and Type II muscle fibres (Sinha-Hikim *et al.*, 2004). Testosterone (T) can be converted into a more bioactive metabolite, di-hydrotestosterone (DHT), by 5-alpha reductase (Van der Merwe *et al.*, 2009). Although the mechanisms by which T increases skeletal muscle mass are poorly understood, cell types that express androgen receptor (AR) protein are the targets of androgens such as T and DHT. In human skeletal muscle, AR protein is expressed in several cell types, including satellite cells, fibroblasts, vascular endothelial cells, smooth muscle cells and mast cells; with satellite cells and myonuclei being the predominant sites of AR expression (Sinha-Hikim *et al.*, 2004). These observations support the hypothesis that T and DHT increase skeletal muscle hypertrophy by acting on multiple cell types within the muscle to up-regulate their AR expression *in vivo* and regulate the differentiation of mesenchymal precursor cells (eg. satellite cells) (Sinha-Hikim *et al.*, 2004).

Van der Merwe *et al.* (2009) reported no effect of Cr supplementation (loading dosage and 14 days of maintenance dosage) on resting serum T levels in well-trained rugby players. However, levels of DHT increased significantly (56%) after seven days of Cr loading and remained 40% above baseline after a maintenance dosage was sustained for 14 days. The ratio of DHT:T also increased by 36% after Cr supplementation was kept up for seven days; moreover the ratio remained 22% above baseline after the maintenance regimen. The increase in DHT and DHT:T ratio after seven days of Cr loading was not seen at Day 21. Thus, there may be a dose-response to the amount of Cr ingested, and the maintenance dosage may not have been high enough to maintain the increased ratio (Van der Merwe *et al.*, 2009). In light of the foregoing it would seem that increases in T, DHT or the DHT:T ratio could be instrumental in the causation of positive effects of Cr on muscle mass and strength (Van der Merwe *et al.*, 2009).

In summary, Van der Merwe *et al.* (2009) and Vatani *et al.* (2011) contend that it is unlikely that increases in body weight and fat-free weight, secondary to Cr supplementation, are hormonally mediated. However, it has been well established that Cr supplementation can enhance the ability to perform resistance training workouts (Volek *et al.*, 1999; Op't Eijnde & Hespel, 2001; Willoughby & Rosene, 2001; Brose *et al.*, 2003; Burke *et al.*, 2003; Chilibeck *et al.*, 2004; Olsen *et al.*, 2006; Souza-Junior *et al.*, 2011). This "*ergogenic"* action of Cr intake may conceivably

enhance the acute hormonal responses to heavy resistance training and thereby facilitate the resulting physiological adaptations (Op't Eijnde & Hespel, 2001). Rahimi *et al.* (2010), who demonstrated elevated serum T and GH levels during post-training recovery, lend support to this theory. Even the study by Van der Merwe *et al.* (2009) supports this theory when the increase in DHT and DHT:T ratio reported is interpreted in light of the results pertaining to androgens, AR expression and muscle hypertrophy as explained by Sinha-Hikim *et al.* (2004). However, the persistent dearth of research results on the possible indirect humoral-mediated anabolic effect on muscle mass of Cr supplementation combined with resistance training precludes finality on the matter.

2.6.5.6 CONCLUSION

Most of the studies reporting on the effect of Cr supplementation on body weight found an increase of between 0.5 - 5 kg in both untrained and trained individuals under comparable conditions. The largest increases in body weight seem to be reported when longer-term Cr supplementation is combined with resistance training. Authors regularly attribute this increase in body weight to the osmotic nature of Cr, evident in the causation of body water retention. Since cellular hydration may act as an anabolic trigger it is conceivable that longer-term Cr supplementation may bring about increases in FFW through increased muscle protein synthesis. Short-term Cr loading, however, does not seem effective in significantly increasing the body water compartments or measures of skeletal muscle protein balance.

Increases in muscle fibre hypertrophy and muscle protein content have been observed with Cr supplementation. However, results mostly indicate that Cr does not stimulate muscle hypertrophy through endocrine mechanisms. Engaging in exercise, especially resistance training, seems to be a prerequisite for Cr-induced muscle hypertrophy and amplified hypertrophy. Louis *et al.* (2003*c*) found that, even when Cr was combined with resistance exercise, it induced a change of only ~2%/wk in muscle fibre cross-sectional area. They postulated that the methods used to determine muscle protein metabolism may not be sensitive enough to detect such small changes. Thus, smaller Cr-induced effects may not be detected unless studies incorporate much larger groups of subjects. To demonstrate this point Louis *et al.* (2003*c*) calculated that, to have detected a change in muscle protein synthesis or breakdown of 15% in their study, they would have required 27 and 58 subjects, respectively, to detect them with a power of 85% and a probability of 5%. Thus, it is clear that studies incorporating much larger population samples are needed.

The effect of Cr supplementation combined with resistance training on muscle hypertrophy seems to occur in two stages: an early stage and a late response. The early myogenic response seems to involve the interaction of various MRF (eg. myostatin and GASP-1) that may activate satellite cells to donate their nuclei to damaged and/or enlarging myofibres, thereby supporting and enhancing muscle repair and remodeling in response to resistance training. The late myogenic response (long-term hypertrophic response) may involve increased muscle protein synthesis and/or cell differentiation by the expression of IGF-I, IGF-II, androgens and/or other unknown factors that may shift the cellular status to its anabolic pole. However, the exact physiological reasons/mechanisms by which these factors operate in response to Cr supplementation need to be elucidated.

A variety of theories offer possible explanations for the concomitant increase in muscle strength that is demonstrated by many studies reporting increased body weight, FFW and/or muscle size with Cr supplementation: (i) increased cellular hydration status may stimulate increased muscle contractile protein (actin and myosin) synthesis, thereby increasing muscle contraction force; (ii) Cr supplementation in combination with resistance training may lead to an acute elevation in T. T can directly interact with the contractile apparatus of the muscle cell, thereby increasing contractile strength (Baechle & Earle, 2008); (iii) acute post-exercise elevations in androgens (eg. T and/or DHT) may lead to increased AR protein expression and/or AR protein interaction, thereby up-regulating satellite cell activity. This response may then enhance muscle fibre hypertrophy and post-exercise muscle fibre repair and remodeling, leading to enhanced recovery from exercise; and (iv) the long-term enhanced post-exercise recovery due to Cr supplementation may lead to better quality training sessions, thereby enhancing the training effect of exercise.

It remains important to note that the findings of studies undertaken to probe the effect of Cr supplementation on muscle hypertrophy, hormone levels and other MRF are inconsistent. Furthermore, the mechanisms behind these effects are largely unknown. The said inconsistency may be attributable to differences in subjects, blood sampling time, exercise mode, intensity, and duration, all of which present obstacles to comparisons between the studies (Saremi *et al.*, 2010).

2.6.5.7 RECOMMENDATIONS

- More research is needed before a clear verdict can be passed on the hydration theory of Cr supplementation and its link to muscle protein synthesis (Volek *et al.*, 2001).

- Future studies should continue to investigate the effects of Cr supplementation, and of Cr supplementation in combination with training, on different population samples (ie. trained and untrained) and various methods of exercise (Vatani *et al.* 2011).

- The possible impact of Cr intake on the intracellular signaling pathways involved in the regulation of muscle protein synthesis and breakdown needs to be addressed in greater detail (Saremi *et al.*, 2010).

- Future studies on the putative anabolic effects of Cr supplementation should be more comprehensive in terms of potential humoral and intramuscular effects (Van der Merwe *et al.*, 2009).

- Research on the possible additive hormonal effects of Cr supplementation in combination with training is limited. Areas that show promise and warrant further investigation include DHT, GH and T.

3. SAFETY OF CREATINE SUPPLEMENTATION

3.1 Profile of Cr users

Galen (180 AD.), who served as physician to the gladiators of ancient Rome, said: *"He (it) cures most successfully in whom the people have the greatest confidence"* (Greydanus & Patel, 2010 : 729). Today's athletes are taking various products in ever-increasing amounts because they are driven more or less obsessively by a desire to compete successfully in contemporary society (Greydanus & Patel, 2010). Also, as addressed later in this section, it is clear that recreational athletes, including university students, are consuming a variety of nutritional supplements to achieve the perceived glories of increased strength and performance and a desirable physique.

The term "doping" is derived from the Dutch word ''dop'' in reference to the practice of providing race horses with an opium mixture to act as a stimulant and enhance competitive performance of the animal "in the running" (Greydanus, 2009). Sports doping refers to an attempt to by fair

means or foul improve or stimulate sports performance in *Homo sapiens* in the eternal quest to emerge the victor from a bout of competitive sport (Greydanus & Patel, 2010). The promise of having a drug that is a true sports doping chemical often veils an underlying eternal quest for a product with an ergogenic quality, and this term is derived from the Greek words *érgon* (to work) and *gennan* (to gain) (Greydanus & Patel, 2010). Cr and other nutritional supplements are not drugs. These are, however, ingested to obtain a competitive advantage over opponents.

When comparing the results of different studies on the profile of Cr users, one has to be aware of the different methodologies used to obtain this data (Tscholl *et al*., 2010). In some studies (Dascombe *et al.*, 2010; Goston & Toulson Davisson Correia, 2010) participants were interviewed or data were verified by a researcher, but in the majority by far (Sheppard *et al.*, 2000; Froiland *et al.*, 2004; Jackson *et al.*, 2010; Tsitsimpikou *et al.*, 2011) a self-administered questionnaire was used and no objective measurements were included to cross-check the reported information, thus increasing the risk of inconsistent or incomplete data. The substance usage reported therefore have been underestimated as a result of participants' ignorance and - perhaps to a lesser extent – their negligence (Tscholl *et al.*, 2010). In an extensive study on world-class athletes, Tscholl *et al.* (2010) analysed the doping control forms of 3887 track and field (TF) athletes competing in 12 World-Championship events held under the regis of the International Association of Athletics Federations (IAAF) and one out-of-competition season for nutritional supplement intake. The study figures were derived from only a sub-selection of athletes - those who were submitted to doping control. The studies concerned differ according to the sporting codes at issue in each case, as follows: baseball (Froiland *et al.*, 2004), basketball (Froiland *et al.*, 2004), bowling (Froiland *et al.*, 2004), diving (Froiland *et al.*, 2004), field hockey (Dascombe *et al.*, 2010), football (Froiland *et al.*, 2004), gymnastics (Froiland *et al.*, 2004), kayaking (Dascombe *et al.*, 2010), netball (Dascombe *et al.*, 2010), rifle shooting (Froiland *et al.*, 2004), rowing (Dascombe *et al.*, 2010), soccer (Froiland *et al.*, 2004), softball (Froiland *et al.*, 2004), swimming (Froiland *et al.*, 2004; Dascombe *et al.*, 2010), tennis (Froiland *et al.*, 2004), track and field (Froiland *et al.*, 2004; Tscholl *et al.*, 2010; Dascombe *et al.*, 2010); volleyball (Froiland *et al.*, 2004), waterpolo (Dascombe *et al.*, 2010), wrestling (Froiland *et al.*, 2004), and the level of participation: international-level sport (Tscholl *et al.*, 2010), elite sport (Dascombe *et al.*, 2010), sport at university level (Froiland *et al.*, 2004) and physical exercise at recreational/health-club level (Sheppard *et al.*, 2000; Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010; Tsitsimpikou *et al.*, 2011).

The data elicited from a number of participants who took part in the survey covering a sporting/physical activity as a sub-section were duly included in the statistical analyses reported in individual studies. The subjects from the relevant studies included one boxer (Froiland *et al.*, 2004) and 3887 track and field athletes. Understandably therefore, the effectiveness of generalisations ventured in regard to nutritional supplements and Cr use remains speculative.

3.1.1 Cr use in athletes

Tscholl *et al.* (2010) analysed the out-of-competition doping control forms of TF athletes during one season (2007, 1 646 forms), as well as the forms relating to three consecutive outdoor world championships for adults (2003 - 2007, 1 292 forms), various indoor world championships for adults (2003 - 2008, 488 forms), three junior world championships (2004 - 2008, 367 under-20 TF athletes), and three youth world championships (2003 - 2007, 94 under-18 TF athletes). In total 67% of the athletes whose forms were perused reported using nutritional supplements. This amounted to 0.93 of a supplement per individual youth and junior athlete, and 1.78 supplements per adult athlete. It was found that Cr supplementation had increased significantly with age. The highest use of Cr was reported by male athletes participating in combined events (0.21 Cr supplement), power sports (0.19), and power/sprint sports (0.16 Cr supplement) per athlete. Athletes originating from Africa and Asia reported the lowest rate of Cr use. The researchers (Tscholl *et al.*, 2010) could not ascertain whether the athletes from these countries had been undersupplied with these substances, or whether athletes from other countries (not in Africa) had been oversupplied. No association was found between a higher use of supplement and a better ranking in the championships (Tscholl *et al.*, 2010). This study (Tscholl *et al.*, 2010) revealed a lower than expected rate of nutritional supplement (including Cr) use by international-level athletes.

Dascombe *et al*. (2010) reported on the nutritional supplementation habits, perceptions and knowledge of elite athletes (aged 21.9 ± 3.9 years) associated with a state-based sporting institute (Western Australia Institute of Sport). The vast majority (88%) of surveyed athletes (n = 72) in that instance reported using nutritional supplements, with no difference between female (86%) and male (89%) athletes. This is considerably more than the incidence reported by Tscholl *et al.* (2010). In that instance significantly more male athletes (22%) reported using Cr. The researchers (Dascombe *et al*., 2010) support the suggestion that the use of nutritional supplementation is rising among elite athletes.

As far as university-level male and female sports participants go, Froiland *et al*. (2004) reported 89% percent of the subjects (n = 203) had or were currently using nutritional supplements. Of these, 37% were consuming Cr.

In a study on male team-sport athletes (n = 100) between the ages of 15 and 18 years at selected high schools for boys in Johannesburg (South Africa), Gradidge (2010) found that 30% used performance-enhancing substances, including a fraction of 32% who reported using Cr for that purpose.

Thus, it is estimated that most junior and adult, male and female, elite athletes use nutritional supplements. Indeed, Cr consumption seems particularly popular with junior and senior male athletes participating in combined events, power sports and power/sprint sports. More research is needed to investigate the extent of Cr use in high school boys.

3.1.2 Cr use in non-athletes

Cr and nutritional supplement use in regularly exercising gymnasium members have been investigated in relatively large study populations. Two hundred and twenty nine members (male and female) of health clubs (civilians, n = 96, aged 33 ± 1 years and military personnel, n = 133, aged 30 ± 1 years) completed questionnaires on the use of Cr and other supplements (Sheppard *et al*., 2000). Of these participants, 57% of civilians and 29% of military personnel reported using of Cr or Cr-containing supplements.

In a more recent study done by Goston and Toulson Davisson Correia (2010) supplement intake was assessed in people who exercised regularly in gymnasiums (n = 1102, male and female participants aged 29 ± 11 years) in the city of Belo Horizonte, Brazil. Supplementation was reported by 37% of participants. Men consumed significantly more supplements than women (45% *versus* 28%). Eight percent reported Cr consumption (men only and predominantly in the age group < 30 years).

A closed-ended, anonymous questionnaire was answered by 329 subjects (180 men and 149 women) aged 30.6 ± 12 years from randomly selected gymnasium centres in Athens, Greece (Tsitsimpikou, *et al*., 2011). Forty-one percent of the participants reported using supplements of which the most popular were proteins/amino acids and vitamins (63% and 50%, respectively).

Younger male participants reported more consumption of nutritional supplements. Thirteen percent of the participants (only males) used Cr.

Jackson *et al.*, (2010) recently surveyed 200 university students (a representative sample including male and female participants) who were users of a campus recreation facility to assess the prevalence of nutritional supplementation, the types of supplements being used, and where users obtained information regarding these supplements. Dietary supplementation was reported at 44% of which Cr accounted for 29%. Ony one Cr user was female.

Thus it is established that the use of dietary supplements is prevalent among male and female users of gymnasium facilities. However, Cr use was found to be more prevalent among male than female respondents.

3.1.3 Polysupplementation

Results from the study by Jackson *et al.* (2010) confirmed that male university gymnasium users are likely to take a protein supplement as well as some type of Cr supplement, generally to increase muscle mass when used in conjunction with strength training (Jackson *et al.*, 2010). Polysupplementation (ie. the use of Cr and other anabolic dietary supplements) is prevalent among regular gymnasium visitors who use nutritional supplements (Sheppard *et al.*, 2000; Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010; Tsitsimpikou *et al.*, 2011). Goston and Toulson Davisson Correia (2010) found that 40% of participants in their study were using two or more products simultaneously. This rate of usage constitutes a potential public health concern (Sheppard *et al.*, 2000) because of the potential adverse effects of the other anabolic supplements (eg. protein/amino acids, dehydro-epiandrosterone (DHEA), beta-hydroxy beta-methylbutyrate (HMB) and anabolic-androgenic steroids) that may form part of this cocktail and whose safety is suspect. Also, elite athletes are in danger of testing positive for doping to the irretrievable detriment or ruin of a sports career. Anabolic androgenic steroids have been banned from sports for some time, and DHEA and its metabolites 7α-hydroxy-DHEA, 7β-hydroxy-DHEA and 7-keto-DHEA have recently been added to the World Anti-doping Agency's (WADA) list of banned substances (World Anti-doping Agency, 2012). Although the reported dosages of individual ingredients were often too low to patently achieve the purpose sought when taken individually, it is suggested that it is reasonable to expect that a combination of relatively low doses of several ingredients (eg. caffeine, Cr, branched-chain amino acids, whey

protein, and others) may well bring about increases in exercise performance, training volume and the maintenance of lean mass (Smith *et al.*, 2010).

3.1.4 Reasons for supplement use

Reasons for nutritional supplementation, including Cr, can be summarised as follows:

- Believing that performance (particularly strength and power) will be enhanced (Sheppard *et al.*, 2000; Froiland *et al.*, 2004; Dascombe *et al.*, 2010; Goston & Toulson Davisson Correia, 2010; Gradidge, 2010; Greydanus & Patel, 2010; Jackson *et al.*, 2010; Tscholl *et al.*, 2010; Tsitsimpikou *et al.*, 2011);
- Believing that heavy training increases supplement or nutrient requirements (Froiland *et al.*, 2004; Dascombe *et al.*, 2010);
- Idolising famous athletes (Calfee & Fadale, 2006; Boone, 2010); and
- A desire for muscle gain (Sheppard *et al.*, 2000; Froiland *et al.*, 2004; Calfee & Fadale, 2006; Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010).

As young athletes begin to model themselves on sport icons, they start to believe that ergogenic drugs are entirely acceptable, indeed necessary perhaps, to reach record-breaking performances in sports such as track and field, American football, and basketball (Calfee & Fadale, 2006). Ergogenic compounds that are commonly used by youths today include anabolic-androgenic steroids, steroid precursors (androstenedione and dehydro-epiandrosterone), growth hormone, Cr, and ephedra alkaloids (Calfee & Fadale, 2006; Gradidge, 2010). Reviewing the literature to date, it is clear that children are exposed to these substances at declining ages with use starting as early as middle school (Calfee & Fadale, 2006; Gradidge, 2010; Sobolewski *et al.*, 2011). The American College of Sports Medicine (ACSM) has recommended that Cr is not used by the pediatric population (anyone below 18 years of age) (Terjung *et al.*, 2000).

3.1.5 Users' knowledge of products

When reviewing reports on supplement use, it is evident that the large majority of both athletes and non-athletes do not consult with professionals (eg. dieticians and scientific support staff) when deciding on product use (Sheppard *et al.*, 2000; Dascombe *et al.*, 2010; Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010; Tsitsimpikou *et al.*, 2011). In the most recent study by Tsitsimpikou *et al.* (2011) regular users of gymnasium facilities professed that they considered their personal trainers and fellow gymnasium users to be the main purveyors of

trustworthy advice to them regarding nutritional supplement use. The media (internet, popular magazines and advertisements) are also considered a major credible source of information for athletes (Froiland *et al.*, 2004; Dascombe *et al.*, 2010; Gradidge, 2010) and non-athletes (Sheppard *et al.*, 2000; Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010; Tsitsimpikou *et al.*, 2011), followed by coaches (Froiland *et al.*, 2004; Dascombe *et al.*, 2010; Gradidge, 2010; Jackson *et al.*, 2010), shop assistants selling nutritional supplements (Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010), and friends and family (Froiland *et al.*, 2004; Dascombe *et al.*, 2010; Gradidge, 2010). Twenty-four percent of male and 27% of female participants in the relevant studies considered the advice or opinion of a fitness instructor, or employees at fitness facilities that they attended to be "experts" who could give definitive advice on any supplement they considered using (Jackson *et al.*, 2010). A considerable proportion of athletes (17%) (Dascombe *et al.*, 2010) and non-athletes (34%) (Goston & Toulson Davisson Correia, 2010) listed themselves as influential figures (ie. reliable sources of advice) on effective supplement usage.

3.1.6 CONCLUSION

More than a decade ago, Sheppard *et al.* (2000) described the typical Cr user as a young male engaged in resistance training primarily to improve strength, muscle mass and power. This statement remains true for regular users of gymnasium facilities. However, the extent of Cr supplementation (expressed as a percentage of overall nutritional supplement use) seems to have declined, possibly as a result of increased marketing (and subsequent consumption of) protein/amino acid powders. Cr use in athletes remains prevalent. In their extensive comparison of literature on Cr users, Sobolewski *et al.* (2011) conclude that university level-team sportsmen and elite male power athletes worldwide are the greatest consumers of Cr.

It seems that in the years that passed since Cr was first introduced to the South African market (1994), very limited progress had been made in educating consumers on safe and effective supplement use. Consumers worldwide still have limited specific knowledge of the proven effects, mechanisms of action and side-effects of Cr. Polysupplementation is widely practiced by athletes (Froiland *et al.*, 2004; Dascombe *et al.*, 2010; Tscholl *et al.*, 2010), team-sport athletes (Bishop, 2010) and non-athletes (Sheppard *et al.*, 2000; Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010; Tsitsimpikou *et al.*, 2011). Supplements that have been duly tested and found safe and efficacious when ingested individually, may have adverse effects when combined with other supplements (Bishop, 2010). Furthermore, athletes and non-athletes often

look to unqualified associates, and not to health-care professionals, for information on and assistance with supplementation (Sheppard *et al.*, 2000; Dascombe *et al*., 2010; Goston & Toulson Davisson Correia, 2010; Gradidge, 2010; Jackson *et al.*, 2010; Tsitsimpikou *et al.*, 2011). This ignorance, combined with largely unregulated manufacturing procedures within the nutritional supplement industry (Boone, 2010; Jackson *et al.*, 2010; Tscholl *et al.*, 2010), may endanger the health of consumers.

3.1.7 RECOMMENDATIONS

- Scientific and support staff at sporting institutes (Dascombe *et al*., 2010), as well as lecturers and health-care professionals at universities and schools (Boone, 2010), need to be more proactive and explicit in impressing on intending users the potential and actual risks and unfounded perceptions associated with attempts made at performance enhancement with the aid of nutritional supplements (Dascombe *et al*., 2010).

- Sport institutes need to ensure that athletes seek advice from scientific and support staff when planning their nutritional supplementation routine (Dascombe *et al*., 2010).

- The teenage athlete should be carefully counseled that there are few substances (if any) that consistently and safely improve the performance of a well-trained individual (Greydanus & Patel, 2010).

- The importance of balanced nutrition and scientific training in all population groups (with particular reference to the use of nutritional supplements), should be emphasised by all gymnasium personnel, health-care professionals, coaches and teachers.

- In accordance with the statement made by the ACSM (Terjung *et al.*, 2000), Cr supplements should not to be used by anyone who is younger than 18 years.

- More research is required to investigate the effects of polysupplementation on both performance and health (Bishop, 2010).

3.2 Product manufacturing and promotion

For thousands of years, humans have sought the use of medicines, herbs, and chemical substances reputed to have special properties to improve their lives in various ways (Greydanus

& Patel, 2010). In the same manner, for as long as competitive sports have existed, athletes have attempted to improve their performance by ingesting a variety of substances (Bishop, 2010). An early (well-documented) example that readily comes to mind is the chewing of coca leaves by Peruvian Indians serving as runners to convey messages across the Inca empire.

A considerable number of nutritional substances are being marketed specifically for athletes (Van der Merwe & Grobbelaar 2005). Nutritional supplementation is not deemed necessary for professional athletes with an adequate diet unless they are on a regimen of restricted energy intake (Tscholl *et al.*, 2010). A well-designed diet that meets the energy and nutrient intake needs and incorporates the proper timing of meals, is the essential foundation for the development of optimal training and performance (Bishop, 2010). However, athletes' declarations in the media or in antidoping control forms reveal that the use of dietary supplements is increasing among athletes (Baume *et al.*, 2006; Dascombe *et al.*, 2010; Tscholl *et al.*, 2010). It is common cause that ingestion of dietary supplements can enhance sport performance if it is used judiciously in conjunction with well-designed training (Bishop, 2010).

The term *"dietary supplement"* is defined as any product taken by mouth, in addition to common foods, that is reputed to have a performance-enhancing effect, while the term *"nutritional supplement"* implies that there is some nutritional value to the supplement (Bishop, 2010). These two terms are, however, often used synonymously. Athletes assume that these supplements (including Cr) do not contain prohibited substances because they are readily available without prescription and are sold legally by pharmacies and health shops (Van der Merwe & Grobbelaar 2005). As will be seen in this section, this may be a dangerous, or at least an injudicious assumption.

3.2.1 Production of Cr: regulations

In 1994 the Food and Drug Administration (FDA) of the United States government was mandated to enforce a newly promulgated statute called the Dietary Supplement and Health Education Act. Enforcement of this act allows the FDA to remove any dietary supplement from the market in the event that the product can be deemed unsafe for the consumer (Jackson *et al.*, 2010). Although this act provides some protection for the health of the consumer (also the consumer in South Africa), the manufacturer is relieved by this means of the responsibility of providing a safe product (Jackson *et al.*, 2010). The manufacturer will be inspected and held accountable only if and when consumers become ill or suffer side-effects that can be traced

back directly to the specific product. In such an event, in South Africa, the Medical Control Council (MCC) will inspect the premises to determine whether or not the guidelines for current Good Manufacturing Practice (cGMP) were adhered to. They will also test the quality of the product, and if it is deemed unsafe, will bring a lawsuit against the manufacturer and endeavour to remove the product from the shelves of suppliers. Thus, the manufacturers of dietary supplements are not held accountable for the purity/quality of their products *via* routine MCC inspections, which would be a prohibitive task, hence consumers are exposed to the major implied risk. Therefore individual manufacturers have to take responsibility for and set their own standards for product research and production (Jackson *et al.*, 2010).

The Consumer Protection Act (CPA) came into effect on 24 October 2010 and introduced radical and comprehensive law reform whereby the manufacturer, importer, distributor, or retailer of dietary supplements is held liable for damage (death or illness and economic loss) caused by unsafe or defective products, irrespective of the presence or absence of negligence (Neethling & Potgieter, 2008). Strict liability was thus introduced, but again can only be implemented if the consumer has been harmed in any way. However, a consumer who wishes to approach a court for damages must first try to enforce the relevant claim before other bodies (such as the commission, tribunal, ombud, consumer court, or alternative dispute resolution agent) (Neethling & Potgieter, 2008). A court has the authority to assess whether any harm has been proved and adequately mitigated; to determine the extent and monetary value of the damages, including economic loss; and to apportion liability among persons who are jointly and/or individually liable (Neethling & Potgieter, 2008). The CPA should make coaches, biokineticists and sports scientists think twice before selling/providing dietary supplements to athletes or other consumers.

The MCC proposed draft regulations for standards of manufacturing of complementary medicines (this includes herbal preparations, nutritional/dietary supplements and traditional medicines) to comply with the existing regulations for cGMP for medicines in South Africa (Medicines Control Council, 2010). South Africa is currently one of the countries that uphold the Pharmaceutical Inspection Cooperation Scheme (PIC/S). The South African version of the cGMP guide adheres to the prescriptions of the PIC/S (Medicines Control Council, 2010). The Minister of Health therefore proposes that complementary “medicines”/preparations be regarded and registered as medicines/drugs and comply with the same strict manufacturing rules and inspections that govern the production of pharmaceutical medicines. The parties to be affected

by these regulations (ie. the general public, manufacturers of nutritional/dietary/herbal supplements, distributors and suppliers) were given until 1 November 2011 to comment on and/or propose changes to the document. South Africa is, therefore, headed towards strict control of the contents, labeling and research requirements of dietary supplements. However, the date of final implementation of these regulations is still unknown.

3.2.2 The commercial product: purity concerns

A number of studies have investigated the purity of dietary supplements and Cr *via* mass spectrometric detection, gas chromatographic analysis and/or ultra-violet (UV) detection.

Baume *et al.* (2006) randomly purchased 103 dietary supplement products on the internet from websites in Europe and the USA. The supplements were screened for contamination with major anabolic steroid parent compounds, stimulants and traces of testosterone, nandrolone and their precursors. Results revealed that 18 products had been contaminated. The number of miss-labeled supplements represented 18% of the 103 products analysed. The most prevalent contaminant was the testosterone parent molecules (ie. precursors of testosterone). Five pro-hormone contaminants were detected in these samples. The two Cr products that were tested declared a content of Cr pyruvate and magnesium stearate on their labels. After these contaminated Cr products were ingested for three days by male volunteers at the recommended dosage specified on the packaging (5.25 g), the presence of the two main metabolites of nandrolone (19-norandrosterone and 19-noretiocholanolone) was detected in their urine with concentrations close to the official WADA limit of 2 ng/ml. Baume *et al.* (2006) concluded that with more prolonged treatment, the concentrations of these metabolites would certainly have exceeded the WADA limit and the urine samples would have been deemed positive for the purposes of declaring the outcome of anti-doping tests.

Tsitsimpikou *et al.* (2011) also detected synthetic anabolic steroid contamination in two of the 12 dietary supplements they tested (Cr was a component of some of these products). The presence of the contaminant (in both cases, stanozolol) was not stated on the products' labels.

In their extensive survey on the quality of 33 commercial Cr products sold in Italy and produced in different European countries and in the USA, Moret *et al.* (2011) found no significant contamination of Cr with heavy metals. They reported that contamination of Cr products with heavy metals have various causes: the raw materials, the reagents and the solvents used to

produce them, the tubing, the equipment and the instrumentation (reaction containers, electrodes, etc.) that can come into contact with the product (eg. lead is used in metallic alloys), and containers in which stocked or packed (eg. mercury and cadmium are employed in the plastic industry). However, they (Moret *et al.*, 2011) concluded that possible contamination of Cr products with heavy metals seems not to be of particular concern.

Crn is the major organic contaminant of Cr supplements (Moret *et al.*, 2011), probably because it is so reabily formed from Cr in non-optimised production or conservation conditions, for example in water under acidic conditions or at high temperatures (Moret *et al.*, 2011). Thus, the Crn content of Cr products may indicate the quality of these products. In the study by Moret *et al.* (2011) CrM supplements showed a high variability in their Crn content. Six out of the 23 tested samples (about 25% of total CrM samples) contained Crn levels above 100 mg/kg (maximum acceptable value). CrC demonstrated even higher instability, probably due to the higher instability of Cr at acidic pH, while the CrA samples had a higher Crn content on average than the CrM samples. The CrA products were also the most contaminated with dicyandiamide (a potentially toxic substance), while CrC had the lowest contamination level.

Research (Moret *et al.*, 2011) indicates that CrM powder (stored in unopened containers) remains stable for a long time (> 36 months), and that probably most of the Crn discovered in analysis is already present in the product before packaging. Thus, organic contamination is due to poor quality of raw materials.

Van der Merwe and Grobbelaar (2005) indicate that the intake of a prohibited substance in a nutritional supplement in the order of micrograms can cause an athlete to fail an anti-doping test. Products contaminated with 19-nor-4-androstenedione and 4-androstenedione were identified in a current study that has been screening over-the-counter nutritional supplements. Five healthy male volunteers received one capsule of one of these contaminated nutritional supplements to determine if the ingestion of these small amounts (0.02 - 0.06% of the usual dose of 50 mg) of prohibited anabolic substance would result in a positive anti-doping test. All the volunteers showed urinary concentrations of 19-norandrosterone above the WADA threshold of 2 ng/ml two hours post-administration. In two volunteers 19-norandrosterone above the threshold value could be detected at 36 hours post-administration. These results (Van der Merwe & Grobbelaar, 2005) were obtained after the ingestion of only one capsule of contaminated product. However, the recommended dosage according to the manufacturer was

four capsules three times per day, which can result in even higher and more persistent concentrations of 19-norandrosterone.

3.2.3 Industry ethics: the promotion of Cr products

The common belief that, when duly integrated with well-designed training, the appropriate ingestion of dietary supplements can enhance sport performance has given rise to a multibillion dollar industry that aggressively markets its products to athletes as performance enhancing, often without objective, scientific evidence to support such claims (Bishop, 2010). Also, the general public are still primarily reliant on the mass media which may or may not distribute accurate information on the effects of dietary supplements (Buford et al., 2007). In an industry that is not regulated very vigorously it can be safely assumed that numerous companies are likely to cut corners (ie. take risks) to maximise profits (Jackson *et al.*, 2010).

Studies that investigated the financial investment made by users of gymnasium facilities in dietary supplements (including Cr) reported that women spent less money than men (Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010). In university students, nearly three times more men (24%) than women (9%) were likely to spend more than US $50 per month on supplements (Jackson *et al.*, 2010). Goston and Toulson Davisson Correia (2010) reported that most Brazilian women spent up to US $30 per month, while the men spent more than US $30 per month on dietary supplements.

As explained before, Cr products are readily available as dietary supplements and are regulated by the USA Food and Drug Administration (FDA). In 1994 specifically, President Bill Clinton signed into law the Dietary Supplement Health and Education Act (DSHEA), also implemented in South Africa under the MCC, which allows manufacturers/companies/brands to make structure-function claims. However, the law strictly prohibits disease claims for dietary supplements. Thus, product-effect claims can be made for Cr in advertising. These claims are regulated by the Advertising Standards Agency of South Africa (ASA) which monitors the media in South Africa and is obliged by law to investigate and rule on any complaints that are made regarding deceptive/wrongful advertising of products. The permission to make structure-function claims in the advertisements of Cr-containing supplements is potentially dangerous considering the risk of contamination (Baume *et al.*, 2006; Moret *et al.*, 2011; Tsitsimpikou *et al.*, 2011) and mislabeling (Van der Merwe & Grobbelaar, 2005; Baume *et al.*, 2006) of these products. Thus,

as stated before, strict control of the contents, labeling and research requirements of dietary supplements are needed.

The International Society of Sports Nutrition declares in its position statement on Cr supplementation and exercise, that supplementation is an inexpensive and efficient means of increasing dietary availability of Cr without excessive fat and/or protein intake, since large amounts of fish and meat must be consumed in order to obtain gram quantities of Cr (Buford *et al.*, 2007). This position would have been acceptable if the purity of Cr products were undisputed. The same organisation also states (Buford *et al.*, 2007 : 1) that: "*If proper precautions and supervision are provided, Cr supplementation in young athletes is acceptable and may provide a nutritional alternative to potentially dangerous anabolic drugs*". The acceptability of this proposition is certainly debatable and it contradicts the ACSM's position that Cr users should at least be 18 years of age (Terjung *et al.*, 2000). It seems as if the committee members (Buford *et al.*, 2007) aim to have children use Cr rather than anabolic steroids. However, encouraging dietary supplement use in children, whether it be Cr or not, is a dubious practice fraught with safety and moral implications.

The search for an effective alternative to young athletes' current use of androgenic anabolic steroids (AAS) is understandable. According to the head of the South African Institute for Drug Free Sport, mr Khalid Galant, the use of AAS among young sportspeople has risen markedly in the past few years (Eckard, 2012). The reasons given for this tendency include: (i) the ever-increasing pressure on school rugby players to do their utmost to defend the honour of their schools; (ii) players' knowledge that only a few years at top-rugby can provide them with financial security for life; and (iii) a desire to enhance physical appearance (Eckard, 2012).

An interesting perspective on the marketing of sport supplements such as Cr is proposed by Boone (2010 : 2) who argues that the profession of exercise physiology forms part of the problem of promoting false and exaggerated claims about sport supplements: "*In a nutshell, the sport nutrition course is part of the exercise physiology curriculum. The instructor is typically an exercise physiologist with an interest in sport nutrition. More often than not, the sport nutritionist participated in athletics (eg. weight-lifting or football). Either past athletic experiences with using supplements or the present necessity for grant money renders the instructor vulnerable to the sport supplement industry. The shared values between the instructor and the industry set the stage for the promotion of supplement products within the sports nutrition course. The key point*

is that the sport nutrition instructor becomes an unannounced paid consultant for the supplement industry. As college teachers, they have a tremendous influence over student-athletes". It is beyond the scope of this thesis to elaborate further on the potential dangers of this byproduct of nutritional supplementation, except to say that the sport supplement industry and tertiary educational institutions more specifically, may be entering the epicentre of an ethical minefield.

3.2.4 CONCLUSION

The purity of most Cr products is not known, but their use remains popular. Research (Moret *et al*., 2011) indicates that the stability and efficacy of Cr products is highest in CrM but vary considerably in CrC and the patented form CrA. Given that large numbers of athletes and other members of the public are using Cr and dietary supplements on a regular basis (Dascombe *et al*., 2010; Goston & Toulson Davisson Correia, 2010; Jackson *et al*., 2010; Tsitsimpikou *et al*., 2011) while we know full well that the supplement industry is poorly regulated (Bishop, 2010; Jackson *et al*., 2010), biokineticists, sport scientists and consumers alike should be concerned. A shadow of suspicion is hanging over the quality and purity of sport supplements, including Cr, for reasons such as the possibility of contamination. It is therefore advisable that consumers give their preference to products obtained by manufacturers that ensure the highest quality-control standards and certify the maximum limit to which contaminants will be found in their products (Moret *et al*., 2011).

Cr supplementation is not currently banned by any athletic organisation. However, in the USA the National Collegiate Athletics Association (NCAA) does not allow institutions to provide Cr or other "muscle building" supplements to their student-athletes (eg. protein, amino acids, HMB, etc.) (Buford *et al*., 2007), thus obliging athletes to purchase Cr-containing supplements on their own (Buford *et al*., 2007). This rule may protect athletes from unscrupulous coaches or nutritionists providing them with contaminated products or benefiting financially from sales, and it certainly protects the institutions concerned from exposure to risk of procecution in the event of failing an anti-doping test. However, this arrangement does little to empower student-athletes with knowledge that will help them to select sport supplements judiciously for their personal use.

The fact that some Cr supplements are found to contain unlisted anabolic pro-hormones (Baume *et al*., 2006) and synthetic anabolic steroids (Tsitsimpikou *et al*., 2011), may lead to several and unintentional consequences with respect to the morphological appearance

(physique) and behaviour (characteristic conduct) of the users (Baume *et al.*, 2006). Depending on the time lapse after ingestion, however, these psychological and physiological effects may be dangerous and irreversible (Baume *et al.*, 2006).

