Effect of creatine monohydrate supplementation for 3 weeks on testosterone conversion to dihydrotestosterone in young rugby players

Johann van der Merwe

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Supervisor: Prof KH Myburgh
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Declaration

I, Johann Marius van der Merwe declare that:

1. The work on which this thesis is based is original and that neither the whole work nor part thereof is or will be used for any other purpose.

2. The theory investigated is original.

3. The subjects all participated without any financial incentive.

4. No third party had any interest in the study as a whole.

5. The Ethics Committee of the Sub-committee “C” of Research Administration of the University of Stellenbosch approved the study.

Johann Marius van der Merwe
Abstract

Background.

Creatine monohydrate is widely used for its purported ergogenic and anabolic properties. The mechanism by which creatine supplementation enhances muscle growth is not understood. This study was undertaken to determine whether creatine monohydrate supplementation increases the conversion rate of testosterone to dihydrotestosterone. An increase in dihydrotestosterone could partly explain the beneficial effect of creatine monohydrate on muscle hypertrophy.

Methods.

Subcommittee C of the research committee of the University of Stellenbosch approved the study. Project number 2001/C045.

The study was designed as a double blind crossover with subjects (n = 20) in each leg of the study. Group 1 (n = 10) taking creatine monohydrate and group 2 (n = 10) glucose during the first leg of the study. In accordance with crossover study design the groups were reversed in the second leg of the study.

Gelatin capsules were filled with 5g of either creatine monohydrate or 5g of glucose. Subjects taking creatine monohydrate also took 25g of glucose to improve absorption of creatine. Subjects took creatine monohydrate 25g plus 25g of glucose (ten capsules in all) or glucose ten capsules per day for seven days in the loading phase. In the maintenance phase they took 5g of creatine monohydrate plus 25g of glucose (six capsules in all) per day or six capsules of glucose, for 14 days.

The groups were reversed after a six-week washout period and the dosages repeated as per crossover study design.

Blood samples were taken on day zero of the study as baseline measurements, repeated on day 7, (after the loading phase), and again on day 21, (after the maintenance phase). These were again repeated in the second leg of the study as per crossover design. Serum was separated within one hour of collection and stored at minus 70°C.
Testosterone and dihydrotestosterone concentrations were determined using a radio-immunoassay kit by an accredited university laboratory. The percentage conversion of testosterone to dihydrotestosterone was calculated.

The results were statistically analyzed: A paired $t$-tests at the beginning of each leg of the study and repeated measure analysis of variance, for the pooled data for each condition over the whole study.

Results.

The difference in blood levels of testosterone and dihydrotestosterone on both days 0, were not statistically significant. This made the pooling of the data possible.

The difference in the percentage conversion of testosterone to dihydrotestosterone over the study period between the creatine monohydrate condition and the glucose condition, was however significant ($p < 0.0001$).

In this small study highly significant statistical results were obtained. The answer to how creatine taken as a supplement exerts its effect may lie in the increased rate of conversion of testosterone to dihydrotestosterone.

Conclusion.

With the known greater androgenic effect of dihydrotestosterone as opposed to testosterone, the increase in testosterone conversion to dihydrotestosterone could explain how creatine supplementation exerts its anabolic effect in susceptible individuals. A larger study should be done to confirm these results and answer the questions arising from the findings.
Opsomming

Agtergrond.

Die gebruik van kreatien monohidraat vir sy beweerde ergogeniese en anaboliese eienskappe kom wydverspreid voor.

Die mekanisme waardeur kreatien spier hipertrofie veroorsaak is nog nie uitgewerk nie. Hierdie studie is gedoen om te bepaal of kreatien monohidraat as supplement, ’n vermeerdering in die hoeveelheid testosteroon wat omgesakel word na dihidrotestosteroon beinvloed. Verhoging in dihidrotestosteroon vlakke mag die mekanisme van anabolisme van kreatien monohidraat verklaar.

Goedkeuring.

Die studie is goedgekeur deur die Navorsingsadministrasie van die Universiteit van Stellenbosch, se Subkomitee C, projek nommer 2001/C045.

Metode.

Die sg. dubbelblinde oorkruis studie formaat is gebruik, met dieselfde (n = 20) manlike studente in elke been van die studie. In die eerste been van die studie het groep 1 (n = 10) kreatien monohidraat geneem en groep 2 (n = 10) glukose. Na ’n ses weke uitwas periode is die groepe omgekeer soos in die studie formaat beskryf.

Gelatien kapsules is gevul met of 5g kreatien monohidraat of 5g glukose sodat die dubbelblinde deel van die studie formaat nagekom kon word. Vyg en twintig gram glukose is saam met die kreatien monohidraat gegee om die kreatien absorpsie te verbeter. Die studente het 25g kreatien monohidraat en 25g glukose (tien kapsules in totaal), of tien kapsules glukose per dag vir 7 dae geneem gedurende die ladingsfase.

Vir die volgende 14 dae het groep 1, 5g kreatien monohidraat en 25g glukose, (ses kapsules in total), geneem en groep 2, ses kapsules glukose, die instandhoudingsfase. Na ’n ses weke uitwas periode is dieselfde prosedure gevolg met groepe omgeruil.
Bloedmonsters is op dag nul as basislyn vir hormoonvlakke geneem, op dag sewe herhaal, (die hormoonvlak bepalings na die ladingsfase) en weer op dag 21 geneem (die hormoonvlak bepalings na die instandhoudingsfase).

Hierdie bepalings is herhaal in been 2, die omgekeerde deel van die ondersoek formaat.

Die serum is in alle gevalle binne een uur geskei en teen minus 70 grade Celsius gestoor.

Die testosteroon en dihidrotestosteroon vlakke is gemeet met behulp van ’n kommersieël beskikbare RIA Kit deur ’n geakrediteerde universiteits laboratorium.