The contamination of dietary supplements is a vexed problem for athletes, sport federations and anti-doping laboratories. Approximately one in five supplements on sale are contaminated - whether accidental or deliberate - with products that are not declared on the label (Baume *et al.*, 2006). It is proposed that in the South African market 7% of supplements are mislabeled or contaminated with prohibited substances (Van der Merwe & Grobbelaar, 2004). Track and field athletes seem to be at particular risk, given that they reportedly use dietary/nutritional supplements more than twice as often as soccer players and participants in other multisport events, which unfortunately increases their risk of failing anti-doping tests (Tscholl *et al.*, 2010). It is suggested (Hall & Judkins, 2008) that the sub-set of products that supplement the usual intake of substances that would enhance sporting performance (commonly referred to as *"sport supplements"*) are the first concern when addressing the issue of supplements that may be contaminated with substances prohibited by WADA. Other supplements that athletes may use, including weight-loss products, muscle-regulator products, vitamin C, multivitamins, magnesium and skimmed-milk products, have all tested positive for WADA banned substances in the past. The most widely prevalent among these contaminants were the anabolic steroid precursors DHEA and androstenedione (Hall & Judkins, 2008). Authorities, sports federations, team physicians and other clinicians prescribing supplements to competitors should be aware of these problems and should educate athletes on the risks involved with sport/dietary supplement use, as the intake of prohibited substances contained in contaminated supplements in amounts in the order of micrograms can cause an athlete to fail an anti-doping test (Van der Merwe & Grobbelaar, 2005).

University students and student-athletes consume significant amounts of dietary supplements and are thus prime targets for marketing campaigns. If we consider that the supplement industry is marketing its products by pandering to athletes' desire to win and recreational athletes' desire to build an impressive physique that signals physical powers to the observer, then such conduct must be regarded as ethically indefensible, since it is patently informed by a disregard for the user's safety. Whether it is marketing a product, teaching a sports nutrition course, or giving a patient a prescription drug, the need to adopt and maintain an ethically responsible course remains inescapable in all these matters (Boone, 2010).

Thus, many factors may be to blame for the increasing risk of health problems and/or positive anti-doping tests in the general public and/or athletes. An assumption that the supplement does as it claims, taking more of a supplement than is recommended in hopes of greater or faster results, and unknowingly ingesting synergistic combinations that contain the same or similar products are just a few of the ways that unregulated nutritional supplementation may be dangerous to the user (Jackson *et al.*, 2010).

3.2.5 RECOMMENDATIONS

- Product purity should be established before a clinical trial on Cr or Cr-containing supplements is conducted.

- Pure CrM is still the preferred (and safest) form of Cr supplementation.

- Products containing CrC and CrA should be avoided.

- All forms of dietary supplementation should be considered a risk factor that could lead to positive anti-doping tests that could seriously jeopardise the careers of elite athletes as a direct result of inadvertent contamination of raw materials and/or cross-contamination within the manufacturing process (Hall & Judkins, 2008).

- Nutritional supplements should be taken with due regard to and acceptance of WADA's strict liability rule, which states that an athlete is ultimately responsible for substances found in his/her body fluids irrespective of their origin. There is no guarantee that taking a nutritional supplement will not result in a positive doping case (Van der Merwe & Grobbelaar, 2005).

- Hall and Judkins (2008) propose that athletes and their coaches use the Informed-Sport web-directory (www.Informed-Sport.com) to choose products which have been screened for banned substances.

- Coaches, sports institutes, biokineticists and sports scientists should not sell/provide dietary supplements (including Cr) to athletes or other consumers.

- Selected nutritional supplements should be systematically reviewed by educated support staff at sports institutes to ensure physiological and performance-enhancement validity, financial viability for athletes, and to minimise the likelihood of attracting an anti-doping charge from the sport regulatory authorities (Dascombe *et al*., 2010).

- Athletes and support staff should steer clear of companies/suppliers that make exaggerated claims about the benefits to be derived from using their supplements.

- It is in the best interest of anyone proposing to take a dietary supplement to research the risks, benefits and potential side-effects associated with the active components in the product (Jackson *et al*., 2010).

- Strict and efficient regulation of the dietary-supplement and complementary medicine market in South Africa is long overdue. The government and MCC should prioritise implementation of the proposed legislation.

3.3 Possible side-effects of Cr supplementation

The dangers and adverse effects of some diet/energy supplements (ie. those used to assist weight loss and/or enhance physical performance) have been well documented over the years (Jackson *et al*., 2010) and have been a major cause for concern to the extent that consumers have been strongly advised against taking them (eg. ephedra, ma huang, DHEA and synephrine) (Williams, 2010). As explained in the previous section, any dietary supplement is attended by the risk of negative side-effects even if the prescribed regimen is followed. Furthermore, when excessive dosages are consumed in the hopes of quicker and better results the potential for health-threatening effects increases exponentially (Jackson *et al*., 2010).

Side-effects from Cr supplementation have been reported both anecdotally (Benzi, 2000; Benzi & Ceci, 2001; Persky & Brazeau, 2001; Shao & Hathcock, 2006; Buford *et al*., 2007) and in the scientific literature (Vandenberghe *et al*., 1996*a*; Persky & Brazeau, 2001; Buford *et al*., 2007; Bender *et al*., 2008). It is understandable, given its popularity, its widespread use, and use as an ergogenic aid, that the safety of Cr supplementation has been questioned in order to instill caution (Sobolewski *et al*., 2011).

3.3.1 Anecdotal claims

Anecdotal claims of side-effects experienced by those who supplement with Cr include dehydration, heat intolerance, muscle cramping, musculoskeletal injury and gastrointestinal distress (Shao & Hathcock, 2006; Buford *et al*., 2007; Sobolewski *et al.*, 2011). According to Buford *et al*. (2007) many of these have been attributable to the media and data taken from case studies, and are not supported by the scientific literature.

3.3.2 Side-effects reported in research studies

Of the scientific trials reviewed in this thesis, some report a complete absence of side-effects in both healthy (Kreider *et al.*, 1998; Mujika *et al.*, 2000; Bemben *et al.*, 2001; Op'T Eijnde & Hespel, 2001; Volek *et al.*, 2001; Kambis & Pizzedaz, 2003; Ostojic, 2004; Deldicque *et al*., 2008; Gotshalk *et al.*, 2008; Hadjicharalambous *et al.*, 2008) and diseased (Chang *et al.*, 2002; Cornelissen *et al.*, 2010) human populations. Some studies do not address the issue of side-effects (Volek *et al.*, 1997; Chwalbiñska-Moneta, 2003; O'Connor & Crowe, 2003; Gradidge, 2010; Smith *et al.*, 2010; Tsitsimpikou *et al.*, 2011), or report no difference in the incidence of side-effects between Cr and placebo groups (Greenwood *et al.*, 2003).

The two primary side-effects reported in clinical trials are weight gain and gastrointestinal upset. Weight gain, which is addressed fully in section 2.6.5 on the effects of Cr supplementation on body composition, is not considered to be a side-effect for the purpose of this thesis. Research subjects who reported gastrointestinal distress typically did so during the loading phase of Cr supplementation (Vandenberghe *et al.*, 1996*a*; Vandebuerie *et al.*, 1998; Sheppard *et al*., 2000; Kohler, 2001; Parise *et al.*, 2001; Bender *et al.*, 2008; Benton & Donohoe, 2011). In the study by Sheppard *et al*. (2000) the authors concluded that, within the limits of the study, the purported side-effects could not be attributed specifically to Cr supplementation since there was no association between Cr ingestion (users of Cr and other supplements *vs* users of Cr only) and reported gastrointestinal problems.

Shao and Hathcock (2006) attribute the gastrointestinal problems reported as the outcome of clinical trials dealing with the malabsorption of large doses of Cr, while Silber (1999 : 186) explains the incidence of diarrhea as follows: "*Uploading with pure Cr monohydrate will consequently increase its extra-cellular concentration. At critical moments, the mechanism of Cr self-regulation will trigger the down-regulation of the body's own Cr biosynthesis. The net effect is secondary hyper-ornithinemia and a drop of glutamine concentration in the intestinal mucosa,*

which is clinically manifested and commonly referred to as 'irritated stomach". According to Silber (1999) this metabolic distortion (the down-regulation of Cr biosynthesis and ensuing diarrhea) can be prevented by restricting Cr intake to 2 g/day.

The timing of Cr ingestion may also influence its effect. Since it has been known to result in post-exercise distress and even syncope, it is considered inadvisable to ingest large doses of Cr while exercising (Vandebuerie *et al.*, 1998).

3.3.3 Other concerns

3.3.3.1 Thermoregulation

Dehydration (the process of losing TBW) can impair aerobic performance, exertion of maximal strength, and anaerobic muscular power (González-Alonso *et al.*, 2008; Horswill & Janas, 2011; Sobolewski *et al.*, 2011). It also presents challenges for thermoregulation. Sobolewski *et al.* (2011) note that it was shown in a landmark article by Montain and Coyle (1992) that the extent of dehydration was proportional to the increase in cardiovascular strain and core temperature. Thus, when environmental conditions (hot and humid) heighten core temperature, dehydration will further increase cardiovascular strain (González-Alonso *et al.*, 1997; González-Alonso *et al.*, 2008; Sobolewski *et al.*, 2011). Cardiovascular strain is typified by reductions in cardiac output (heart rate and stroke volume), skin and locomotor-muscle blood flow, and systemic and muscle-oxygen delivery, together with marked dehydration and hyperthermia during participation in the prolonged and intense exercise of summer sports (González-Alonso *et al.*, 2008).

Perhaps the greatest safety concern of Cr is its potential impairment of hydration status and thermoregulation during exercise (Sobolewski *et al.*, 2011). Concerns are based on the premise that Cr is an osmotically active substance (as explained in section 2.6.5) causing an alteration in fluid balance by increasing intracellular fluid volume (Persky & Brazeau, 2001; Hadjicharalambous *et al.*, 2008) and preventing fluid from entering the extracellular environment to aid thermoregulation during exercise performed while high ambient temperature prevail (González-Alonso *et al.*, 1997; Sobolewski *et al.*, 2011). The ACSM therefore currently recommends that high-dosage Cr supplementation (ie. 20 g/day) be avoided during periods of increased thermal stress, such as sports activities performed while high ambient temperature and humidity prevail (Terjung *et al.*, 2000).

However, Sobolewski *et al.* (2011) argue that exercising while ambient temperature and humidity conditions are high the increase in intracellular fluid volume may result in one of two possibilities:

- *Impaired thermoregulation:* As dehydration reduces plasma volume, intra-cellular Cr will continue to act as an osmotically active substance holding fluid within the cell and preventing fluid availability to the extracellular compartment for thermoregulation. The risk of heat illness is therefore increased; or
- *Improved thermoregulation:* As dehydration reduces plasma volume, the hyper-osmotic activity of the extracellular fluid will be greater than the osmotic activity of Cr. The hyper-hydrated muscle cells will thus release fluid into the extracellular compartment for thermoregulation, thereby decreasing the risk of heat illness.

Studies that report on the physiological effects of high-dosage Cr supplementation on thermoregulatory responses indicate either no difference (Volek *et al.*, 2001; Sobolewski *et al.*, 2011) or improved ability of healthy subjects to tolerate exercise in high ambient temperatures (Greenwood *et al.*, 2003; Hadjicharalambous *et al.*, 2008; Sobolewski *et al.*, 2011). It is therefore suggested that Cr does not significantly influence temperature regulation, ie. rectal temperature (Volek *et al.*, 2001; Hadjicharalambous *et al.*, 2008), heart rate (Volek *et al.*, 2001; Hadjicharalambous *et al.*, 2008), blood pressure (Volek *et al.*, 2001) and sweat rate (Volek *et al.*, 2001) during exercise in a hot and humid environment and may actually have a positive influence on core temperature and heart rate responses (Hadjicharalambous *et al.*, 2008; Sobolewski *et al.*, 2011).

However, a small decrement in plasma volume is expected due to the apparent cellular fluid retention effects of Cr (Terjung *et al.*, 2000; Sobolewski *et al.*, 2011). The ACSM thus advises combative athletes who need to "*make weight*" (ie. lose 5% of body mass in 5 days), or individuals wishing to control weight and who are subjected to strenuous exercise and/or high ambient temperatures, to avoid Cr supplementation (Terjung *et al.*, 2000).

It was previously reported (Davis & Bailey, 1997) that a high brain [5-HT] : [DA] ratio increases effort perception (ie. central fatigue) during prolonged exercise, while a low [5-HT] : [DA] ratio may favour increased arousal and central neural motivation. In their study on the effect of Cr supplementation on the performance to exhaustion of endurance-trained males when ambient temperatures and humidity are high, Hadjicharalambous *et al.* (2008) reported that Cr

influenced key modulators of brain serotonin (brain 5-HT) and dopamine (DA) function. Since participants reported a significant reduction in effort-perception during exercise following Cr supplementation when high ambient temperatures prevail, the authors concluded that the increased hydration status brought about by the Cr ingestion lowered plasma free-tryptophan concentration (free-[Trp]), and subsequently lowered brain 5-HT synthesis. Thus, the resulting higher DA levels protected against central fatigue development (Hadjicharalambous *et al.*, 2008).

To conclude, when recommended amounts of Cr are consumed and normal body hydration is maintained, Cr supplementation does not present additional heat risk or hinder thermoregulation and may actually lower body core temperature and heart rate responses during exercise in warm and humid conditions (Sobolewski *et al.*, 2011).

3.3.3.2 Muscle and other injuries

As explained above, anecdotal claims of side-effects experienced by those who supplement with Cr include muscle cramping. However, in the clinical studies reviewed for this thesis, only three specifically reported on muscle cramps as a side-effect. Sheppard *et al.* (2000) reported that 15% of military personnel and civilian members of health clubs that used Cr experienced muscle cramping, while the same side-effect was evident in only one rugby player (Kohler, 2001). Hickner *et al.* (2010) reported muscle cramps at rest in two cyclists ingesting Cr at a low dosage (3 g/day for 28 days). Deldicque *et al.* (2008) argue that the higher occurrence of muscle cramps in basal conditions may be due to a bias related to the level of physical activity of sports people during the week preceding their sports event or exercise session. Thus, since Cr supplementation can be reasonably expected to enhance exercise intensity, athletes may over-exert themselves during training and thereby bring about muscle cramping due to muscle fatigue or electrolyte imbalances. However, this last statement is contradicted by Greenwood *et al.* (2003) as explained later in this section.

Proponents of the theory that Cr may promote a higher incidence of muscle injuries postulate that because Cr supplementation may promote rapid gains in strength and body weight the athlete may run a risk of exposure to enhanced stress on muscles, bones, joints, ligaments and connective tissues (Greenwood *et al.*, 2003). It was also hypothesised that the rapid fluid retention and "*dry matter growth*" evident after Cr supplementation may cause an increase in musculo-tendonous stiffness (Watsford *et al.*, 2003). One would expect either one, or both, of

these scenarios to increase the chance of injury during exercise. However, in the study conducted by Watsford *et al.* (2003) musculo-tendonous stiffness of the triceps did not occur after high-dosage Cr ingestion. In fact, performance indices that may have been negatively affected by musculo-tendonous stiffness, namely countermovement jump height and 20 cm-drop jump height, significantly improved after Cr loading. The investigators (Watsford *et al.*, 2003) concluded that their study did not support anecdotal evidence that Cr supplementation causes muscular strain injuries.

One clinical study indicates that Cr supplementation may reduce the frequency of muscle and other injuries in sportsmen. Greenwood *et al.* (2003) investigated the effects of Cr supplementation (0.3 g/kg/day for five days followed by 0.03 g/kg/day for the rest of the season) on the incidence of muscle cramping and injury observed during one season of NCAA division 1A football training and competition. Cr users (n = 38) had significantly less heat illness or dehydration, muscle tightness, muscle cramps and total all-cause injuries than non-users. The impression was gained that Cr supplementation allowed the athletes to tolerate training to a higher degree, thus lessening the incidence of injury (Greenwood *et al.*, 2003).

Finally, Chang *et al.* (2002) reported a decrease in the frequency of haemodialysis-associated muscle cramps (HAMC) in patients ingesting 12 mg CrM before each dialysis session for four weeks. Muscle cramp is a common complication of haemodialysis. One of the possible mechanisms of HAMC is the disturbance of muscle energy metabolism (Chang *et al.*, 2002). The investigators (Chang *et al.*, 2002) reported that the frequency of HAMC decreased by 60% in the CrM treatment group. Thus, the increase in the ability of skeletal muscle to regenerate ATP rapidly due to improved TCr stores after Cr loading (Rawson *et al.*, 2004; Deldicque *et al.*, 2005; Hadjicharalambous *et al.*, 2008) may benefit this specific group of patients. The same mechanism may be partly responsible for the decrease in the incidence of muscle cramps reported by Cr-consuming athletes.

In summary, anecdotal evidence suggesting that Cr supplementation causes muscle cramps and muscular strain injuries is not supported by clinical studies. Evidence suggests that Cr supplementation may even lessen the incidence of these conditions in athletes.

3.3.4 CONCLUSION

Overall, Cr ingestion in the recommended dosages is well tolerated. The ACSM (Terjung et al., 2000), the ISSN (Buford *et al.*, 2007), and results from clinical studies (Volek *et al.*, 2001; Greenwood *et al.*, 2003; Watsford *et al.*, 2003; Shao & Hathcock, 2006; Hadjicharalambous *et al.*, 2008; Sobolewski *et al.*, 2011) maintain that media reports of links between Cr use and muscle strains, muscle cramps, heat intolerance, and other side-effects are not supported by scientific literature. It appears that the type of activities and conditions in which athletes train and compete may expose them to a greater risk of dehydration, cramping or injury than Cr supplementation (Greenwood *et al.*, 2003).

3.3.5 RECOMMENDATIONS

- Biokineticists, sport scientists and sports physicians should keep in mind that they are working with human subjects, and thus need to evaluate statistically significant data on the safety of Cr supplementation by striking a healthy balance between skepticism and open-mindedness (Juhn, 2000).

- Professionals involved in the training and medical supervision of athletes (ie. coaches, athletic trainers, researchers, strength and conditioning coaches, nutritional consultants, administrators and sport governing bodies) should re-examine the methods used to train and manage athletes (ie. training in extreme climates, exhaustive conditioning drills, hydration practices, etc.) and make changes where appropriate to protect athletes' health (Greenwood *et al.*, 2003).

- If gastrointestinal upset is experienced, Cr intake should be reduced to 2 g/day (Silber, 1999).

- Athletes should consume healthy amounts of water and electrolytes when supplementing with Cr and training in conditions of high temperatures and humidity (Juhn, 2000; Terjung *et al.*, 2000).

- Severe or recurrent cramps should be investigated to eliminate untoward clinical conditions such as: electrolyte disorders, muscle enzyme deficiency and sickle-cell trait (Terjung *et al.*, 2000).

- Athletes who need to "*make weight*" and individuals wishing to control weight and who are subjected to strenuous exercise and/or hot environments, should avoid Cr supplementation (Terjung *et al.*, 2000).

- According to the ACSM, the lack of adverse effects of Cr supplementation does not equal safety. Research must be performed on a continuous basis to eliminate any possibility of theoretical complications (Terjung *et al*., 2000).

3.4 Clinical chemical pathology of Cr supplementation

As the body of evidence supporting the beneficial effect of Cr supplementation on performance continues to grow (Shao & Hathcock, 2006) its use has spread among both sportspeople (Dascombe *et al*. 2010; Gradidge, 2010; Tscholl *et al.*, 2010) and the physically active (Sheppard *et al.*, 2000; Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010; Tsitsimpikou *et al.*, 2011). Although there is no conclusive evidence concerning the possible health risks of long-term Cr supplementation (Schröder *et al.* 2005), charges to that effect persist (Shao & Hathcock, 2006). Since cases of drug abuse by professional athletes have been attracting more public notice in recent years, the interest of scientists and the general public has become increasingly focused on the potential risk of harmful effects that may be caused by ergogenic supplements (Schröder *et al.*, 2005).

3.4.1 Clinical safety

3.4.1.1 Kidneys

In clinical studies several marker compounds are used to assess renal function, namely blood creatinine (S-Crn) and urea (S-Urea) levels (Bishop *et al.*, 2000; Hathcock *et al.*, 2006; Shao & Hathcock, 2006), and urinary albumin and inulin clearance (Shao & Hathcock, 2006).

S-Crn is a relatively insensitive monitor of kidney function, hence kidney function may deteriorate more than 50% before the change shows up in measurements (Bishop *et al.*, 2000). Because of the difficulties encountered in analysing the small amount of Crn normally present, several authors have suggested that the use of S-Crn does not provide needed sensitivity in the detection of mild renal dysfunction (Bishop *et al.*, 2000). Despite these problems, S-Crn remains the most commonly used monitor of renal function (Bishop *et al.*, 2000; Hathcock *et al.*, 2006; Shao & Hathcock, 2006). When S-Crn is elevated above normal, the renal glomerular filtration

rate (ie. Crn clearance) is reduced, indicating renal damage: Crn clearance = urine Crn ÷ plasma Crn (Bishop *et al.*, 2000).

Reference intervals for S-Crn vary with assay type, age and sex (Bishop *et al.*, 2000). In the present study, the reference ranges for males aged 18 – 20 years were established at 62 – 115 µmol/ℓ (Niehaus & Ungerer, 2003).

As indicated above, the degradation of Cr and PCr in humans is, for the most part, a spontaneous non-enzymatic process (Wyss & Kaddurah-Daouk, 2000). *In vivo*, once this irreversible conversion happens (Greenhaff, 1995), Crn passively diffuses out of the body cells. It is removed from circulation by glomerular filtration in the kidney (Bishop *et al.*, 2000) and excreted into the urine (Wyss & Schulze, 2002; Brudnak, 2004). Thus, S-Crn levels reflect both the rate of Cr turnover and renal function (Bishop *et al.*, 2000; Hathcock *et al.*, 2006).

It is expected that Cr supplementation would increase the daily rate of Cr turnover (as a result of the dietary-induced increase in muscle Cr stores), and thereby Crn clearance (Hultman *et al.*, 1996; Kreider *et al.*, 1998; Robinson *et al.*, 2000; Schröder *et al.*, 2005; Taes & De Vriese, 2005). Therefore, concerns have been raised that Cr supplementation may increase renal stress or may lead to renal dysfunction (Benzi, 2000; Kreider *et al.*, 2003; Schröder *et al.*, 2005; Buford *et al.*, 2007). Indeed, Derave *et al.* (2004) demonstrated that if the total daily load of Cr is: 8.8 g (by oral supplementation) plus a presumed dietary intake of 0.5 – 2 g, plus an unknown amount of endogenously synthesised Cr, a minimum of 9.3 g of Cr will be added to the TCr pool and between 5.2 g and 6.7 g will be excreted as Cr and Crn. After one, 10, and 20 weeks of Cr supplementation the fractional excretion will increase to ~100% of the total amount of dietary Cr, thus indicating a saturation of the body Cr content (Derave *et al.*, 2004). In light of this abundance of body Cr and nearly complete excretion of ingested Cr (Derave *et al.*, 2004), it is reasonable to expect strain to be placed on the kidneys. In addition, Poortmans and Francaux (1999) postulated that the high nitrogen content (32%) of Cr could induce renal hyper-filtration and thus induce further strain on the kidney if taken in large excess for a long period of time.

Elevated S-Crn levels in athletes should be interpreted with care, however. The regular and high-intensity training performed by this population may induce protein degradation and thereby lead to an increase in S-Crn (Schröder *et al.*, 2005) regardless of whether they are using Cr or not (Kreider *et al.*, 2003). A number of studies have demonstrated baseline Crn levels at the

upper end of normal, or above normal values in athletes (Mayhew *et al.*, 2002; Kreider *et al.*, 2003; Schröder *et al.*, 2005). The increased baseline S-Crn levels found in these studies may be interpreted as the ability of healthy, well-trained athletes to maintain a greater training volume rather than as a direct consequence of Cr supplementation (Kreider *et al.*, 1998; Mayhew *et al.*, 2002). Also, large variations in renal markers are apparently normal for this population (Kreider *et al.*, 2003).

Several publications have investigated the effect of repeated doses of Cr on S-Crn levels. Some investigators (Kreider *et al.*, 1998; Robinson *et al.*, 2000; Schröder *et al.*, 2005; Armentano *et al.*, 2007) reported significant increases in fasting S-Crn levels after Cr loading (15.75 - 20 g/day for 5 - 28 days), and after subsequent supplementation with the maintenance dosage (3 - 5 g/day) (Robinson *et al.*, 2000; Schröder *et al.*, 2005; Armentano *et al.*, 2007). Studies have also indicated no difference in fasting S-Crn levels between the athletes included in placebo/control groups and athletes who are supplementing with Cr (Poortmans & Francaux, 1999; Parise *et al.*, 2001; Mayhew *et al.*, 2002; Kreider *et al.*, 2003; Mendes *et al.*, 2004). Longer-term studies relating to changes in fasting Crn levels over time between Cr and control/placebo groups demonstrated no significant change even though levels were initially elevated in pre-tests (Schröder *et al.*, 2005), or remained elevated (Robinson *et al.*, 2000; Kreider *et al.*, 2003) during post-tests.

It is generally accepted (Kreider *et al.*, 1998; Robinson *et al.*, 2000; Mayhew *et al.*, 2002; Hathcock *et al.*, 2006; Shao & Hathcock, 2006; Deldique *et al.*, 2008) that these elevations are not indicative of renal damage but probably reflect an increased rate of muscle Crn formation as a result of the dietary induced increase in muscle Cr stores. According to the risk assessment of Shao and Hathcock (2006), concerns about high-dosage Cr usage causing kidney damage are based solely on a total of two published case reports. Both comprehensive literature reviews and expert panels have maintained that there is no conclusive evidence to support the notion that Cr may adversely affect kidney function in healthy individuals (Shao & Hathcock, 2006).

The adverse side-effects of Cr supplementation on renal function cannot be excluded entirely unless all kidney function parameters (eg. urinary Crn, 24 h urinary Crn clearance and urinary albumin) are measured (Bishop *et al.*, 2000; Schröder *et al.*, 2005). Although administering some of these measures (eg. 24 h urinary Crn clearance) poses significant difficulties, more studies are necessary to address this important issue (Schröder *et al.*, 2005).

Urea constitutes the major excretory product of protein metabolism. After synthesis in the liver it is carried in the blood to the kidney, where it is readily filtered from the plasma by the glomerulus (Bishop *et al.*, 2000). The level of serum urea (S-Urea) is governed by renal function and perfusion, the protein content of the diet, and the amount of protein catabolism (Bishop *et al.*, 2000). The term *"blood urea nitrogen"* (BUN) is commonly used when referring to urea measurement because historical assays for urea were based on nitrogen measurement (Bishop *et al.*, 2000). Because of the amino acid structure of Cr, concerns have been raised about the possibility of renal dysfunction resulting from its catabolism (Benzi, 2000). In the present study, the reference ranges for S-Urea in males aged 19 - 20 years were established at 3.5 - 7.5 mmol/ℓ (Niehaus & Ungerer, 2003).

Robinson *et al.* (2000) reported increased S-Urea levels six weeks after Cr loading at 20 g/day for 5 days. This elevation was evident, however during the maintenance stage (3 g/day for 9 weeks) of the study. The authors (Robinson *et al.*, 2000) suggested that the increase in S-Urea was of little clinical significance and unlikely to be a direct result of Cr supplementation. They (Robinson *et al.*, 2000) support this statement by citing the lack of any difference in S-Urea concentration that was demonstrated both on the day after the Cr loading regimen and after more chronic supplementation (9 weeks). Other studies (Poortmans & Francaux, 1999; Crowe *et al.*, 2003) found no significant differences in S-Urea levels between athletes in placebo/control groups compared to groups supplementing with Cr (varying dosages of Cr for periods of 10 months to 5 years, and 3 g/day Cr ingested routinely for 6 weeks ingested as a Cr-and-HMB combination supplement).

Microalbuminuria (specifically, the loss of glomerular membrane integrity) is an early indicator of renal disease. The term *"microalbuminuria"* is used to describe unusually large albumin concentrations in the urine that are nevertheless not detectable with common dipstick assays (Bishop *et al.*, 2000). As the glomerular lesions become more severe, proteins of all sizes will pass into the urine (Bishop *et al.*, 2000). In the present study reference intervals for U-Microalbumin in males aged 18 - 20 years were established at 0 - 19 mg/ℓ (Niehaus & Ungerer, 2003).

In conclusion, there have been studies that reported renal dysfunction accompanying oral Cr supplementation in humans (Pritchard & Kalra, 1998; Revai *et al.*, 2003), but these were case reports of kidney impairment in a patient with: (a) pre-existing renal disease (Pritchard & Kalra,

1998) and (b) a history of continuously taking methandion in large quantities (Revai *et al.*, 2003). This persuasive circumstantial evidence linking Cr supplementation to renal dysfunction has been questioned and is thus considered to be inconclusive (Greenhaff, 1998; Bizzarini & De Angelis, 2004; Hathcock *et al.*, 2006).

With respect to clinically relevant markers of kidney function, none of the studies reviewed (Kreider *et al.*, 1998; Poortmans & Francaux, 1999; Robinson *et al.*, 2000; Crowe *et al.*, 2003; Kreider *et al.*, 2003; Schröder *et al.*, 2005; Hathcock *et al.*, 2006; Shao & Hathcock, 2006; Armentano *et al.*, 2007) showed clinically relevant changes in S-Crn or S-Urea that would indicate kidney damage or functional restriction. Individuals who ingest Cr will frequently have elevated Crn levels. This is normal and represents an increased rate of muscle Cr conversion to Crn, rather than abnormalous kidney function (Hathcock *et al.*, 2006). However, none of the longer-term studies have reported on the safety of Cr use for university students who participate in sport and/or attend campus gymnasiums. These individuals are most at risk as they represent the target population for dietary supplement and Cr sales (Sobolewski *et al.*, 2011). Clientele visiting gymnasiums for regular exercise sessions also tend to use multiple supplements simultaneously (Sheppard *et al.*, 2000; Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010; Tsitsimpikou *et al.*, 2011).

3.4.1.2 Electrolyte balance

Electrolyte homeostasis is essential for health since a disturbance of this balance can have severe clinical repercussions (Schröder *et al.*, 2005). Sodium is the primary extracellular cation in the human body and is excreted principally through the kidneys (Bishop *et al.*, 2000). Potassium is the main intracellular cation. Precise regulation of its concentration, which is critical for cellular metabolism, is controlled chiefly by renal means (Bishop *et al.*, 2000). Thus, an electrolyte imbalance can be considered an indirect indicator of renal dysfunction (Schröder *et al.*, 2005). Potassium is particularly important in the pathogenesis of cardiovascular disease and sudden cardiac death (Schröder *et al.*, 2005).

In the present study, the reference ranges for electrolytes in males >13 years were set as follows (Niehaus & Ungerer, 2003):
S-Sodium: 133 – 148 mmol/ℓ
S-Potassium: 3.5 – 5.1 mmol/ℓ.

It has been suggested that Cr supplementation alters fluid balance and/or electrolyte status (Kreider *et al.*, 2003). This concern is based on studies that reported increases in TBW after Cr loading (Volek *et al.*, 2001; Hadjicharalambous *et al.*, 2008), which suggested that short-term Cr supplementation may increase fluid retention. It has been speculatively argued that this potential increase in fluid retention dilutes electrolytes and predisposes athletes to muscle cramps (Kreider *et al.*, 2003).

Robinson *et al.* (2000) and Kreider *et al.* (2003) have reported increases in serum sodium and potassium levels in response to Cr ingestion. However, results from these studies do not support the electrolyte-dilution hypothesis. Differences between Cr and control groups were negligible and of no physiological or clinical significance, and were unlikely to be attributable to supplementation (Robinson *et al.*, 2000; Kreider *et al.*, 2003). Schröder *et al.* (2005) reported no alterations in plasma concentrations of magnesium, potassium and calcium. Plasma potassium values were constant, and in the normal clinical range, between all time points in the study (Schröder *et al.*, 2005).

In conclusion, findings (Robinson *et al.*, 2000; Kreider *et al.*, 2003; Schröder *et al.*, 2005) indicate that changes caused in electrolyte status by Cr supplementation are not clinically significant.

3.4.1.3 Liver

Serum levels of the enzymes aspartate amino-transferase (AST), alanine amino-transferase (ALT) and lactate dehydrogenase (LDH) are often referred to as "*liver function tests*" because concentrations of substances are so often elevated in liver disease (Korones *et al.*, 2001). S-AST is considered to be "*liver specific*" and is highest in acute hepato-cellular disorders (liver damage) and hepato-biliary disease (liver disease) (Bishop *et al.*, 2000). Gamma glutamyl-transferase (GGT) is used in association with S-AST to indicate liver damage (Bishop *et al.*, 2000). Because of the effect of alcohol on S-GGT activity, GGT assays are considered sensitive indicators of alcoholism. Enzyme elevations in alcoholics and heavy drinkers are 2 - 3 times the upper normal range (Bishop *et al.*, 2000). Thus, any interpretation of GGT levels must be done with due allowance for the related effects of drugs and alcohol (Bishop *et al.*, 2000). S-LDH levels are elevated in a variety of disorders (eg. cardiac, hepatic, skeletal muscle and renal diseases). An elevated total S-LDH value is therefore a rather non-specific finding (Bishop *et al.*, 2000).

In the present study, the reference ranges for liver enzymes in males were established as follows (Niehaus & Ungerer, 2003):
S-AST: 7 - 41 U/ℓ
S-ALT: 7 - 40 U/ℓ
S-GGT: 11 - 49 U/ℓ
S-LDH: 266 - 500 U/ℓ

Prothrombin is a coagulation protein. Prothrombin coagulation time (PT) (ie. the velocity of blood clotting) is commonly prolonged in liver disease and is usually measured to quantify the severity of liver damage (Robert & Chazouillères, 1996; Bishop *et al.*, 2000).

Variability in thromboplastin reagents used to perform PT analysis leads to large inter-laboratory differences in PT results (Robert & Chazouillères, 1996), with the result that it has been recommended that PT be standardised on the basis of an international normalisation ratio (INR). In patients with liver failure, the INR fails to yield a PT expression independent of the thromboplastin used and only activity percentage expression (%) provides a common international scale for PT reporting (Robert & Chazouillères, 1996).

PT is increased (ie. coagulation time is delayed) when the damaged liver is unable to manufacture adequate amounts of blood clotting factors (eg. prothrombin). In the present study reference intervals for prothrombin time in males aged 18 - 20 years were established as follows (Niehaus & Ungerer, 2003):

Prothrombin index (percentage activity): 70 - 130%
Prothrombin INR: 0.85 - 1.15.

To date there are no known studies that measured PT as indicator of liver pathology after/during Cr supplementation.

In summary, concerns have been raised that Cr supplementation may increase liver damage (Kreider *et al.*, 1998; Juhn & Tarnopolsky, 1998; Kreider *et al.*, 2003). It is reasonable to assume that the adverse effects of Cr supplementation, if any, would alter plasma concentrations or activity occurring at levels above clinical markers (Schröder *et al.*, 2005).

Kreider *et al.* (1998) demonstrated that exercise, with or without Cr supplementation, elevates efflux of liver enzymes (S-ALT and S-LDH). These findings indicate that even mildly elevated liver enzyme values found in athletes may be indicative of training intensity and not of liver damage (Kreider *et al.*, 1998). Indeed, studies have been reported according to which all hepatic function indices remained within normal ranges after Cr loading (Robinson *et al.*, 2000), nor did the findings reveal any increase between time points recorded during the course of the study (Schröder *et al.*, 2005). Results thus suggest that no risk of liver damage attaches to either short-term or long-term Cr supplementation.

3.4.1.4 Muscles

Serum creatine kinase (S-CK) is frequently elevated in disorders of cardiac and skeletal muscle. S-CK is predominantly found in muscle and is released into the blood circulatory system during muscular lesions (Totsuka *et al.*, 2002). It is a very sensitive indicator of acute myocardial infarction (MI) and muscular dystrophy (Bishop *et al.*, 2000). However, it is not specific, since elevated S-CK levels are evident as basic elements of various other abnormalities (Bishop *et al.*, 2000). Commonly accepted mechanisms of CK release are damage to muscle tissue or changes in myocyte membrane permeability (Totsuka *et al.*, 2002). During exercise, the muscle repeatedly contracts and uses energy substrates, thereby increasing the activity/concentration of this enzyme (Mitchell *et al.*, 1996; Totsuka *et al.*, 2002). When exercise intensity increases the resulting muscular force or tension development temporarily decreases the stability of muscle cell membranes (leading to increased CK "*leak*") and/or erodes the muscle cell membrane integrity (resulting in more "*holes*") (Mitchell *et al.*, 1996). CK is thus released from the active muscle into the extracellular space. It should be noted in light of this effect of muscle activity on S-CK levels that physically well-trained people tend to have elevated baseline levels of this enzyme (Bishop *et al.*, 2000). The high activities of S-CK that are observed in highly trained professional athletes may be attributable to the large amounts of eccentric loads they have to bear on a daily basis (Schröder *et al.*, 2005). S-CK levels are commonly used to judge the severity of muscle damage (Clarkson *et al.*, 2006). However, clinical data (Clarkson *et al.*, 2006) indicate a genetically determined high degree of individual variability in the expression of this indicator in response to strenuous exercise.

The clinical significance of S-CK activity depends more on iso-enzyme fractionisation than on total levels (Bishop *et al.*, 2000). There are four major CK iso-enzymes, which have been named for the tissues from which they were historically isolated (McLeish & Kenyon, 2005). The

iso-enzymes have been characterised on the basis of differences in gene and amino acid sequence, tissue localisation and immunogenicity. There are two cytosolic forms, the muscle (CK-MM) and brain (CK-BB) forms, which exist as dimers under physiological conditions (McLeish & Kenyon, 2005). The myocardium is essentially the only tissue that contains significant quantities of the iso-enzyme hybrid type CK-MB (Bishop *et al.*, 2000). For many years only the plasma form of CK was known (S-CK) and the main physiological role ascribed to it was the maintenance of energy homeostasis at sites of high energy turnover such as rapidly contracting skeletal muscle (McLeish & Kenyon, 2005). The high levels of CK ensured that ADP and ATP levels remained almost constant, effectively buffering the cell against rapid depletion of ATP (McLeish & Kenyon, 2005). The discovery of the mitochondrial iso-enzymes showed that CK was located in individual compartments as explained in section 2.3.2 above.

As a final note on clinical indicators of muscle damage, Korones *et al.* (2001) state that although AST, ALT and LDH are present in large amounts in the liver, they are not specific to the liver. Heart and muscle also contain substantial quantities of all three enzymes, and insult to either of these organs can result in striking elevations of serum AST, ALT, and LDH (Korones *et al.*, 2001). Whenever unexplained high levels of these liver enzymes are encountered, physicians should consider determining S-CK levels because many children with muscular dystrophy initially present with elevated liver enzymes. Thus, elevated liver enzymes may be a warning for further investigation into possible muscle dystrophy diseases (Korones *et al.*, 2001).

In the present study the reference ranges for S-CK as indicator of non-specific muscle damage in males are 38 – 174 U/ℓ (Niehaus & Ungerer, 2003).

Results of studies on S-CK levels in response to Cr or placebo supplementation in athletes demonstrated baseline S-CK levels above clinical norms for untrained individuals (Kreider *et al.*, 2003; Schröder *et al.*, 2005; Armentano *et al.*, 2007). No significant differences, or an increased trend, were observed between Cr and non-Cr groups in S-CK over time, even though levels were initially elevated during the pre-test (Kreider *et al.*, 2003; Schröder *et al.*, 2005). Robinson *et al.* (2000) reported that S-CK levels remained unchanged during Cr supplementation of 20 g/day for 5 days, followed by 3 g/day for 9 weeks. Crowe *et al.* (2003) reported unchanged S-CK levels during low-dosage Cr supplementation (3 g/day Cr for 6 weeks, ingested as a Cr-and-HMB combination supplement).

In conclusion, both high-dosage and low-dosage Cr supplementation appear not to induce muscle damage in athletes. However, in its position statement on the physiological and health effects of Cr ingestion, the ACSM maintains that "…*an elevated plasma CK activity should be investigated, not simply attributed to Cr supplementation, to identify the cause, ie. eccentric exercise, cardiac ischemia, metabolic myopathy, inflammatory myopathy, etc."* (Terjung *et al*., 2000 : 712).

Because methods of analysis for serum creatine (S-Cr) are not available in most laboratories, its measurement has been replaced by the measurement of CK levels for the diagnosis of muscle disease (Bishop *et al*., 2000). According to Silber (1999), experimental data and calculation show a leakage of Cr from the muscle cell into the blood plasma during maximal muscle contractions. However, this efflux of Cr from the muscle cell has the tendency to slow down in step with the ongoing adaptation of skeletal muscle to extreme exercise (Silber, 1999). The kidney effectively salvages Cr from the urine through simple diffusion. Notable quantities are only excreted (creatinuria) in certain diseases, under muscle-mass reducing conditions, and also fasting or high dietary intake of Cr (Silber, 1999; Terjung *et al*., 2000; Hadjicharalambous *et al*., 2008).

3.4.1.5 Heart

CK catalyses the reversible transfer of a phosphoryl group from ATP to Cr, producing PCr and ADP. As stated earlier, the myocardium is essentially the only tissue that contains significant quantities of the iso-enzyme CK-MB. It is, therefore, of major clinical interest, particularly as a marker for myocardial damage (Bishop *et al*., 2000; McLeish & Kenyon, 2005). Niehaus & Ungerer (2003) indicate that a CK-MB Index above 2.5% in the presence of raised CK-MB mass is suggestive of CK of cardiac origin (myocardial damage). Demonstration of elevated levels of CK-MB higher than or equal to 6% of total CK is considered the most specific indicator of myocardial damage, particularly acute MI (Bishop *et al*., 2000).

In the present study the reference ranges for CK-MB markers in males were set as follows (Niehaus & Ungerer, 2003):

CK-MB (mass) : 0.0 - 5.0 ng/ml
CK-MB (mass) index [100 x CK-MB mass (ng/ℓ) / total CK (U/ℓ)] : 0.0 - 2.5%

Ischemia of the heart is accompanied by hepato-cellular damage (due to decreased blood flow), hence S-AST is also a cardiac marker. It was actually first used for laboratory diagnosis of acute MI, but it lacks cardiac specificity and presently has no clinical significance for acute MI diagnosis (Bishop *et al.*, 2000).

Cardiac troponin T (TnT) is a plasma protein that may be monitored to provide significant information about the extent of myocardial damage (Krüger *et al.*, 2004). It allows for both early and late diagnosis of acute MI (Bishop *et al.*, 2000). After MI, serum levels of TnT increase and remain elevated for an extended period (Davé *et al.*, 2000). Some researchers consider it to be less than optimal for use in the early diagnosis of acute MI because it is also expressed in skeletal muscle (Löfberg *et al.*, 1996; Davé *et al.*, 2000). Apple *et al.* (2003) showed TnT levels above the 99th percentile, and/or a CK-MB value exceeding the 99th percentile, to be indicative of myocardial necrosis. In the present study, the reference ranges for TnT were 0.0 - 0.1 ng/ml (Niehaus & Ungerer, 2003).

There are no known studies to date that have monitored TnT or CK-MB as indicators of potential myocardial damage during Cr supplementation. Although Cr supplementation appears safe relative to the absence of any major cardiovascular toxicities, its use should be monitored carefully (Dhar *et al.*, 2005).

3.4.1.6 Haematological indices (including blood lipids)

Studies that reported on the effect of both short-term and relatively long-term Cr supplementation on haematological indices (ie. full blood count including haemoglobin, haematocrit, RBC count, WBC count, etc.), demonstrated no clinically significant change in these markers (Robinson *et al.*, 2000; Crowe *et al.*, 2003; Kreider *et al.*, 2003).

Several researchers have examined whether Cr supplementation would lower blood lipid profiles. One theory states that Cr supplementation may enhance the quality of training, thereby accentuating the positive effects of exercise on blood lipid profiles (Kreider *et al.*, 2003). However, Kreider *et al.* (2003) reported no significant differences between Cr and non-Cr users in a one-year analysis. These findings suggest that the possible influence of Cr on lipid profiles in athletes with normal blood lipids is either transient or non-existent (Volek *et al.*, 2000; Crowe *et al.*, 2003; Kreider *et al.*, 2003).

Thus there seems to be no health risk associated with Cr supplementation to haematological indices in healthy people when it is ingested in quantities that have been scientifically proven to increase muscle Cr stores. However, additional research should investigate the potential influence of Cr supplementation on lipid profiles, particularly in individuals with elevated cholesterol and/or triglycerides (Kreider *et al.*, 2003).

3.4.1.7 Other safety concerns

In his letter to the editor of the Clinical Journal of Sport Medicine, Archer (1999) pointed out that under the conditions that exist in the human stomach (high acidity, supply of nitrite from food and saliva) Cr can react to form N-nitrosarcosine. He (Archer, 1999) argued that the deleterious effects of the presence of this carcinogenic compound would only become apparent after many years, and thus warranted consideration by those advocating the use of Cr as an ergogenic compound. Tarnopolsky (1999) indicated in his reply to these comments that under ideal conditions approximately one gram of N-nitrosarcosine can be formed *in vivo* from five grams of Cr. This would amount to one sixteenth of the dose used to induce carcinoma in experiments (Tarnopolsky, 1999). The author did, however, recommend that detailed pathologic studies be performed to determine whether an increase in carcinogenesis is associated with Cr supplementation in humans (Tarnopolsky, 1999).

Rhabdomyolysis is a serious condition that results from a breakdown of the muscle cell wall, which leads to cell necrosis (Juhn, 2000). Two case studies have raised concerns about the possibility of this serious adverse effect of Cr supplementation. Robinson (2000) reported acute quadriceps compartment syndrome and rhabdomyolysis in a weight-lifter using high-dosage Cr supplementation. Potteiger *et al.* (2001) also reported elevated lower-leg anterior compartment pressure in a healthy physically active male subject during and after Cr supplementation, although rhabdomyolysis was absent. Both authors (Robinson, 2000; Potteiger *et al.*, 2001) attributed the increased compartmental pressure to the potential water retention properties of Cr. Thus, individuals susceptible to compartment syndrome and rhabdomyolysis due to dehydration, illicit drug use, trauma or strenuous exercise (Juhn, 2000) should be closely monitored during Cr supplementation. According to Juhn (2000) the question they should be asking is: *"Is it really worth it?"*

In conclusion, although Cr use is currently considered safe, consumers should always err on the cautious side when choosing a supplementation regimen.

3.4.1.8 Safety limits for Cr intake (ULS and OSL)

The emerging importance of the bioactive components of food, also known as non-essential nutrients, and their increased use in food supplements is recognised by research institutes, industry and regulatory officials (Hathcock *et al.*, 2006). The acquisition of longer-term data on the safety of non-essential nutrients such as Cr, is difficult for multiple reasons. Human studies seldom have safety measures as primary endpoints, and longer-term studies are almost always planned to detect possible long-term benefits, not risks (Hathcock & Kriengsinyos, 2011). While many bioactive substances, such as Cr, are generally considered to be non-toxic, safety limits of use need to be determined (Hathcock *et al.*, 2006).

Policy and regulatory authorities have two basic approaches to determining the safety of food products and components – (a) risk assessment based on toxicological or other appropriate data, and (b) evaluation of whether there is a ‘‘*history of safe use”* (Hathcock & Kriengsinyos, 2011). Both these approaches were utilised by Hathcock *et al.* (2006) when drafting the International Alliance of Dietary/Food Supplement Associations (IADSA) report on the safety of bioactive substances found in dietary supplements. The report was developed by members of the IADSA scientific group with the guidance of experts in the bioactive substances field to provide guidance to governments and scientific bodies on safe nutrient intake (Hathcock *et al.*, 2006).

For nutrients and related substances, the Tolerable Upper Intake Level (UL) has become the internationally accepted risk assessment criterion (Hathcock & Kriengsinyos, 2011). The major limitation of the ULS method as applied by authoritative groups thus far is that no UL has been set for nutrients without established adverse effects (Hathcock & Kriengsinyos, 2011). In contrast to the limitations inherent in the UL method, an alternative approach is available; it identifies a risk assessment value termed the Highest Observed Intake (HOI) (Hathcock & Kriengsinyos, 2011). In the absence of a ULS, the HOI is the highest intake with adequate data to show, with acceptable confidence, the absence of adverse effects up to that intake (Hathcock & Kriengsinyos, 2011).

For some nutrients, without established hazard at high intakes, the toxic potential is so low that there is no credible evidence of adverse effects at any level of intake that has been consumed or used in a clinical trial (Hathcock *et al.*, 2006). For these nutrients, such as Cr, the maximum level with sufficient evidence of safety can be identified as an OSL, and this OSL can be used

as a ULS. That is, ULS = OSL (the highest level with convincing evidence of safety, if there are no established adverse effects at any level) (Hathcock *et al.*, 2006).

The recommended human OSL (5 g/day) set for Cr, is based on the results of only one randomised controlled trial (RCT), namely that of Derave *et al.* (2004), because the authors of the IADSA report (Hathcock *et al.*, 2006) considered the study to be of substantial duration (20 weeks) and exposure (20 g/day of Cr for 5 days, followed by 5 g/day for 19 weeks), although they admitted that the study sample size (n = 8) was relatively small. Other relevant studies (Robinson *et al.*, 2000; Bennet *et al.*, 2001; Greenwood *et al.*, 2003; Kilduff *et al.*, 2003; Kreider *et al.*, 2003; Van Loon *et al.*, 2003; Watsford *et al.*, 2003), although not RCT, provided support and confidence in the selected OSL and by implication the safety of low-dosage Cr supplementation, although these studies also employed relatively small sample sizes for short periods.

Since the 5 g/day dosage was administered to subjects on normal diets, no correction of the OSL for dietary intake is needed, therefore OSL = ULS = 5 g/day (Hathcock *et al.*, 2006; Shao & Hathcock, 2006).

3.4.1.9 CONCLUSION

Currently there is no conclusive evidence to support the notion that normal Cr intakes (< 25 g/day) in healthy adults/athletes cause renal dysfunction, hepatocellular damage, myocardial necrosis or haematological abnormalities. It should be considered that the interpretation of both baseline and post-test results on S-Crn levels may be misread when athletes supplementing with Cr are also undergoing intense exercise training. The increased S-Crn levels in these athletes can be wrongly interpreted as indicative of renal dysfunction (Kuehl *et al.*, 2000; Kreider *et al.*, 2003; Hathcock *et al.*, 2006); therefore measurements of renal function should be done seperately from S-Crn levels by measuring the albumin excretion rate (Poortmans, 2000) and by applying the gold-standard tests for glomerular filtration rate (GFR), namely non-ionic contrast techniques (Kuehl *et al.* 2000).

Although critical analyses of the available literature revealed that Cr supplementation did not adversely affect kidney function (Hathcock *et al.*, 2006; Shao & Hathcock, 2006; Gualano *et al.*, 2011), some concerns have remained (Pritchard & Kalra, 1998; Kuehl *et al.*, 2000) due to conflicting results from case reports and animal studies (Gualano *et al.*, 2011). Consequently

some regulatory agencies (eg. the French Sanitary Agency and the Brazilian National Agency for Sanitary Vigilance) are restricting the sale of Cr-based nutritional supplements to healthy adults (Gualano *et al.*, 2011).

Finally, the application of risk assessment methodology to the available published human clinical trial data involving Cr supports a high level of confidence in the safe use of Cr in dietary supplements (Shao & Hathcock, 2006). Evidence from well-designed, randomised, controlled human clinical trials indicates the ULS for Cr to be 5 g/day (Shao & Hathcock, 2006).

3.4.1.10 RECOMMENDATIONS

- Confidence in Cr as a safe supplement would be enhanced with high-quality research (RCTs) incorporating larger sample sizes and longer periods of Cr supplementation to determine whether and what levels adverse effects of Cr supplementation can be expected in human subjects (Hathcock & Kriengsinyos, 2011).

- Homogeneous, standardised research protocols need to be drafted and executed to determine the safety of Cr supplementation to humans.

- Renal function should be measured without reference to S-Crn levels by measuring, amongst others, the albumin excretion rate (Poortmans & Francaux, 1998; Poortmans, 2000; Kuehl *et al.*, 2000; Yoshizumi & Tsourounis, 2004).

- Future studies should investigate the effects of Cr supplementation in several kidney diseases as well as in the elderly, type-2 diabetics and hypertensive individuals, whose tendency to renal dysfunction is well-described (Gualano *et al.*, 2008).

- Additional study is needed to determine the health implications and potential interactions when Cr is ingested with other supplements (ie. "stacking") (Mayhew *et al.*, 2002).

- Regular clinical monitoring of athletes is recommended in the case of long-term Cr usage (Benzi, 2000; Bizzarini & De Angelis, 2004; Dhar *et al.*, 2005; Schröder *et al.*, 2005).

- It would be wise to seek the advice of a medical professional before engaging in any form of supplementation (Mayhew *et al.*, 2002).

3.4.2 Clinical use

Cr has established itself as an ergogenic aid in sports and has gained increasing attention in the medical and lay press as a possible therapeutic agent for various diseases (Taes & De Vriese, 2005). According to Benzi (2000), Cr can be used either at supplementary or therapeutic levels as a function of the dosage. Benzi (2000 : 260) explains this as follows: *"Supplementary doses of nutritional factors usually are in the order of the daily turnover, while therapeutic ones are three or more times higher. In a subject with a body weight of 70 kg with a total Cr pool of 120 g, the daily turnover is approximately 2 g. Thus, in healthy subjects nourished with a fat-rich, carbohydrate-, protein-poor diet and participating in daily recreational sport, the oral Cr supplementation should be in the order of the daily turnover, ie. less than 2.5 - 3 g per day, bringing the gastrointestinal absorption to account. In healthy athletes submitted daily to high-intensity strength- or sprint-training, the maximal oral Cr supplementation should be in the order of two times the daily turnover, ie. less than 5 - 6 g per day. The oral administration of more than 6 g per day of Cr should be considered as a therapeutic intervention because the dosage is more than three times higher than the Cr daily turnover and more than six times higher than the Cr daily allowance."*

Evidence of benefits from this supplement as an adjunctive treatment have been reported in connection with a broad range of diseases (Gualano *et al.*, 2011). The therapeutic benefits of Cr relates to its characteristics of increasing the muscles' TCr pool (Greenhaff *et al.*, 1994; Hultman *et al.*, 1996; Deldicque *et al.*, 2005; Hadjicharalambous *et al.*, 2008; Rawson *et al.*, 2008; Safdar *et al.*, 2008), thereby acting as an energy buffer for tissue with a high energy turnover such as cardiac, neural, muscle and brain cells (Walker, 1979; Harris, 1993; Ponticos *et al.*, 1998; McLeish & Kenyon, 2005). This general strengthening of cellular energetic turnover makes the cells and tissues more resistant to metabolic and environmental challenges (Wyss & Schulze, 2002). Cr is also capable of exerting multiple, non-energy related effects on diverse and relevant cellular targets (Sestili *et al.*, 2011), as will be briefly explained in the following section. These effects include a mild antioxidant activity (Sestili *et al.*, 2011).

3.4.2.1 Brain disorders, cognitive function and mood

PCr plays a vital role in cerebral energy provision (Rawson *et al.*, 2008; Gualano *et al.*, 2011; Allen, 2012). Although Cr can cross the blood-brain barrier, its permeability is limited (Wyss & Schulze, 2002). Cr biosynthesis in this organ therefore adds to its TCr and PCr content (Wyss & Schulze, 2002).

Low-dosage Cr supplementation (4 g/day for 8 weeks) was successful in significantly raising brain PCr concentrations in adolescent females with SSRI-resistant (selective serotonin re-uptake inhibitor resistant) major depressive disorder (Kondo *et al.*, 2011). The participants' rating of depression eased 56% after Cr supplementation (Kondo *et al.*, 2011). Lyoo *et al.* (2003) demonstrated Cr supplementation in healthy adults to raise brain Cr and PCr levels by 9% and 3%, respectively. The researchers therefore suggest that Cr may modify brain energy metabolism (Lyoo *et al.*, 2003; Kondo *et al.*, 2011; Allen, 2012) thereby potentially exerting a positive effect in patients with mood disorders (Kondo *et al.*, 2011; Allen, 2012).

According to Benton and Donohoe (2011), studies conducted to determine the impact of Cr supplementation on cognitive functioning have produced inconsistent findings. Cr supplementation (0.03 g/kg/day for 6 weeks) failed to improve neurocognitive functioning (simple reaction time, code substitution, logical reasoning, mathematical processing, running memory and memory recall) in college-aged individuals (Rawson *et al.*, 2008). Acute Cr administration (100 mg/kg 1½ h before each trial) did, however, ameliorate decreased performance of a simple repeat skill in sleep-deprived elite rugby players (Cook *et al.*, 2011). Thus, acute Cr supplementation may offer a practical and viable option prior to training and competition of elite athletes to assist their sport-skill performance when sleep deprived (Cook *et al.*, 2011). These results seem to corroborate the theory posited by Rawson *et al.* (2008), namely that potentially, Cr supplementation only improves cognitive processing and psychomotor performance in individuals who have impaired cognitive processing abilities. However, Cr supplementation (20 g/day for 5 days) resulted in better memory in female undergraduates who were given to vegetarian dietary regimens, compared to others who were meat eaters (Benton & Donohoe, 2011). Before supplementation the cognitive capacities of the vegetarians were on par with those of the meat eaters. Thus, vegetarians were found to be more sensitive to Cr supplementation (Benton & Donohoe, 2011).

The exact mechanisms underlying the effects of Cr supplementation on mood and neurocognitive functioning remain to be elucidated (Gualano *et al.*, 2011). PCr can be expected to play a role in supplying energy to metabolically active areas of the brain (Benton & Donohoe, 2011; Gualano *et al.*, 2011; Allen, 2012). However, the uneven distribution of Cr phosphokinase indicates that, rather than having a general function, it is important in some aspects of functioning rather than others (Benton & Donohoe, 2011). The high concentration in the hippocampus is of particular interest, given the known importance for memory of this area of the brain (Benton & Donohoe, 2011).

3.4.2.2 Disorders of the neuromuscular system

Myopathies are genetic or acquired disorders of skeletal muscle that lead to varying degrees of weakness, atrophy and exercise intolerance (Tarnopolsky, 2011). They lead to disability either by altering the contractile apparatus and thus to progressive weakness (ie. muscular dystrophy and congenital myopathy), or by interfering with energy pathways and thus to contractile failure (ie. mitochondrial myopathy and McArdle's disease) (Tarnopolsky, 2011). In theory, Cr supplementation could have a number of beneficial effects that could enhance function in myopathy patients, including increased muscle mass and strength, endurance enhancement, lower calcium levels, antioxidant effects and reduced apoptosis (Tarnopolsky, 2011).

Low-dosage (3 - 5 g/day) Cr supplementation for extended periods of months to years has proved effective in extending exercise endurance (Komura *et al.*, 2003), muscle strength (Tarnopolsky *et al.*, 1997; Louis *et al.*, 2003*a*) and resistance to fatigue (Louis *et al.*, 2003*a*) in myopathic populations. The neuroprotective effect of Cr supplementation has proven beneficial to upper- and lower-body strength (Stout *et al.*, 2001), as well as functional strength (Smith *et al.*, 2006) in patients with neuromuscular diseases. In patients with cystic fibrosis, Cr supplementation (12 g/day for 7 days followed by 6 g/day for 12 weeks) proved effective in increasing muscle strength and general well-being (Braegger *et al.*, 2003). However, no improvements in muscle TCr concentrations or high-intensity exercise capacity were found in patients with multiple sclerosis after Cr supplementation (20 g/day for 5 days) (Lambert *et al.*, 2003). The authors attributed this finding to a lower concentration of the skeletal muscle Cr transporter in patients with multiple sclerosis (Lambert *et al.*, 2003).

In conclusion, the current body of research points to Cr supplementation as a promising adjunctive therapy to combine with exercise training as an aid to increasing muscular

performance in patients with disorders of the neuromuscular system. Also, some important long-term outcomes (eg. deferring wheelchair use) could be influenced positively by this combination (Tarnopolsky, 2011). However, more research on these populations is needed.

3.4.2.3 Cardiovascular disease

Cornelissen *et al.* (2010) recently investigated the effect of Cr supplementation in conjunction with an exercise programme on physical fitness in patients with coronary artery disease or chronic heart failure. Measures of cardiorespiratory fitness and muscle function were determined at baseline and following three months of cardiac rehabilitation in both the placebo and Cr groups. Measures were increased in both groups after training. However, no difference in these improvements was found between the placebo and the Cr group. HDL cholesterol was significantly increased and plasma triglycerides were significantly decreased after physical training whereas total and LDL cholesterol remained statistically unchanged. Again, no difference was found where these changes were concerned between the placebo and Cr groups. Thus, Cr supplementation in combination with exercise training exerted no additional effect on the improvement in physical performance, health-related quality of life or lipid profile in patients with coronary artery disease or chronic heart failure.

In conclusion, it seems that the future of Cr supplementation lies in its application as adjunctive therapy to supplement medication and exercise in treating neuromuscular and brain/cognitive disorders.

CHAPTER 3

SAFETY OF CREATINE MONOHYDRATE SUPPLEMENTATION AND ITS EFFECTS ON EXERCISE PERFORMANCE AND BODY COMPOSITION IN ULTRADISTANCE RUNNERS

3.1 INTRODUCTION

Numerous studies have indicated that Cr supplementation provides significant performance improvements during repeated bouts of short-duration, high-intensity exercise (Bennett *et al.*, 2001; Preen *et* al., 2001; Wiedermann *et al.*, 2001; Izquierdo *et al.*, 2002; Warber *et al.*, 2002; Ziegenfuss *et al.*, 2002; Van Loon *et al.*, 2003; Peyrebrune *et al.*, 2005; Jäger *et al.*, 2008; Rawson *et al.*, 2011). A number of studies have investigated the effects of Cr supplementation on aerobic endurance performance (ie. cycling), mostly with negative results (Van Loon *et al.*, 2003; Van Schuylenbergh *et al.*, 2003; McConell *et al.*, 2005; Stout *et al.*, 2006); or maximal aerobic capacity (Jones *et al.*, 2002; Van Loon *et al.*, 2003; Reardon *et al.*, 2006; Graef *et al.*, 2009). Balsom *et al.* (1993*b*) reported a decrease in performance levels attained during a 6 km terrain run in the Cr-supplementation group (20 g/d for 6 days). As this group's body mass increased significantly (~2kg) after Cr loading, the lower levels of endurance performance may be attributable to a less efficient running economy (greater mechanical effort required to support the larger body mass) during the time trial. However, Engelhardt *et al.* (1998) reported that Cr supplementation significantly decreased fatigue during bouts of high-intensity exercise that were interspersed with endurance running. They also demonstrated that blood glucose levels could be better maintained with the aid of Cr supplementation during endurance exercise (Engelhardt *et al.,* 1998). Since endurance running typically takes place over undulating terrain, and thus incorporates bouts of high-intensity effort, these athletes might benefit from Cr supplementation.

No studies have been published, however, on the effect of Cr supplementation on the performance of ultradistance athletes who should benefit from Cr supplementation for the same reasons as other endurance athletes: (i) enabling slow-twitch muscle fibres to rely for longer on oxidative metabolism, as would be evident from an increase in oxygen uptake during exercise testing; (ii) increasing muscle TCr and PCr content, and consequently buffering larger amounts

of ADP at myofibrillar sites in muscle-fibre types I as well as II (ie. facilitating the PCr energy shuttle), as would be depicted by an increase in work output and/or a delayed onset of fatigue during exercise testing (Rico-Sanz & Marco, 2000); and (iii) reducing intramuscular lactate accumulation by decreasing the reliance on anaerobic glycolysis during periods of increased exercise intensity/pace (Stout *et al.*, 2006) and/or buffering H^+ accumulation during incremental aerobic exercise to fatigue (Stout *et al.*, 2006).

Concerns remain among endurance and ultradistance runners that Cr supplementation may significantly increase body weight, thus exerting a negative performance effect due to reduced mechanical efficiency, quite apart from a possible risk of organ (especially renal) dysfunction, with both Cr loading and lower-dosage, longer-term supplementation (Benzi, 2000; Kreider *et al.*, 2003; Schröder *et al.*, 2005; Buford *et al.*, 2007).

In light of the above the goals of the study under review were to determine whether or not both short-term moderate-dosage Cr intake and moderate-term maintenance supplementation in well-trained ultradistance runners would:

- lead to an improved training effect, and thus enhanced performance during an incremental test for aerobic capacity;
- change the pattern of substrate utilisation during an incremental test for aerobic capacity;
- decrease blood lactate accumulation during an incremental test for aerobic capacity;
- change body weight, lean body weight (LBW) and other parameters of body composition;
- increase sub-maximal running economy; and
- prove safe for organ function (ie. skeletal muscle, myocardium, blood, liver and kidneys).

3.2 PARTICIPANTS, MATERIALS AND METHODS

3.2.1 Participants

The study was approved by the ethics committees of the University of the Witwatersrand (since the study was initially designed, and the researcher registered for a PhD (Med) degree, under the supervision of Professor G Rogers), the Tshwane University of Technology (where the researcher was a lecturer and utilised their sport sciences laboratory for testing) and the University of Pretoria (where the researcher assumed a lecturer's appointment, and the study was completed under the supervision of Professor PE Krüger).

The study incorporated a double-blind, placebo-controlled experimental research design. Seventeen male ultradistance runners who were training for the Comrades Marathon (89 km over undulating terrain) were recruited for the study.

Participant inclusion criteria:

- male gender;
- healthy (without any illness or chronic diseases);
- competing in distance running events >32 km as required for build-up to the Comrades Marathon;
- well-trained (training distance of ≥50 km/week);
- taking no medication (prescription and/or over-the-counter preparations for colds and flu) for at least 7 days before commencement of the study;
- not supplemented with Cr-containing products for 35 days before commencement of the study;
- owning a heart-rate monitor to enable him to train according to a predetermined intensity; and
- dedicated to adhere to the prescribed training schedule and maintain the daily nutrition and training logs.

Participant exclusion criteria:

- not complying with the criteria listed in participant inclusion criteria;
- consciously suffering from any disease;
- taking any form of supplementation that has not been approved by the investigator;
- non-adherence to the prescribed training schedule; and
- failing to maintain the daily training and nutrition logs.

The participants were paired and then, in a double-blind fashion, assigned to either a group consuming Cr (CRE group; 38 ± 7.8 yrs; n = 9) or a group consuming placebo (PLA group; 37.7 ± 8.2 yrs; n = 8). The following information and measurements were used in the pairing of the participants after the baseline testing:

- VO_2 max score (Appendix C); and
- state of aerobic conditioning (fitness%). Percentage endurance fitness was determined as the % VO_2 max achieved at 4 mmol/ℓ blood lactate (Bogdanis *et al.*, 1996).

The nature of the investigation and possible risks involved were explained to each subject before his written consent to participate was obtained. A full medical screening was also conducted.

3.2.1.1 Nutrition log

No dietary restrictions were imposed on the participants, but they were requested not to change their habitual eating and drinking patterns during the course of the study. To monitor their compliance with this request participants received a nutritional logbook after baseline testing so that they could record daily food intake.

3.2.1.2 Training log

After baseline testing, participants received a training schedule designed by a Comrades Marathon coach. The training programme was designed to fit the training needs of a Comrades Marathon runner at the time of year (March - May) leading up to the Marathon. A standardised training programme (Table 3-1) was followed in consonance with the individual's heart rate at OBLA (4 mmol/ℓ blood lactate).

Table 3-1 Training programme in preparation for the Comrades Marathon (March – May)

Type of run	Number of sessions per week	Running intensity (mmol/ℓ blood lactate)	Personal target heart rate range (bpm) as determined by VO_2 max testing
Recovery run	1 to 2	< 1.5	
Long training runs (LSD)	1	< 2.5	
Sub-maximal	1 to 2	2.5 to 3.5	
Time trials	1	± 4	
Fartlek and long intervals	1 to 2	3.5 to 6	
Short intervals	1	6 to 12	

The participants adhered to the programme without demur. They also received a training logbook to record on a daily basis the following:

- wake-up heart rate (bpm);
- number of hours slept the previous night;
- running distance covered (km);

- running time (h : min);
- running intensity (running pace at mmol/ℓ blood lactate as prescribed after VO_2 max testing, and monitored *via* heart-rate monitor);
- characteristics of the training route;
- rating of fatigue; and
- minor ailments experienced.

3.2.2 Anthropometric data

The participants refrained from (i) eating or drinking for four hours prior to testing, (ii) exercise for 12 hours prior to testing, and (iii) alcohol or caffeine consumption for 24 hours prior to testing. The height and body weight of subjects were recorded on the day they reported for the VO_2 max test, using a calibrated height gauge (Seca, model 220) and calibrated medical balance scale (Seca, model 713), respectively. Skin-fold measurements were taken with a Harpenden skin-fold caliper at the right biceps, triceps, sub-scapula, supra-iliac, mid-thigh and calf. Measurements for bone structure were recorded at the humerus and knee with a spreading caliper. Circumferences of the biceps (tensed), sub-gluteal, mid-thigh, calf and pre-patella were determined by means of a Rabone-Chesterman steel tape. The Heath-Carter formula as described in detail by Carter (1980) was then used to determine:

- fat% (proportion of total weight that is fat weight);
- lean body weight; and
- somatotype components:
    - endomorphy (relative fatness),
    - mesomorphy (relative musculoskeletal robustness), and
    - ectomorphy (relative linearity).

Data was again collected by the same researcher after Cr loading (six supplementation days; post-test 1) and maintenance feeding (at the end of week 10; post-test 2), respectively.

3.2.3 Blood sampling

A qualified nurse collected urine and three blood samples from the antecubital vein of participants during baseline testing (pre-test), after the 6 day loading period (post-test 1) and again after 10 weeks of supplementation (post-test 2). The samples were stored on ice until they were analysed (all on the same day) by the clinical hematology laboratory of Niehaus & Ungerer Pathologists for parameters reflecting:

- Liver function

  - Serum AST, ALT, GGT
  - Prothrombin index
  - Prothrombin INR
- Renal function
  - Serum creatinine
  - Serum LDH
  - Urine microalbumin
- Skeletal muscle integrity
  - Serum creatine kinase (CK)
- Heart muscle integrity
  - Serum CK-MB (mass)
  - Serum CK-MB (mass) index

Chemical pathological analyses (Table 3-2) were performed on blood serum (ie. biochemistry), full blood (ie. haematology) and urine using standard methods (Niehaus & Ungerer, 2003).

Table 3-2 Chemical pathology analyses

TEST	METHODOLOGY	UNITS	NORMAL RANGE: MALES (18 – 30yrs)
BIOCHEMISTRY			
GGT	Beckman-CX7/LX	U/ℓ	11 – 49
ALT	Beckman-CX7/LX	U/ℓ	7 – 40
AST	Beckman-CX7/LX	U/ℓ	7 – 41
LDH	Beckman-CX7/LX	U/ℓ	266 – 500
Creatinine	Beckman-CX7/LX	mmol/ℓ	62 – 115
CK	Beckman-CX7/LX	U/ℓ	38 – 174
CK-MB (mass)	Access	ng/ml	0.0 – 5.0
CK-MB (mass) Index	Access	%	0.0 – 2.5
HAEMATOLOGY			
Prothrombin control: • Prothrombin index • Prothrombin INR	Behring coagulation timer	%	 70 – 130 0.85 – 1.15
URINE			
Microalbumin	Behring BNII	mg/ℓ	0 – 19

3.2.4 Supplementation

After baseline testing (pre-test) participants were instructed to ingest four capsules of supplement (moderate dosage) three times per day (6 g/day) with a carbohydrate containing beverage (eg. grape juice) for six days. The capsules contained Cr Monohydrate (500 mg per capsule) and were supplied by Biomox Pharmaceuticals Pty Ltd (Silverton, South Africa). After post-test 1 (on day 7), the maintenance dosage (low dosage) was ingested in capsule form (3 g/day) for 9 weeks. The placebo capsules contained 500 mg potato starch.

3.2.5 Exercise testing protocols

3.2.5.1 Maximal aerobic capacity (VO_2 max)

Participants reported to the sport science laboratory one week prior to baseline testing. They were familiarised with the VO_2 max testing protocol by completing three workload intervals and a short sprint on the motorised treadmill while wearing the appropriate facial mask and nose clamp.

One week later baseline testing (pre-test) was conducted on the same day and time as the familiarisation session. Measurements for indices reflecting aerobic/endurance exercise performance and capacity of the ultradistance runners were recorded by means of a continuous incremental test for maximal aerobic capacity (VO_2 max) conducted on a Quinton 0-65 motorised treadmill (Quinton Instrument Company, Seattle, USA).

After anthropometric and blood sampling procedures the participant was instrumented with 12 electrodes and linked to the Med Graphics CardiO_2 combined 12-lead VO_2/ECG analyser system (Medical Graphics Corporation, St Paul, Minnesota, USA). The participant was then instrumented with a nose clamp and a bidirectional differential-pressure pre-vent pneumotach. Expired air was collected by means of a sample line and analysed for fractions of O_2 and CO_2 by using a Zircoma-type oxygen analyser and a NDR-type carbon dioxide analyser. The gas samples were drawn at the end of each 30-second exercise period using a patented gas drying sample circuit with a warm-up time of 30 minutes from cold start. Expiratory gas volumes collected by the Med Graphics spirometer were analysed and data for VO_2 max and RER were calculated by computer (Med Graphics System). The gas analyser was calibrated manually and electronically using Analar grade standard bottled gas concentrations prior to testing.

The participant performed a warm-up phase (4 min) at 8 km/h and 0% treadmill gradient, whereafter the test commenced at a treadmill speed of 10km/h and 0% gradient. The treadmill speed was kept constant at 10 km/h for the duration of the test. The treadmill gradient was increased every two minutes by 2.5% until exhaustion. Cardio-respiratory measurements were recorded continuously. Heart rate (HR) response was measured throughout the test. The test was terminated when the participant indicated he was no longer able to maintain the pace (at which point strong verbal encouragement to finish the stage was given), whereupon the participant's heart rate levelled off and the RER values exceeded 1.0. The testing period did not exceed 13 minutes.

Measurements for blood lactate concentration (mmol/ℓ) were taken *via* finger prick initially during rest, then continuously at the end of each 2-minute increment (within 20 seconds) and at 5 min recovery. Enzymatic analyses were performed with an Eppendorf ESAT 6661 lactate analyser (Kayser *et al.*, 1993; Rossouw & Rossouw, 2000*b*).

Endurance fitness was determined as the percentage VO_2 max achieved at 4 mmol/ℓ blood lactate (Bogdanis *et al.*, 1996). The lactate concentration of 4 mmol/ℓ blood was defined as OBLA, while the power output which corresponds to a lactate concentration of 4 mmol/ℓ blood was defined as W_{OBLA} as described by Tesch *et al.* (1983).

In summary, data collected during the incremental test for VO_2 max included:

- **VO_2 max:** maximal oxidative capacity (Appendix C);
- **fitness%:** level of endurance fitness (Bogdanis *et al.*, 1996);
- **HR_{OBLA}:** heart rate at blood lactate concentration of 4 mmol/ℓ;
- **W_{OBLA}:** the power output identifiable with a lactate concentration of 4 mmol/ℓ blood (Tesch *et al.*, 1983);
- **HR_{max}:** maximum HR achieved during the test;
- **peak RER:** peak RER value achieved during the test;
- **end lactate:** blood lactate concentration at the end of the last workload;
- **LA5min:** blood lactate at 5 min recovery; and
- **HR5min:** HR at 5 min recovery.

3.2.5.2 Sub-maximal running economy

On a separate visit to the laboratory (two days after the VO_2 max test), a test for sub-maximal running economy was performed on each participant while he was wearing a nose clamp and breathing out through a bidirectional differential-pressure pre-vent pneumotach. The participant was instrumented with 12 electrodes and linked to the Med Graphics CardiO_2 combined 12-lead VO_2/ECG analyser system (Medical Graphics Corporation, St Paul, Minnesota, USA).

A 5 min warm-up was performed at a comfortable pace (8 km/h). The treadmill gradient was set at 1% and remained constant throughout the test. After warm-up the treadmill speed was increased to 10 km/h and the test commenced. The participant ran at this pace for five minutes, whereafter the speed was increased to 12 km/h (5 min) and again to 15 km/h (5 min). Heart rate was monitored continuously. Expiratory gas volumes were collected by the Med Graphics spirometer and analysed by computer (Med Graphics System) as explained above. Data collected included:

- **running economy:** steady-state oxygen consumption (VO_2, expressed as ml/kg/min) at each stage (ie. 10 km/h, 12 km/h and 15 km/h);
- **HR:** steady-state heart rate at each running stage (ie. 10 km/h, 12 km/h and 15 km/h); and
- **HR5min:** heart rate at 5 min recovery.

All exercise testing was conducted in a closed laboratory environment at temperatures of 20 – 22 °C and a relative humidity of 45 - 50%. The post-tests were conducted under exactly the same conditions as the pre-test.

3.2.6. Statistical methodology

The data were captured onto Microsoft Excel and converted to SPSS (Statistical Package for the Social Sciences) in order to do the analysis (Field, 2000). The data analysis had the following aims:

- to determine whether or not significant differences existed and/or developed over time between the two groups for all variables measured; and
- to determine whether or not significant differences existed and/or developed over time between the pre- and post-test measurements within the same group.

Since the participant sample was relatively small the data were analysed with non-parametric statistics. Non-parametric tests, also known as distribution-free tests, are a class of tests that do not rely on a parameter estimation and/or distribution assumptions (Howell, 1992). The major advantage attributed to these tests is that they do not rely on any seriously restrictive assumptions concerning the shape of the sampled populations and thus accommodate small samples, as in this study.

3.2.6.1 Descriptive statistics

The mean, standard deviation, minimum and maximum scores for each measurement per group were determined for reference purposes. These scores reflect the results of all subjects regardless of whether complete data were available.

3.2.6.2 Inferential statistics

3.2.6.2.1 The Mann-Whitney U-test

The Mann-Whitney U-test is used to test differences between means when there are two conditions (ie. experimental and placebo) and different subjects have been used in each (Field, 2000). This test is a distribution-free alternative to the independent-samples t-test. Like the t-test, Mann-Whitney tests the null hypothesis that two independent samples (groups) come from the same population (not just populations with the same mean). Rather than being based on parameters of a normal distribution like mean and variance, Mann-Whitney statistics are based on ranks. The Mann-Whitney statistic is obtained by counting the number of times an observation from the group with the smaller sample size precedes an observation from the larger group. It is especially sensitive to population differences in central tendency (Howell, 1992). The rejection of the null hypothesis is generally interpreted to mean that the two distributions had different central tendencies. This test was used to determine significant differences between the control group and the experimental group for all variables measured (ie. between-group differences).