Die persentasie omskakeling van testosteroon na dihidrotestosteroon is bereken. Die resultate is statisties ontleed: Gepaarde $t$-toets analise is gedoen op dag 0 van elke been van die studie tov testosteroon, dihidrotestosteroon en die persentasie omskakeling van testosteroon na dihidrotestosteroon en herhalende waarneemings analise van variansie, vir die saamgevoegde data oor die volle studie.

Resultate.

Op dag 0 was die testosteroon en dihidrotestosteroon vlakke van die twee groepe nie statisties verskillend nie. In die omgekeerde been op dag 0 was die verskil weereens nie statisties betekenisvol nie. Hierdie bevindings maak die saamvoeging van die data uit die twee bene van die studie moontlik.

Die persentasie omskakeling van testosteroon na dihidrotestosteroon in die kreatien monohidraat groep versus die plasebo groep was statisties betekenisvol ($p < 0.0001$).

Gevolgtrekking.

Die hoogs betekenisvolle resultate verkry, mag die verklaring wees vir kreatien supplementasie se werkings meganisme in vatbare persone. Dit lei egter ook tot meer vrae. ’n Groter studie behoort onderneem te word om hierdie bevindings te bevestig.
Chapter 1. Introduction.


In this literature review, I will briefly address testosterone, its biochemistry, and physiology, medical and non-medical uses. This will be followed by a more detailed discussion of testosterone’s metabolism to dihydrotestosterone and their mechanisms of action in respect of muscle hypertrophy. Creatine supplementation, its role as an ergogenic and myogenic aid, conflicting results of efficacy and mechanisms of action will be reviewed. Finally it will be argued that the inconsistent and sometimes conflicting results on creatine’s effectiveness as an ergogenic and myogenic aid, stems, in part from a lack of understanding of the mechanism of action of creatine use. This is the issue that this project will attempt to address.
Chapter 2. Literature review

2.1 Testosterone.

2.1.1 Introduction.

Testosterone was isolated as the hormone secreted by the testis in 1935 by Ernst Laquer. Testosterone is androgenic, anabolic and myogenic, as well as the precursor of dihydrotestosterone, and estradiol.

Figure 1

2.1.2 Production.

Testosterone production, 95% of which comes from the testis and 5% from the adrenal glands in males and what testosterone is secreted in females from the ovaries, is initially independent of luteinizing hormone produced in the pituitary gland. In later life the production of testosterone comes under the control of luteinizing hormone, which in turn is controlled by the hypothalamus through gonadotropin releasing hormone and inhibited by testosterone concentration via a negative feedback mechanism (Hall, 1988, Stocco, 1996).

The androgenic steroid testosterone and its metabolites, dihydrotestosterone and estradiol are major determinants of body composition in mammalian males, and to a lesser degree in females. The normal range of plasma testosterone in males is 300 to 1000 ng/dl, (Ito and Horton 1971, Bagatell and Bremner, 1996), but the average value declines by age 80 to
approximately 50% of that at age 20 (Snyder, et al., 1999). In females, the circulating testosterone concentrations are typically about 10% of those observed in men (Wilson, 1996). On average 4-6% of the testosterone is converted to dihydrotestosterone (Ito and Horton 1971, Ishimaru, et al., 1978, Santner, et al., 1998.).

The major circulating androgen is testosterone.

2.1.3 Function.


2.1.4 Deficiency.

2.1.5 Supplementation.

Testosterone replacement increases nitrogen retention in castrated males of several animal species (Kochakian, 1950), in eunechoidal men, in boys before puberty, and women (Kenyon, et al., 1940).


2.1.6 Non- Medical uses.

Humans have been trying to enhance body composition, muscle strength and efficiency for a variety of reasons, (Eichner, 1997, Evans, 2004), employing various substances, i.e. anabolic steroids, nutritional supplements and exercise to achieve this (Elashoff et al., 1991, Balsom et al., 1993, Balsom et al., 1993 Cooke et al., 1995, Hultman et al., 1996, Catlin and Murray, 1996, Redondo et al., 1996, Mujika and Padilla, 1997). Among these substances the anabolic steroids and the nutritional supplement creatine are the most widely used, although their effect and mechanism of action is still poorly understood. The historical aspects of the use of androgenic anabolic steroids have been extensively reviewed (Wilson, 1988, Bardin, 1996). The use of anabolic steroids amongst all concentrations of the community, legal or otherwise, is widespread (Buckley et al., 1988, Yesalis, 1993, Yesalis, et al., 1997, Lambert et al., 1998, Evans, 2004). Athletes use anabolic steroids in an effort to enhance athletic performance, despite decades of controversy as to its effectiveness and its possible mode of action. Even governments have been involved in a systematic program of androgenization of athletes (Franke and Berendonk 1997). Various studies to test the validity of athlete’s claims have been done, with mostly inconclusive results, due amongst others, to poor study design, and low doses of supplementation (Cowart, 1987, Wilson, 1988, Elashoff, 1991, Bhasin, 1996, Casaburi, 1996). It has now been shown that at the supra physiological doses that the athletes use, there may be some benefit, at least in the strength requiring sports (Griggs, et al., 1989, Martin, 1992, Urban, 1995, Bardin, 1996, Bhasin, 1996, Casaburi, 1996, Katznelson, 1996, Tincello, 1997, Snyder, 1999, Tenover, 2000, Bhasin, et al., 2001).
Although dihydrotestosterone is a more efficient androgen than testosterone, (Ishimaru, et al., 1978, Deslypere, et al., 1992, Zhou, et al., 1995, Santner, et al., 1998,), no study could be found that looked at possible mechanisms for enhancing the conversion of testosterone to dihydrotestosterone. This, in my view, is a shortcoming in the field.