3.2.6.2.2 Friedman's rank test for κ-correlated samples

This test is the distribution-free analogue of the one-way repeated-measures analysis of variance. "*It is a test on the null hypothesis that the scores of each treatment were drawn from identical populations, and it is especially sensitive to population differences in central tendency*" (Howell, 1992 : 624). This test was used to determine whether statistically significant differences existed between the scores obtained at the different testing intervals within the same group.

3.3 RESULTS

3.3.1 Results of comparing the CRE group with the PLA group across various measurements (between-group data analyses)

Differences between scores of the CRE group compared to that of the PLA group - at each test stage - were analysed for statistical significance. These scores, explored with Mann-Whitney U-tests, are reported in this section. However, these analyses do not explain the changes revealed within each group. These within-group changes in scores were explored with Friedman tests, and will be addressed in the next section (3.3.2).

3.3.1.1 Body composition

At the onset of the study the PLA group's averaged body weight was somewhat, but not significantly, greater than that of the CRE group (Table 3-3). As explained earlier, due to their being ultradistance athletes the participants were paired after baseline testing according to their aerobic status (ie. VO_2 max values and fitness%) and not according to their body weight or body composition.

No statistically significant differences in either body weight or body composition scores were found at any of the test stages between the CRE group and the PLA group (Table 3-3). Thus, when comparing them at each measurement, the groups did not differ significantly from one another (Table 3-3).

3.3.1.2 Maximal aerobic capacity

At the onset of the study no statistically significant differences were found between the aerobic capacity scores (ie. VO_2 max, fitness%, aerobic peak work capacity, HR_{max}, RER and blood lactate response) of the CRE and PLA groups (Table 3-4). Thus, when compared at each measurement, the groups did not differ significantly.

Table 3-3: Between-group analysis of the change in body composition of the CRE group compared to that of the PLA group

Variable	Test	Mean		Standard Deviation		p-value
		PLA	CRE	PLA	CRE	
Weight (kg)	**Pre**	81.50	75.24	7.60	5.39	0.067
	Post 1	80.88	75.18	7.64	5.27	0.054
	Post 2	80.23	75.42	7.17	5.11	0.059
Fat%	**Pre**	17.26	14.94	4.95	4.42	0.248
	Post 1	17.13	15.08	4.58	3.58	0.336
	Post 2	16.49	14.37	4.08	4.22	0.229
Endomorphy	**Pre**	3.59	2.93	1.42	1.29	0.289
	Post 1	3.55	3.01	1.29	1.06	0.386
	Post 2	3.34	2.86	1.21	1.24	0.267
Mesomorphy	**Pre**	5.48	5.18	0.97	1.31	0.563
	Post 1	5.41	5.37	1.04	1.23	0.596
	Post 2	5.30	5.37	0.86	1.03	0.809
Ectomorphy	**Pre**	1.93	2.26	0.61	1.22	0.664
	Post 1	1.99	2.26	0.60	1.14	0.595
	Post 2	2.00	2.20	0.57	1.12	0.528
LBW (kg)	**Pre**	66.96	63.84	4.66	3.19	0.102
	Post 1	66.84	62.96	5.04	4.99	0.102
	Post 2	66.81	64.46	4.27	3.56	0.178

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

Supplement consumption did not significantly influence between-group changes in any of the measurements of maximal aerobic capacity, except for RER and LA5min scores during post-test 1 (Table 3-4).

As demonstrated in Table 3-4, short-term moderate-dosage Cr consumption increased the difference in peak RER score reached during post-test 1 on the 10% level of significance ($p = 0.053$). It also significantly increased the difference in peak blood lactate level reached at 5 min recovery on the 5% level of significance ($p = 0.034$). Both the CRE and PLA groups increased their scores for peak work and HR_{max} after Cr loading and maintenance feeding, respectively, but without significant change in the between-group differences (Table 3-4).

Table 3-4 **Between-group analysis of the change in recorded scores associated with maximal aerobic capacity of the CRE group compared to that of the PLA group**

Variable	Test	Mean		Standard Deviation		p-value
		PLA	CRE	PLA	CRE	
VO_2 max (ml/kg/min)	**Pre**	52.11	57.23	5.72	5.29	0.178
	Post 1	54.21	56.48	4.82	5.76	0.500
	Post 2	53.70	55.81	4.65	6.54	0.630
Fitness%	**Pre**	83.80	83.60	8.21	9.15	0.847
	Post 1	82.80	88.52	6.38	6.33	0.123
	Post 2	84.81	82.82	4.30	8.06	1.000
HR_{OBLA} (bpm)	**Pre**	163.50	162.22	9.50	12.99	0.885
	Post 1	163.63	163.56	7.78	12.95	0.772
	Post 2	166.25	163.67	8.26	12.56	0.469
W_{OBLA} (W)	**Pre**	6.56	8.33	2.96	1.77	0.201
	Post 1	7.50	9.17	1.89	2.17	0.101
	Post 2	8.13	9.72	2.91	2.92	0.232
Peak work (W)	**Pre**	11.25	12.50	2.67	1.77	0.244
	Post 1	12.19	14.17	2.48	2.17	0.141
	Post 2	12.81	14.17	2.81	2.17	0.267
HR_{max} (bpm)	**Pre**	177.50	174.78	10.25	13.25	0.531
	Post 1	178.38	176.44	6.52	12.90	0.288
	Post 2	180.50	177.56	9.68	10.28	0.735
Peak RER	**Pre**	1.14	1.14	0.05	0.05	0.846
	Post 1	1.14	1.19	0.06	0.04	0.053
	Post 2	1.16	1.17	0.04	0.05	0.310
End lactate (mmol/ℓ)	**Pre**	9.41	7.92	2.53	3.47	0.123
	Post 1	11.14	10.82	2.66	3.46	0.700
	Post 2	9.30	10.69	2.48	4.10	0.500
LA5min (mmol/ℓ)	**Pre**	9.98	10.32	2.46	2.14	0.773
	Post 1	9.39	11.29	1.96	1.34	0.034*
	Post 2	9.74	10.38	2.08	4.01	0.629
HR5min (bpm)	**Pre**	104.75	99.89	9.98	15.16	0.177
	Post 1	99.25	100.44	8.92	13.82	0.847
	Post 2	102.25	102.33	8.51	16.47	1.000

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

3.3.1.3 Sub-maximal running economy

Although the body weight of the CRE group was lower than the PLA group at the onset of the study (Table 3-3), there were no statistically significant differences in their baseline running economy (VO_2, expressed as ml/kg/min) at all test speeds (Table 3-5). Both groups increased their running economy scores at post-tests 1 and 2 (Table 3-5). However, the running economy of the CRE group had increased significantly more than that of the PLA group at 10 months of supplementation for treadmill speeds of 10 km/h ($p = 0.027$) and 12 km/h ($p = 0.016$).

The differences in the steady-state HR scores between the CRE group and the PLA group were significant during the baseline tests (ie. 10 km/h, 12 km/h and 15 km/h) in that the scores of the CRE group were lower than the PLA group's scores (Table 3-5). The differences in steady-state HR scores between the CRE group and the PLA group were significant during both post-tests for running speeds of 10 km/h and 12 km/h. In both instances the scores of the CRE group were lower than that of the PLA group (Table 3-5). Thus, it appears from the recorded scores as indicated above that the two groups differed significantly in terms of their steady-state heart-rate response at all three running speeds during baseline testing. This difference in between-group scores remained significant during both post-tests for running speeds of 10 km/h and 12 km/h (Table 3-5).

Table 3-5 Between-group analysis of the change in recorded scores associated with the sub-maximal running economy of the CRE group compared to those of the PLA group

Variable	Test	Mean		Standard Deviation		p-value
		PLA	CRE	PLA	CRE	
RE10 (mlO_2/kg/min)	**Pre**	34.88	34.81	2.14	1.93	0.923
	Post 1	31.11	30.69	6.44	2.10	0.228
	Post 2	33.23	29.90	2.83	1.97	0.027*
RE12 (mlO_2/kg/min)	**Pre**	42.01	41.45	3.33	2.75	0.563
	Post 1	38.86	38.79	6.41	3.64	0.413
	Post 2	40.04	36.81	3.11	2.35	0.016*
RE15 (mlO_2/kg/min)	**Pre**	48.05	50.00	1.23	5.05	0.102
	Post 1	45.18	47.47	7.20	3.15	0.923
	Post 2	46.64	45.61	2.89	2.78	0.470

Variable	Test	Mean		Standard Deviation		p-value
		PLA	CRE	PLA	CRE	
HR10 (bpm)	**Pre**	158.00	138.44	8.25	13.26	0.006*
	Post 1	147.25	132.67	12.81	8.56	0.026*
	Post 2	149.00	133.33	13.70	10.15	0.021*
HR12 (bpm)	**Pre**	169.63	154.11	9.64	13.05	0.027*
	Post 1	163.50	148.67	11.61	9.92	0.009*
	Post 2	164.13	148.22	12.89	11.00	0.012*
HR15 (bpm)	**Pre**	181.25	170.78	8.60	9.54	0.047*
	Post 1	179.00	171.67	10.28	7.68	0.083
	Post 2	180.38	172.78	11.21	8.45	0.100
HR5min (bpm)	**Pre**	114.88	97.89	12.53	15.04	0.018*
	Post 1	104.13	96.89	9.55	11.89	0.178
	Post 2	110.13	100.78	12.73	8.89	0.101

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

3.3.1.4 Blood and urine chemical pathology

At the onset of the study no statistically significant differences were found between the blood and urine chemical-pathology scores of the CRE and PLA groups (Table 3-6). Thus, no significant difference between them was found when they were compared at each clinical marker.

Supplementation did not result in statistically significant between-group changes in the scores for chemical pathology (Table 3-6).

Table 3-6 Between-group analysis of the change in recorded scores associated with the serum chemical pathology of the CRE group compared to those of the PLA group

Variable	Test	Mean		Standard Deviation		p-value
		PLA	CRE	PLA	CRE	
BIOCHEMISTRY						
Creatinine (mmol/ℓ)	**Pre**	95.77	97.75	10.40	10.04	0.563
	Post	97.13	103.50	20.52	13.97	0.318
CK (U/ℓ)	**Pre**	223.67	274.38	137.15	300.81	0.500
	Post	172.38	207.50	59.17	100.49	0.529

Variable	Test	Mean		Standard Deviation		p-value
		PLA	CRE	PLA	CRE	
CK-MB mass (ng/ml)	**Pre**	3.52	5.23	1.59	4.43	0.665
	Post	3.50	3.89	1.61	1.96	0.563
CK-MB index (%)	**Pre**	1.90	2.18	1.09	0.78	0.469
	Post	2.01	1.99	0.50	0.76	0.635
BIOCHEMISTRY						
ALT (U/ℓ)	**Pre**	25.56	22.13	6.62	6.03	0.335
	Post	26.63	24.13	7.67	10.68	0.246
AST (U/ℓ)	**Pre**	29.44	27.25	7.49	5.50	0.885
	Post	26.50	26.50	4.34	3.70	0.672
GGT (U/ℓ)	**Pre**	23.22	26.50	8.50	8.55	0.500
	Post	20.88	18.75	6.81	6.63	0.494
LDH (U/ℓ)	**Pre**	478.89	491.63	47.35	111.72	0.630
	Post	444.88	473.25	49.53	77.75	0.563
HAEMATOLOGY						
Prothrombin index (%)	**Pre**	11.76	12.08	0.49	0.71	0.469
	Post	11.99	12.20	0.68	0.81	0.862
Prothrombin INR	**Pre**	11.56	11.66	0.47	0.44	0.490
	Post	11.80	11.59	0.41	0.10	0.455
URINE						
Microalbumin (mg/ℓ)	**Pre**	6.64	2.35	5.88	13.26	0.357
	Post	11.43	2.50	18.64	7.07	0.206

Pre- : baseline test

Post- : at 10 weeks of supplementation

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

3.3.2 Results of the comparison of the pre- and post-tests of the same group across various recorded scores (within-group data analyses)

As indicated earlier, post-tests were performed one week after the administration of moderate-dosage Cr supplementation (post-test 1), and once again after 9 weeks at lower-dosage maintenance ingestion (post-test 2). Within-group changes in scores were explored with Friedman's rank tests.

3.3.2.1 Body composition

The body weight and fat% of the PLA group decreased over time (Table 3-7). The change in body weight of this group reached significance (p = 0.032) at 10 weeks (post-test 2). Both the body weight and fat% of the CRE group remained relatively stable as the study progressed (Table 3-7).

The mesomorphic component of the CRE group increased significantly from the pre-test to the two consecutive post-tests (Table 3-7). Thus, the relative musculoskeletal robustness of the CRE group increased significantly over the 10-week period, while no improvement was evident in the PLA group. As can be seen in Table 3-7, the mesomorphic component of the PLA group showed a steady decrease (albeit below statistical significance) from pre-test to post-test 2. The CRE group showed a tendency towards increased LBW, but again, this value did not rise to significance (Table 3-7).

Table 3-7 Comparison of the change in body composition of ultradistance runners in the CRE and PLA groups across test intervals

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
Weight (kg)	**Pre**	81.50	75.24	7.60	5.39
	Post 1	80.88	75.18	7.64	5.27
	Post 2	80.23	75.42	7.17	5.11
	p-value	0.032*	0.539		
Fat%	**Pre**	17.26	14.94	4.95	4.42
	Post 1	17.13	15.08	4.58	3.58
	Post 2	16.49	14.37	4.08	4.22
	p-value	0.078	0.682		
Endomorphy	**Pre**	3.59	2.93	1.42	1.29
	Post 1	3.55	3.01	1.29	1.06
	Post 2	3.34	2.86	1.21	1.24
	p-value	0.355	0.666		
Mesomorphy	**Pre**	5.48	5.18	0.97	1.31
	Post 1	5.41	5.37	1.04	1.23
	Post 2	5.30	5.37	0.86	1.03
	p-value	0.331	0.042*		

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
Ectomorphy	**Pre**	1.93	2.26	0.61	1.22
	Post 1	1.99	2.26	0.60	1.14
	Post 2	2.00	2.20	0.57	1.12
	p-value	0.104	0.446		
LBW (kg)	**Pre**	66.96	63.84	4.66	3.19
	Post 1	66.84	62.96	5.04	4.99
	Post 2	66.81	64.46	4.27	3.56
	p-value	0.882	0.368		

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

3.3.2.2 Maximal aerobic capacity

The VO_2 max of the PLA group increased significantly over time ($p = 0.036$), while that of the CRE group decreased, although not significantly (Table 3-8). The workload at which OBLA was reached also increased significantly in the PLA group ($p = 0.022$). Their fitness%, heart rate at OBLA, peak work and maximum heart rate increased over time, but not significantly (Table 3-8).

A tendency towards both HR_{OBLA} and HR_{max} was also evident in the CRE group (Table 3-8). However, the CRE group demonstrated a significant increase in both peak work and end lactate (Table 3-8). The significant improvement in peak work was already reached after Cr loading (post-test 1), and then maintained by maintenance ingestion ($p = 0.006$).

Table 3-8 Comparison of the change in recorded values associated with maximal aerobic capacity of ultradistance runners in the CRE and PLA groups across test intervals

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
VO_2 max (ml/kg/min)	**Pre**	52.11	57.23	5.72	5.29
	Post 1	54.21	56.48	4.82	5.76
	Post 2	53.70	55.81	4.65	6.54
	p-value	0.036*	0.459		
Fitness (%)	**Pre**	83.80	83.60	8.21	9.15
	Post 1	82.80	88.52	6.38	6.33
	Post 2	84.81	82.82	4.30	8.06
	p-value	0.882	0.196		

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
HR_{OBLA} (bpm)	**Pre**	163.50	162.22	9.50	12.99
	Post 1	163.63	163.56	7.78	12.95
	Post 2	166.25	163.67	8.26	12.56
	p-value	0.093	0.761		
W_{OBLA} (W)	**Pre**	6.56	8.33	2.96	1.77
	Post 1	7.50	9.17	1.89	2.17
	Post 2	8.13	9.72	2.91	2.92
	p-value	0.022*	0.210		
Peak work (W)	**Pre**	11.25	12.50	2.67	1.77
	Post 1	12.19	14.17	2.48	2.17
	Post 2	12.81	14.17	2.81	2.17
	p-value	0.093	0.006*		
HR_{max} (bpm)	**Pre**	177.50	174.78	10.25	13.25
	Post 1	178.38	176.44	6.52	12.90
	Post 2	180.50	177.56	9.68	10.28
	p-value	0.066	0.139		
Peak RER	**Pre**	1.14	1.14	0.05	0.05
	Post 1	1.14	1.19	0.06	0.04
	Post 2	1.16	1.17	0.04	0.05
	p-value	0.140	0.166		
End lactate (mmol/ℓ)	**Pre**	9.41	7.92	2.53	3.47
	Post 1	11.14	10.82	2.66	3.46
	Post 2	9.30	10.69	2.48	4.10
	p-value	0.882	0.050*		
LA5min (mmol/ℓ)	**Pre**	9.98	10.32	2.46	2.14
	Post 1	9.39	11.29	1.96	1.34
	Post 2	9.74	10.38	2.08	4.01
	p-value	0.417	0.368		
HR5min (bpm)	**Pre**	104.75	99.89	9.98	15.16
	Post 1	99.25	100.44	8.92	13.82
	Post 2	102.25	102.33	8.51	16.47
	p-value	0.393	0.819		

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

3.3.2.3 Sub-maximal running economy

Table 3-9 shows that the sub-maximal running economy (RE) of the CRE group had improved significantly at post-test 1, and then further improved over the research period for running speeds of 10 km/h ($p = 0.001$), 12 km/h ($p = 0.008$) and 15 km/h ($p = 0.018$). No significant changes in RE were evident in the PLA group.

Steady-state HR decreased significantly in both the CRE and PLA groups at running speeds of 10 km/h and 12 km/h (Table 3-9). These changes were evident at post-test 1 and then remained significant for the duration of the research period (Table 3-9). Thus, a decreased steady-state HR was recorded for both the CRE and PLA groups for a given sub-maximal running speed over the research period.

Table 3-9 Comparison of the change in recorded scores associated with sub-maximal running economy of ultradistance runners in the CRE and PLA groups across test intervals

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
RE10 ($mlO_2/kg/min$)	**Pre**	34.88	34.81	2.14	1.93
	Post 1	31.11	30.69	6.44	2.10
	Post 2	33.23	29.90	2.83	1.97
	p-value	0.072	0.001*		
RE12 ($mlO_2/kg/min$)	**Pre**	42.01	41.45	3.33	2.75
	Post 1	38.86	38.79	6.41	3.64
	Post 2	40.04	36.81	3.11	2.35
	p-value	0.072	0.008*		
RE15 ($mlO_2/kg/min$)	**Pre**	48.05	50.00	1.23	5.05
	Post 1	45.18	47.47	7.20	3.15
	Post 2	46.64	45.61	2.89	2.78
	p-value	0.197	0.018*		
HR10 (bpm)	**Pre**	158.00	138.44	8.25	13.26
	Post 1	147.25	132.67	12.81	8.56
	Post 2	149.00	133.33	13.70	10.15
	p-value	0.016*	0.016*		

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
HR12 (bpm)	**Pre**	169.63	154.11	9.64	13.05
	Post 1	163.50	148.67	11.61	9.92
	Post 2	164.13	148.22	12.89	11.00
	p-value	0.023*	0.044*		
HR15 (bpm)	**Pre**	181.25	170.78	8.60	9.54
	Post 1	179.00	171.67	10.28	7.68
	Post 2	180.38	172.78	11.21	8.45
	p-value	0.772	0.572		
HR5min (bpm)	**Pre**	114.88	97.89	12.53	15.04
	Post 1	104.13	96.89	9.55	11.89
	Post 2	110.13	100.78	12.73	8.89
	p-value	0.010*	0.717		

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

3.3.2.4 Blood and urine pathology

The CRE group demonstrated a significantly elevated S-Creatinine level after 10 weeks of Cr ingestion ($p = 0.050$), while the PLA group showed no significant change (Table 3-10). S-CK activity decreased significantly in the PLA group during 10 weeks of ultradistance training ($p = 0.036$).

Table 3-10 Comparison of the change in recorded scores associated with chemical pathology of ultradistance runners in the CRE and PLA groups across test intervals

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
BIOCHEMISTRY					
Creatinine (mmol/ℓ)	**Pre**	95.77	97.75	10.40	10.04
	Post	97.13	103.50	20.52	13.97
	p-value	0.833	0.050*		
CK (U/ℓ)	**Pre**	223.67	274.38	137.15	300.81
	Post	172.38	207.50	59.17	100.49
	p-value	0.036*	0.575		

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
CK-MB mass (ng/ml)	**Pre**	3.52	5.23	1.59	4.43
	Post	3.50	3.89	1.61	1.96
	p-value	0.674	0.726		
CK-MB index (%)	**Pre**	1.90	2.18	1.09	0.78
	Post	2.01	1.99	0.50	0.76
	p-value	0.051	0.396		
CK-MB index (%)	**Pre**	1.90	2.18	1.09	0.78
	Post	2.01	1.99	0.50	0.76
	p-value	0.051	0.396		
ALT (U/ℓ)	**Pre**	25.56	22.13	6.62	6.03
	Post	26.63	24.13	7.67	10.68
	p-value	0.292	0.352		
AST (U/ℓ)	**Pre**	29.44	27.25	7.49	5.50
	Post	26.50	26.50	4.34	3.70
	p-value	0.173	0.733		
GGT (U/ℓ)	**Pre**	23.22	26.50	8.50	8.55
	Post	20.88	18.75	6.81	6.63
	p-value	0.931	0.028*		
LDH (U/ℓ)	**Pre**	478.89	491.63	47.35	111.72
	Post	444.88	473.25	49.53	77.75
	p-value	0.050*	0.401		
HAEMATOLOGY					
Prothrombin index (%)	**Pre**	11.76	12.08	0.49	0.71
	Post	11.99	12.20	0.68	0.81
	p-value	0.553	0.799		
Prothrombin INR	**Pre**	11.56	11.66	0.47	0.44
	Post	11.80	11.59	0.41	0.10
	p-value	0.309	1.000		
URINE					
Microalbumin (mg/ℓ)	**Pre**	2.35	6.64	5.88	13.26
	Post	11.43	2.50	18.64	7.07
	p-value	0.109	0.317		

Pre- : baseline test

Post- : at 10 weeks

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

S-GGT levels decreased significantly after Cr ingestion, while the decrease recorded for the PLA group was insignificant (Table 3-10). S-LDH levels of the PLA group decreased significantly over the research period, while the decrease demonstrated by the CRE group did not reach statistical significance.

As demonstrated by Table 3-10, large differences between individuals - as evidenced by high standard deviation scores - were prevalent for indices of chemical pathology. This is especially true for S-Crn, S-CK, S-CK-MB and U-microalbumin scores.

Two individuals in the CRE-group were referred to a cardiologist after baseline testing, because they demonstrated both S-CK and S-CK-MB mass scores above the normal range. The cardiologist found no structural or functional restrictions of the myocardium, and thus cleared them for participation.

None of the participants reported side-effects (eg. gastrointestinal upset) that could be attributable to Cr supplementation.

3.4 DISCUSSION

Cr supplementation is associated with an increase in body weight ranging between 0.5 kg and 5 kg, as reflected in Table 2-3. Cr is an osmotically active substance, hence increases in body weight are usually attributed to water retention (Rawson & Clarkson, 2000; Persky & Brazeau, 2001; Mendes *et al.*, 2004; Ahmun *et al.*, 2005; Gotshalk *et al.*, 2008; Safdar *et al.*, 2008; Rahimi *et al.*, 2010; Vatani *et al.*, 2011). A comparison of differences between the CRE group and the PLA group led to no indication that either short-term moderate-dosage Cr supplementation, or longer-term low-dosage Cr ingestion caused a significant change in body weight, musculoskeletal robustness (mesomorphy) or LBW scores of well-trained ultradistance runners (Table 3-3).

However, when changes that occurred within the groups were analysed the PLA group was found to have sustained a significant weight loss over the 10-week supplementation period,

while no significant change in body weight was recorded for the CRE group (Table 3-7). The weight loss sustained by the PLA group could be partially attributable to the combined tendencies towards a loss of relative musculoskeletal robustness (mesomorphy) and fat% as shown in Table 3-7. These changes are attributable to the effect of the long-distance endurance training programme followed in preparation for the Comrades Marathon. The participants covered training distances ranging between 57 km/week to 130 km/week. These sessions included long training runs ("long, slow, distance runs"), time trials and interval-training (3.2.1.2). Thus, the tendency to sustain a loss in musculoskeletal robustness and fat% was to be expected. The loss of muscle mass in endurance athletes is usually attributed to increased protein oxidation due to the body's high energy needs (Fielding & Parkington, 2002; Tarnopolsky, 2006), and/or decreased muscle protein synthesis due to the activation of intra-cellular signaling pathways (Nader, 2006).

A major finding of this study was that Cr supplementation prevented the decrease in body weight and musculoskeletal component that were found in the PLA group. The CRE group improved their mesomorphic component during intensive endurance training, without significantly impacting on their body weight (Table 3-7). The increase in mesomorphy was already evident after short-term moderate-dosage supplementation, and was maintained for the duration of the study. The LBW of the group increased on average by ~0.6 kg after 10 weeks of Cr supplementation, but this was not statistically significant. The reason for these increases is unclear, but may be attributable to cell hydration (Rawson & Clarkson, 2000; Persky & Brazeau, 2001; Mendes *et al.*, 2004; Ahmun *et al.*, 2005; Gotshalk *et al.*, 2008; Safdar *et al.*, 2008; Rahimi *et al.*, 2010; Vatani *et al.*, 2011) and/or changes in intracellular signaling pathways. Cellular hydration (swelling) can be considered an anabolic proliferative signal (Häussinger *et al.*, 1993; Schliess & Häussinger, 2002), as it favours the synthesis and inhibits the degradation of muscle protein (Häussinger *et al.*, 1993; Schliess & Häussinger, 2002; Brilla *et al.*, 2003). Because body hydration levels were not determined in the current study, the increase in mesomorphy cannot be attributed beyond a reasonable doubt to the osmotic characteristic of Cr (cellular swelling), which exerts an anabolic effect on the cellular environment, although the possibility remains.

Endurance exercise, however, is associated with activation of AMP-activated protein kinase (AMPK) signaling within cells (Nader, 2006). AMPK is activated by a decrease in the energy charge of the muscle cell, that is, an increase in the ADP/ATP ratio. Activation of AMPK

signaling reduces muscle protein synthesis by inhibiting other anabolic molecular pathways (Nader, 2006). Thus, hypothetically, Cr supplementation during prolonged high-intensity endurance training may improve the sustainability of the energy charge of the muscle cell by increasing the availability of high-energy phosphates. AMPK signaling during training will potentially be reduced, thereby maintaining or improving muscle protein synthesis and therefore resulting in a maintained/increased musculoskeletal robustness of the ultradistance runner.

Very few studies have reported on the effects of Cr supplementation on the body weight and/or body composition of endurance athletes (Rico-Sanz & Marco, 2000; Jones *et al.*, 2002; Van Loon *et al.*, 2003; McConell *et al.*, 2005; Reardon *et al.*, 2006; Hadjicharalambous *et al.*, 2008; Graef *et al.*, 2009; Hickner *et al.*, 2010). None of these studies incorporated Cr ingestion for longer than 6 weeks. Three studies reported significant increases in body weight after Cr ingestion at high-dosage loading (Hadjicharalambous *et al.*, 2008); after a loading dosage followed by low-dosage supplementation (Van Loon *et al.*, 2003); and after longer-term low-dosage supplementation (Hickner *et al.*, 2010). Van Loon *et al.* (2003) attributed the increases in body weight respectively after Cr loading and maintenance ingestion, to significant increases in muscle TCr and PCr that exerted an osmotic effect on muscle cells and/or up-regulated the anabolic status of the cells. The remaining four studies reported no significant changes in body weight and/or composition after Cr loading (Rico-Sanz & Marco, 2000; McConell *et al.*, 2005), moderate-dosage supplementation (Graef *et al.*, 2009) or low-dosage ingestion (Reardon *et al.*, 2006). The studies clearly differed significantly in their Cr supplementation regimens, which presents an obstacle to comparison. However, it seems that results from the current study are consonant with most of the relevant research that has shown no significant effect of Cr supplementation on the body weight of endurance athletes.

Researchers have paid far less attention to the effect of Cr supplementation on aerobic endurance performance (Balsom *et al.* (1993*b*; Jones *et al.*, 2002; Ööpik *et al.*, 2002; Van Loon *et al.*, 2003; Van Schuylenbergh *et al.*, 2003; McConell *et al.*, 2005; Reardon *et al.*, 2006; Stout *et al.*, 2006; Hadjicharalambous *et al.*, 2008; Graef *et al.*, 2009) than to anaerobic performance, especially where performance during repeated bouts of maximal exercise is concerned (Greenhaff *et al.*, 1993*b*; Vandenberghe *et al.*, 1996*a*; Rossouw *et al.*, 2000; Bennett *et al.*, 2001; Preen *et* al., 2001; Wiedermann *et al.*, 2001; Izquierdo *et al.*, 2002; Warber *et al.*, 2002; Ziegenfuss *et al.*, 2002; Van Loon *et al.*, 2003; Ahmun *et al.*, 2005; Peyrebrune *et al.*, 2005; Glaister *et al.*, 2006; Pluim *et al.*, 2006; Jäger *et al.*, 2008; Rawson *et al.*, 2011). However, the

undulating terrain over which endurance events usually take place naturally will cause a shift to anaerobic energy provision when athletes encounter a hill and/or increase their pace (Stroud *et al.*, 1994; Rico-Sanz & Marco, 2000). Furthermore, research indicates that substrate utilisation during aerobic activity (Stroud *et al.*, 1994) and during rest (Huso *et al.*, 2002) may be modified by Cr use. These arguments lend support to the potential benefit of Cr supplementation to endurance performance (> 150s).

At the onset of the study the groups did not differ significantly with regard to maximal aerobic capacity scores (Table 3-4). As the study progressed, however, significant intragroup changes occurred in several recorded scores (Table 3-8). When these changes were analysed it was found that they reflected significantly improved scores for both groups as regards maximal aerobic work capacity. The significant increase in both VO_2 max and $Work_{OBLA}$ recorded for the PLA group (Table 3-8) is attributable to the combined effect of the progressive training programme followed and the significant decrease in their mean body weight and fat% (Table 3-7). These changes improved the efficiency of their aerobic system. The tendency towards a progressive decrease in the mean VO_2 max of the CRE group was an unexpected result (Table 3-8). The decrease was not significant, however, and did not detract from their capacity for aerobic work. A significant improvement in their peak work had already been demonstrated after Cr loading, which was followed by a maintenance regime lasting throughout the course of the study. The CRE group also demonstrated a significant increase in end lactate, thereby supporting the finding of an increased capacity for peak work output during an incremental test for maximal aerobic capacity.

Thus, the participants' training programme was effective in improving the measurements reflecting maximal aerobic work capacity of both groups (Table 3-8). Furthermore, both moderate-dosage short-term Cr ingestion and low-dosage longer-term supplementation proved effective in increasing the capacity for peak work in well-trained ultradistance runners during a test for maximal aerobic capacity. This may be attributable to the fact that Cr supplementation increased the availability of muscle free Cr and PCr, thus resulting in a higher rate of ATP resynthesis and, consequently, lower cellular AMP and IMP accumulation (Rico-Sanz & Marco, 2000). The increase in muscle free Cr should potentially favour changes in oxidative phosphorylation in contracting muscle fibres due to an amplification of mitochondrial-CK activity and, consequently, the PCr shuttle (Rico-Sanz & Marco, 2000; McConell *et al.*, 2005). Energy is shuttled out of the mitochondria by the transporter PCr, and is then transferred back to ADP in

the cytosol, thereby making ATP available at the actin-myosin cross-bridge interface (Rico-Sanz & Marco, 2000; McConell *et al.*, 2005). Energy balance within the muscle cells should be better maintained by means of these mechanisms during endurance exercise. However, explained earlier in comparing differences between the CRE group and the PLA group, the findings suggest that neither short-term moderate-dosage Cr supplementation, nor longer-term low-dosage Cr ingestion enhanced the CRE group's training adaptation for peak work above that demonstrated by the PLA group (Table 3-4).

Unfortunately, by far the greatest part of studies conducted to determine the effect of Cr supplementation on aerobic endurance performance have focused on cycling. Recent findings (post-2000) report performance benefits for both sub-maximal (Ööpik *et al.*, 2002; Hadjicharalambous *et al.*, 2008; Graef *et al.*, 2009) and maximal (McConell *et al.*, 2005) aerobic cycling exercise. However, the majority of studies report no benefit to either sub-maximal (Van Loon *et al.*, 2003; Van Schuylenbergh *et al.*, 2003; McConell *et al.*, 2005; Stout *et al.*, 2006) or maximal (Jones *et al.*, 2002; Van Loon *et al.*, 2003; Reardon *et al.*, 2006; Graef *et al.*, 2009) aerobic performance. Consonant with these findings, Balsom *et al.* (1993*b*) reported no performance enhancement or increase in peak oxygen uptake following a supramaximal treadmill run to exhaustion (~ 4 min). Thus, the present study is the first to demonstrate an increase in peak work output during a test for maximal aerobic capacity on a treadmill. The increase in peak work of well-trained ultradistance runners may be attributable to the combined effects of the progressive training programme and an increase in mesomorphy. It is reasonable to suggest that - without a significant change in body weight, and with enhanced cellular energy balance (McLeish & Kenyon, 2005; Nader, 2006) - the CRE group could significantly increase their peak work output as a result of the increase in musculoskeletal component.

The speculative rationale has been raised that Cr supplementation might increase the RER during rest (Huso *et al.*, 2002) and endurance exercise (Jones *et al.*, 2002; Van Loon *et al.*, 2003; McConell *et al.*, 2005; Hadjicharalambous *et al.*, 2008), thereby indicating a shift towards greater CHO oxidation and thus more efficient oxygen utilisation. The RER is a value that provides information regarding the proportion (%) of energy derived from various nutrients at rest and during steady-state sub-maximal exercise as indicated in Table 2-2 (Franklin *et al.*, 1989; Wilmore & Costill, 2008). It indicates the ratio between the amount of carbon dioxide produced and the amount of oxygen consumed. Values exceeding 1.0 indicate that the subject's metabolism is beginning to rely mainly on anaerobic processes (Morgan *et al.*, 1989; McArdle *et*

al., 2001). An accurate estimate of the fuel type being used is no longer possible when the RER value exceeds 1.0, as when the individual approaches exhaustion (Wilmore & Costill, 2008). Although the body reaps more energy when it metabolises a given amount of fat than when it metabolises the same amount of CHO, it uses proportionally more oxygen to oxidise fat (Wilmore & Costill, 2008).

To date, none of the studies measuring RER at rest (Huso *et al.*, 2002) or during endurance exercise of varying intensity (Jones *et al.*, 2002; Van Loon *et al.*, 2003; McConell *et al.*, 2005; Reardon *et al.*, 2006; Hadjicharalambous *et al.*, 2008; Hickner *et al.*, 2010; Beis *et al.*, 2011) have revealed a significant change in RER, and thus in CHO oxidation, in response to Cr supplementation. The present study revealed that, when differences in peak RER scores between the CRE and the PLA group were compared, a change at the 10% level of confidence ($p = 0.053$) was evident after short-term moderate-dosage Cr supplementation in well-trained ultradistance runners (Table 3-3). The increase in peak RER score indicates a greater reliance on anaerobic energy sources during maximal aerobic work (Morgan *et al.*, 1989). As it is suspected that Cr increases cell hydration and therefore cell volume it would be reasonable to expect a concomitant increase in muscle glycogen content (Huso *et al.*, 2002). Thus, the greater availability of CHO after Cr loading may have resulted in a greater anaerobic use of CHO at high-intensity aerobic exercise, thereby significantly increasing both peak work within the CRE group (Table 3-8) and between-group differences in peak blood lactate concentrations (Table 3-4). The significant increase in peak RER may further be attributable to greater/more efficient anaerobic ATP regeneration *via* the ATP-PCr system in response to increased muscle TCr and PCr concentrations, thereby increasing peak work output during an incremental test for maximal aerobic capacity. However, when adhering to the 5% level of confidence, Cr supplementation did not alter peak RER significantly during aerobic exercise testing.

In summary, it is reasonable to propose that both Cr supplementation protocols employed in this study were effective at raising muscle TCr and PCr levels, as no treatment response would have been apparent if the supplementation protocol had been ineffective (Van Schuylenbergh *et al.*, 2003; Stout *et al.*, 2006). It is therefore suggested that the increase in muscle TCr and PCr content, and consequently the enhanced buffering of large amounts of ADP at myofibrillar sites in muscle fibre types I as well as II (ie. facilitating the PCr energy shuttle), was the reason for the increase in work output during maximal aerobic exercise testing (Rico-Sanz & Marco, 2000). The increase in the musculoskeletal component of the CRE group may have further contributed

to this effect. Furthermore, the presence of an adequate exercise stimulus during the supplementation period, previously proposed to be essential but lacking in other endurance study designs (Reardon *et al.*, 2006), may be one of the reasons for the benefits to maximal aerobic performance demonstrated by Cr supplementation in ultradistance athletes.

Running economy (RE) is typically defined as the energy demand for a given velocity of sub-maximal running, and is determined by measuring the steady-state consumption of oxygen (Saunders *et al.*, 2004). There is a strong association between RE and distance running performance (Saunders *et al.*, 2004). At the onset of the current study the groups did not differ significantly with regard to sub-maximal RE at test speeds of 10, 12 and 15km/h (Table 3-4). Even though both groups increased their RE at post-tests 1 and 2 - as can be expected from aerobic training - the RE of the CRE group had increased significantly more than that of the PLA group at 10 months of supplementation for treadmill speeds of 10 km/h and 12 km/h. Thus, when compared to the changes that took place in the PLA group's scores, longer-term Cr supplementation significantly lowered the volume of oxygen consumed to maintain a steady running pace at 10 km/h and 12 km/h, respectively (Table 3-5). The CRE group thus demonstrated an aerobic training adaptation above the level found in the PLA group.

Higher body weight and/or gains in body weight could, theoretically, increase the oxygen cost of running (Beis *et al.*, 2011). However, the lower (though not statistically significant) body weight of the CRE group cannot explain their significantly increased RE through the course of the study, as the baseline RE of the groups were similar (Table 3-5). Also, even though the PLA group's body weight decreased significantly over the course of the study, this did not translate into improved RE, thus lending support to the theory that RE is an inherent ability (ie. economical running pattern) that will remain relatively stable in controlled environments (Morgan *et al.*, 1989; Morgan & Craib, 1992; Saunders *et al.*, 2004). However, RE may improve with training due to metabolic adaptations within the muscle, such as increased mitochondria and oxidative enzymes, improved ability of the muscle to store and release elastic energy, and more efficient mechanics leading to less energy wasted on braking forces and excessive vertical oscillation (Saunders *et al.*, 2004). Also, cardiorespiratory fitness and RE are inversely related (Sawyer *et al.*, 2010). These adaptations could account for the improved RE of both groups after standardised endurance training. It does not, however, account for the enhanced training adaptation demonstrated by the CRE group. It therefore seems reasonable to suggest that Cr consumption itself was the sole agency eliciting this effect.

The significantly lower oxygen cost of running at a fixed sub-maximal velocity after prolonged Cr supplementation (Table 3-5) may be attributable to factors other than muscle energy balance (McConell *et al.* 2005; Hadjicharalambous *et al.*, 2008). Jones *et al.* (2002) postulated that the effect of Cr supplementation on sub-maximal endurance performance could be related to changes in individuals' motor unit recruitment patterns or the volume of muscle activated. They (Jones *et al.*, 2002) proposed that Cr loading might increase recruitment of type II muscle fibres during intense sub-maximal endurance exercise, and/or cause a reduction in the total amount of muscle mass recruited to perform this type of exercise. Another theory holds that RE may be enhanced by increased muscle stiffness. Dumke *et al.* (2010 : 255) postulate that muscle stiffness is significantly related to RE at speeds that approximate endurance competition: *"The conversion of energy to motion involves the recoil of some elastic energy in muscle and tendon. Muscle and tendon are two springs in series. In the translation of energy expenditure to ground force during running more energy is stored in the compliant spring. As stiffness increases, less muscle activation is required (to maintain a fixed running velocity), and therefore energy expenditure is spared. Since muscle is the more compliant of the two springs, it is conducive to its relationship with RE... A "stiffer" muscle is thus better at transferring energy economically…"*. Cr supplementation may increase muscle stiffness through increased cellular hydration and/or muscle accretion. Thus - since muscle Cr accumulation is improved in muscles that are exercised (Greenhaff, 1995) and may thereby have improved RE - the increase in musculoskeletal robustness demonstrated by the CRE group may have given rise to stiffer skeletal muscle of the lower body. Finally, an increase in muscle strength and power has been shown to increase RE (Spurrs *et al.*, 2003). Therefore, the increase in peak work demonstrated after Cr supplementation (Table 3-8) may have contributed to an increase in RE. However, given the limitations of the current study design, the exact cellular and/or molecular mechanisms involved have not been elucidated.

Heart rate and ventilation are two physiological indices reflecting the oxygen demand of the exercising muscles (Morgan & Craib, 1992). It also acts as cardio-pulmonary markers of exercise intensity (Faulkner *et al.*, 2012). Both groups' HR-response to sub-maximal running decreased significantly at post-test 1, and then remained at that lower level. The mechanism causing the drop in HR may be an enhanced RE (ie. improved efficiency in oxygen utilisation due to training) and, for that reason, less cardiopulmonary strain. However, the specific

relationship between HR, ventilation and RE has yet to be explained (Morgan & Craib, 1992; Braun & Paulson, 2012).

In summary, for the CRE as well as the PLA group improved sub-maximal RE in response to progressive, high-intensity, high-volume aerobic training was recorded. However, short-term moderate-dosage Cr supplementation followed by longer-term low-dosage ingestion elevated the training effect to a level above that resulting from placebo ingestion. The enhanced training effect brought about by Cr ingestion was demonstrated in the performance of single-effort strength/power activities after participants had taken part in structured resistance training (Peeters *et al.*, 1999; Stout *et al.*, 1999; Volek *et al.*, 1999; Arciero *et al.*, 2001; Bemben *et al.*, 2001; Huso *et al.*, 2002; Brilla *et al.*, 2003; Brose *et al.*, 2003; Burke *et al.*, 2003; Ostojic, 2004; Olsen *et al.*, 2006; Law *et al.*, 2009). The current study supports findings by Chwalbinska-Moneta (2003) that short-term higher-dosage Cr ingestion in conjunction with sport-specific endurance training will increase peak power output during a sport-specific laboratory test for maximal aerobic capacity. However, since the study protocol of Chwalbinska-Moneta (2003) did not incorporate longer-term Cr ingestion, the current study is the first to demonstrate an enhanced sport-specific endurance training effect after prolonged Cr ingestion.

The safety of Cr supplementation remains a matter for concern since the use of Cr has become widespread among both sports practitioners (Dascombe *et al*. 2010; Gradidge, 2010; Tscholl *et al.*, 2010) and people engaging in comparable levels of physical activity (Sheppard *et al.*, 2000; Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010; Tsitsimpikou *et al.*, 2011). No conclusive evidence exists concerning the possible health risks of long-term Cr supplementation (Schröder *et al.* 2005) - even though the recommended ULS has been set at 5 g/day (Shao & Hathcock, 2006) - and, therefore, accusations of adverse effects continue (Shao & Hathcock, 2006).

The degradation of Cr and PCr in humans is, for the most part, a spontaneous non-enzymatic process (Wyss & Kaddurah-Daouk, 2000). The resulting product, Crn, passively diffuses out of the body cells. It is removed from the circulatory system by glomerular filtration in the kidney (Bishop *et al.*, 2000) and excreted into the urine (Wyss & Schulze, 2002; Brudnak, 2004). It is expected that Cr supplementation would increase the daily rate of Cr turnover (as a result of the dietary induced increase in muscle Cr stores), and thereby Crn clearance (Hultman *et al.*, 1996; Kreider *et al.*, 1998; Robinson *et al.*, 2000; Schröder *et al.*, 2005; Taes & De Vriese, 2005).

Concerns have therefore been raised that Cr supplementation may increase renal stress or may lead to renal dysfunction (Benzi, 2000; Kreider *et al.*, 2003; Schröder *et al.*, 2005; Buford *et al.*, 2007).

S-Crn levels reflect both the rate of Cr turnover and renal function (Bishop *et al.*, 2000; Hathcock *et al.*, 2006). Even though S-Crn is a relatively insensitive monitor of kidney function and may not increase measurably until renal function has deteriorated more than 50% (Bishop *et al.*, 2000), it remains the most commonly used monitor of renal function (Bishop *et al.*, 2000; Hathcock *et al.*, 2006; Shao & Hathcock, 2006). When S-Crn is elevated above normal, renal glomerular filtration rate (ie. Crn clearance) decreases, indicating renal damage. There have been studies that reported renal dysfunction in association with oral Cr supplementation in humans (Pritchard & Kalra, 1998; Revai *et al.*, 2003). These instances, however, were reported as cases of kidney impairment in a patient with (a) pre-existing renal disease (Pritchard & Kalra, 1998), and (b) a history of continuously taking heavy dosages of methandion (Revai *et al.*, 2003). Although critical analyses of literature revealed that Cr supplementation did not adversely affect kidney function (Hathcock *et al.*, 2006; Shao & Hathcock, 2006; Gualano *et al.*, 2011), some concerns have remained (Pritchard & Kalra, 1998; Kuehl *et al.*, 2000) due to conflicting results reflected in case reports and animal studies (Gualano *et al.*, 2011).

Whereas the CRE group was found to have a significantly elevated mean S-Crn level, after 10 weeks of Cr ingestion ($p = 0.050$), the PLA group showed no such change (Table 3-10). S-Crn in the CRE group was elevated towards the upper-end of the normal range. These findings are consistent with studies in the literature that report no clinically relevant changes in S-Crn that would indicate kidney damage or functional restriction (Kreider *et al.*, 1998; Poortmans & Francaux, 1999; Robinson *et al.*, 2000; Crowe *et al.*, 2003; Kreider *et al.*, 2003; Schröder *et al.*, 2005; Hathcock *et al.*, 2006; Shao & Hathcock, 2006; Armentano *et al.*, 2007). It is generally accepted (Kreider *et al.*, 1998; Robinson *et al.*, 2000; Mayhew *et al.*, 2002; Hathcock *et al.*, 2006; Shao & Hathcock, 2006; Deldique *et al.*, 2008) that S-Crn elevations in healthy athletes are not indicative of renal damage but probably reflect an increased rate of muscle Crn formation as a result of the supplement-induced increase in muscle Cr stores.

No statistically significant changes in other markers for renal function (ie. S-LDH and U-microalbumin concentrations) were discovered in the CRE group. Thus, longer-term Cr supplementation did not result in renal damage. However, the adverse side-effects of Cr

supplementation on renal function cannot be dismissed completely, unless all kidney function parameters (eg. urinary Crn, 24 h urinary Crn clearance and urinary albumin) are measured (Bishop *et al.*, 2000; Schröder *et al.*, 2005). Although some of these recorded values (eg. 24 h urinary Crn clearance) pose significant administrative difficulties, more studies are necessary to address this important issue (Schröder *et al.*, 2005).

Concerns have been raised that prolonged Cr supplementation may cause liver damage (Kreider *et al.*, 1998; Juhn & Tarnopolsky, 1998; Kreider *et al.*, 2003). This may potentially occur due to cytotoxic effects of Cr ingestion (Yu & Deng, 2000; Wyss & Kaddurah-Daouk, 2000) and/or the presence of contaminants in the commercial preparation of the product (Baume *et al.*, 2006; Tsitsimpikou *et al.*, 2011). Yu and Deng (2000) hypothesised that chronic administration of large quantities of Cr could increase the production of methylamine and subsequently formaldehyde, which is potentially cytotoxic. Tarnopolsky *et al.* (2000) report the development of inflammatory lesions (identical to those of chronic hepatitis) in the liver of mice fed Cr in concentrations similar to human consumption. They attributed the inflammatory changes to the significant swelling (due to the osmotic effects of Cr) that was seen in the hepatocytes of the mice aged 56 days. However, dietary Cr supplementation did not induce inflammation in the liver of rats that were fed supraphysiological doses of Cr (Tarnopolsky *et al.*, 2000). The potential toxicity of Cr may thus be species specific.

The purity of most Cr products is not known, and their stability may vary considerably (Moret *et al.*, 2011). Investigations into the purity of commercially available Cr products have revealed contamination with synthetic anabolic steroids (Tsitsimpikou *et al.*, 2011) and anabolic steroid parent compounds (Baume *et al.*, 2006). The presence of significant amounts of either cytotoxic byproducts or anabolic steroid compounds may induce strain on the liver due to an increased demand for catabolism and detoxification of these substances. If inflammatory changes were to occur in the liver, the activity of the cytochrome P450 enzyme system – a family of drug-metabolising enzymes (Coon, 2005) – may be reduced, thus leading to organ damage (Carcillo *et al.*, 2003).

Serum levels of the enzymes AST, ALT and LDH are often elevated in instances of liver disease (Korones *et al.*, 2001). S-AST is considered to be "*liver specific*" and is highest in acute hepato-cellular disorders and hepato-biliary disease (Bishop *et al.*, 2000). S-GGT is used in association with S-AST to detect liver damage (Bishop *et al.*, 2000). PT (ie. clotting rate of blood) tends to

be prolonged in liver disease and is usually measured to quantify the extent of liver damage (Robert & Chazouillères, 1996; Bishop *et al.*, 2000). It is reasonable to assume that the adverse effects of Cr supplementation, if any, would alter plasma concentrations or activity of these clinical markers (Schröder *et al.*, 2005).

Results from the present study are consistent with other studies in which it was found that indices of hepatic function remained within the normal range after Cr loading (Robinson *et al.*, 2000), instead of increasing between time points as supplementation continued (Schröder *et al.*, 2005).

As indicated, researchers have hypothesised that the rise in muscle Cr concentration associated with supplementation could increase the osmolarity of the intracellular milieu, thereby drawing water into the cell (Francaux *et al.*, 2000; Bemben *et al.*, 2001; Jówko *et al.*, 2001; Brilla *et al.*, 2003; Safdar *et al.*, 2008; Saremi *et al.*, 2010). Thus, due to the potential of cellular swelling, the maintenance of skeletal muscle integrity may be of concern during Cr supplementation.

S-CK is found predominantly in muscle and is released into the circulation during the formation of muscular lesions (Totsuka *et al.*, 2002). It is considered to be a sensitive indicator of acute MI and muscular dystrophy (Bishop *et al.*, 2000). The main physiological role ascribed to CK is the maintenance of energy homeostasis at sites of high energy turnover, such as rapidly contracting skeletal muscle (McLeish & Kenyon, 2005). The high concentrations of CK ensure that ADP and ATP levels remain almost constant, effectively buffering the cell against rapid depletion of ATP (McLeish & Kenyon, 2005). Commonly accepted mechanisms of CK release are damage to muscle tissue or changes in myocyte membrane permeability (Totsuka *et al.*, 2002). During high-intensity exercise CK is released from the active muscle into the extracellular space as a result of tension development that temporarily destabalises muscle cell membranes and/or the integrity of the muscle-cell membrane (Mitchell *et al.*, 1996). Thus, physically well-trained individuals tend to have elevated baseline levels of this enzyme (Bishop *et al.*, 2000; Schröder *et al.*, 2005).

Baseline levels of S-CK were found to be above the clinical norm in studies investigating athletes' response to Cr or placebo supplementation (Kreider *et al.*, 2003; Schröder *et al.*, 2005; Armentano *et al.*, 2007). These findings are consonant with those of the study under review.

Furthermore, as demonstrated in previous studies (Kreider *et al.*, 2003; Schröder *et al.*, 2005), neither significant differences between, nor an upward trend in S-CK over time was observed in the CRE and PLA groups, even though levels were initially elevated during the pre-test. Thus, Cr supplementation for 10 weeks did not compromise skeletal muscle integrity in well-trained ultradistance runners.

The significant decrease in S-CK levels in the PLA group may indicate that Cr supplementation supported/maintained the activity of the ATP-PCr energy system during extreme endurance training, while placebo ingestion resulted in a significant deterioration of the contributions to whole-body energy supply of the ATP-PCr system. Cr supplementation thus increased the ability of ultradistance athletes to cope with the increased energy demands during intense endurance training.

The myocardium is essentially the only body tissue that contains significant quantities of the iso-enzyme hybrid type CK-MB (Bishop *et al.*, 2000). It is therefore a major source of clinical interest, particularly as a marker for myocardial damage (Bishop *et al.*, 2000; McLeish & Kenyon, 2005). Niehaus and Ungerer (2003) indicate that an S-CK-MB index above 2.5% in the presence of raised S-CK-MB mass is suggestive of CK of cardiac origin (ie. indicative of myocardial damage). During baseline testing the mean scores for the S-CK-MB index and S-CK-MB mass of the CRE group were elevated towards the upper end of the normal range in the presence of large standard deviation scores (Table 3-10). As noted earlier, two participants from this group were referred to a cardiologist (and subsequently cleared for participation) after baseline testing due to raised levels of these indices. Given the small sample size, their scores affected the mean and standard deviation scores of the group.

Previous studies have demonstrated elevated baseline S-CK-MB scores in endurance-trained athletes (Jaffe *et al.*, 1984; Apple & Tesch, 1989; Siegel *et al.*, 1997; Shave *et al.*, 2010). It is generally accepted that a substantial fraction of athletes who engage in unusually vigorous endurance training have elevations of plasma CK-MB that should not be interpreted as indicative of myocardial ischemic injury (Jaffe *et al.*, 1984; Lippi *et al.*, 2008; Shave *et al.*, 2010). Instead these elevations reflect a favourable metabolic adaptation of CK activity allowing for the muscle to cope with increased energy demands during prolonged endurance exercise (Apple & Tesch, 1989). If the elevated S-CK and S-CK-MB levels were attributable to rhabdomyolysis arising from skeletal muscle injury sustained by way of physical exertion, the researcher would

expect to see abnormally high amounts of other biochemical markers, including S-AST, S-LDH and myoglobin (Lippi *et al.*, 2008). It is recommended that a more specific marker for myocardial damage (ie. cardiac troponins) be evaluated before myocardial injury is diagnosed (Siegel *et al.*, 1997). After 10 weeks of Cr supplementation both the CK-MB index and CK-MB mass scores of the two groups were within the normal range. Thus, longer-term Cr supplementation did not negatively impact on myocardial integrity in well-trained ultradistance runners.

As demonstrated in Table 3-10, large individual variations in the scores for clinical markers were evident in both groups. In athletes, increased baseline S-Crn levels may be interpreted as the ability of healthy, well-trained individuals to maintain a greater training volume rather than as a direct consequence of Cr supplementation (Kreider *et al.*, 1998; Mayhew *et al.*, 2002). Also, large variations in renal markers are apparently normal for this population (Kreider *et al.*, 2003). Exercise training may be the reason for variations in S-CK and S-CK-MB levels, as previously explained. Clinical data (Clarkson *et al.*, 2006) also indicate a high degree of genetically based individual variability in the expression of this indicator in response to strenuous exercise.

In summary, no significant difference in chemical pathology was found between the two groups when they were compared at each clinical marker during baseline testing (Table 3-5). Supplementation by administering either Cr or a placebo also did not result in significant between-group differences for these markers. However, when changes that occurred within the CRE and the PLA group were investigated the results seemed to suggest that short-term moderate-dosage Cr supplementation followed by longer-term low-dosage Cr ingestion had caused significant change in some indices of clinical chemical pathology (Table 3-10). Most of these scores remained within the normal range, but those that did not (ie. S-CK) can be attributed to characteristics peculiar to the participants (ie. being well-trained athletes participating in intense training). Thus, results from the present study showed that longer-term normal Cr intake should not cause renal dysfunction, liver damage, skeletal muscle cytolysis or myocardial necrosis in healthy ultradistance runners.

3.5 CONCLUSION

This study demonstrated that peak aerobic work and sub-maximal aerobic RE achieved by well-trained ultradistance runners benefited from short-term moderate-dosage Cr supplementation, as well as from and short-term supplementation followed by longer-term maintenance ingestion. The body weight of participants who ingested Cr, as compared to placebo ingestion, did not

increase significantly. However, the body composition component indicating musculoskeletal robustness in the relevant participants increased significantly, thereby contributing to increased peak work output during an incremental test for aerobic capacity. Furthermore, the ultradistance runners ingesting Cr demonstrated an enhanced training effect during progressive high-intensity, high-volume sport-specific endurance training. Findings of the present study are consistent with the findings of studies in the literature to the effect that Cr supplementation effectively heightened training intensity and augmented physiological adaptations to training, thus resulting in a *"super-training"* effect (Law *et al.*, 2009; Saremi *et al.*, 2010; Souza-Junior *et al.*, 2011). However, since the authors subscribing to the super-training reported on anaerobic exercise performance, the present study is the first to demonstrate the effect in well-trained ultradistance athletes participating in a standardised, individually-tailored, sport-specific endurance training programme. Longer-term low-dosage Cr supplementation seems to be a safe practice, not harmful to bodily organs.

The first of two limitations of this study is that the body water compartments were not included in the measurements taken to determine body composition; the second is the small sample size of participants. Small sample sizes are a constant limitation that hampers the effectiveness of research studies conducted to determine the effects of supplementation on human exercise performance, organ systems and body composition (Lemon, 2002; Hathcock *et al.*, 2006; Reardon *et al.*, 2006). As a research study incorporating human subjects continues it becomes increasingly difficult to prevent drop-out due to personal time constraints, sickness, injuries, and/or non-adherence to study prescriptions. The non-measurement of body water compartments was addressed in the study protocol determined for the next chapter of this thesis. Another limitation (not relevant in the context under review) was that cellular signaling pathways - which may play a role in mediating the effects of Cr on muscle cells - were not investigated.

On the other hand, a major strength of the study is that the longer-term safety of Cr supplementation was demonstrated for a number of different organs, namely the kidneys, liver, skeletal muscle and myocardium. A contribution was made to remedy the lack of conclusive evidence of the effects of longer-term Cr supplementation on body composition and performance in well-trained endurance athletes. Specifically, no other study has addressed the potential benefits of Cr supplementation for ultradistance athletes and/or athletes who participate in high-mileage training programmes.

3.6 RECOMMENDATIONS

- More research is needed before a clear verdict can be given on the hydration theory of Cr supplementation and its link to muscle protein synthesis (Volek *et al.*, 2001).

- More research is needed before consensus will be reached as to the effect of Cr supplementation on endurance performance of continuous or variable intensity (Hickner *et al.*, 2010).

- The long-term safety of Cr supplementation remains a matter for concern, especially in young people.

- All forms of dietary supplements should be considered as potential sources of a positive drugs test for elite athletes given the risk of inadvertent contamination of raw materials and/or cross-contamination within the manufacturing process (Hall & Judkins, 2008).

CHAPTER 4

SAFETY OF LONG-TERM CREATINE MONOHYDRATE SUPPLEMENTATION AND ITS EFFECTS ON ISOKINETIC STRENGTH, BODY COMPOSITION, BODY WATER COMPARTMENTS AND MOOD IN HIGHLY ACTIVE MALE UNIVERSITY STUDENTS

4.1 INTRODUCTION

Classically, Cr has been successfully used for its ergogenic properties by individuals involved in single-effort, explosive exertion (Harris *et al.*, 1993*a*; Vandenberghe *et al.*, 1996*b*; Bosco *et al.*, 1997; Kreider *et al.*, 1998; Rossouw *et al.*, 2000; Ostojic, 2004; Gotshalk *et al.*, 2008; Saremi *et al.*, 2010) or repeated maximal-effort exercise (Bennett *et al.*, 2001; Preen *et* al., 2001; Wiedermann *et al.*, 2001; Izquierdo *et al.*, 2002; Warber *et al.*, 2002; Ziegenfuss *et al.*, 2002; Van Loon *et al.*, 2003; Peyrebrune *et al.*, 2005; Jäger *et al.*, 2008; Rawson *et al.*, 2011). However, Cr supplementation is popular with young people involved in both recreational (Sheppard *et al.*, 2000; Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010; Tsitsimpikou *et al.*, 2011) and sports activities (Froiland *et al.*, 2004; Dascombe *et al.*, 2010; Gradidge, 2010; Tscholl *et al.*, 2010). It seems that all/most of the "*long-term*" studies and "*long-term*" retrospective studies have reported on the clinical effects in highly trained athletes (Parise *et al.*, 2001; Mayhew *et al.*, 2002; Kreider *et al.*, 2003; Mendes *et al.*, 2004; Schröder *et al.*, 2005), or in people with existing disease (Pritchard & Kalra, 1998; Louis *et al.*, 2003*a*; Braegger *et al.*, 2003; Cornelissen *et al.*, 2010), and not in university students who participate in sport and/or visit campus gymnasiums for workout sessions. This population group is a prime target market for those in the sport supplement industry (Jackson *et al.*, 2010; Sobolewski *et al.*, 2011).

Human studies seldom have safety measures as their primary object, and longer-term studies are almost always planned to discover possible long-term benefits, rather than risks (Hathcock & Kriengsinyos, 2011). Although there is no conclusive evidence of health risks attaching to long-term Cr supplementation (Schröder *et al.*, 2005), claims to that effect persist (Shao & Hathcock, 2006). As the nutritional supplement industry is not subject to strict regulation

(Bishop, 2010; Jackson *et al.*, 2010) the potential risk for users is ever present. An area that is particularly crying out for experimental research is the safety and effects of both short-term and long-term Cr use by students at tertiary institutions.

In light of this background, therefore, the goals set for the study under review were to determine whether or not short-term Cr loading and/or long-term maintenance consumption of Cr by male students of Human Movement Sciences who follow intensive regimens of physical exercise would:

- undergo a change in body weight and/or body composition;
- experience enhanced isokinetic strength;
- experience normal, healthy organ function; and
- undergo a change in mood state.

4.2 PARTICIPANTS, MATERIALS AND METHODS

4.2.1 Participants

Twenty-three second-year male Human Movement Sciences students volunteered to take part in the study. They were healthy and highly active - participating regularly in a combination of various sports/activities (eg. touch rugby, rugby, athletics, tennis, cycling, running and gymnasium training). They reported no family history of kidney or liver disease - attested in virtue of a medical history questionnaire. Students who volunteered chose whether they wanted to participate in a regimen of Cr supplementation [Experimental group (EX group): 20.5 ± 1.9 yrs; n = 11)] or not [Control group (CO group): 20.3 ± 2.2 yrs; n = 12]. Informed consent was obtained from each participant in writing before baseline testing was conducted. The study was approved by the Ethics Committee in the Faculty of Humanities at the University of Pretoria.

No dietary restrictions were imposed on the participants, but they were requested not to change their habitual eating and drinking patterns during the course of the study. To monitor their compliance with this request participants were asked to complete a recall dietary questionnaire at baseline testing (pre-test), on day 7 (post-test 1), and again whenever they collected their monthly supply of Cr. The CO group completed the same questionnaire at the same time intervals. Subjects taking vitamin and mineral supplements were asked not to change their dosages.

Inclusion criteria:

- male;
- second-year Human Movement Sciences (HMS) students;
- participation in common physical activities as required by their studies, namely swimming, gymnastics, and soccer/rugby;
- participation in other sports besides required HMS activities, at club/national level (eg. athletics, touch rugby, tennis, etc.);
- healthy; and
- no family history of kidney or liver disease.

Exclusion criteria:

- supplementation with Cr in the past two months before the study commenced; and
- noncompliance with the inclusion criteria.

4.2.2 Anthropometric data

The height and body weight of participants were recorded on the day they reported for the Cybex test, using a calibrated height gauge (Seca, model 220) and a calibrated medical balance scale (Seca, model 713). The activity level of the participant was estimated by using the table drafted for this purpose (Bodystat® Ltd., 2000 : 64), as well as the value entered into the bio-electrical impedance analyser (Table 4-1).

Table 4-1 Participant activity level

Level of Activity	General	Activities
Very Low *Movement restricted*	Generally active	Reclining at ease, standing, driving
Low/Medium *Office/Light work*	Recreational activities for spells and at low intensity	Cycling (9 km/h), bowling, golf, hiking
Medium *Weekend recreation*	Sporadic involvement in recreational activities for short spells and at moderate intensities	Aerobics (low intensity), badminton, cycling (14 km/h), tennis
Medium/High *Moderate exercise*	Moderate employment activity and moderate exercise 3 times per week	Basketball, cycling (18 - 22 km/h), running (8 - 10 km/h), canoeing (vigorously)
Very High *Vigorous exercise at competitive level*	Consistent job activity and vigorous exercise 4 times per week	Cycling (24 - 32 km/h), circuit weight training, field hockey, squash, soccer, running (11 - 14 km/h), swimming (46 - 64m/min)

Body water compartments (Figure 4.1) were estimated using a multifrequency bio-impedance analyser (Quadscan 4000, Bodystat® Ltd., Isle of Man). The Quadscan 4000 bio-electrical impedance analysis (BIA) technology has been used successfully in a number of studies reporting on body composition in clinical settings (Guerreiro *et al.*, 2007; Meas *et al.*, 2008; Ozturk *et al.*, 2008) as well as evaluations of the effect of Cr supplementation (Francaux *et al.*, 2000; Kilduff *et al.*, 2007; Beis *et al.*, 2011).

The physical principle behind the BIA technique is that the body's lean compartment, comprising of approximately 73% electrolytic water, conducts electricity far better than the body's fat compartment which is very low in body water (BW) content (~5 – 10%) (Bodystat® Ltd, 2000). Lean weight or fat-free weight (FFW) is defined as everything that is not fat, and includes the skeleton, muscles, viscera (ie. brain, heart, liver and intestines) and total body water (TBW) content (Bodystat® Ltd., 2000). Water is a good conductor of electricity and thus, the less fluid there is in the body, the higher the impedance (resistance factor) will be (Bodystat® Ltd, 2000; Horswill & Janas, 2011). This will result in a higher impedance value being registered by the apparatus and hence higher fat% figures and lower body fluid volumes (Bodystat® Ltd, 2000; Horswill & Janas, 2011). Thus, the impedance value reflects the degree of resistance to the flow of current in the body.

The participants refrained from the following before tests: eating or drinking for four hours, exercise for 12 hours, and alcohol or caffeine consumption for 24 hours. On the day of the baseline test the participant's height and body weight were accurately determined, whereupon he was requested to lie supine (without any part of the body touching any other) on a non-conductive exam table. Disposable electrodes were placed on the right side of the body to ensure non-passage of battery current (low voltage) through the body on the side where the heart is located.

The two electrodes on the upper body were placed on the right-hand side behind the knuckles and on the wrist next to the ulnar head, respectively. Those on the lower body were placed centrally on the ankle between the medial and lateral malleoli and behind the toes, respectively. After five minutes a multiple-frequency sinusoidal current was applied and the respective impedance values recorded. The 50 kHz frequency was used to predict the value of total body water (TBW) and FFM. At 200 kHz the current penetrated the cell membranes and both extra- and intracellular water determined (ECW and ICW, respectively). These values, together with

the details of age, height, body weight and gender were used *via* a regression equation (Bodystat® software) to produce a personal body composition analysis.

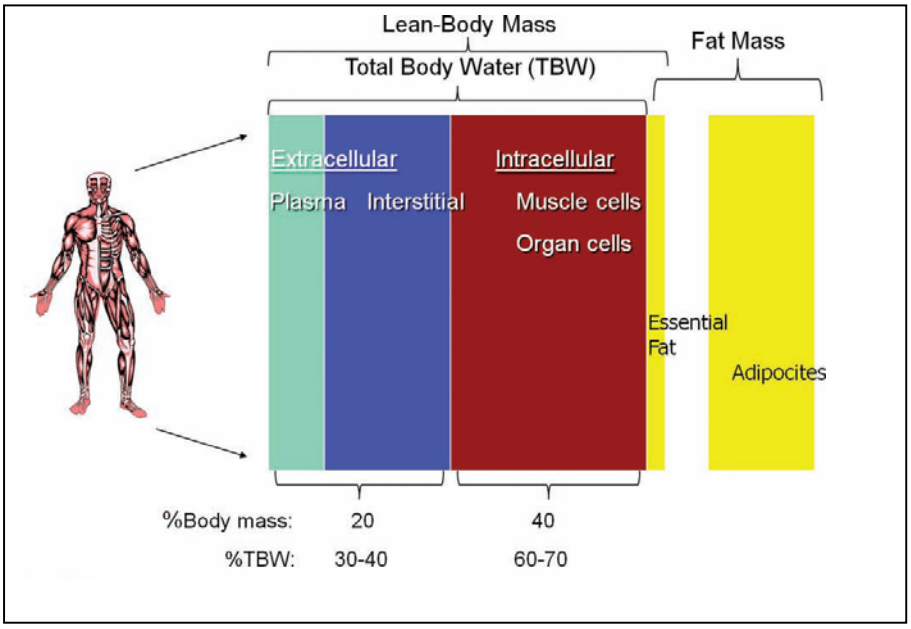


Figure 4-1 Fluid compartments of the body (Horswill & Janas, 2011)

The Quadscan 4000 BIA analyser was calibrated prior to use according to the manufacturer's instructions (Bodystat® Ltd, 2000). Indices measured by BIA included:

- **Fat%:** proportion of total body weight that is fat weight;
- **FFW:** body components that are not fat, including the skeleton, muscles, viscera (ie. brain, heart, liver and intestines) and total body-water (TBW) content (Bodystat® Ltd, 2000);
- **FFW%:** fat-free weight as % of body weight;
- **TBW:** total body water content (comprising blood volume, and intracellular, extracellular and interstitial fluid) as measured in litres (*ℓ*) at an impedance of 200 kHz (Bodystat® Ltd, 2000);
- **TBW%:** TBW expressed as % of body weight;
- **ECW:** extracellular water as measured at an impedance of 5 kHz;
- **ECW%:** Extracellular water as % of TBW;
- **ICW**: Intracellular water (ICW): Calculated from the equation TBW = ICW + ECW (Bodystat® Ltd, 2000);

- **ICW%:** intracellular water as % of TBW;
- **Body cell mass:** weight of the body cells (kg);
- **ECW/weight:** ECW expressed as a fraction of body weight (ℓ/kg);
- **ICW/weight:** ICW expressed as a fraction of body weight (ℓ/kg);
- **Body mass index (BMI):** BMI = body mass (kg) / [height (m)]^2; and
- **TBW/weight:** TBW expressed as a fraction of body weight (ℓ/kg).

The researcher again collected data, first for seven days (six supplementation days) and then for 10 months after the baseline test. Data collection took place meticulously on the same weekday and time of day as the baseline test (pre-test).

4.2.3 Blood sampling

A qualified nurse collected three 10 ml blood samples from the antecubital vein of participants during baseline testing, first after the 6-day loading period and again after 10 months of supplementation. The samples were stored on ice pending analysis on the same day by the clinical haematology laboratory of Niehaus & Ungerer (Pretoria, South Africa). The following chemical pathological analyses were performed on full blood (ie. haematology) and serum (ie. biochemistry), respectively, using standard methods (Table 4-2) (Niehaus & Ungerer, 2003):

Table 4-2 Haematology and biochemistry performed on full blood

TEST	METHODOLOGY	UNITS	NORMAL RANGE: MALES AGED 18 - 30 yrs
HAEMATOLOGY			
Haemoglobin	ADVIA 120	g/dℓ	14 – 18
Haematocrit	ADVIA 120	%	41 – 51
Red blood cell (RBC) count	ADVIA 120	10^12/ℓ	4.4 – 6.0
White blood cell (WBC) count	ADVIA 120	10^9/ℓ	4 – 11
Platelets	ADVIA 120	10^9/ℓ	140 – 450
Neutrophils%	ADVIA 120	%	40 – 75
Lymphocytes%	ADVIA 120	%	20 – 45
Monocytes%	ADVIA 120	%	2 – 10
Eosinophils%	ADVIA 120	%	0 – 7
Basophils%	ADVIA 120	%	0 – 2
Neutrophils (absolute)	ADVIA 120	10^9/ℓ	2.0 – 7.5
Eosinophils (absolute)	ADVIA 120	10^9/ℓ	0.0 – 0.6
Basophils (absolute)	ADVIA 120	10^9/ℓ	0.0 – 0.1
Lymphocytes (absolute)	ADVIA 120	10^9/ℓ	1.5 – 4.0
Monocytes (absolute)	ADVIA 120	10^9/ℓ	0.2 – 0.8

TEST	METHODOLOGY	UNITS	NORMAL RANGE: MALES AGED 18 - 30 yrs
BIOCHEMISTRY			
Sodium	Beckman-CX7/LX	mmol/ℓ	133 – 148
Potassium	Beckman-CX7/LX	mmol/ℓ	3.5 – 5.1
Phosphate	Beckman-CX7/LX	mmol/ℓ	0.87 – 1.45
GGT	Beckman-CX7/LX	U/ℓ	11 – 49
ALT	Beckman-CX7/LX	U/ℓ	7 – 40
AST	Beckman-CX7/LX	U/ℓ	7 – 41
Creatinine	Beckman-CX7/LX	mmol/ℓ	62 – 115
BUN-Urea	Beckman-CX7/LX	mmol/ℓ	3.5 – 7.5
CK	Beckman-CX7/LX	U/ℓ	38 – 174
CK-MB (mass)	Access	ng/ml	0.0 – 5.0
CK-MB (mass) index	Access	%	0.0 – 2.5
Troponin T quantitative	Elecsys	ng/ml	0.0 – 0.1

4.2.4 Supplementation

After baseline testing participants in the EX group were instructed to dissolve five grams of pure Cr monohydrate powder in a carbohydrate containing beverage (eg. grape juice) four times daily for six days. After post-test 1 (day 7), the maintenance dosage was ingested in capsule form (3 x 1 g = 3 g/day Cr) for 10 months. The CO group consumed no Cr for the duration of the study. Participants collected their supply of Cr capsules (Table 4-3) from the researcher at monthly intervals.

Table 4-3 Product information per 2 capsules *Creatine Forte* (NRF Sport, Centurion)

INGREDIENTS	AMOUNTS
CREATINE MONOHYDRATE	1000mg
CARBOHYDRATE	50mg
CHROMIUM (ELEMENTAL)	100mcg
MAGNESIUM (ELEMENTAL)	63mg

4.2.5 Mood state testing

On the day of baseline testing, after completing the medical history questionnaire, the participant completed a Profile of Mood State (POMS) questionnaire (Potgieter, 2002) to determine how he “felt” during the entire week. The POMS questionnaire was again completed after Cr loading and then at monthly intervals to determine whether Cr supplementation exerted an effect on mood. Since both the EX group and CO group were HMS students they could be

expected to experience periods of stress (ie. from tests, written exams, practical exams and sports participation) that could elicit changes in mood, at roughly the same time. As explained in 3.4.2.1 Cr can penetrate the blood-brain barrier (Wyss & Schulze, 2002) and may modify brain energy metabolism (Lyoo *et al.*, 2003; Kondo *et al.*, 2011), thereby potentially exerting an effect on mood.

The POMS consists of a 65-item five-point adjective scale that measures six mood states (Potgieter, 2002). It is scored on a 0 – 4 Likert scale and measures the individual's perception of mood (McMorris *et al.*, 2006):

- **Fatigue:** feelings of tiredness, weariness and inertia;
- **Depression:** feelings of inadequacy and worthlessness;
- **Tension:** emotions of unease and restlessness;
- **Confusion:** feelings of bewilderment;
- **Anger:** feelings of aggression and hostility; and
- **Vigour:** emotions concerning readiness to undertake physical and mental work.

A total mood disturbance score can be calculated by adding the scores on the negative mood states, namely tension, depression, fatigue, anger and confusion, and then subtracting the positive factor of vigour from the negative total (Potgieter, 2002). A constant of 100 is usually added to eliminate a negative score. The POMS has been used in sport settings (Greenwood *et al.*, 1990; Williams *et al.*, 1991; Fry *et al.*, 1995; McMorris *et al.*, 2006), and its concurrent validity is supported by positive relationships between conceptually similar tests (McNair & Doppelman, 1992).

4.2.6 Exercise testing protocol

A maximal work test was performed on an isokinetic dynamometer (Cybex III) in a controlled laboratory environment. After five minutes of warm-up (cycling at a leisurely pace on a cycle ergometer), the participant assumed a sitting position on the dynamometer. A verbal introduction to the isokinetic concept of exercise was given, whereupon the dominant knee was aligned as closely as possible with the axis of rotation of the lever arm. Gravity correction procedures were performed to account for the weight of the lever arm and the limb being tested. The participant was stabilised with straps at both the waist and chest, and his arms were placed across his chest to eliminate contribution from the upper extremities during the test. A familiarisation trial (two sub-maximal repetitions, followed by one maximal repetition) was

performed at a constant velocity of 60 °/second followed by a rest period of 60 seconds. The exercise test consisted of five consecutive unilateral dynamic contractions of the knee extensors and flexors at the predetermined velocity. Exercise was performed with the dominant leg starting from 90° knee flexion to full knee extension, and again from full extension to 90° knee flexion. The isokinetic dynamometer was calibrated before each test. Strong verbal encouragement to keep up maximal speed of movement was given throughout the test.

Knee extension-and-flexion torque was measured during each contraction and digitised by an on-line computer. Indices measured included:

- ***Peak torque*** - the point in the range of motion tested where the greatest force or torque was produced. The computer selected peak torque from among the first repetitions at the work test speeds;
- ***Total work*** - total energy output (i.e programmed number of repetitions at programmed speed); and
- ***Average power*** - total work done (ie. aggregate of test repetitions) divided by the total contraction time.

The post-tests were performed on day 7 (six supplementation days), and again at 10 months after the pre-test.

4.2.7 Statistical methodology

As explained (section 3.2.6), test data were analysed to determine:

- whether or not significant differences existed and/or developed over time between the two groups for all variables measured; and
- whether or not significant differences existed and/or developed over time between the pre- and post-test measurements within the same group.

4.3 RESULTS

During the pre-test (baseline testing), measurements of all the participants (EX group - n = 11; CO group - n = 12) were analyzed for anthropometry and blood pathology. However, isokinetic data pertaining to one participant in the EX group could not be included for analysis. Thus, for the purpose of isokinetic measurements the EX group consisted of 10 participants, while the CO group remained at 12 participants.