2.1.7 Metabolism.

Testosterone undergoes metabolism to both bioactive and inactivated metabolites. Around 4% - 6% (Ishimaru, et al., 1978, Santner, et al., 1998), of circulating testosterone is metabolized in target tissue by the enzyme 5 alpha reductase to the more potent pure androgen dihydrotestosterone and via a diversification pathway by the enzyme aromatase to estradiol, capable of activating estrogen receptors (Ito and Horton 1971). Testosterone is irreversibly metabolized by the intracellular NADPH dependent enzyme 5 α reductase to dihydrotestosterone, and aromatize to estradiol.

**Figure 2**

![Diagram of 5α reductase pathway]

2.2 Dihydrotestosterone.

2.2.1 Introduction.

Dihydrotestosterone, the most potent naturally occurring androgen, is formed by the enzyme 5 alpha reductase from testosterone in males and from the very weak androgen androstenedione in females (Ito and Horton 1971). Congenital absence of 5 alpha reductase leads to the development of pseudo hermaphrodites if untreated with exogenous testosterone (Imperato-McGinley et al., 1979, Wilson, et al., 1993, Imperato-McGinley and Zhu 2002).
2.2.2 Physiology.

This conversion of testosterone to dihydrotestosterone is essential for the initiation of the differentiation and development of the urogenital sinus into the prostate, the male external genitalia and some of the secondary male sexual characteristics (Quigley, et al., 1995). The enzyme 5 alpha reductase originates from two distinct genes (Normington and Russel, 1992), as two isozymes, 5 alpha reductase types 1 and 2 exist. 5 alpha reductase type 1, located in the SRD5A1 gene, on chromosome 5 at band 5p15, is expressed in liver, kidney, skin, and brain. 5 Alpha reductase type 2, located in the SRD5A2 gene, on chromosome 2 at band p23 is characteristically expressed strongly in the prostate but also at lower concentrations in skin, liver, and muscle (Thigpen, et al., 1992, Russell and Wilson 1994). The 5 alpha-reductase type 1 and 2, catalyzing the conversion of testosterone to 5 alpha-dihydrotestosterone, differs in its amino acid composition, kinetics, biochemical properties, substrate specificity, tissue distribution and pH optimum. Type 1 being more efficient at pH 6-8.5, and type 2, at pH 4-5. The binding affinity of dihydrotestosterone for the androgen receptor is 2-5 times that of testosterone, has a 3-10 fold greater molar potency than testosterone and is a more efficient transactivator of the androgen receptor (Ishimaru, et al., 1978, Deslypere et al., 1992, Zhou, et al., 1995, Zhu et al., 1998, Santner, et al., 1998).
2.2.3 Function.

If a way could be found to increase the conversion of testosterone to dihydrotestosterone, this greater efficiency and molar potency could be beneficial in muscular development. As the myogenic mechanism of action of the supplement creatine monohydrate is still unclear, is this not one of the mechanisms involved in this process?

2.2.4 Mechanism of action.

The mechanism by which testosterone regulates body composition is poorly understood. At present it is thought that testosterone increases muscle mass, at least in part, by stimulating protein synthesis (Kenyon, et al., 1940, Brodsky, et al., 1996, Urban, et al., 1995, Ferrando, et al., 2002). Testosterone administration increases nitrogen retention in boys before puberty, in eunuchoidal men, and in women (Kenyon, et al., 1940) as well as in castrated male rats (Kochakian, 1950), and stimulates protein synthesis in young hypogonadal men (Brodsky, et al., 1996), and older men with low testosterone concentrations (Urban, et al., 1995, Ferrando, et al., 2002). However this hypothesis does not explain the decrease in fat mass, or the increase in myonuclear and satellite cell numbers associated with testosterone administration (Hawke and Garry, 2001).

All steroid hormones exert their effect via receptor binding, the androgen receptor for testosterone and dihydrotestosterone and the estrogen receptor for estradiol and estrogen. The human androgen receptor gene localizes to the X chromosome at Xq11–12 and is encoded in 8 exons (Brown, et al., 1989, Quigley et al., 1995, Heinlein and Chang, 2002, Liu et al., 2004).

Androgen receptor expression can be up regulated by exercise (Bamman, et al., 2001, 2003), and by supra-physiological doses of testosterone (Bhasin, et al., 2001).

The hypertrophy of skeletal muscle induced by exercise can be suppressed by androgen receptor antagonists (Inoue, et al., 1994), suggesting that exercise in itself is not responsible for muscle hypertrophy.
Testosterone’s effect on muscle and fat mass are related to its blood concentrations making it an attractive substance to use for increasing muscle mass and strength, more so at supra physiological doses (Bhasin, et al., 1996, Bhasin, et al., 2001).

Testosterone administration is associated with hypertrophy of both type I and II muscle fibers (Sinha-Hikim, et al., 2002), and significant increases in myonuclear and satellite cell numbers (Sinha-Hikim, et al., 2003).

During postnatal development and in muscle hypertrophy, growth and regeneration is dependent on the addition of myonuclei to muscle fibers. Because the nuclei in the muscle fibers are post- mitotic, new myonuclei must be contributed by satellite cells. An increase in satellite cell number is an antecedent of an increase in myonuclear number and muscle fiber hypertrophy (Schultz, 1989, Mitchell and Pavlath, 2001, Hawke and Garry, 2001).

Testosterone supplementation increases satellite cell number in the levator ani muscle of rats (Nnodim, 2001), and in skeletal muscle of men (Sinha-Hikim, et al., 2003). During muscle regeneration or hypertrophy, uncommitted, pluripotent stem cells of mesodermal origin within the muscle, serve as reservoirs for the generation of new satellite cells or myoblasts (Grounds, et al., 2002). These cells also serve as reservoirs of adipocytes in muscle and adipose deposits throughout the body (Jankowski, et al., 2002). Pluripotent, mesenchymal C3H 10T1/2 (10T1/2) cells capable of differentiating into muscle, fat, cartilage, and bone cells are widely used as a model for studying the regulation of myogenic and adipogenic lineage determination (Taylor, et al., 1979, Fischer, et al., 2002). In a study by Singh et al., 2003, it was found that both testosterone and dihydrotestosterone were effective in stimulating myogenisis and inhibiting adipogenesis. In this study, it was shown that this effect is mediated through an androgen receptor mediated pathway, and that dihydrotestosterone was more potent than testosterone in this model. These effects were observed at physiological concentrations of testosterone and dihydrotestosterone, and were associated with greater stimulation of myogenisis and greater inhibition of adipogenesis at supra-physiological doses of testosterone and dihydrotestosterone.