During post-test 1 - after Cr loading - data were obtained from all the participants (EX group - n = 11; CO group - n = 12) for all test measurements. During the remainder of the study, another participant in the EX group withdrew on the grounds of ill health, while five participants from the CO group withdrew either on the grounds of ill health, or because they, presumably, had lost interest. Thus, at post-test 2 (after 10 months of supplementation) participant numbers were: EX group = 10 and CO group = 7.

4.3.1 Descriptive statistics during baseline testing (pre-test)

Descriptive statistics for general measurements of the study participants are reported in Table 4-4.

Table 4-4 Descriptive statistics for general measurements during baseline testing of the EX group and CO group

Group		Minimum	Maximum	Mean	Std Deviation
Experimental	Age (yrs)	18.00	24.00	20.56	1.97
	Waist (cm)	74.00	91.00	84.55	6.04
	Body Weight (kg)	67.00	99.90	81.71	8.61
	Height (cm)	176.00	194.00	181.18	5.31
	Hip (cm)	94.00	111.00	100.91	4.93
Control	Age (yrs)	19.00	27.00	20.33	2.23
	Waist (cm)	74.00	93.00	82.50	5.18
	Body Weight (kg)	66.30	89.40	79.54	6.90
	Height (cm)	170.00	191.00	182.42	6.39
	Hip (cm)	96.00	104.00	100.17	2.63

4.3.2 Results of comparing the EX group with the CO group across various measurements (between-group data analyses)

As indicated previously, Mann-Whitney U-tests were used to determine whether or not the differences in scores that existed (pre-test) and developed over time (post-tests 1 and 2) between the two groups were statistically significant or not. Measurement scores that did not indicate a significant difference between groups are not shown, but mean scores and p-values are incorporated in Appendix A. Measurement scores that did demonstrate significant between-group differences are indicated in the figures and tables of this chapter. The changes that took

place within each group were explored with Friedman's rank tests and are reported in section 4.3.3.

4.3.2.1 Body composition

No statistically significant differences were found between measurement scores recorded for body composition and body water in the EX and the CO group at the pre-test, post-test 1 or post-test 2. Thus, at the onset of the study the EX group and the CO group did not differ significantly with regard to these measurements, and no significant between-group changes in body composition or body water components were demonstrated during the course of the study. Thus, in light of a comparison of changes in the EX group with those occurring in the CO group it appears that Cr consumption did not result in significant changes in either body composition or body water compartments (Appendix A).

4.3.2.2 Haematological pathology

No statistically significant differences were found in the pre-test results between the EX and the CO group for any of the haematological indices. Thus, at the onset of the study the two groups did not differ significantly with regard to these measurements. No significant changes in blood chemical pathology took place throughout the course of the study. Thus, neither Cr loading, nor maintenance feeding resulted in any significant differences in the clinical haematological pathology of the EX group compared to that of the CO group (Appendix A). All mean scores for haematological pathology remained within the normal range throughout the study.

4.3.2.3 Serum biochemical pathology

At the onset of the study the EX and the CO group did not differ significantly with regard to biochemical pathology scores (Table 4-5). Thus, when comparing them at each clinical marker the groups did not differ significantly. All mean baseline biochemical pathology scores, except S-CK, were within the normal range for the gender and age of the participants (Table 4-5). This score was elevated above the normal range in both groups, and remained so (ie. all S-CK scores remained above normal throughout the study).

Statistically significant differences between the biochemical pathology scores obtained for the EX and the CO-group at post-test 1 were evident for: S-Creatinine ($p = 0.004$), S-ALT ($p = 0.016$), S-AST ($p = 0.011$), S-CK ($p = 0.011$) and S-CK-MB mass index ($p = 0.000$). With the exception of the S-CK-MB mass index score, which was lower in the EX group, the EX group scored higher overall than the CO group (Table 4-5).

Table 4-5 **Between-group analysis of the change in recorded scores associated with the serum biochemical pathology of the EX group compared to that of the CO group**

Variable	Test	Mean		Standard Deviation		p-value
		CO	EX	CO	EX	
Sodium (mmol/ℓ)	**Pre**	139.15	138.82	1.52	1.99	0.596
	Post 1	138.77	139.00	1.17	2.93	0.769
	Post 2	139.80	139.57	1.40	1.51	0.878
Potassium (mmol/ℓ)	**Pre**	4.48	4.34	0.31	0.25	0.349
	Post 1	4.38	4.27	0.30	0.21	0.366
	Post 2	4.22	4.24	0.30	0.24	0.922
Urea (mmol/ℓ)	**Pre**	3.53	4.12	0.95	1.46	0.417
	Post 1	4.58	5.05	0.80	1.03	0.192
	Post 2	4.93	5.43	1.40	1.33	0.434
Creatinine (mmol/ℓ)	**Pre**	92.31	95.91	13.12	8.35	0.182
	Post 1	94.77	109.09	13.97	10.42	0.004*
	Post 2	96.70	106.14	7.75	8.91	0.045*
GGT (U/ℓ)	**Pre**	19.46	19.64	15.74	11.12	0.749
	Post 1	19.46	19.27	16.60	11.15	0.793
	Post 2	18.20	13.29	9.48	2.29	0.113
ALT (U/ℓ)	**Pre**	23.85	26.73	9.06	10.65	0.384
	Post 1	22.62	36.00	7.60	19.90	0.016*
	Post 2	25.00	19.86	10.60	5.93	0.328
AST (U/ℓ)	**Pre**	26.46	28.18	5.83	10.20	0.705
	Post 1	26.77	60.18	9.26	64.51	0.011*
	Post 2	24.50	23.71	6.15	3.99	0.695
CK (U/ℓ)	**Pre**	227.00	308.09	109.17	332.38	0.931
	Post 1	253.38	1,531.09	236.65	1,802.61	0.011*
	Post 2	183.80	251.86	96.32	112.99	0.242
CK-MB mass (ng/ml)	**Pre**	3.12	2.95	1.15	1.59	0.524
	Post 1	3.48	5.56	1.70	2.96	0.056
	Post 2	3.48	3.32	1.99	1.04	0.696

Variable	Test	Mean		Standard Deviation		p-value
		CO	EX	CO	EX	
CK-MB mass index (%)	**Pre**	1.52	1.36	0.68	0.57	0.839
	Post 1	1.61	0.69	0.58	0.35	0.000*
	Post 2	1.99	1.41	0.58	0.31	0.077
Troponin T ng/ml	**Pre**	< 0.01	< 0.01	n.a.	n.a.	n.a.
	Post 1	< 0.01	< 0.01	n.a.	n.a.	n.a.
	Post 2	< 0.01	< 0.01	n.a.	n.a.	n.a.

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

n.a.: Not applicable to this measurement. Cardiac Troponin T is reported as < 0.01 if no myocardial damage is present (Niehaus & Ungerer, 2003)

After Cr loading the S-CK-MB mass index scores decreased significantly ($p = 0.000$) when compared to changes that took place in the CO group (Table 4-5). Thus, Cr loading caused a significant decrease in S-CK-MB mass expressed as a fraction of the total S-CK level when compared to changes that took place in the CO group.

Cr loading significantly elevated scores for S-ALT and S-AST ($p = 0.016$ and $p = 0.011$, respectively) when changes in the EX group are compared to those occurring in the CO group (Table 4-5). S-ALT scores were elevated towards the upper-end of normal, while S-AST was elevated above the normal range (Table 4-3).

At post-test 2 the difference in S-Creatinine scores between the groups remained unchanged, in that the score of the EX group was significantly higher than that of the CO group ($p = 0.045$). However, no statistically significant differences between the two groups were found in scores for S-ALT, S-AST, S-CK, S-CK-MB mass and S-CK-MB mass index (Table 4-5). Thus, long-term low-dosage Cr supplementation did not significantly alter these scores when compared to the CO-group. Also, the previous elevations in these scores of the EX group returned close to baseline levels.

Neither short-term high-dosage Cr supplementation nor long-term low-dosage ingestion significantly influenced changes that occurred between the EX and CO groups' scores for S-sodium, S-Potassium, S-Urea, S-GGT and Troponin T.

4.3.2.4 Isokinetic strength/power

Statistically significant differences were found to exist for all the pre-test isokinetic measurement scores obtained for the EX and CO groups. The EX group scored higher than the CO group across all the isokinetic strength/power measurements (Table 4-6). The implication is that differences in the post-test 1 and post-test 2 scores between the EX and the CO group may be traceable to the initial differences, rather than being a result of the intervention. It is interesting to note, however, that these between-group differences were not evident at post-test 1; instead were only evident for peak torque (concentric flexors) at post-test 2 (Table 4-6).

Table 4-6 Between-group analysis of a change in measurement scores associated with isokinetic strength/power expressed relative to body weight for the EX group compared to the CO group

Variable	Test	Mean		Std Deviation		p-value
		CO	EX	CO	EX	
Peak torque (Nm) - concentric flexors	**Pre**	148.3	172.5	24.9	15.8	0.027*
	Post 1	175.1	180.6	29.3	23.1	0.477
	Post 2	187.2	215.2	23.1	34.1	0.046*
Total work (J) - concentric flexors	**Pre**	199.5	237.7	32.6	42.2	0.048*
	Post 1	248.9	247.0	61.3	33.1	0.831
	Post 2	245.8	273.8	47.9	46.1	0.317
Average power (W) - concentric flexors	**Pre**	97.0	120.1	19.1	18.7	0.016*
	Post 1	123.9	129.6	24.2	20.5	0.434
	Post 2	129.5	149.7	21.9	23.9	0.116
Peak torque (Nm) - concentric extensors	**Pre**	287.6	337.4	41.6	34.1	0.008*
	Post 1	287.9	323.5	43.1	51.4	0.088
	Post 2	288.8	350.6	53.4	63.6	0.086
Total work (J) - concentric extensors	**Pre**	382.5	428.4	53.7	49.7	0.041*
	Post 1	389.3	400.8	66.1	57.3	0.619
	Post 2	385.9	424.4	58.8	67.9	0.253
Average power (W) - concentric extensors	**Pre**	179.9	210.5	22.9	11.3	0.002*
	Post 1	194.6	213.2	29.3	33.4	0.155
	Post 2	203.6	227.1	30.9	38.1	0.199

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

4.3.3 Results of comparing the pre- and post-tests of the same group across various measurements (within-group data analyses)

Friedman's rank tests were used to determine whether or not statistically significant changes had taken place between pre- and post-tests within the same group regarding the various measurement scores. As indicated earlier, post-tests were performed one week after administering high-dosage Cr (post-test 1), and once again after lower-dosage maintenance ingestion at 10 months (post-test 2).

4.3.3.1 Body composition

No statistically significant changes in body weight occurred within either the EX group or the CO group at post-test 1 or post-test 2 (Appendix B). Thus, neither Cr loading nor long-term maintenance feeding resulted in a significant increase in body weight of the EX group. No significant changes in either fat% or LBW were discovered in either group at any test stage (Appendix B).

There was, however, a statistically significant change in the TBW scores of the EX group (Figure 4.2). Both the TBW and TBW% scores increased from the pre-test to the two consecutive post-tests. The biggest increase in the TBW score was detected between the pre-test and post-test 1 scores. Thereafter, at post-test 2, the score declined minimally but remained

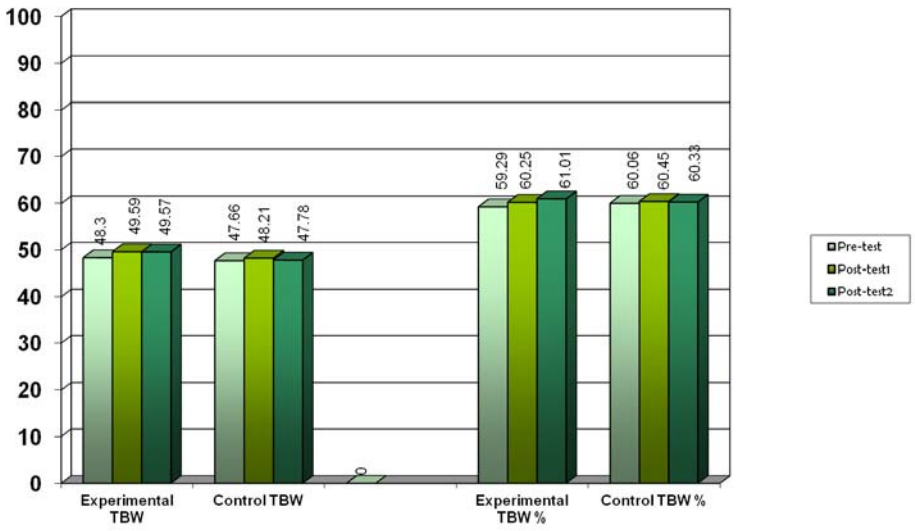


Figure 4-2 Differences in TBW and TBW% within the EX and CO groups, as measured first on day 7 (post-test 1), and again at 10 months (post-test-2)

significantly higher than the pre-test score (p = 0.028). The TBW% score increased steeply into the significant range after Cr loading, and in fact continued to increase with maintenance feeding (p = 0.018). Small changes in the TBW and TBW% scores of the CO group did not amount to a substantial difference from the corresponding pre-test scores obtained for the same group (Figure 4-2).

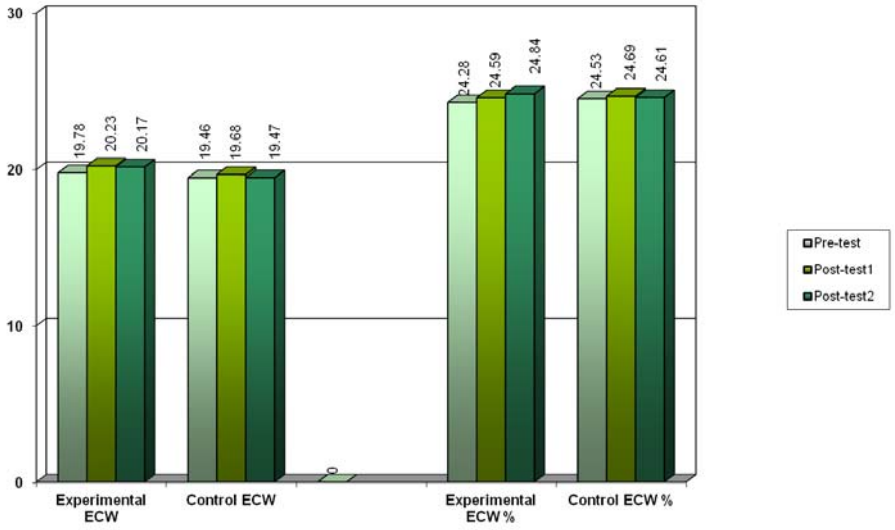


Figure 4-3 **Differences in ECW values recorded within the EX and the CO group, respectively, first on day 7 (post-test 1), and again after 10 months (post-test 2)**

A statistically significant change was found in the pre- and post-test scores of the EX group for ECW% (p = 0.028). The score increased from the pre-test to the two consecutive post-tests (Figure 4.3). The ECW% score increased significantly from pre-test to post-test 1, and then continued to increase for the remainder of the supplementation period (p = 0.028). Figure 4.4 demonstrates that the same statistically significant trend for ICW and ICW% scores was found in the EX group (p = 0.018 and p = 0.054 respectively). No statistically significant changes in either ECW or ICW scores were evident in the scores of the CO group (Figures 4.3 and 4.4).

There was a statistically significant change in the third-space water scores of the EX group (Figure 4-5). The score increased steeply from the pre-test to post-test 1 (p = 0.011), whereafter it declined considerably below baseline levels in accordance with low-dosage Cr ingestion. However, maintenance feeding did not maintain this score and it returned to below baseline

level. The third-space water score of the CO group increased steadily over the research period, but without reaching statistically significant levels (Figure 4-5).

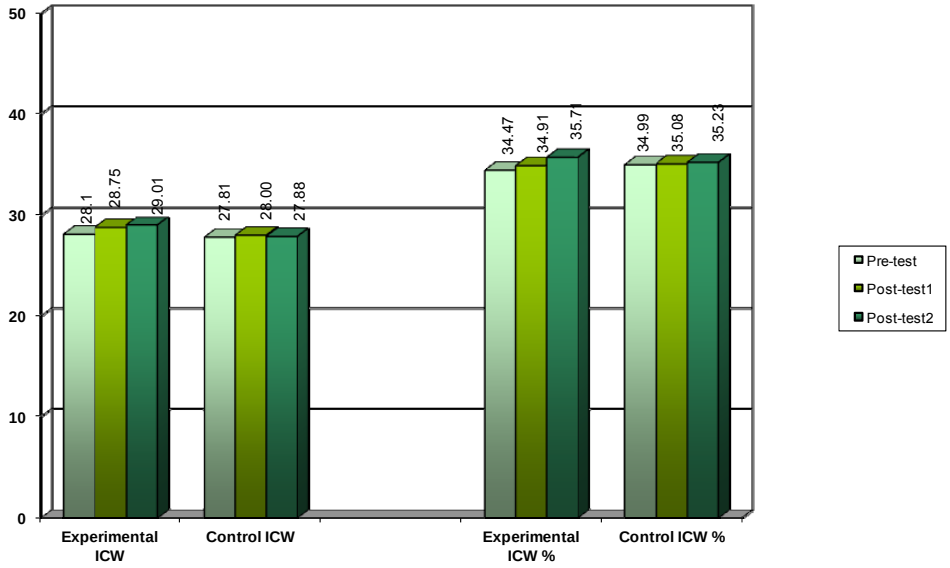


Figure 4-4 **Differences in ICW values recorded within the EX and the CO group, respectively, first on day 7 (post-test 1), and again after 10 months (post-test 2)**

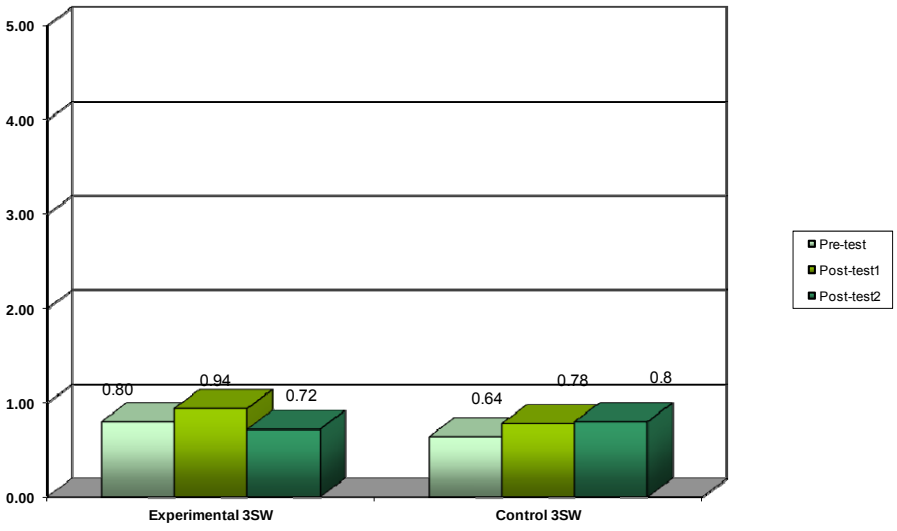


Figure 4-5 **Differences in third-space water scores recorded for the EX and the CO group, respectively, first on day 7 (post-test 1), and again after 10 months (post-test 2)**

There was a statistically significant difference between the pre- and post-test scores for both the TBW/weight and the ECW/weight scores of the EX group (Figure 4-6). The scores increased significantly from the pre-test across the two consecutive post-tests ($p = 0.021$ and $p = 0.028$, respectively). No statistically significant changes in these scores were found in the CO group - the scores remained relatively unchanged (Figure 4-6).

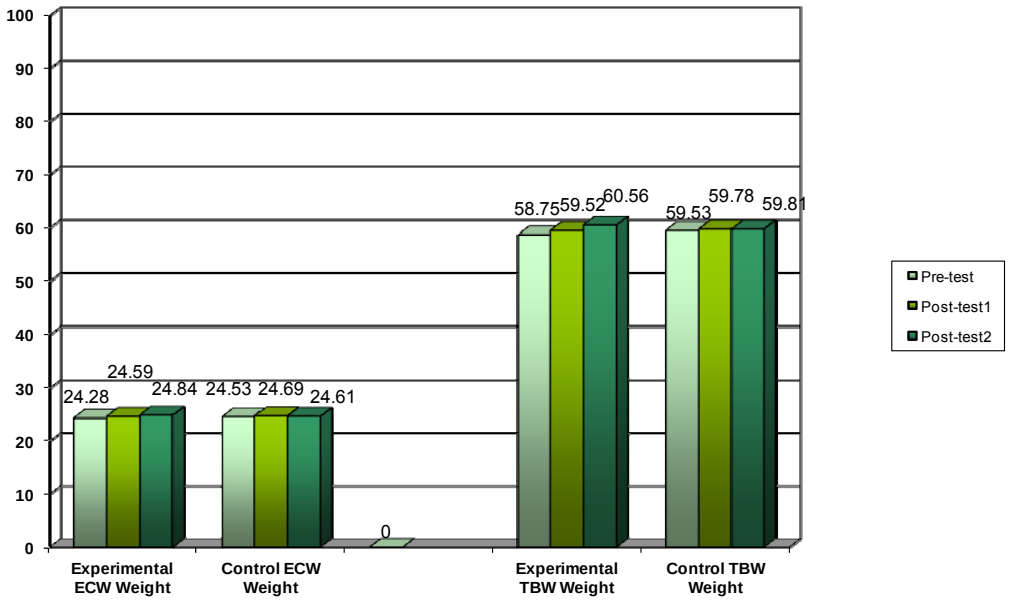


Figure 4-6 **Differences in ECW/TBW and TBW/weight occurring within the EX and the CO group, respectively, measured first on day 7 (post-test 1), and again at 10 months (post-test 2)**

The same tendency was found for the ICW/Weight scores of the EX group on the 10% level of significance ($p = 0.054$). The ICW/Weight scores of the CO group remained relatively unchanged (Appendix B).

No statistically significant changes were found in values recorded for BMI and waist/hip ratio for either group (Appendix B).

4.3.3.2 Haematological chemical pathology

Figure 4-7 indicates that the blood-platelets scores recorded for the EX group declined significantly over the trajectory from pre-test to post-test 2 ($p = 0.018$). The blood-platelets score of the CO group increased at first, but then decreased significantly at post-test 2 ($p = 0.025$). Thus, both groups demonstrated a significant decrease in blood-platelets scores over the

research period. However, all scores remained within the normal range for the participants' age and gender.

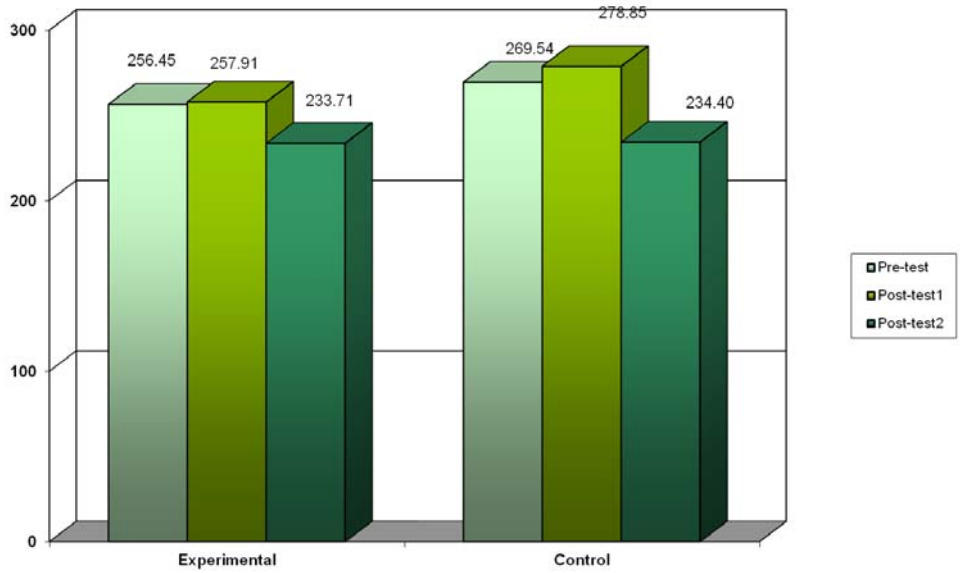


Figure 4-7 **Differences in blood platelet count occurring within the EX and the CO group, respectively, measured first on day 7 (post-test 1), and again at 10 months (post-test 2)**

There was a statistically significant change in the absolute lymphocyte scores of the EX group (Figure 4-8). The score increased continuously from the pre-test across the two consecutive post-tests ($p = 0.028$). The largest increase occurred between the pre-test and post test-1 scores, whereafter a further increase occurred at post-test 2. The score recorded for the CO group increased initially but then fell back until post-test 2 was reached, but none of these shifts were significant (Figure 4-8). All scores remained within the normal range for the age and gender of the participants.

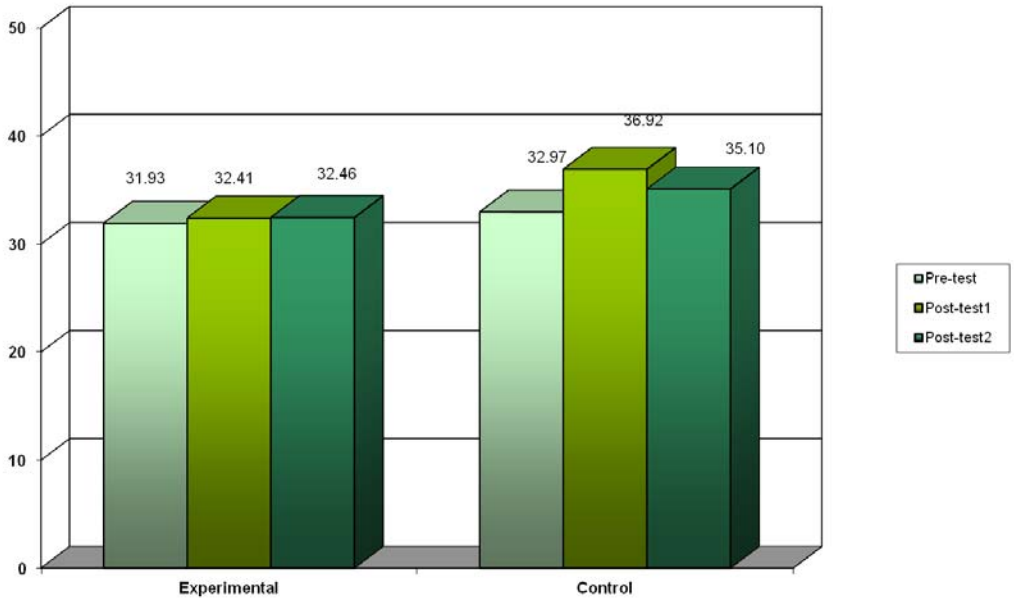


Figure 4-8 **Differences in absolute lymphocyte count within the EX and the CO group, respectively, recorded first on day 7 (post-test 1), and again at 10 months (post-test 2)**

4.3.3.3 Biochemical pathology

Figure 4-9 indicates a significant change in the S-Urea score of the CO group. The score increased from the pre-test across the two consecutive post-tests ($p = 0.008$). The same tendency was found for the EX group, but the increase was not statistically significant. All participant scores remained within the normal range for the age and gender of the participants.

The S-Creatinine pre-test score of the EX group was significantly lower than the post-test scores (Figure 4-9). This difference was significant on the 5% level of significance ($p = 0.004$). However, the CO group showed a significant difference on the 10% level of significance in that the post-test 2 score was significantly higher than the preceding two scores ($p = 0.061$). Although the scores of the CO group demonstrated the same tendency, the increase was less pronounced and did not reach statistical significance (Figure 4-9). All participant scores remained within the normal range.

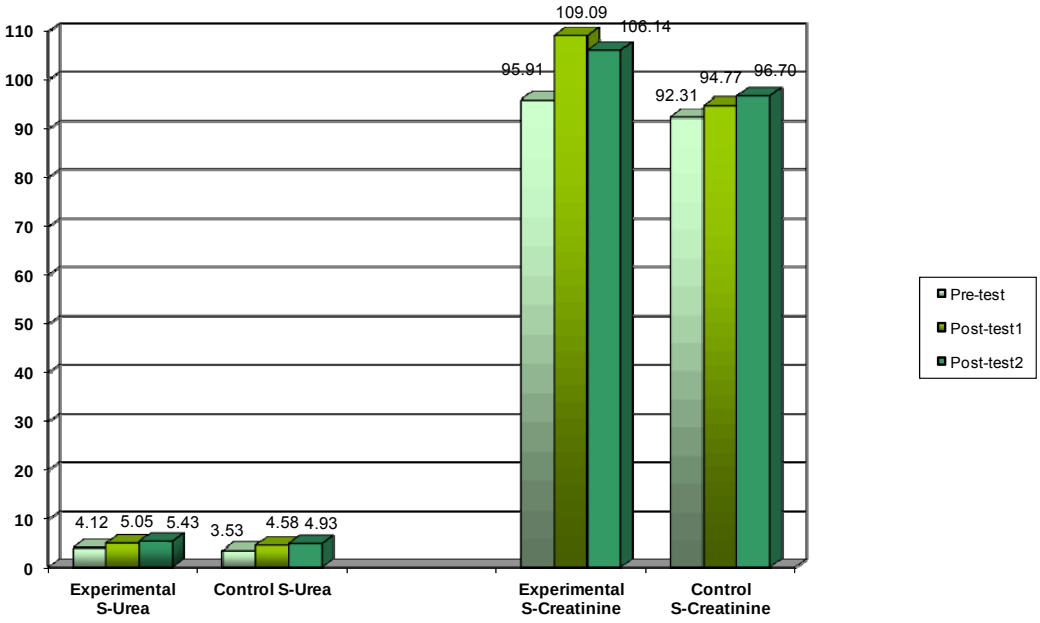


Figure 4-9 **Differences in S-Urea and S-Creatinine scores recorded within the EX and the CO group, respectively, first on day 7 (post-test 1), and again at 10 months (post-test 2)**

Figure 4-10 indicates significant changes in the S-AST and S-CK scores of the EX group. The S-AST score dramatically increased from the pre-test to post-test 1 ($p = 0.004$), and then decreased quite steeply again to below the baseline value at post-test 2. On closer inspection it appears that the standard deviation of the post-test 1 score is very large (± 64.51), with the maximum value for S-AST reported at 248 U/ℓ (normal range: 7 - 41 U/ℓ). The mean score and maximum score returned to within normal range (23.71 ± 3.99 U/ℓ respectively) at post-test 2. No significant changes occurred in the S-AST scores of the CO group.

The baseline S-CK scores for both groups were elevated above the normal range (Appendix B). The EX group underwent a dramatic and statistically significant increase in mean S-CK at post-test 1 ($p = 0.018$) in the presence of large individual response variations (SD ± 1 802.61), while a minor and insignificant increase in S-CK at post-test 1 was recorded for the CO group in the presence of large individual variations (SD ± 236.65). At post-test 2 a S-CK score below baseline was recorded for both groups (Figure 4-10). The individual differences in scores had shrunk, but still remained relatively large (SD = ± 96.32 and 112.99, respectively). All mean S-CK scores remained outside of the normal range (38 – 174 U/ℓ) for the duration of the study.

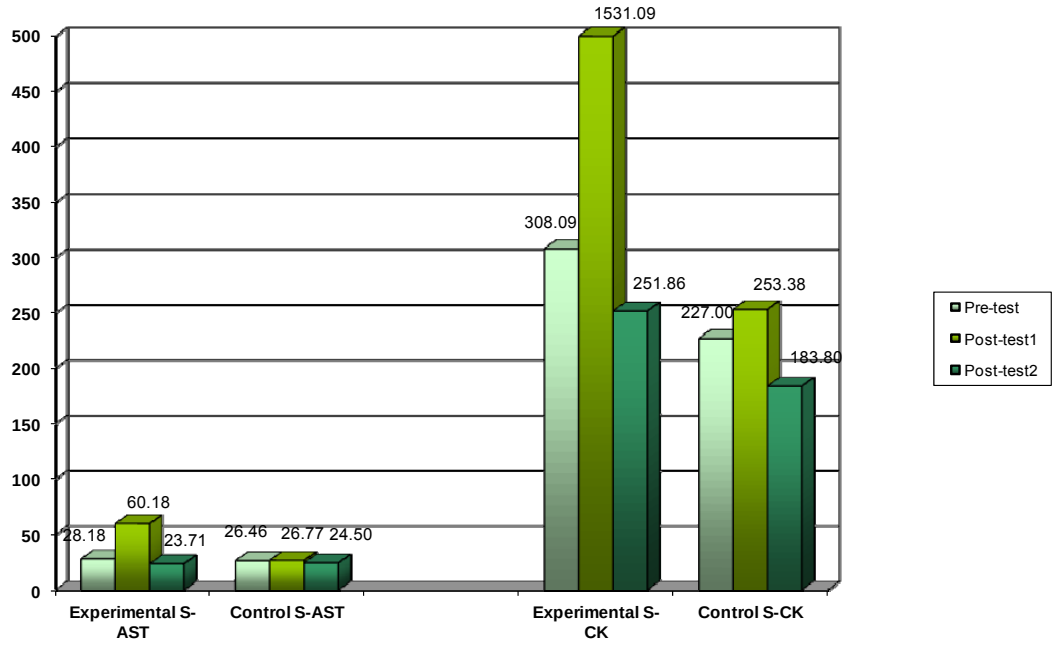


Figure 4-10 **Differences in S-AST and S-CK scores within the EX and the CO group, respectively, measured first on day 7 (post-test 1), and again at 10 months (post-test 2)**

Figure 4-11 indicates significant changes in the S-CK-MB mass and S-CK-MB mass index scores of the EX group. The S-CK-MB mass score increased significantly from the pre-test to post-test 1 ($p = 0.018$). It then decreased, but remained higher than baseline at post-test 2 (although not significantly so). The S-CK-MB mass score of the CO group increased at post-test 1 and stabilised at the higher level. However, these scores underwent no statistically significant changes.

The S-CS-MB mass index score at post-test 1 in the EX group was significantly lower than the baseline score ($p = 0.014$). The score returned to a level close to baseline at post-test 2 (Figure 4-11). The S-CK-MB mass index scores of the CO group increased from the pre-test across the two consecutive post-tests at the 10% level of significance ($p = 0.058$), but the mean score remained within the normal range (0.0 - 2.5%).

Serum cardiac troponin T scores remained at < 0.01 ng/ml for all participants and during all test stages. Cr supplementation did not result in any significant changes in scores for S-Sodium, S-Potassium, S-Urea, S-GGT or S-ALT. Large individual differences - as indicated by large

standard deviation scores - were prevalent for some indices of biochemical pathology. This is especially true for S-GGT, S-ALT, S-AST, S-CK and S-CK-MB scores (Appendix B).

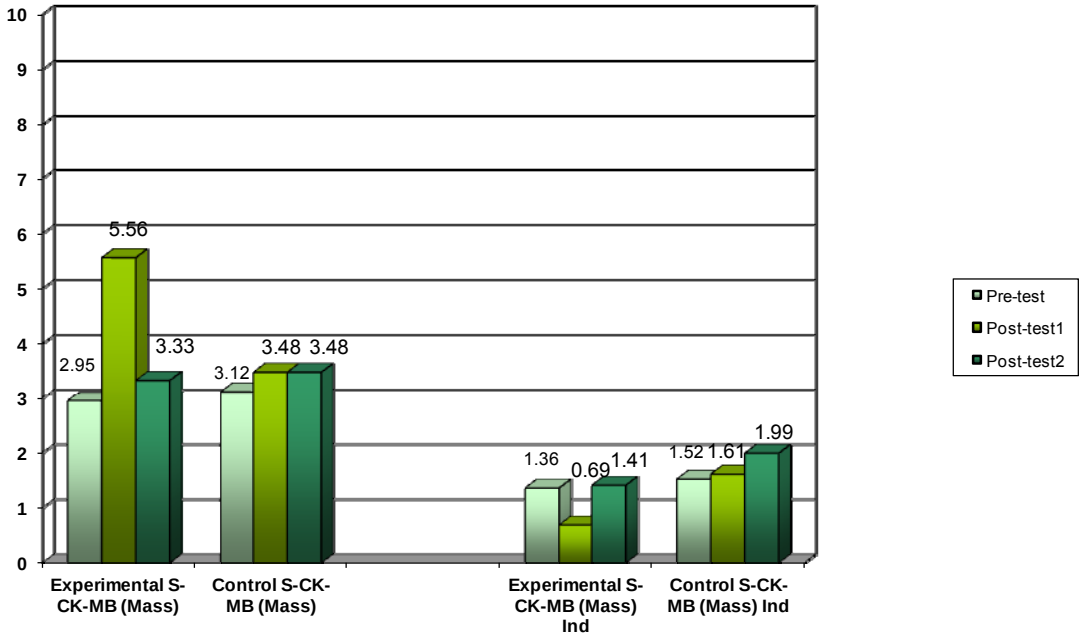


Figure 4-11 **Differences in S-CK-MB mass and S-CK-MB mass index scores measured within the EX and the CO group, respectively, first on day 7 (post-test 1), and again at 10 months (post-test 2)**

4.3.3.4 Isokinetic strength/power

Measurements for isokinetic strength/power are expressed relative to the participant's body weight. All isokinetic measurements for the CO group increased from the pre-test across the two consecutive post-tests (Appendix B). The same tendency was found in the EX group, but the increase did not reach statistical significance. Scores for peak torque - concentric flexors ($p = 0.012$), average power - concentric flexors ($p = 0.018$), total work - concentric extensors ($p = 0.012$) and average power - concentric extensors ($p = 0.021$) increased significantly over time in the CO group (Appendix B).

4.3.4 Activity levels

The activity levels of all participants whose data were included for statistical analyses remained constant during the course of the study, namely at either medium/high or very high levels.

4.3.5 Food intake

The food intake of all participants whose data were included for statistical analysis, as reflected in the recall-nutrition questionnaires, remained constant during the course of the study. Thus, participants did not alter their weekly/daily food intake significantly while the study was in progress.

4.3.6 Mood state (POMS)

The POMS questionnaire was completed by the participants at the following intervals:

Measurement	Research stage
1	Pre-test
2	Post-test 1
3	Month 2
4	Month 3
5	Month 4
6	Month 5
7	Month 6
8	Month 7
9	Month 8
10	Month 9
11	Post-test 2 (Month 10)

Mood disturbance scores were calculated for tension, depression, fatigue, anger, confusion and vigour (Potgieter, 2002). Results of this section have been published previously (Steyn & Rossouw, 2007).

4.3.6.1 Fatigue

Figure 4-12 presents the mean fatigue scores for the EX and the CO group respectively. At the onset of the study no statistically significant difference existed between the fatigue scores of the EX group and those of the CO group. Comparison of the first 10 measurements reveals that a statistically significant change at the 10% level of confidence occurred within the EX group. The fatigue scores of the EX group decreased towards the end of the study ($p = 0.072$), with the largest difference in scores reported at measurements 6 and 9. No significant changes were found in the fatigue scores of the CO group (Figure 4-12).