These observations are not surprising given the fact that up regulation of receptors, in this case, the androgen receptor is possible (Bamman, et al., 2001), and the known greater affinity of dihydrotestosterone for the androgen receptor, as well as its greater molar efficacy at the

Dihydrotestosterone is more anabolic in target tissue, has a greater affinity for the androgen receptor, and has a greater molar potency at the androgen receptor than testosterone. No research could be found into factors that could possibly increase the conversion rate of testosterone to dihydrotestosterone. (Ishimaru, et al., 1978, Deslypere, et al., 1992, Zhou, et al., 1995, Santner, et al., 1998, Heinlein and Chang, 2002). Could this be the mechanism of action in people who respond to creatine supplementation?


2.3 Creatine.

2.3.1 Introduction.

Creatine, discovered in 1832, by the French scientist Michel-Eugene Chevreul is a natural amino acid compound found in meat and fish, and synthesized in the human body. Creatine is normally obtained from dietary sources that provide approximately 1-2 g of creatine per day. In addition, another 1-2 g per day is synthesized from amino acids, arginine, glycine, and methionine in the liver, kidney, and pancreas (Walker, 1979, Wyss and Kaddura-Daouk, 2000). Supplementation of creatine, a $200 million per year industry (Schnirring, 1998), has been shown to reduce endogenous production in humans, however, normal rates return upon termination of supplementation (Walker, 1979). Creatine phosphate is an important reservoir of high-energy phosphate groups in muscle. Chemically it is methyl guanidine-acetic acid:

\[
\text{NH}_2 - \text{C (NH)} - \text{NCH}_2 (\text{COOH}) - \text{CH}_3
\]

Creatine and the phosphorylated form, phosphocreatine is distributed throughout the body, with 95% stored in skeletal muscle and 5% in the brain, liver, kidney and testes (Walker, 1979). Creatine phosphate and free creatine exist in a reversible equilibrium in skeletal
muscle. In the body, there is little creatine found at the site of production, and therefore creatine must be transported from areas of synthesis to areas of storage and utilization. Creatine uptake into muscles occurs via a sodium / chloride dependent transporter, (CreaT) against a concentration gradient, regulated by the intracellular concentration of creatine (Willmot et al., 1999). Creatine uptake is muscle fiber-type dependent. Type 2 fibers have higher concentrations of creatine and phosphocreatine (Casey et al., 1996, Casey and Greenhaff, 2000).

In humans, intramuscular concentrations of creatine have been found to be 110-160 mmol kg$^{-1}$ dry muscle with ~60% of total creatine in the form of phosphocreatine (Harris et al., 1992, Balsom et al., 1995, Casey et al., 1996, Hultman et al., 1996, Guerrero-Ontiveros and Wallimann, 1998). Since creatine is only produced in certain organs but utilized in others, it must enter the blood to reach other tissues such as skeletal muscle. The cellular uptake of creatine by organs is critical due to the potential down-regulation of these systems with chronic exposure to creatine (Guerrero-Ontiveros and Wallimann, 1998). It has however been shown that exercise can stimulate the muscle uptake and content of creatine (Harris et al., 1992, Volek, et al., 1999). In other studies, both insulin and carbohydrate increased total creatine accumulation in both humans and rodents (Green et al., 1996). It is possible that exercise may increase the translocation of the creatine transporter to the muscle membrane similarly to the effects of exercise on GLUT-4 translocation (Volek, et al., 1999). Creatine phosphate serves as a phosphate donor to either AMP or ADP, reconstituting ADP and ATP respectively.

mitochondrial cytopathies, neuropathic disorders, dystrophies, congenital myopathies and inflammatory myopathies. In all the diseases studied there seems to be some benefit to creatine monohydrate supplementation.

Presently, creatine is a legal substance under both Amateur Athletic Association and the International Olympic Committee regulations, but banned in France.

2.3.2 Effectiveness.


Creatine exerts various effects upon entering the muscle. It is these effects that elicit improvements in exercise performance and may be responsible for the improvements of muscle function and energy metabolism seen under certain disease conditions.

Several mechanisms have been proposed to explain the increased exercise performance seen after acute and chronic creatine intake.

2.3.3 Energy Metabolism.

Adenosine tri-phosphate (ATP) concentrations maintain physiological processes and protect tissue from hypoxia-induced damage. Creatine is involved in ATP production through its involvement in phosphocreatine energy system. This system can serve as a temporal and spatial energy buffer as well as a pH buffer. As a spatial energy buffer, creatine and phosphocreatine are involved in the shuttling of ATP from the inner mitochondria into the cytosol (Earnest et al., 1995, Casey et al., 1996, Vandenberghe et al., 1997, Volek et al., 1997). In the reversible reaction catalyzed by creatine kinase, creatine and ATP form phosphocreatine and adenosine diphosphate (ADP). It is this reaction that can serve as both a temporal energy
buffer and pH buffer. This energy and pH buffer is one mechanism by which creatine works to increase exercise performance.

2.3.4 Protein Synthesis.

One beneficial effect of creatine supplementation in young, healthy males is enhanced muscle fiber size and increased lean body mass. Typically, creatine loading of 20 g/day for 4 to 28 days in humans increases total body mass from 1 to 2 kg (Balsom, et al., 1993, Greenhaff, et al., 1994, Earnest, et al., 1995, Green, et al., 1996, Vandenbergehe, et al., 1999, Kreider, et al., 2004) with increases coming from fat-free mass (Vandenbergehe, et al., 1997, Volek, et al., 1999, Becque, et al., 2000). Volek, et al., (1999) found after 12 weeks of resistance training in men, creatine supplementation increased muscle fiber diameter in both Type 1 and Type 2 muscle fibers by 35%. Resistance-trained subjects not supplemented with creatine had fiber-type increases of 6 to 15%. Subjects both trained and supplemented had fat-free mass increases of 1.5 kg after 1 week and 4.3 kg after 12 weeks compared with the trained-only group that had a fat-free mass increase of 2.1 kg after 12 weeks. In patients with gyrate atrophy, a 42% increase in Type 2 muscle fibers were found after 1 year of supplementation with 1.5 g/day of creatine monohydrate without resistance training (Sipila, et al., 1981).

The increases in muscle mass may result from increased protein synthesis or reduced protein catabolism. Studies using cell culture by Ingwall and colleagues (Ingwall, 1975), they support the theory that exogenous creatine can increase protein synthesis both in vitro and in vivo. It was hypothesized by the authors that creatine, an end product of contraction, may serve as a stimulus of protein synthesis and muscle hypertrophy. They found the rate of myosin and actin synthesis in chick embryo myoblasts increased in the presence of creatine, but the degradation rate of the muscle proteins remained unchanged. However, using a similar model to Ingwall, Fry and Morales (1980) did not find an effect of creatine on protein synthesis in cell culture. Recently, Tarnopolsky's group (Parise et al., 2001) found no increase in protein synthesis, but a possible decrease in protein catabolism. The results from cell culture and the human study offer conflicting results as far as the role of creatine and regulation of protein metabolism. Is it not because the cell cultures had no androgens to complete the combination of factors, needed for protein synthesis? I find this a surprising omission in the study. The regulation of protein metabolism by an osmotic agent like creatine is supported by studies investigating the effect of cell swelling on protein synthesis. When creatine accumulates in cells, water drag occurs.
and increases cell hydration. Hyper-hydration can act as an anabolic signal stimulating protein synthesis (Haussinger, et al., 1994) or the hypo-osmolality can act as a protein-sparing signal and reduce protein degradation (Berneis, et al., 1999). This theory of creatine-induced hydration affecting protein synthesis is still under debate because it has not been directly investigated.

Another mechanism by which creatine may increase muscle mass is by involvement in satellite cell activity (Dangott, et al., 2000). Dangott and colleagues examined the effect of creatine on compensatory hypertrophy in the rodent. There was no difference between supplemented and non-supplemented groups with regard to muscle mass and fiber diameter for muscles that underwent compensatory hypertrophy. However, the combination of creatine and increased functional loading did increase satellite cell mitotic activity.

2.3.5 Membrane Stabilization.

Creatine can potentially prevent tissue damage by two possible mechanisms. The first mechanism involves stabilization of cellular membranes and the second involves maintenance of ATP. Creatine, more specifically phosphocreatine, may stabilize membranes due to the zwitterion nature of phosphocreatine with negatively charged phosphate and positively charged guanidino groups. Phosphocreatine binds to the phospholipid head groups and thus decreases membrane fluidity and decreases loss of cytoplasmic contents such as intracellular enzymes creatine kinase. Sharov, et al., (1987) administered phosphocreatine to attenuate ischemic damage to cardiomyocytes of rabbit. They found that phosphocreatine decreased the elevation in inulin diffusible space seen in untreated cardiomyocytes indicating maintenance of membrane integrity and reduced necrotic zone size. Recently studies have examined whether creatine supplementation would reduce exercise-induced muscle damage. No difference was found in the indirect indicators of muscle damage in a double-blind placebo study in males between the creatine supplement groups and non supplemented control (Rawson, et al., 2001). However, oxidative damage markers were not measured, and it may be possible that creatine attenuated oxidative stress by maintaining mitochondrial energy homeostasis.

The second mechanism of protection relates to ATP production. In cases of transient ischemia, the ability to generate ATP through oxidative pathways is reduced resulting in cell
Since creatine supplementation increases phosphocreatine, there is a higher reserve of ATP, thus providing the energy until eupoxic conditions are re-established (Balestrino, 1999). It has now been fairly well established that creatine monohydrate supplementation is beneficial to athletes engaging in anaerobic activities, (Casey, 1996, Demant and Rhodes, 1999, Graham and Hatton, 1999, Becque, 2000, Benzi, 2000), but not of benefit during aerobic activities (Kamber, 1999, Becque, 2000). However, creatine may be of benefit in aerobic activities requiring intermittent bouts or short bursts of high-intensity anaerobic activity (Mujika, et al., 1996, Thompson, et al., 1996 Mujika and Padilla, 1997).

Creatine has also been shown to buffer lactic-acid build-up, thus possibly delaying fatigue associated with increased lactic acid production during aerobic activities (Eichner, 1997). Some questions arise regarding creatine’s effectiveness in highly trained athletes where it is theorized that muscle creatine stores may already be at full capacity (Mujika, et al., 1996, Mujika and Padilla, 1997).

There is evidence that creatine may enhance muscle size giving rise to its popularity among body builders. This was shown in research involving subjects with neuromuscular disease and may not carry over to benefit athletes (Tarnopolsky and Martin, 1999). Studies have now been published showing some anabolic benefit from taking creatine monohydrate supplementation combined with resistance training (Willoughby, et al., 2001).

From the above discussion, it seems logical to do a study to determine whether creatine monohydrate supplementation exerts its effect by increasing the conversion of testosterone to dihydrotestosterone in responsive subjects.