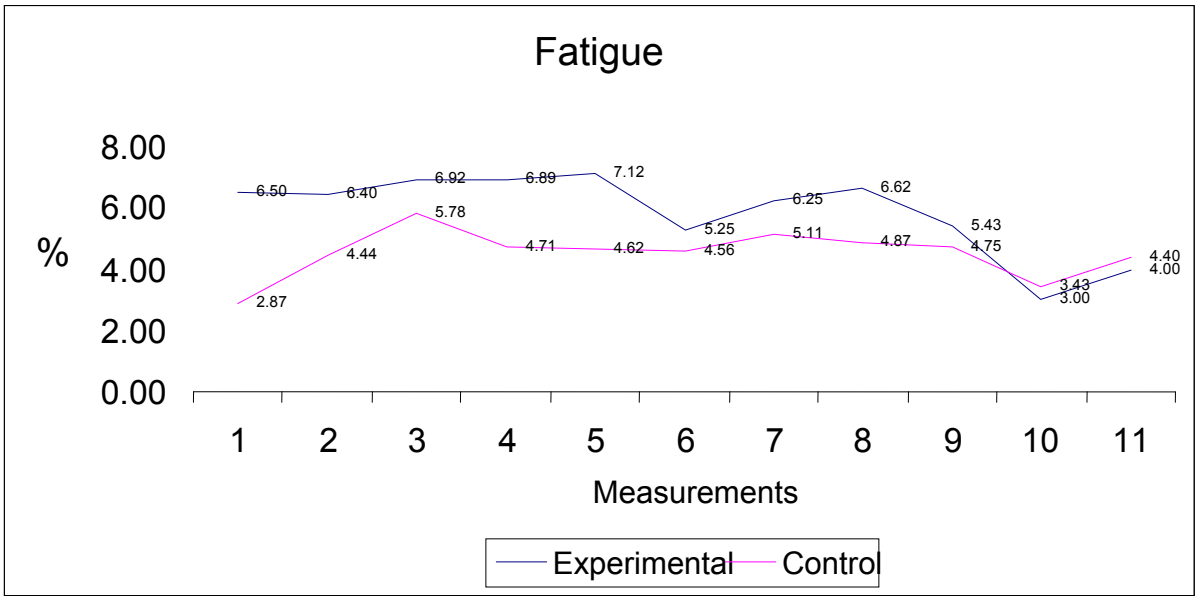


Figure 4-12 **Mean fatigue scores of the EX group and the CO group at the different measurement stages**

4.3.6.2 Tension-anxiety

No statistically significant differences were found between the tension-anxiety pre-test results of the EX and the CO groups (Figure 4-13). Thus, at the onset of the study the two groups did not differ significantly with regard to this measurement. No significant differences were noted in changes of tension-anxiety scores that occurred throughout the study, except for measurement 8 (at the 10% level of confidence). At this measurement stage the tension-anxiety score of the EX group was significantly higher than that of the CO group ($p = 0.063$). No statistically significant within-group changes occurred in either the EX group or the CO group throughout the course of the study.

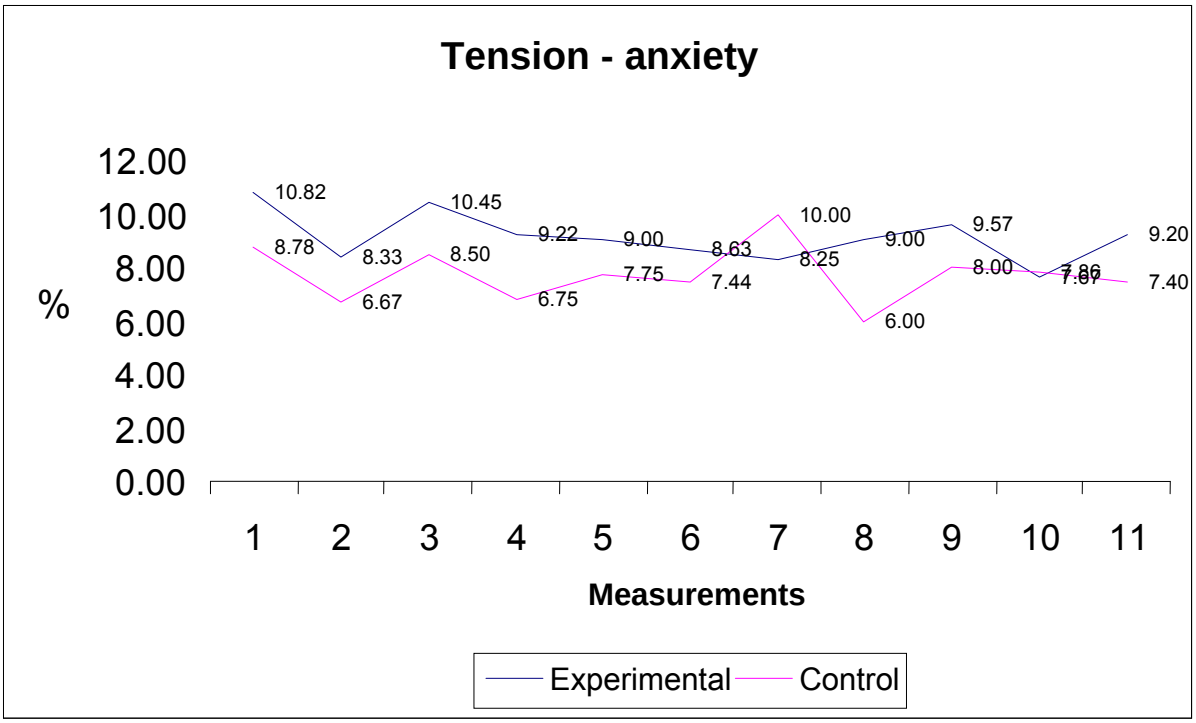


Figure 4-13 **Mean tension-anxiety scores of the EX and the CO group at the different measurement stages**

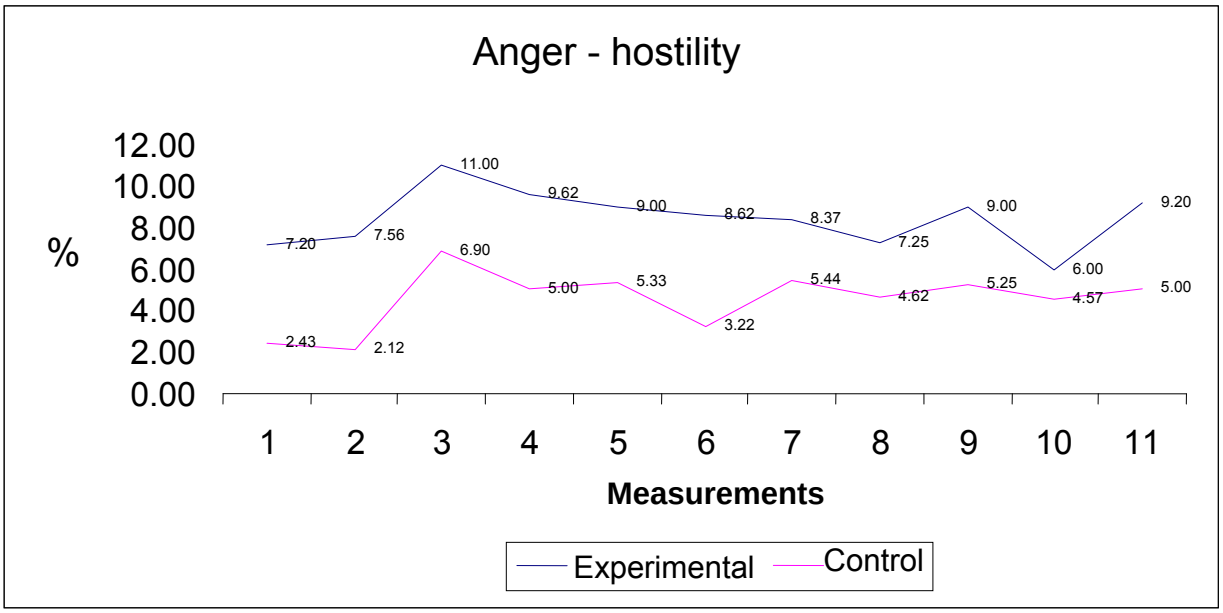


Figure 4-14 **Mean anger-hostility scores of the EX and the CO group at the different measurement stages**

4.3.6.3 Anger-hostility

No statistically significant differences were found in the anger-hostility pre-test results between the EX and the CO group (Figure 4-14). Thus, at the onset of the study the two groups did not differ significantly with regard to this measurement. However, significant differences in anger-hostility scores were evident between the groups at measurement stages 2 and 6 (at the 10% level of confidence). At these measurements, the anger-hostility scores of the EX group were significantly higher than those of the CO group ($p = 0.040$ and $p = 0.030$). No statistically significant within-group changes occurred in either the EX group or the CO group throughout the course of the study.

4.4 DISCUSSION

Increases in body weight are frequently reported side-effects of Cr supplementation (Table 2-3). These increases may range between 0.5 kg and 5 kg and are usually attributed to water retention (Rawson & Clarkson, 2000; Persky & Brazeau, 2001; Mendes *et al.*, 2004; Ahmun *et al.*, 2005; Gotshalk *et al.*, 2008; Safdar *et al.*, 2008; Rahimi *et al.*, 2010; Vatani *et al.*, 2011). It is argued that the increased concentration in muscle free Cr and PCr that results from effective Cr supplementation (Greenhaff *et al.*, 1994; Söderlund *et al.*, 1994; Balsom *et al.*, 1995; Brose *et al.*, 2003; Tarnopolsky *et al.*, 2003*b*; Rawson *et al.*, 2004; Deldicque *et al.*, 2005; Hadjicharalambous *et al.*, 2008; Hickner *et al.*, 2010) is likely to increase the osmolarity of the intra-cellular milieu despite the large number of mechanisms allowing the cell to regulate its own osmolarity, thereby drawing water into the cell (Francaux *et al.*, 2000; Bemben *et al.*, 2001; Jówko *et al.*, 2001; Brilla *et al.*, 2003; Safdar *et al.*, 2008; Saremi *et al.*, 2010). Cellular hydration (swelling) can be considered an anabolic proliferative signal (Häussinger *et al.*, 1993; Schliess & Häussinger, 2002; Baechle & Earle, 2008) that favours the synthesis and inhibits the degradation of muscle protein (Häussinger *et al.*, 1993; Schliess & Häussinger, 2002; Brilla *et al.*, 2003). Factors such as hormones, substrates (ie. Cr) and oxidative stress can change the cellular hydration status within minutes, thereby affecting protein turnover (Häussinger *et al.*, 1993; Schliess & Häussinger, 2002; Brilla *et al.*, 2003). It is also postulated that short-term alterations of cell volume - induced by hormones (ie. insulin), cumulative substrate uptake (eg. Cr) and/or oxidative stress - may activate independent, volume-sensitive signaling cascades which contribute to the regulation of metabolic cell function and gene expression (Häussinger *et al.*, 1993; Schliess & Häussinger, 2002; Safdar *et al.*, 2008).

If the hyper-hydration hypothesis is correct, then the increase in body weight observed in juxtaposition with Cr supplementation could be a short-term effect of water retention (Francaux *et al.*, 2000; Mendes *et al.*, 2004; Ahmun *et al.*, 2005; Gotshalk *et al.*, 2008; Safdar *et al.*, 2008; Rahimi *et al.*, 2010; Saremi *et al.*, 2010; Vatani *et al.*, 2011). Protein synthesis could be a longer-term phenomenon (Francaux *et al.*, 2000; Saremi *et al.*, 2010). Unfortunately, to date not many studies have included body water analyses in their study designs.

At the onset of the study the EX and the CO group did not differ significantly with regard to body weight, body composition or body water compartments. A comparison of changes occurring during the course of the study suggested that neither short-term Cr loading nor long-term low-dosage Cr ingestion changed the values recorded for body weight, body composition or body water compartments significantly compared to no Cr ingestion (Appendix A). Furthermore, analysis of changes that occurred within the groups revealed no statistically significant changes in body weight within either the EX or the CO group. Thus, neither Cr loading nor long-term maintenance feeding resulted in a significant increase in the body weight of highly active male HMS students.

Previously, Balsom *et al.* (1993*a*) found a significant increase in the body weight (+1.1 kg) of physical education students after Cr loading. Findings from the present study are consistent, however with studies in the literature that reported no significant increase in the body weight of physical education students after Cr loading (Op ‘T Eijnde & Hespel, 2001; Louis *et al.*, 2003*b*; Louis *et al.*, 2003*c*; Glaister *et al.*, 2006). The same studies did not report on changes in fat% and LBW. No significant changes in either fat% or LBW were demonstrated in either of the groups, and at any test stage in the present study (Appendix B). When results from the present study were compared to others undertaken with well-trained young males, Cr loading achieved mixed results. A study conducted with young men of similar activity level and age (18 - 24 yrs) produced a significant increase of 0.7 kg in body weight (Rahimi *et al.*, 2010), but in another similar instance no such increase occurred (Ahmun *et al.*, 2005). Thus, the findings gained from the present study are consistent with the majority of studies undertaken with well-trained or highly trained young men who did not report significant increases in body weight in response to Cr loading (Hamilton *et al.*, 2000; Op ‘T Eijnde & Hespel, 2001; Louis *et al.*, 2003*b*; Louis *et al.*, 2003*c*; Ahmun *et al.*, 2005; Glaister *et al.*, 2006).

It is interesting to note that the generally accepted cause/effect principle that an increase in body weight follows Cr loading seems to be most consistently demonstrated in studies where (i) male participants were described as *"healthy"* or *"physically active"* (Greenhaff *et al.*, 1994; Balsom *et al.*, 1995; Green *et al.*, 1996; Jakobi *et al.*, 2000; Jakobi *et al.*, 2001; Preen *et al.*, 2001; Volek *et al.*, 2001; Kinugasa *et al.*, 2004), and (ii) male and female participants were included (with their results grouped together) (Mujika *et al.*, 1996; Kirksey *et al.*, 1999; Mihic *et al.*, 2000; Ziegenfuss *et al.*, 2002; Mendes *et al.*, 2004). The reason why no significant increases occurred in the body weight of well-trained/highly active young men could be that they did not respond to Cr loading in the same manner as so-called *"healthy"*, *"physically active"* or *"recreationally active"* men. Other possibilities: (i) subjects were recruited in the competitive season after initial pre-season strength-orientated training and concomitant increase in body weight (Van der Merwe *et al.*, 2009); (ii) differences in researchers' comprehension of what "*well-trained*" means; and/or (iii) difficulties that arise from mismatches caused by grouping results together inappropriately and incorrectly drawing comparisons between people who participate in a variety of athletic codes (ie. effectively comparing apples and pears).

The present study did not demonstrate significant changes in the body weight, fat%, BMI and waist/hip ratio of highly active male HMS students in response to long-term Cr feeding (ie. Cr loading at 20 g/day for 6 days, followed by maintenance ingestion at 3 g/day for the remainder of 10 months). Long-term studies on Cr supplementation in humans are rare, probably due to challenges to research logistics and availability of dedicated subjects. Studies that incorporated long periods of supplement ingestion (> 6 months) have reported on effects in older men (Op 'T Eijnde *et al.*, 2003) and patients suffering from chronic disease (Verbessem *et al.*, 2003; Escolar *et al.*, 2005; Groeneveld *et al.*, 2005). No studies could be found on highly active young men; two studies that investigated the long-term safety of Cr ingestion in young athletes did not report on changes in body weight and body composition (Poortmans & Francaux, 1999; Kreider *et al.*, 2003).

The present study is the first to analyse the effect of Cr ingestion on all body water compartments. Both short-term Cr loading and long-term maintenance ingestion significantly affected the majority of measurements for body hydration. Measurements for TBW, TBW%, ECW%, ICW, ICW% and BCM demonstrated the largest increase in scores after Cr loading. The scores then continued to increase for the remainder of the supplementation period. Even though there were some small changes in the scores of the CO group, these did not differ

significantly from their respective baseline scores. Short-term Cr loading significantly increased the third-space body water compartment. However, maintenance feeding did not maintain this score and it returned to below baseline level. The third-space water score of the CO group steadily increased over the research period, but these changes were not statistically significant. The scores for TBW/weight and ECW/weight increased significantly over the period of Cr supplementation, while the scores of the CO group remained relatively unchanged. A tendency on the 10% level of significance ($p = 0.054$) was found for the ICW/weight scores of the EX-group to increase over the supplementation period, while the scores of the CO group remained relatively unchanged.

The effect of Cr loading on the body water compartments seems variable. Three of the five studies that reported on the effect of Cr loading on the body water compartments (Table 6-2) demonstrated no changes (Francaux *et al.*, 2000; Rawson & Clarkson, 2000; Armentano *et al.*, 2007). However, Volek *et al.* (2001) and Hadjicharalambous *et al.* (2008) reported significant increases in both body weight and TBW in *"endurance-trained"* and *"healthy"* men, respectively. Findings of the present study are thus consistent with those reporting that Cr loading was followed by a significant increase in TBW (Volek *et al.*, 2001; Hadjicharalambous *et al.*, 2008). Thus Cr loading significantly increased all body water compartments, without being accompanied by significant changes in the CO group, thus supporting the hypothesis contained in existing literature that Cr loading has whole-body and/or cellular hydration effects (Rawson & Clarkson, 2000; Persky & Brazeau, 2001; Mendes *et al.*, 2004; Ahmun *et al.*, 2005; Gotshalk *et al.*, 2008; Safdar *et al.*, 2008; Rahimi *et al.*, 2010; Vatani *et al.*, 2011). In the present study, the increase in hydration status was associated with a negligible mean increase in body weight (+ 0.86 kg).

Increases of 1.0 to 3.2 ℓ in TBW have been reported when Cr loading was followed by long-term maintenance feeding (Francaux & Poortmans, 1999; Arciero *et al*, 2001; Bemben *et al*, 2001; Jówko *et al.*, 2001; Kutz & Gunter, 2003; Safdar *et al.*, 2008). The findings of the present study are thus consistent with these results. The significant increase in BCM score that occurred over the research period (Appendix B) does show an increase in the weight of the body's cells (+ 1.3 kg). An increase in FFW ($p = 0.067$) was also demonstrated (Appendix B). However, the question remains whether these increases were attributable to contractile protein accretion, and if so, to what extent.

Muscle protein accretion is usually associated with increases in strength and/or power (Volek *et al.*, 1999; Willoughby & Rosene, 2001; Brose *et al.*, 2003; Burke *et al.*, 2003; Chilibeck *et al.*, 2004; Olsen *et al.*, 2006; Souza-Junior *et al.*, 2011). Results from the present study indicate that both groups increased their isokinetic strength through the course of the study. However, a statistically significant increase was demonstrated only by the CO group, and no significant between-group changes were observed. Thus, the significant increase in scores for body hydration, and the concomitant increase in FFW, cannot be attributed definitively to muscle protein accretion. Although FFW is commonly used as a surrogate measure of muscle mass, it does not always accurately reflect specific changes in muscle mass or differences in muscle mass between individuals (Andreoli *et al.*, 2001). Further studies are thus warranted to determine whether or not the hypothesis of Kraemer and Volek (1999) is correct in that the impact of Cr supplementation on body composition would be mediated more readily by the long-term use of higher intensities in weight-training, rather than the acute effects of short-term water influx to maintain a proper solute to hydration relationship in the muscle cell. Since resistance training in conjunction with Cr supplementation for longer periods (6 - 15 weeks) has proved successful in increasing muscle strength and hypertrophy (Volek *et al.*, 1999; Burke *et al.*, 2003; Chilibeck *et al.*, 2004; Olsen *et al.*, 2006; Souza-Junior *et al.*, 2011), it may be that Cr supplementation without active participation in structured heavy-resistance weight training would not translate into significant muscle protein accretion. Further investigation is needed to show whether the EX group of highly active students could have demonstrated significant gains in FFW and strength above those of the CO group if both groups had participated in a structured resistance-training programme for 10 months. Also, conclusive evidence has yet to be adduced that Cr supplementation is accompanied by increases in ICW (Francaux & Poortmans, 1999; Jówko *et al.*, 2001; Bemben *et al.*, 2001) of sufficient magnitude to positively influence measures of protein synthesis or breakdown (Volek & Rawson, 2004).

Further research is therefore needed to clarify whether oral Cr supplementation has *direct* effects on cells that may result in muscle protein accretion by influencing: (i) the expression of MRFs and MHC (Willoughby & Rosene, 2001; Willoughby & Rosene, 2003; Louis *et al.*, 2004; Deldicque *et al.*, 2005; Saremi *et al.*, 2010); (ii) myonucleus number (Olsen *et al.*, 2006); (iii) myostatin and GASP-1 protein levels (Saremi *et al.*, 2010); (iv) satellite cell activation and differentiation (Saremi *et al.*, 2010); and (v) muscle gene expression (Deldicque *et al.*, 2005; Safdar *et al.*, 2008), or whether the effect is *indirectly* mediated through a greater training volume that serves to increase the stimulus for muscle hypertrophy (Burke *et al.*, 2003; Louis *et*

al., 2003*b*; Chilibeck *et al.*, 2004; Volek & Rawson, 2004; Rahimi *et al.*, 2010; Souza-Junior *et al.*, 2011). The latter may suggest either that acute exercise leads to the expression of some anabolic effect of Cr not seen at rest (Schedel *et al.*, 2000) or that, because Cr increases force development through increased muscle PCr stores, work output during training can be increased during Cr supplementation, with a concomitant benefit to muscle accretion (Louis *et al.*, 2003*c*; Souza-Junior *et al.*, 2011).

In summary, results from the present study support the postulated osmotically active nature of Cr when ingested as a supplement. Cr loading resulted in an expressed increase in all markers for body hydration which was then, with the exception of third-space water, maintained and/or improved upon for the duration of the study. However, neither short-term Cr loading nor long-term ingestion resulted in significant changes in body weight and isokinetic strength of highly active male HMS students.

Globally the use of Cr is prevalent in young men attending university or the equivalent. Froiland *et al*. (2004) reported that 37% of university level sport participants who were using dietary supplements, were consuming Cr. Gradidge (2010) reported that 32% of male team-sport athletes at high-schools for boys (Johannesburg, South Africa) who used performance enhancing substances, supplemented with Cr. Jackson *et al.* (2010) report that Cr was ingested by 29% of surveyed users of a university-campus recreation facility who used dietary supplements. Recently Sobolewski *et al.* (2011) concluded that, world-wide, university level team sportsmen and male elite power athletes were the greatest consumers of Cr. Young athletes and sport participants tend to model themselves after sport icons, and believe that ergogenic aids are acceptable, if not indispensable, to reach record-breaking performances in sport (Calfee & Fadale, 2006). Since they tend to idolise famous athletes (Calfee & Fadale, 2006; Boone, 2010) and desire muscle gain (Sheppard *et al.*, 2000; Froiland *et al*., 2004; Calfee & Fadale, 2006; Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010) they may consume Cr in higher-than-recommended dosages and/or for prolonged periods of time. To date no long-term, controlled, safety studies on a homogeneous population of young males have been conducted. Therefore, the safety of Cr supplementation for organ function remains an unresolved concern (Juhn & Tarnopolsky, 1998; Kreider *et al.*, 1998; Pritchard & Kalra, 1998; Kuehl *et al.*, 2000; Kreider *et al.*, 2003; Schröder *et al.* 2005; Gualano *et al.*, 2011). The ULS has been set at 5 g/day (Shao & Hathcock, 2006), but this level is exceeded during high-dosage and moderate-dosage Cr ingestion.

Participants in the present study reported no side-effects (ie. gastrointestinal upset, thermal stress, dehydration, and muscle cramping) to Cr ingestion. This finding is consistent with scientific trials that reported an absence of side-effects in healthy participants (Kreider *et al.*, 1998; Mujika *et al.*, 2000; Bemben *et al.*, 2001; Op'T Eijnde & Hespel, 2001; Volek *et al.*, 2001; Kambis & Pizzedaz, 2003; Ostojic, 2004; Deldicque *et al.*, 2008; Gotshalk *et al.*, 2008; Hadjicharalambous *et al.*, 2008). However, one participant in the EX group sprained his ankle playing touch-rugby during Month 8. Proponents of the theory that Cr may promote a higher incidence of muscle injuries postulate that, because Cr supplementation may promote rapid increases in strength and body weight, an athlete may be more inclined to place additional stress on muscles, bones, joints, ligaments and connective tissues (Greenwood *et al.*, 2003), thus increasing the risk of sustaining exercise-related injuries. However, no significant, or rapid, increases in body weight and strength were demonstrated in the present study. Thus, the extent to which Cr supplementation contributed to the injury - if at all - remains unclear.

At the onset of the present study the two groups did not differ significantly with regard to indices of haematological and biochemical pathology. Mean scores were within the normal range for the gender and age of the participants, with the exception of S-CK (Table 4-5). Baseline S-CK scores were elevated above normal in both groups, and all mean S-CK scores remained outside the normal range throughout the duration of the study. These findings are consistent with reports that physically well-trained people tend to have elevated baseline levels of this enzyme (Bishop *et al.*, 2000 Kreider *et al.*, 2003; Schröder *et al.*, 2005; Armentano *et al.*, 2007). The high activities of S-CK that are observed in highly-trained individuals may be a consequence of the large amounts of eccentric loads they endure on a daily basis (Schröder *et al.*, 2005). The large inter-individual variations in the baseline scores of both groups (Appendix B), are consistent with clinical data that indicate a genetically based high degree of individual variability in the expression of this indicator (Clarkson *et al.*, 2006).

Short-term Cr loading dramatically increased S-CK scores within the EX group. This increase was statistically significant when compared to the CO group (Table 4-5). A commensurate significant elevation in S-Crn of the EX group was also demonstrated. Elevations of these serum markers are associated with an increased Cr turnover rate after Cr ingestion (Bishop *et al.*, 2000; Hathcock *et al.*, 2006), and with an increased reliance on the ATP-PCr energy system during periods of high-intensity exercise in well-trained athletes (Schröder *et al.*, 2005). Cr

supplementation is expected to increase the daily rate of Cr turnover (as a result of the dietary-induced increase in muscle Cr stores), and thereby Crn clearance (Hultman *et al.*, 1996; Kreider *et al.*, 1998; Robinson *et al.*, 2000; Schröder *et al.*, 2005; Taes & De Vriese, 2005). During exercise, the muscle repeatedly contracts and uses energy substrates thereby increasing the activity/concentration of S-CK (Mitchell *et al.*, 1996; Totsuka *et al.*, 2002). High levels of this enzyme ensures that ADP and ATP levels remain almost constant, effectively buffering the cell against rapid depletion of ATP (McLeish & Kenyon, 2005). However, elevated S-CK levels may also be associated with a loss of skeletal muscle membrane integrity or damage to muscle tissue (Bishop, 2000; Totsuka *et al.*, 2002). During the loading phase of Cr ingestion, the influx of Cr into the cell may be accompanied by a concomitant influx of water (Brudnak, 2004). The net effect - a swelling of the cells and associated tissues – may predispose them to damage (Brudnak, 2004).

Studies investigating the effect of Cr supplementation on muscle enzymes have demonstrated neither significant differences, nor an upward trend, in S-CK between Cr and non-Cr groups over time (Robinson *et al.*, 2000; Rawson *et al.*, 2001; Crowe *et al.*, 2003; Kreider *et al.*, 2003; Schröder *et al.*, 2005). Moreover, Korones *et al.* (2001) have stated that although S-AST and S-ALT are present in large amounts in the liver, they are not specific to the liver. Muscle also contains substantial quantities of these enzymes. Therefore, insult to skeletal or cardiac muscle tissue may result in striking elevations of these serum markers (Korones *et al.*, 2001). The significant and dramatic elevations in S-CK and S-AST in response to Cr loading may thus indicate muscle damage - possibly incurred due to cellular and/or whole-body hyperhydration (Appendix B).

Although S-CK-MB mass increased significantly to 5.56 ± 2.96 ng/ml - a level that may indicate myocardial damage - the S-CK-MB mass index declined significantly to 0.69 ± 0.35%. This change indicates that the S-CK-MB mass score was elevated due to CK leaking from skeletal muscle (Mitchell *et al.*, 1996), and not due to myocardial damage. Furthermore, cardiac troponin T levels remained below 0.1 ng/ml in all participants, thereby excluding myocardial damage as the cause of elevated S-CK-MB mass levels.

The significant decrease in S-CK-MB mass index may point to a cardio-protective effect in highly active individuals. After investigating results from animal and human studies, Persky and Brazeau (2001) postulated that the myocardium may derive a therapeutic benefit from Cr

supplementation. It has been hypothesised that Cr supplementation may protect the myocardium during periods of high energy flux through increased availability of PCr. However, the benefits for humans seem to derive only from intravenous administration of PCr to patients presenting with heart failure (Gordon *et al.*, 1995; Kreider, 1998; Gualano *et al.*, 2010). There has been speculation that Cr supplementation may increase energy provision to cardio-myocytes, which could therefore result in improved cardiac function in these patients (Gordon *et al.*, 1995; Gualano *et al.*, 2010). Under normal physiological conditions, however, myocardial PCr levels are not depleted to the same extent as those in skeletal muscle (Field, 1996). The relatively minor changes in levels of myocardial PCr after Cr loading should therefore not affect cardiac function in healthy subjects (Field, 1996). Further study is needed to determine whether or not Cr supplementation would "protect" the cardiac muscle tissue of highly active individuals and athletes.

S-AST is considered to be a "*liver specific*" enzyme marker of pathology, and high levels are indicative of acute liver damage and liver disease (Bishop *et al.*, 2000). S-GGT is used in association with S-AST to indicate liver damage (Bishop *et al.*, 2000). Results from the present study show S-AST levels above the normal range in response to Cr loading. However there was no commensurate rise in mean S-GGT levels (Appendix B). It is thus possible that instead of hepato-cellular damage, the abnormally elevated S-AST levels were attributable to a loss of integrity of skeletal muscle membrane, or to muscle damage (Bishop *et al.*, 2000; Korones *et al.*, 2001).

In summary, the reason(s) for the dramatic elevations in muscle enzymes - S-CK, S-CK-MB mass and S-AST - in response to Cr loading remain unclear, but may be attributable to: (i) elevated Cr turnover due to increased Cr ingestion; (ii) increased activity of (and reliance on) the ATP-PCr energy system during physical activity due to increased muscle TCr and PCr stores; (iii) skeletal muscle damage caused by cellular hyper-osmolarity; (iv) increased skeletal muscle membrane permeability; and/or (v) interaction between, or a combination of, these effects.

Findings from the present study are consistent with others in the literature that did not show any clinically relevant changes in S-Crn or S-Urea that would indicate kidney damage or functional inhibition (Kreider *et al.*, 1998; Poortmans & Francaux, 1999; Robinson *et al.*, 2000; Crowe *et al.*, 2003; Kreider *et al.*, 2003; Schröder *et al.*, 2005; Hathcock *et al.*, 2006; Shao & Hathcock, 2006; Armentano *et al.*, 2007). The increase in S-Crn after short-term and long-term

supplementation was statistically significant and the mean values were still within the normal range (Table 4-5), thus reflecting a possible increased release and cycling of intra-muscular Cr as a consequence of enhanced Cr turnover in response to Cr ingestion, rather than a pathological causation (Kreider, 1998; Kreider *et al.*, 1998; Mayhew *et al.*, 2002). Furthermore, results do not support the electrolyte-dilution hypothesis (Kreider *et al.*, 2003). Thus, Cr loading - although significantly increasing water retention - did not alter electrolyte balance. These findings are consistent with others in the literature that reported no clinically relevant alterations in plasma concentrations of electrolytes in response to Cr ingestion (Robinson *et al.*, 2000; Kreider *et al.*, 2003; Schröder *et al.*, 2005).

S-Urea is a marker of protein degradation (Kreider, 1998). The significant increase in S-Urea of the CO group during the course of the study indicated an increased catabolic state in those affected (Kreider, 1998). The high activity levels of the students caused an increase in S-Urea in both groups, but statistical significance was reached only within the CO group (Appendix B). Thus, Cr supplementation may have protected the EX group from the catabolic consequences of high-activity states (Kreider, 1998; Kreider *et al.*, 1998).

The elevated S-CK and S-AST levels evident in the EX group returned to below baseline levels at post-test 2, as did the levels of the CO group. The drop in muscle enzyme levels in both groups may indicate that the participants were less active towards the end than at the beginning of the study, possibly because Month 10 coincided with the start of the students' year-end exams. The significant drop in these muscle enzymes also indicates that, if strain had been put on the muscles and/or liver during Cr loading, the effects were not permanent and dissipated during prolonged low-dosage (3 g/day) supplementation. The fact that S-Crn levels of the EX group remained significantly elevated at post-test 2 served to indicate that a higher Cr turnover rate was maintained by low-dosage Cr supplementation.

Large interindividual variations in biochemical pathology scores were prevalent at all stages and in both groups during the research period, probably indicating a genetically based high degree of individual variability in the expression of indicators for biochemical pathology in highly active male HMS students (Clarkson *et al.*, 2006).
Analysis of haematological changes that occurred within the groups revealed that both groups underwent a significant decrease in blood platelets count over the research period. However, all scores remained within the normal range for the participants' age and gender. The EX group

demonstrated a significant decrease in absolute lymphocyte count after short-term Cr loading and long-term maintenance ingestion, respectively. The reason for this decrease remained unclear, but all scores were within the normal range. However, the decrease in absolute lymphocyte count during Cr supplementation may indicate an immunoprotective function, possibly due to the proposed anti-oxidant activity of the supplement (Sestili *et al.*, 2011). The protective effects of Cr appear only when cellular free Cr levels are significantly increased, that is when a threshold level is attained upon adequate supplementation (Sestili *et al.*, 2011). Thus, short-term high-dosage Cr supplementation followed by long-term low-dosage supplementation may have been adequate to elicit an antioxidant and/or immuneprotective effect.

PCr plays a vital role in cerebral energy provision (Rawson *et al.*, 2008; Gualano *et al.*, 2011). Although Cr can cross the blood-brain barrier its permeability for Cr is limited (Wyss & Schulze, 2002). Cr biosynthesis in this organ therefore adds to its TCr and PCr content (Wyss & Schulze, 2002). Cr supplementation can significantly raise brain PCr concentrations in humans (Lyoo *et al.*, 2003; Kondo *et al.*, 2011). To date, results from studies in the literature seem to corroborate the theory proposed by Rawson *et al.* (2008) to the effect that, potentially, Cr supplementation only improves cognitive processing and psychomotor performance in individuals who either have impaired cognitive processing abilities (Cook *et al.*, 2011), or who suffer from depressive disorders (Kondo *et al.*, 2011). However, high-dosage Cr supplementation (20 g/day for 5 days) improved vegetarian female undergraduates' memory compared to that of meat eaters (Benton & Donohoe, 2011). The cognitive capacity of the vegetarians before supplementation, compared to meat eaters, remained unaffected. Thus, vegetarians were found to be more sensitive to Cr supplementation (Benton & Donohoe, 2011).

The exact mechanisms underlying the effects of Cr supplementation on mood and neurocognitive functioning have yet to be elucidated (Gualano *et al.*, 2011). PCr can be expected to play a role in supplying energy to metabolically active areas of the brain (Benton & Donohoe, 2011; Gualano *et al.*, 2011). However, the uneven distribution of Cr phosphokinase indicates that, rather than having a general function, it is important in some aspects of functioning rather than others (Benton & Donohoe, 2011). The high concentration in the hippocampus is of particular interest, given the known importance of this area of the brain for memory (Benton & Donohoe, 2011).

Findings from the present study indicate that short-term high-dosage Cr ingestion followed by long-term low-dosage supplementation may decrease the mood state of fatigue towards the latter part of the intervention. It is important to note that a decrease in fatigue during high-intensity exercise is quite different from the total mood state. A mood state of less fatigue would mean that the HMS students consuming Cr experienced less feelings of being worn-out, listless, exhausted, sluggish, tired and/or weary over a much longer period of time (Steyn & Rossouw, 2007). The finding of decreased fatigue as a mood state is promising, but warrants further investigation since increased tension-anxiety and anger-hostility scores were also demonstrated. However, all changes in mood states were significant at the 10% level of confidence and did not occur consistently over the course of the study. Thus, no definitive conclusions regarding the effect - if any - of Cr supplementation on mood states could be made. Previous studies relating to the effect of short-term high-dosage Cr ingestion on the mood state of well-trained individuals demonstrated no significant changes (Warber *et al.*, 2002; McMorris *et al.*, 2006).

4.5 CONCLUSION

This study is the first to investigate the safety and effects of long-term Cr supplementation in the recommended dosages (Becque *et al.*, 2000; Huso *et al.*, 2002; Burke *et al.*, 2003; Kutz & Gunter, 2003; Preen *et al.*, 2003; Olsen *et al.*, 2006; Stout *et al.*, 2006; Van der Merwe *et al.*, 2009; Gualano *et al.*, 2011) in a relatively homogeneous population of young male students incorporating a control group. Results demonstrate that both short-term high-dosage Cr supplementation, as well as short-term Cr loading followed by long-term maintenance ingestion, first increased and then maintained markers for body hydration (ie. TBW, ECW, ICW, and BCM). However, neither short-term Cr loading nor long-term ingestion (10 months) resulted in any significant changes in body weight and isokinetic strength of highly active male HMS students.

Dramatic elevations in serum enzyme markers for muscle damage (ie. S-CK, S-CK-MB and S-AST) in response to the Cr loading regimen were demonstrated. These may be due to: (i) elevated Cr turnover as a result of increased Cr ingestion; (ii) increased activity (and reliance) on the ATP-PCr energy system during physical activity as a function of increased muscle TCr and PCr stores; (iii) skeletal muscle damage as a result of cellular hyperosmolarity; (iv) increased skeletal muscle membrane permeability; and/or (v) interaction between, or a combination of, these effects. The above-normal elevation in S-AST levels may also indicate

that Cr loading placed considerably more strain on the liver of highly active male university students than non-intake did. This strain, however, did not result in liver damage since: (i) enzyme values returned to the normal range after prolonged low-dosage ingestion of Cr; and (ii) S-GGT levels remained within the normal range. To conclude, findings of the present study indicate an increase in muscle and liver enzyme efflux in highly active male HMS students who did ingest Cr in high dosages, compared to those who did not use the supplement.

No clinically significant changes in serum markers for renal integrity and function were demonstrated during the course of the study. Interestingly, a tendency towards a protective effect of Cr supplementation for highly active young men was demonstrated in terms of immune system status and/or muscle catabolism. However, no definitive conclusion could be drawn regarding the effects of Cr supplementation on mood states.

4.6 RECOMMENDATIONS

- University students who participate in sport and/or attend campus gymnasiums throughout the world represent the target population for dietary-supplement and Cr sales (Sobolewski *et al.*, 2011). Moreover, frequenters of gymnasium facilities tend to use multiple supplements simultaneously (Sheppard *et al.*, 2000; Goston & Toulson Davisson Correia, 2010; Jackson *et al.*, 2010; Tsitsimpikou *et al.*, 2011). These considerations are strong indicators that more research is needed to determine and monitor the safety of Cr supplementation in the population group referred to (ie. university student population).

- More research should be brought to bear on the effect of Cr supplementation on all the body water compartments.

- Continued research should be conducted on the possible mechanisms (direct and indirect) whereby Cr supplementation may result in muscle protein accretion.

- The safety of Cr supplementation for organ function remains a concern (Juhn & Tarnopolsky, 1998; Kreider *et al.*, 1998; Pritchard & Kalra, 1998; Kuehl *et al.*, 2000; Kreider *et al.*, 2003; Schröder *et al.* 2005; Gualano *et al.*, 2011) and should remain under research scrutiny.