**Chapter 3: Aims of the study.**

The double blind placebo controlled cross over study was done to determine whether creatine monohydrate used as a supplement, changes the conversion rate of testosterone to dihydrotestosterone in young men.
Chapter 4: Methods and Materials.

4.1 Subjects and ethical approval.

Twenty four male students, aged between 18.17 and 19.83 years (average 18.99 years), attending the South African Rugby Institute at Stellenbosch University were recruited for this study. All the subjects were actively participating in rugby and gave written informed consent for the study. See Appendix 1. The study was approved by the University of Stellenbosch ethics “C” committee. See Appendix 2. None of the subjects had taken any supplements to their normal diet for a period of six weeks prior to the onset of the study. All subjects continued with their normal training schedules. The subjects were randomized into two groups of twelve. Four subjects, two from each group withdrew during the study. One student left the University after one week of the study; one was hospitalized due to rugby injury during the maintenance phase of leg one and two with drew because of a fear of needles at the onset of the study.

4.2 Materials.

Clear gelatin capsules were filled with commercially available creatine monohydrate or glucose powder, the placebo, 5 grams of either per capsule, resulting in identical capsules enabling double blind cross over study. Creatine monohydrate evaluated as the active ingredient with glucose acting as the placebo. Glucose was given with both creatine monohydrate and glucose to improve absorption of creatine (Green, et al., 1996). The daily allocation of capsules was ingested between 07h00 and 08h00.

For dosages see 4.3 paragraph 3 page 23.

4.3 Study design.

The study was designed as a double blind placebo controlled cross over study, with two groups of 10 subjects, along the following time line.

[Day 0 loading period. Day 7 Maintenance period. Day 21]
Wash out 6 weeks.

Cross over.

[Day 0 loading period. Day 7 Maintenance period. Day 21.]

Blood samples and anthropometric measurements were taken on day 0, 7, 21 in each leg of the study. Anthropometrical data consisted of 7 skinfold measurements (triceps, biceps, sub scapular, supra iliac, abdominal, thigh and mid calf), as well as height and weight. The measurements were taken by two experienced biokineticists, using a Harpenden caliper, and the average recorded. Although 7 skinfolds were measured, only six were used in the anthropometrical calculations (Heyward V, 2001). Two blood samples were taken from the anti-cubital fossa using a standard gel Vacutainer™ test tube, allowed to clot, and placed on ice. The serum was then separated by centrifuging at 3000 rpm with the temperature set at 4°C before freezing at minus 70°C. All samples and measurements were made and collected between 16h00 and 17h00 at each point, and the serum separated and frozen within one hour of collection.

In the first leg of the study, group one took 25g (five identical capsules) of creatine monohydrate with 25g (five identical capsules) of glucose as loading dose for 7 days, the active ingredient. This was followed by 5 grams (one capsule) of creatine monohydrate and 25grams (five identical capsules) of glucose for 14 days, the maintenance phase (Casey, 2000, Francaux, 2000, Hultman, 1996). Group two received ten identical capsules of glucose daily for 7 days as a loading dose, the placebo. Six identical capsules of glucose followed this for 14 days, the maintenance phase.

After completion of this part of the study the subjects took no supplements for six weeks, the recommended washout period for creatine, (Ziegenfuss, et al., 1998), but continued with their training schedules.

In accordance with the study design the groups were then reversed. Group one now taking placebo and group two, creatine monohydrate, in identical fashion to the first leg of the study.
4.4 Calculations and blood analysis.

4.4.1 Anthropometrical calculations.

Sum 6 = triceps + sub-scapular + supra-spinale + abdominal + front thigh + medial calf. (Heyward V, 2001).

Body density (BD) = 1.10326 – 0.00031(age) – 0.00036(sum 6).

Estimated % Body fat = (495/BD) – 450. (Heyward V, 2001).

Fat free mass = weight – estimated % body fat in kg.

4.4.2 Blood sample analysis.

Testosterone and dihydrotestosterone estimations were done using RIA kits (DSL 4000 and DSL 9600 kits, Diagnostic Systems Laboratories, Inc. Webster, Texas, USA).

The procedure follows the basic principle of immunoassay where there is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of [I-125]-labeled Testosterone/ dihydrotestosterone bound to the antibody is inversely proportional to the concentration of the Testosterone/ dihydrotestosterone present. Decanting or aspirating the antibody-coated tubes easily and rapidly achieves the separation of free and bound antigen.

4.4.2.1. Testosterone.

The DSL testosterone immunoassay uses a sensitive and specific rabbit anti-human testosterone antibody. Although cross-reactivity occurs with dihydrotestosterone and a small number of androgen metabolites, the relative concentrations of these compounds in normal human samples predict that they will have a minimal effect on assay results (Testosterone RIA DSL – 4000, 2003).

4.4.2.2. Dihydrotestosterone.

Measurement of dihydrotestosterone concentrations can be complicated by antibody cross-reactivity to testosterone. The DSL Dihydrotestosterone Radio immunoassay utilizes a sample
oxidation/extraction procedure to remove most of the testosterone, coupled with a relatively specific immunoassay for dihydrotestosterone (Dihydrotestosterone RIA DSL 9600, 2003).

### 4.5 Statistical analysis.

The subjects randomized into two groups at baseline were compared using unpaired $t$ test.

The data collected over the two legs of the study were pooled and analyzed comparing the two conditions over time using repeated measure analysis of variance with confidence levels set at $p < 0.001$. Differences at each time interval were analyzed *post hoc* by means of Tukey test with the limit for significance set at $p < 0.01$.

### Chapter 5. Results.

Twenty-four Caucasian males were recruited from the SA Rugby Institute at Stellenbosch University, of whom four, two from each group, withdrew from the study.