- Future research should focus on the extent to which Cr loading (Harris *et al.*, 1992; Casey *et al.*, 1996; Schedel, *et al.*, 2000; Steenge *et al.*, 2000; Warber *et al.*, 2002; Louis *et al.*, 2003*b*; Mendes *et al.*, 2004; Santos *et al.*, 2004; Hadjicharalambous *et al.*, 2008; Rahimi *et al.*, 2010) may exert an osmotic effect - cause cellular swelling - in muscle and liver cells of humans, and thereby possibly cause cell damage.

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APPENDIX A

Table A-1 **Between-group analysis of the change in recorded scores associated with the body composition and body water compartments of well-trained male HMS students in the EX group compared to that of the CO group**

Variable	Test	Mean		Standard Deviation		p-value
		CO	EX	CO	EX	
Body weight (kg)	**Pre**	79.54	81.72	6.90	8.61	0.601
	Post 1	79.96	82.58	7.35	8.78	0.559
	Post 2	76.71	81.57	17.08	11.70	0.558
Fat%	**Pre**	12.16	13.24	2.99	3.79	0.622
	Post 1	11.84	12.46	3.20	4.22	0.667
	Post 2	12.45	12.30	3.86	4.37	0.845
FFW (kg)	**Pre**	69.83	70.75	5.86	6.54	0.829
	Post 1	70.41	72.10	6.14	6.34	0.601
	Post 2	69.60	71.29	6.56	8.37	0.770
FFW%	**Pre**	87.84	86.76	2.99	3.79	0.622
	Post 1	88.16	87.54	3.20	4.22	0.667
	Post 2	87.55	87.70	3.86	4.37	0.845
TBW (*ℓ*)	**Pre**	47.66	48.30	3.70	4.72	0.951
	Post 1	48.21	49.59	3.94	4.68	0.667
	Post 2	47.78	49.57	3.74	6.03	0.558
TBW%	**Pre**	60.06	59.29	3.38	4.08	0.666
	Post 1	60.45	60.25	6.63	4.46	0.758
	Post 2	60.33	61.01	5.36	4.34	0.464
ECW (*ℓ*)	**Pre**	19.46	19.78	1.28	1.81	0.878
	Post 1	19.68	20.23	1.38	1.74	0.644
	Post 2	19.47	20.17	1.34	2.34	0.625
ECW%	**Pre**	24.53	24.28	1.44	1.50	0.479
	Post 1	24.69	24.59	1.52	1.74	0.805
	Post 2	24.61	24.84	2.20	1.75	0.557

Variable	Test	Mean		Standard Deviation		p-value
		CO	EX	CO	EX	
ICW (ℓ)	**Pre**	27.81	28.10	2.33	2.59	0.975
	Post 1	28.00	28.75	2.46	2.60	0.579
	Post 2	27.88	29.01	2.35	3.40	0.732
ICW%	**Pre**	34.99	34.47	1.30	1.91	0.460
	Post 1	35.08	34.91	1.38	1.93	0.735
	Post 2	35.23	35.71	2.23	1.94	0.434
Body cell mass (kg)	**Pre**	39.72	40.15	3.31	3.69	0.975
	Post 1	40.00	41.08	3.49	3.71	0.579
	Post 2	39.90	41.46	3.33	4.86	0.807
ECW/weight (ℓ/kg)	**Pre**	24.53	24.28	1.44	1.50	0.479
	Post 1	24.69	24.59	1.52	1.74	0.805
	Post 2	24.61	24.84	2.20	1.75	0.557
ICW/weight (ℓ/kg)	**Pre**	34.99	34.47	1.30	1.91	0.460
	Post 1	35.08	34.91	1.38	1.92	0.735
	Post 2	35.20	35.71	2.22	1.94	0.406
TBW/weight (ℓ/kg)	**Pre**	59.53	58.75	2.67	3.39	0.622
	Post 1	59.78	59.52	2.86	3.66	0.833
	Post 2	59.81	60.56	4.39	3.63	0.591
BMI	**Pre**	23.90	24.85	1.59	2.00	0.207
	Post 1	24.01	25.13	1.77	2.12	0.157
	Post 2	23.95	25.31	2.26	2.89	0.129

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

Table A-2 **Between-group analysis of the change in recorded scores associated with the haematological pathology of well-trained male HMS students in the EX group compared to that of the CO group**

Variable	Test	Mean		Standard Deviation		p-value
		CO	EX	CO	EX	
Haemoglobin (g/dℓ)	**Pre**	16.88	17.16	0.71	0.62	0.296
	Post 1	16.33	16.72	0.72	1.03	0.147
	Post 2	16.48	16.50	0.87	1.07	0.845
Red blood cell count (10^12/ℓ)	**Pre**	5.60	5.57	0.30	0.28	0.750
	Post 1	5.51	5.52	0.25	0.45	0.885
	Post 2	5.61	5.39	0.43	0.32	0.172
Haematocrit (%)	**Pre**	47.94	48.67	1.86	1.51	0.182
	Post 1	47.11	48.23	2.51	2.96	0.258
	Post 2	47.28	47.09	2.68	2.86	0.883
Platelets (10^9/ℓ)	**Pre**	269.54	256.45	54.93	44.51	0.750
	Post 1	387.00	257.91	50.69	54.70	0.505
	Post 2	234.40	233.71	32.94	50.70	0.696
White blood cells (10^9/ℓ)	**Pre**	7.05	7.79	1.81	1.28	0.235
	Post 1	9.11	7.35	1.43	0.93	0.505
	Post 2	6.76	7.07	1.82	1.21	0.845
Neurophils%	**Pre**	59.00	60.48	8.26	5.47	0.524
	Post 1	76.30	60.02	10.50	7.95	0.077**
	Post 2	56.72	60.50	8.22	9.51	0.380
Eosinophils%	**Pre**	2.51	2.14	1.76	2.13	0.354
	Post 1	7.00	2.00	2.12	2.71	0.098**
	Post 2	2.41	1.70	1.61	1.06	0.434
Basophils%	**Pre**	0.72	0.70	0.24	0.23	0.977
	Post 1	1.20	0.73	0.24	0.17	0.106
	Post 2	0.68	0.63	0.19	0.16	0.517
Lymphocytes%	**Pre**	32.97	31.93	6.28	4.05	0.622
	Post 1	54.10	32.41	8.98	7.96	0.246
	Post 2	35.10	32.46	6.83	9.11	0.591

Variable	Test	Mean		Standard Deviation		p-value
		CO	EX	CO	EX	
Monocytes%	**Pre**	4.80	4.76	1.16	1.10	0.793
	Post 1	7.80	4.84	1.40	0.81	0.505
	Post 2	5.07	4.73	0.89	1.15	0.406
Neurophils (absolute) (10^9/ℓ)	**Pre**	4.21	4.70	1.48	0.87	0.235
	Post 1	5.91	4.43	1.23	0.90	0.164
	Post 2	3.93	4.31	1.50	1.15	0.495
Eosinophils (absolute) (10^9/ℓ)	**Pre**	0.17	0.17	0.11	0.17	0.561
	Post 1	0.47	0.16	0.13	0.25	0.182
	Post 2	0.16	0.12	0.08	0.07	0.328
Basophils (absolute) (10^9/ℓ)	**Pre**	0.05	0.05	0.02	0.02	0.538
	Post 1	0.10	0.05	0.02	0.02	0.454
	Post 2	0.05	0.04	0.02	0.01	0.725
Lymphocytes (absolute) (10^9/ℓ)	**Pre**	2.29	2.50	0.61	0.55	0.505
	Post 1	3.77	2.36	0.72	0.58	0.400
	Post 2	2.30	2.27	0.49	0.59	0.591
Monocytes (absolute) (10^9/ℓ)	**Pre**	0.33	0.37	0.10	0.08	0.270
	Post 1	0.58	0.35	0.08	0.07	0.749
	Post 2	0.33	0.33	0.06	0.09	1.000

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

APPENDIX B

Table B-1 **Comparison of the change in body composition and body water compartments of well-trained male HMS students in the EX and CO groups across test intervals**

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
Body weight (kg)	**Pre**	79.54	81.72	6.90	8.61
	Post 1	79.96	82.58	7.35	8.78
	Post 2	76.71	81.57	17.08	11.70
	p-value	0.584	0.180		
Fat%	**Pre**	12.16	13.24	2.99	3.79
	Post 1	11.84	12.46	3.20	4.22
	Post 2	12.45	12.30	3.86	4.37
	p-value	0.527	0.121		
FFW (kg)	**Pre**	69.83	70.75	5.86	6.54
	Post 1	70.41	72.10	6.14	6.34
	Post 2	69.60	71.29	6.56	8.37
	p-value	0.497	0.067		
FFW%	**Pre**	87.84	86.76	2.99	3.79
	Post 1	88.16	87.54	3.20	4.22
	Post 2	87.55	87.70	3.86	4.37
	p-value	0.527	0.121		
TBW (*ℓ*)	**Pre**	47.66	48.30	3.70	4.72
	Post 1	48.21	49.59	3.94	4.68
	Post 2	47.78	49.57	3.74	6.03
	p-value	0.527	0.028*		
TBW%	**Pre**	60.06	59.29	3.38	4.08
	Post 1	60.45	60.25	6.63	4.46
	Post 2	60.33	61.01	5.36	4.34
	p-value	0.368	0.018*		
ECW (*ℓ*)	**Pre**	19.46	19.78	1.28	1.81
	Post 1	19.68	20.23	1.38	1.74
	Post 2	19.47	20.17	1.34	2.34
	p-value	0.607	0.089		

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
ECW%	**Pre**	24.53	24.28	1.44	1.50
	Post 1	24.69	24.59	1.52	1.74
	Post 2	24.61	24.84	2.20	1.75
	p-value	0.607	0.028*		
ICW (ℓ)	**Pre**	27.81	28.10	2.33	2.59
	Post 1	28.00	28.75	2.46	2.60
	Post 2	27.88	29.01	2.35	3.40
	p-value	0.497	0.018*		
ICW%	**Pre**	34.99	34.47	1.30	1.91
	Post 1	35.08	34.91	1.38	1.93
	Post 2	35.23	35.71	2.23	1.94
	p-value	0.779	0.054		
Body cell mass (kg)	**Pre**	39.72	40.15	3.31	3.69
	Post 1	40.00	41.08	3.49	3.71
	Post 2	39.90	41.46	3.33	4.86
	p-value	0.497	0.018*		
ECW/weight (ℓ/kg)	**Pre**	24.53	24.28	1.44	1.50
	Post 1	24.69	24.59	1.52	1.74
	Post 2	24.61	24.84	2.20	1.75
	p-value	0.607	0.028*		
ICW/weight (ℓ/kg)	**Pre**	34.99	34.47	1.30	1.91
	Post 1	35.08	34.91	1.38	1.92
	Post 2	35.20	35.71	2.22	1.94
	p-value	0.779	0.054		
TBW/weight (ℓ/kg)	**Pre**	59.53	58.75	2.67	3.39
	Post 1	59.78	59.52	2.86	3.66
	Post 2	59.81	60.56	4.39	3.63
	p-value	0.836	0.021*		
BMI	**Pre**	23.90	24.85	1.59	2.00
	Post 1	24.01	25.13	1.77	2.12
	Post 2	23.95	25.31	2.26	2.89
	p-value	0.656	0.180		

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

Table B-2 **Comparison of the change in haematological pathology of well-trained male HMS students in the EX and CO groups across test intervals**

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
Haemoglobin (g/dℓ)	**Pre**	16.88	17.16	0.71	0.62
	Post 1	16.33	16.72	0.72	1.03
	Post 2	16.48	16.50	0.87	1.07
	p-value	0.223	0.565		
Red blood cell count (10^12/ℓ)	**Pre**	5.60	5.57	0.30	0.28
	Post 1	5.51	5.52	0.25	0.45
	Post 2	5.61	5.39	0.43	0.32
	p-value	0.273	0.651		
Haematocrit (%)	**Pre**	47.94	48.67	1.86	1.51
	Post 1	47.11	48.23	2.51	2.96
	Post 2	47.28	47.09	2.68	2.86
	p-value	0.497	0.651		
Platelets (10^9/ℓ)	**Pre**	269.54	256.45	54.93	44.51
	Post 1	387.00	257.91	50.69	54.70
	Post 2	234.40	233.71	32.94	50.70
	p-value	0.025*	0.018*		
White blood cells (10^9/ℓ)	**Pre**	7.05	7.79	1.81	1.28
	Post 1	9.11	7.35	1.43	0.93
	Post 2	6.76	7.07	1.82	1.21
	p-value	0.670	0.368		
Neurophils%	**Pre**	59.00	60.48	8.26	5.47
	Post 1	76.30	60.02	10.50	7.95
	Post 2	56.72	60.50	8.22	9.51
	p-value	0.122	0.867		
Eosinophils%	**Pre**	2.51	2.14	1.76	2.13
	Post 1	7.00	2.00	2.12	2.71
	Post 2	2.41	1.70	1.61	1.06
	p-value	0.704	0.717		
Basophils%	**Pre**	0.72	0.70	0.24	0.23
	Post 1	1.20	0.73	0.24	0.17
	Post 2	0.68	0.63	0.19	0.16
	p-value	0.069	0.289		

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
Lymphocytes%	**Pre**	32.97	31.93	6.28	4.05
	Post 1	54.10	32.41	8.98	7.96
	Post 2	35.10	32.46	6.83	9.11
	p-value	0.497	0.867		
Monocytes%	**Pre**	4.80	4.76	1.16	1.10
	Post 1	7.80	4.84	1.40	0.81
	Post 2	5.07	4.73	0.89	1.15
	p-value	0.741	0.276		
Neurophils (absolute) (10^9/ℓ)	**Pre**	4.21	4.70	1.48	0.87
	Post 1	5.91	4.43	1.23	0.90
	Post 2	3.93	4.31	1.50	1.15
	p-value	0.905	0.651		
Eosinophils (absolute) (10^9/ℓ)	**Pre**	0.17	0.17	0.11	0.17
	Post 1	0.47	0.16	0.13	0.25
	Post 2	0.16	0.12	0.08	0.07
	p-value	0.670	0.764		
Basophils (absolute) (10^9/ℓ)	**Pre**	0.05	0.05	0.02	0.02
	Post 1	0.10	0.05	0.02	0.02
	Post 2	0.05	0.04	0.02	0.01
	p-value	0.107	0.065		
Lymphocytes (absolute) (10^9/ℓ)	**Pre**	2.29	2.50	0.61	0.55
	Post 1	3.77	2.36	0.72	0.58
	Post 2	2.30	2.27	0.49	0.59
	p-value	0.122	0.028*		
Monocytes (absolute) (10^9/ℓ)	**Pre**	0.33	0.37	0.10	0.08
	Post 1	0.58	0.35	0.08	0.07
	Post 2	0.33	0.33	0.06	0.09
	p-value	0.610	0.751		

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

Table B-3 **Comparison of the change in biochemical pathology of well-trained male HMS students in the EX and CO groups across test intervals**

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
S-Sodium (mmol/ℓ)	**Pre**	139.15	138.82	1.52	1.99
	Post 1	138.77	139.00	1.17	2.93
	Post 2	139.80	139.57	1.40	1.51
	p-value	0.095	0.482		
S-Potassium (mmol/ℓ)	**Pre**	4.48	4.34	0.31	0.25
	Post 1	4.38	4.27	0.30	0.21
	Post 2	4.22	4.24	0.30	0.24
	p-value	0.132	0.756		
S-Urea (mmol/ℓ)	**Pre**	3.53	4.12	0.95	1.46
	Post 1	4.58	5.05	0.80	1.03
	Post 2	4.93	5.43	1.40	1.33
	p-value	0.008*	0.459		
S-Creatinine (mmol/ℓ)	**Pre**	92.31	95.91	13.12	8.35
	Post 1	94.77	109.09	13.97	10.42
	Post 2	96.70	106.14	7.75	8.91
	p-value	0.061	0.004*		
S-GGT (U/ℓ)	**Pre**	19.46	19.64	15.74	11.12
	Post 1	19.46	19.27	16.60	11.15
	Post 2	18.20	13.29	9.48	2.29
	p-value	0.798	0.887		
S-ALT (U/ℓ)	**Pre**	23.85	26.73	9.06	10.65
	Post 1	22.62	36.00	7.60	19.90
	Post 2	25.00	19.86	10.60	5.93
	p-value	0.293	0.152		
S-AST (U/ℓ)	**Pre**	26.46	28.18	5.83	10.20
	Post 1	26.77	60.18	9.26	64.51
	Post 2	24.50	23.71	6.15	3.99
	p-value	0.349	0.004*		
S-CK (U/ℓ)	**Pre**	227.00	308.09	109.17	332.38
	Post 1	253.38	1,531.09	236.65	1,802.61
	Post 2	183.80	251.86	96.32	112.99
	p-value	0.741	0.018		

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
S-CK-MB (mass) (ng/ml)	**Pre**	3.12	2.95	1.15	1.59
	Post 1	3.48	5.56	1.70	2.96
	Post 2	3.48	3.32	1.99	1.04
	p-value	0.482	0.018		
S-CK-MB (mass) index (%)	**Pre**	1.52	1.36	0.68	0.57
	Post 1	1.61	0.69	0.58	0.35
	Post 2	1.99	1.41	0.58	0.31
	p-value	0.058	0.014		

*p ≤ 0.05 : Statistically significant difference at the 5% level of significance

Table B-4 **Comparison of the change in measurement scores associated with isokinetic strength/power expressed relative to body weight of well-trained male HMS students in the EX and CO groups across test intervals**

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
Peak torque (Nm) - concentric flexors	**Pre**	148.30	172.50	24.86	15.79
	Post 1	175.05	180.57	29.34	23.10
	Post 2	187.20	215.27	23.12	34.07
	p-value	0.012*	0.819		
Total work (J) - concentric flexors	**Pre**	199.46	237.66	32.62	42.27
	Post 1	248.85	247.00	61.27	33.14
	Post 2	245.81	273.75	47.86	46.05
	p-value	0.066	0.549		
Average power (W) - concentric flexors	**Pre**	97.02	120.13	19.16	18.65
	Post 1	123.99	129.54	24.19	20.47
	Post 2	129.46	149.73	21.94	23.90
	p-value	0.018*	0.549		
Peak torque (Nm) - concentric extensors	**Pre**	287.58	337.44	41.59	34.09
	Post 1	287.93	323.49	43.14	51.39
	Post 2	288.80	350.62	53.41	63.58
	p-value	0.317	0.549		
Total work (J) - concentric extensors	**Pre**	382.47	428.41	53.65	49.66
	Post 1	389.34	400.83	66.11	57.32
	Post 2	385.99	424.40	58.81	67.89
	p-value	0.012*	0.247		

Variable	Test	Mean		Standard Deviation	
		PLA	CRE	PLA	CRE
Average power (W) - concentric extensors	**Pre**	179.96	210.51	22.98	11.33
	Post 1	194.59	213.16	29.26	33.40
	Post 2	203.59	227.15	30.98	38.09
	p-value	0.021*	0.819		

*$p \leq 0.05$: Statistically significant difference at the 5% level of significance

APPENDIX C

Terminology associated with tests for aerobic endurance capacity as used in the study under review

VO_2 max

The VO_2 max score (ml/kg/min) is determined by a discontinuous progressively greater constant work rate test (Wasserman *et al.*, 2005). It is argued (Wasserman *et al.*, 2005) that although VO_2 max tests are often considered "steady state" tests, this cannot be true at the high work rates necessary to ensure that VO_2 max is reached, and likely at any work rate accompanied by significant lactate accumulation. Taylor *et al.* (1955) argue that the VO_2 peak reached during such a test be identified as the VO_2 max if the progressively incremental constant work rate test is performed on fit participants using large work rate increments, such as a 2.5% grade change at a treadmill speed of 10 – 11 km/h. The VO_2 max can also be defined as the VO_2 when an increase in work rate results in an increase of VO_2 of less than 150 ml/min above the VO_2 from the previous work rate (Taylor *et al.*, 1955).

VO_2 peak

The highest VO_2 reached during a continuous incremental exercise test protocol (Wasserman *et al.*, 2005). The VO_2 peak score (ml/kg/min) can be used in a clinical setting to compare the cardiorespiratory function of special populations (ie. diabetics, cardiac patients, etc) to that of normal responses (Wasserman *et al.*, 2005). It is also appropriate to use the term VO_2 peak instead of VO_2 max when the work increments during a progressively incremental constant work rate test are 15 W per minute or less (Wasserman *et al.*, 2005).

CONTENTS

CHAPTER 4
SAFETY OF LONG-TERM CREATINE MONOHYDRATE SUPPLEMENTATION AND ITS EFFECTS ON ISOKINETIC STRENGTH, BODY WATER COMPARTMENTS AND MOOD IN HIGHLY ACTIVE MALE UNIVERSITY STUDENTS **162**

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Titel: VEILIGHEID EN GEVOLGE VAN KREATIENMONOHIDRAATAANVULLING

Kandidaat: Francè Rossouw

Promotor: Prof. P.E. Krüger

Graad: DPhil (MBK)

Kreatien (Kr) is ‘n stikstofbevattende verbinding wat endogeen in die lewer, niere en pankreas uit die aminosuur arginien, met byvoegings uit glisien en metionien, gesintetiseer word. Optimale vlakke van spierkreatien en fosfokreatien (PKr) kan die aanvang vertraag van vermoeidheid wat voorkom tydens spiersametrekking wat op die ATP-PCr energiestelsel steun. Kr-aanvulling word nodig geag om die vermoë van skeletspiere om energie vinnig te lewer te vergroot en sodoende maksimale oefenwerkverrigting te verbeter. Navorsers het dusver min aandag aan die uitwerking van Kr-aanvulling op aerobiese uithouvermoë geskenk. Cr-aanvulling word ook verbind met ‘n verhoogde sellulêre anaboliese toestand wat moontlik muskulêre atrofie kan voorkom wat met hoë-kilometerinoefening in die geval van ultra-afstandatlete verband hou.

Die doel van hierdie navorsing was om die ergogeniese gevolge en veiligheid van Kr-aanvulling oor die kort-, middel- en langtermyn te bepaal. Twee bevolkingsgroepe is vir hierdie doel aangewys, naamlik goed geoefende manlike ultra-afstandatlete en hoogs aktiewe manlike universiteitstudente.

Sewentien goed geoefende manlike ultra-afstandatlete is gevolglik in pare gedeel en daarna dubbelblindelings toegedeel aan òf ‘n groep wat Kr-monohidraat ingeneem het (CRE-groep; 38 ± 7.8 jr; n = 9) òf ‘n groep wat ‘n skynmiddel ingeneem het (PLA-groep; 37 ± 8.2 jr; n = 8). Deelnemers het 6 g/dag van die aanvulling vir ses opeenvolgende dae ingeneem. Na posttoets 1 (op dag 7) is die volhoudingsdosis teen 3 g/dag oor 9 weke ingeneem. Deelnemers het ‘n individueel uitgewerkte hoëintensiteit-, hoëvolume-, sportspesifieke oefenprogram gevolg.

Die CRE-groep het na aanvulling teen ‘n matige innamekoers hul hoogste aerobiese werkverrigtingpeil oorskry en dié nuwe peil tydens inname teen ‘n lae koers behou ($p = 0.006$). Teen ‘n oefenwedloopekonomie van onderskeidelik 10 km/h ($p = 0.027$) en 12 km/h ($p = 0.016$) het die CRE-groep blyke van ‘n “supernormale oefengevolg” getoon. Liggaamsgewig het geen noemwenswaardige verandering ondergaan nie. By geleentheid van posttoets 1 is bevind dat die mesomorfiese komponent van die CRE-groep heelwat vergroot het. Hierdie waarde ($p = 0.042$) het ook oor die duur van die studie onveranderd gebly, terwyl die ooreenstemmende waarde oor dieselfde tydperk in die geval van die PLA-groep afgeneem het. Uit ‘n kliniese oogpunt is geen noemenswaardige veranderings in bloedpatologie gevind nie.

Drie-en-twintig manlike MBK-tweedejaarstudente het aan die tweede studie deelgeneem. Hulle kon kies of hulle ‘n Kr-aanvullingsprogram [Eksperimentele groep (EX-groep): 20.5 ± 1.9 jr; n = 11)] wou volg of nie [Kontrolegroep (CO-groep): 20.3 ± 2.2 yrs; n = 12]. Die EX-groep het Kr-monohidraat teen 20 g/dag vir ses opeenvolgende dae ingeneem. Na posttoets 1 (dag 7) is die volhoudingsdosis teen 3 g/dag gedurende die oorblywende tydperk van 10 maande ingeneem.

Die gevolg van Kr-lading was ‘n merkbare toename in alle merkers wat op liggaamshidrasie dui. Dié waardes het oor die duur van die studie standhoudend gebly en/of het verbeter. Albei groepe het geen noemenswaardige veranderings in liggaamsgewig ondergaan nie. Deelnemers het geen newe-effekte as gevolg van Kr-inname in hul beriggewing genoem nie. Kr-lading oor ‘n kort termyn het ‘n dramatiese verhoging in S-KK tellings in die EX-groep ($p = 0.018$) tot gevolg gehad. Verder het die EX-groep ‘n soortgelyke veelseggende toename in S-Krn ($p = 0.004$) getoon. Toenames in hierdie serummerkers toon ‘n verbintenis met ‘n verhoogde Kr-omsetkoers na Kr-inname, asook met ‘n verhoogde afhanklikheid van die ATP-PKr-energiestelsel wanneer goed geoefende atlete aan hoëintensiteitoefening deelneem. Daar was geen blyke van miokardiale skade nie. Die besondere afname in die S-KK-MB-massaïndeks in die EX-groep kan op ‘n kardiobeskermende gevolg van Kr-aanvulling dui. Dramatiese toenames is gevind in serumensiemmerkers wat op spierbeskadiging as gevolg van Kr-aanvulling dui. Geen hepatosellulêre skade is opgespoor nie. Kr-lading het egter heelwat meer stremming op die lewer as nie-inname tot gevolg gehad. Dié stremming het egter nie lewerskade veroorsaak nie, en daar was ook geen blyke van nierskade nie.

Om af te sluit, bevindings dui daarop dat Kr-inname ergogenies vir goed geoefende langafstandatlete kan wees as gevolg van ‘n verhoogde vermoë om strawwe oefening en/of die aksies van anaboliese weë te verdra. Gematigde (6 g/dag) Kr-monohidraataanvulling teen ‘n lae

innamekoers (3 g/day) is veilig vir nier-, lewer-, hart- en skeletalespierintegriteit bevind. Aanvulling teen ‘n hoë innamekoers (20 g/dag) kan egter die lewer ooreis en word derhalwe nie aanbeveel nie.

Sleutelwoorde:

KREATIENMONOHIDRAAT
ERGOGENIESE UITWERKINGS/-GEVOLGE
HOOGS AKTIEF
PIEK AEROBIESE WERKVERRIGTING
OEFENWEDLOOPEKONOMIE
VEILIGHEID
SUPERNORMALE OEFENGEVOLG
ULTRA-AFSTANDATLETE
UNIVERSITEITSTUDENTE
GOED GEOEFENDE

Title: SAFETY AND EFFECT OF CREATINE MONOHYDRATE SUPPLEMENTATION

Candidate: Francè Rossouw

Promoter: Prof. P.E. Krüger

Degree: DPhil (HMS)

Creatine (Cr) is a nitrogen-containing compound endogenously synthesised in the kidneys, liver and pancreas from the amino acid arginine, with further additions from glycine and methionine. Optimal levels of muscle Cr and PCr may delay the onset of fatigue during muscle contraction that relies on the ATP-PCr energy system. Cr supplementation is considered necessary to increase skeletal muscle's capacity to generate energy quickly, thereby enhancing maximal exercise performance. Researchers have paid little attention to the effect of Cr supplementation on aerobic endurance performance. Cr supplementation is also associated with an enhanced cellular anabolic state that may potentially prevent or lessen muscle atrophy associated with high-mileage training in ultradistance runners.

The aim of this research was to investigate the ergogenic effects and safety of Cr supplementation over the short-, moderate-, and long term. For this purpose two population groups of interest were identified, namely well-trained male ultradistance runners and highly active male university students.

Seventeen well-trained male ultradistance runners were paired and then, in a double-blind fashion, assigned to either a group consuming Cr monohydrate (CRE group; 38 ± 7.8 yrs; n = 9) or a group consuming placebo (PLA group; 37 ± 8.2 yrs; n = 8). Participants ingested 6 g/day supplement for six days. After post-test 1 (on day 7), the maintenance dosage was ingested at 3 g/day for 9 weeks. Participants adhered to an individually-tailored, high-intensity, high-volume sport-specific training programme.

The CRE group significantly increased their peak aerobic work after moderate-dosage supplementation which was then maintained during low-dosage ingestion ($p = 0.006$). A *"super-training"* effect was demonstrated by the CRE group for running economy at 10 km/h ($p = 0.027$) and 12 km/h ($p = 0.016$). No significant changes in body weight were found. The mesomorphic component of the CRE group increased significantly at post-test 1 and was then maintained for the duration of the study ($p = 0.042$), while that of the PLA group decreased over time. No clinically significant alterations in blood pathology were demonstrated.

Twenty-three second-year male Human Movement Sciences (HMS) students participated in the second study. They chose whether they wanted to participate in a regimen of Cr supplementation [Experimental group (EX group): 20.5 ± 1.9 yrs; n = 11)] or not [Control group (CO group): 20.3 ± 2.2 yrs; n = 12]. The EX group ingested 20 g/day Cr monohydrate for six days. After post-test 1 (day 7), the maintenance dosage was ingested (3 g/day for the remainder of 10 months).

Cr loading resulted in an expressed increase in all markers for body hydration which was then maintained and/or improved upon for the duration of the study. Neither group demonstrated significant changes in body weight. Participants reported no side-effects to Cr ingestion. Short-term Cr loading dramatically increased S-CK scores within the EX group ($p = 0.018$). A commensurate significant elevation in S-Crn of the EX group was also demonstrated ($p = 0.004$). Elevations of these serum markers are associated with an increased Cr turnover rate after Cr ingestion, and with an increased reliance on the ATP-PCr energy system during periods of high-intensity exercise in well-trained athletes. No myocardial damage was indicated. The significant decrease in S-CK-MB mass index in the EX group may point to a cardio-protective effect of Cr supplementation. Dramatic elevations in serum enzyme markers for muscle damage in response to the Cr loading regimen were demonstrated. No hepato-cellular damage was found. However, Cr loading placed considerably more strain on the liver than non-intake did. This strain, however, did not result in liver damage. No kidney damage was found.

To conclude, findings indicate that Cr ingestion may be ergogenic for well-trained ultradistance runners due to an enhanced ability to tolerate greater training volumes and/or the actions of anabolic pathways. Moderate (6 g/day) and low-dosage (3 g/day) of Cr monohydrate supplementation proved to be safe for kidney, liver, heart and skeletal muscle integrity. However, high-dosage (20 g/day) supplementation may place strain on the liver and is therefore not recommended.

List of key words:

CREATINE MONOHYDRATE
ERGOGENIC EFFECTS
HIGHLY ACTIVE
PEAK AEROBIC WORK
RUNNING ECONOMY
SAFETY
SUPER-TRAINING EFFECT
ULTRADISTANCE RUNNERS
UNIVERSITY STUDENTS
WELL-TRAINED

LIST OF ABBREVIATIONS

AAS	-	Anabolic-androgenic steroids
ACSM	-	American College of Sports Medicine
ADP	-	Adenosine diphosphate
AGAT	-	Arginine:glycine amidinotransferase
ALT	-	Alanine amino-transferase
AMP	-	Adenosine monophosphate
AMPK	-	AMP-activated protein kinase
AR	-	Androgen receptor
ASA	-	Standards Agency of South Africa
AST	-	Aspartate amino-transferase
ATP	-	Adenosine triphosphate
~	-	Approximately
Arg	-	Arginine
bpm	-	Beats per minute
BIA	-	Bioelectrical impedance analyzer/analysis
BUN	-	Blood urea nitrogen
cGMP	-	Current Good Manufacturing Practice
CHO	-	Carbohydrates
Cl^-	-	Chlorine
CK	-	Creatine kinase
CK-BB	-	Brain-type creatine kinase isoform
CK-MB	-	Hybrid-type creatine kinase isoform
CK-MM	-	Muscle-type creatine kinase isoform
cm	-	Centimetre(s)
CnP	-	Creatinine phosphate
CO	-	Control
CO_2	-	Carbon dioxide
CPT I	-	Carnitine palmitoyltransferase I
CPA	-	Consumer Protection Act
Cr	-	Creatine
CrA	-	Kre-Alkalyn®
CrC	-	Creatine citrate

CRE	-	Creatine
Crea T	-	Creatine transporter
CrM	-	Creatine monohydrate
Crn	-	Creatinine
Cr-Pyr	-	Creatine pyruvate
d	-	Day(s)
DA	-	Dopamine
DEXA	-	Duel energy x-ray absorptiometry
DHEA	-	Dehydro-epiandrosterone
DHT	-	Di-hydrotestosterone
DNA	-	Dioxyribonucleic acid
DSHEA	-	Dietary Supplement Health and Education Act
ECF	-	Extra-cellular fluid
ECW	-	Extra-cellular water/hydration
eg.	-	For example
et al.	-	And others
etc.	-	*Et cetera*
EX	-	Experimental
FDA	-	Food and Drug Administration
FFA	-	Free fatty acid(s)
FG	-	Fast-twitch glycolytic
fitness%	-	Level of endurance fitness / percentage fitness
FOG	-	fast-twitch oxidative glycolytic
Gly	-	Glysine
g	-	Gram(s)
g/day	-	Gram(s) per day
(g/dℓ)	-	Gram(s) per decilitre
g/kg	-	Gram(s) per kilogram
g/kg/d	-	Gram(s) per kilogram per day
GASP	-	Growth and differentiation factor-associated serum protein
GAMT	-	Guanidinoacetate methyltransferase
GGA	-	Guanidinoacetate
GGT	-	Gamma glutamyl-transferase
GH	-	Growth hormone
h	-	Hour(s)

HAMC	-	Haemodialysis-associated muscle cramps
HDL	-	High-density lipoprotein
HMB	-	Beta-hydroxy beta-methylbutyrate
HMS	-	Human Movement Sciences
HOI	-	Highest Observed Intake
HR	-	Heart rate
HR_{max}	-	Maximum heart rate
HR_{OBLA}	-	Heart rate at blood lactate concentration of 4 mmol/ℓ
HR5min	-	Heart rate at 5 minutes recovery
HR10	-	Steady-state heart rate at 10 km/h
HR12	-	Steady-state heart rate at 12 km/h
HR15	-	Steady-state heart rate at 15 km/h
Hz	-	Hertz
H^+	-	Hydrogen ions
H_2O	-	Water
IAAF	-	International Association of Athletics Federations
IADSA	-	International Alliance of Dietary/Food Supplement Associations
ICF	-	Intracellular fluid
ICW	-	Intracellular water/hydration
ie.	-	*Id est*
IGF	-	Insulin-like growth factor(s)
IMP	-	Inosine monophosphate
INR	-	International normalization ratio
in vitro	-	In a test tube
in vivo	-	In the body
ISSN	-	International Society of Sports Nutrition
J	-	Joule(s)
kg	-	Kilogram(s)
kHz	-	Kilohertz
km	-	Kilometre(s)
km/h	-	Kilometre(s) per hour
kpm	-	Kilopound metre(s)
kpm/min	-	Kilopound metre per minute
ℓ	-	Litre(s)
LA5min	-	Blood lactate at 5 minutes recovery

LBW	-	Lean body weight
LDH	-	Lactate dehydrogenase
LDL	-	Low-density lipoprotein
LSD	-	Long, slow distance running
m	-	Metre(s)
MCC	-	Medical Control Council
Met	-	Methionine
mfMRI	-	Muscle-functional magnetic resonance imaging
mg	-	Milligram(s)
mg/kg	-	Milligram(s) per kilogram
mg/ℓ	-	Milligram(s) per litre
m/min	-	Metre(s) per minute
MHC	-	Myosin heavy chain
Mi	-	Mitochondrial
Mi-CK	-	Mitochondrial creatine kinase
MI	-	Myocardial infarction
min	-	Minute(s)
ml	-	Millilitre(s)
ml/kg/min	-	Millilitre(s) per kilogram per minute
mlO_2/kg/min	-	Millilitre(s) oxygen per kilogram per minute
mmol	-	Millimole(s)
mmol/kg	-	Millimole(s) per kilogram
mmol/ℓ	-	Millimole(s) per litre
MRF	-	Myogenic regulatory factors
Na^+	-	Sodium
n.a.	-	Not applicable
NC	-	No change
NCAA	-	National Collegiate Athletics Association
ng/ml	-	Nanograms per milliliter
Nm	-	Newton Metre(s)
NR	-	Not reported
O_2	-	Oxygen
OBLA	-	Onset of blood lactate accumulation
OSL	-	Highest level of supplement intake with evidence of safety
p	-	Level of significance

PCr	-	Phosphocreatine
PFK	-	Phosphofructokinase
pH	-	Level of acidity (- log [H^+])
Pi	-	Inorganic phosphate
PIC/S	-	Pharmaceutical Inspection Cooperation Scheme
PLA	-	Placebo
Post 1	-	Post-test 1
Post 2	-	Post-test 2
Pre	-	Pre-test
PT	-	Prothrombin coagulation time
RBC	-	Red blood cell
RCT	-	Randomised controlled trial
RE	-	Running economy
RE10	-	Steady-state running economy at 10 km/h
RE12	-	Steady-state running economy at 12 km/h
RE15	-	Steady-state running economy at 15 km/h
RER	-	Respiratory exchange ratio
RM	-	Repitition(s) maximum
RNA	-	Ribonucleic acid
s	-	Second(s)
S	-	Serum
SD	-	Standard deviation
SO	-	Slow-twitch oxidative
SPSS	-	Statistical Package for the Social Sciences
SSRI	-	Selective serotonin re-uptake inhibitor
std	-	Standard
T	-	Testosterone
TBW	-	Total body water
TCr	-	Total creatine
TF	-	Track and field
TnT	-	Cardiac troponin T
Trp	-	Tryptophan
U/ℓ	-	Unit(s) per litre
UL	-	Tolerable Upper Intake Level
ULS	-	Safe Tolerable Upper Intake Level

USA	-	United States of America
USSR	-	Union of Soviet Socialist Republics
US $	-	United States dollar(s)
UV	-	Ultraviolet
via	-	By
VO_2	-	Steady-state oxygen consumption
VO_2 max	-	Maximal oxygen consumption / Aerobic capacity
VO_2 peak	-	Peak oxygen consumption
W	-	Watt(s)
WADA	-	World Anti-doping Agency
WAnT	-	Wingate anaerobic test
WBC	-	White blood cell
wk	-	Week
W_{OBLA}	-	Power output at a lactate concentration of 4 mmol/ℓ blood
yrs	-	Years
µmol/ℓ	-	Micromole(s) per litre
%	-	Percentage
%/wk	-	Percentage per week
°C	-	Degree(s) Celsius
°/second	-	Degree(s) per second
1RM	-	One repetition maximum
5-HT	-	Serotonin
10^9/ℓ	-	Nanolitre / million cubic micrometres
10^12/ℓ	-	Picolitre / thousand cubic micrometres
<	-	Smaller than / Less than
≥	-	Larger than and equal to / More than and equal to
≤	-	Smaller than and equal to / Less than and equal to
±	-	Plus-minus

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