Subject characteristics of Group 1 and 2. Subjects fitted into a very narrow age range (between 18 and 19 years of age) and groups therefore did not differ. Height and body mass varied substantially between subjects. In the group that took creatine monohydrate first, the range was 74.4 to 104.0 kg, whereas in the group taking glucose first it ranged from 75.8 to 107 kg. The subjects were randomized into their groups and the mean body mass did not differ between the two groups (85.3 ± 8.6 kg and 87.9 ± 11.7 kg). Similarly, baseline testosterone did not differ between the two groups of randomly divided subjects (13.2 ± 2.6 and 15.9 ± 3.8 nMol/L; $p = 0.16$), and neither did dihydrotestosterone (0.91 ± 0.08 and 1.11 ± 0.33 nMol/L; $p = 0.23$). In a cross over designed study the groups should not differ at the end of the washout period, which is also the baseline for the second leg or cross over part of the study. In this study the group taking glucose second differed from the group taking creatine second in some respects. Body mass did not differ and neither did the dihydrotestosterone or the % of testosterone converted to dihydrotestosterone ($p = 0.58$ and $p = 0.41$ and $p = 0.19$, respectively). However, with respect to total testosterone concentrations the difference was $p = 0.052$. Therefore, for 3 out of the 4 highly relevant variables, the two groups did differ significantly after wash-out and for the 4th variable the difference was marginal and not within the range of significance that we considered important.
Therefore, the two groups were pooled for all other analyses. This resulted in a comparison of 20 subjects in terms of their responses to taking creatine monohydrate for 21 days (Table 1a) vs. their responses taking glucose for 21 days (Table 1b). Mean and Sd for age was 18.7 ± 0.58 yr and height was 1.81 ± 0.05 m, and these variables were not considered again at the following time points.

Data for the main variables of body mass, testosterone and dihydrotestosterone are presented in Table 1.

**Table 1. Body mass and hormone profiles**

<table>
<thead>
<tr>
<th></th>
<th>Creatine n=20</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 21</td>
<td></td>
</tr>
<tr>
<td>Body mass: kg</td>
<td>87.04 (10.66)</td>
<td>87.83 (10.88)</td>
<td>87.81 (10.82)</td>
<td></td>
</tr>
<tr>
<td>Testosterone: nMol/L</td>
<td>14.44 (2.95)</td>
<td>16.08 (2.86)</td>
<td>16.69 (4.61)</td>
<td></td>
</tr>
<tr>
<td>Dihydrotestosterone: nMol/L</td>
<td>0.98 (0.37)</td>
<td>1.53 (0.50)</td>
<td>1.38 (0.45)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Glucose n=20</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 21</td>
<td></td>
</tr>
<tr>
<td>Body mass: kg</td>
<td>86.84 (9.89)</td>
<td>87.31 (10.11)</td>
<td>87.26 (9.96)</td>
<td></td>
</tr>
<tr>
<td>Testosterone: nMol/L</td>
<td>17.09 (3.42)</td>
<td>17.02 (4.11)</td>
<td>17.04 (5.25)</td>
<td></td>
</tr>
<tr>
<td>Dihydrotestosterone: nMol/L</td>
<td>1.26 (0.52)</td>
<td>1.09 (0.40)</td>
<td>1.06 (0.43)</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as Mean (Sd). Sd = Standard deviation. T = Testosterone. DHT = Dihydrotestosterone.

The variable most influenced by the supplementation with creatine monohydrate was the % of conversion of testosterone to dihydrotestosterone (see Figure 4 for visual display of the interaction effect between groups and time). From these results it is clear that the change in conversion rate of testosterone to dihydrotestosterone (expressed as a percentage of available testosterone) was highly significant (p < 0.0001) in the creatine monohydrate supplemented group. More details of where the groups differed over time are presented in Table 2 with the post hoc analyses.
Figure 4. Change in conversion rate of testosterone to dihydrotestosterone in the creatine monohydrate supplemented group.

% Conversion Testosterone to Dihydrotestosterone

\[ p < 0.0001 \]

Individual data for the change in testosterone, dihydrotestosterone and percentage conversion are presented in the appendix. (Table A1-6 and Figures A1-6).
Table 2. Percentage conversion of testosterone to dihydrotestosterone on days 0, 7 and 21 of the study in both conditions (n=20 subjects in each condition).

<table>
<thead>
<tr>
<th>% Conversion: T to DHT</th>
<th>Creatine</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>6.42</td>
<td>6.84</td>
</tr>
<tr>
<td>Sd</td>
<td>2.11</td>
<td>2.23</td>
</tr>
<tr>
<td>Post hoc Tukey</td>
<td>p = 0.405</td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.71</td>
<td>6.12</td>
</tr>
<tr>
<td>Sd</td>
<td>2.54</td>
<td>1.86</td>
</tr>
<tr>
<td>Post hoc Tukey</td>
<td>p &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Day 21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.85</td>
<td>5.94</td>
</tr>
<tr>
<td>Sd</td>
<td>2.67</td>
<td>2.04</td>
</tr>
<tr>
<td>Post hoc Tukey</td>
<td>p &lt; 0.009</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA: p < 0.0001; T = Testosterone. DHT = Dihydrotestosterone.

I also determined body composition in all subjects at all time points. All subjects were available for all measurements. Skinfolds were taken at six different sites and 3 variables were calculated namely: body density, % body fat and fat free mass.
Table 3. Anthropometry

<table>
<thead>
<tr>
<th>Creatine n=20</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of 6 skinfolds</td>
<td>272 ± 54</td>
<td>269 ± 49</td>
<td>269 ± 43</td>
</tr>
<tr>
<td>Body density</td>
<td>1.068 ± 0.009</td>
<td>1.068 ± 0.009</td>
<td>1.068 ± 0.009</td>
</tr>
<tr>
<td>% body fat</td>
<td>13.43 ± 3.92</td>
<td>13.36 ± 4.02</td>
<td>13.30 ± 3.95</td>
</tr>
<tr>
<td>Fat free mass</td>
<td>75 ± 6.7</td>
<td>75.8 ± 6.8</td>
<td>75.8 ± 6.5</td>
</tr>
<tr>
<td>Glucose n=20</td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 21</td>
</tr>
<tr>
<td>Sum of 6 skinfolds</td>
<td>274 ± 58</td>
<td>274 ± 55</td>
<td>272 ± 55.8</td>
</tr>
<tr>
<td>Body density</td>
<td>1.068 ± 0.009</td>
<td>1.068 ± 0.009</td>
<td>1.068 ± 0.009</td>
</tr>
<tr>
<td>% body fat</td>
<td>13.53 ± 3.95</td>
<td>13.54 ± 3.94</td>
<td>13.47 ± 3.97</td>
</tr>
<tr>
<td>Fat free mass</td>
<td>75 ± 5.7</td>
<td>75. ± 5.8</td>
<td>75 ± 5.8</td>
</tr>
</tbody>
</table>

Data presented as Mean (Sd). Sd = Standard deviation.

Chapter 6. Discussion.

The main aim of this study was to determine whether creatine monohydrate supplementation has an effect on the conversion rate of testosterone to dihydrotestosterone. A secondary aim was to determine if body composition changed during the 21 days of creatine supplementation in young rugby players actively training.

Although anthropometrical data were collected and analyzed these were not statistically significant. Various reasons could be put forward for this e.g. training programmes were not standardized, subjects continued with their normal rugby training with no specific strength training included by the researcher, the supplementation period was too short, and the subject’s diets were not controlled. One of the most convincing studies for the anabolic effect of creatine supplementation in skeletal muscle is that of Volek (1999). Their study lasted 12
weeks and subjects who were already resistance trained were subjected to heavy periodized training during that time.

However, the current study was designed to answer the first aim, was well controlled in respect of this main objective. The possible effect of creatine monohydrate supplementation on testosterone to dihydrotestosterone conversion was investigated in a placebo controlled manner in groups well-matched at baseline and after cross-over for this specific variable. From the results obtained in this study a highly significant (p < 0.0001) increase in the percentage of testosterone converted to dihydrotestosterone was found in the creatine monohydrate supplemented group. Even allowing for the higher concentration of testosterone in the group taking placebo second, this higher concentration did not result in increased conversion of testosterone to dihydrotestosterone without taking creatine. It does however suggest that the generally accepted 6 week washout period for creatine is not enough.

I would suggest that this fact further strengthens the argument and aim of this study that creatine supplementation increases the conversion rate of testosterone to dihydrotestosterone.

It is known that dihydrotestosterone is a more efficient androgen than testosterone. The binding affinity of dihydrotestosterone for the androgen receptor is 2-5 times that of testosterone, has a 3-10 fold greater molar potency than testosterone and is therefore a more efficient transactivator of the androgen receptor (Ito T and Horton 1971). However, it is not the concentration of dihydrotestosterone alone that is important, but rather the ability to convert testosterone to the more efficient dihydrotestosterone.

Pluripotent stem cells can be stimulated, via androgen receptor activation, to develop into lipogenic or myogenic cell lines, thus leading to lipogenic or myogenic stem cells (Singh et al. 2003). The development of fat or muscle tissue is dependent on these cells developing into mature cells. Increasing the number of myogenic stem cells as opposed to the number of fat cells will lead to an increase in fat free mass and muscle hypertrophy. The resident stem cells of skeletal muscle are the satellite cells (Dangott et al., 2000). It has been shown that the combination of creatine and increased loading exercise increases satellite cell mitotic activity in rats. However, in this study no measurements were made of possible hormonal mechanisms influencing this result. It is also known that testosterone will increase the proliferation of satellite cells in human subjects (Sinha-Hikim et al., 2002, 2003).
This greater stimulatory effect of dihydrotestosterone, as compared to testosterone, especially on pluripotent stem cells, although in vitro could be the cellular basis for muscle hypertrophy seen in creatine monohydrate supplementation. It is possible that even greater gains in androgenic effects can be obtained in susceptible individuals if creatine supplementation is used in conjunction with strength training. Strength training per se up regulates androgen receptors (Bamman et al., 2001, Inoue et al., 1994). Creatine monohydrate increases the conversion rate of testosterone to dihydrotestosterone, even without strength training.

The mechanism for creatine’s effect on testosterone conversion to dihydrotestosterone is likely to be through an effect on the activity of type 2, 5 alpha reductase. This could either be by stimulating increased expression or modifying activity.

A decrease in pH increases the efficacy of type 2, 5 alpha reductase. Increased androgen receptors, increased enzyme efficacy, more dihydrotestosterone, could this combination be the basis for the androgenic effect of creatine monohydrate supplementation especially in combination with high intensity resistance exercise?

During the study no subjects reported any undesirable side effect of creatine supplementation.

Chapter 7. Conclusion.

Although this was a small study (n = 20), it simulated actual use of creatine monohydrate as a supplement by the majority of athletes using creatine supplementation. A larger study should be undertaken to further investigate the mechanisms behind the results obtained. Should the findings be confirmed and elucidated serious medical and ethical questions will be raised:

1. Is the long-term effect of creatine supplementation safe, or could it be implicated in an increase in prostate hypertrophy/cancer in men?

2. Is it in the spirit of sport if by supplementation you find a way to increase your naturally produced steroid hormone concentrations?

3. Is the increase in dihydrotestosterone by creatine supplementation “steroid abuse” by the athletes?
4. Is it an unfair advantage to some individuals, as only about 2/3 of creatine users show a beneficial effect from creatine supplementation?

5. Are the tests currently used for the detection of steroid abuse by athletes sensitive enough to allow for increased concentrations of dihydrotestosterone in the urine? Is it possible that false positive “anabolic steroid use” urine tests can result from this increased dihydrotestosterone concentrations in the urine?

This study may have found some answers, but may also have resulted in many more questions needing answers.
References:


