

The effect of magnesium supplementation on high and low dietary magnesium intake on resting, during and recovering from exercise on blood pressure, performance and serum levels of magnesium ( $Mg^{2+}$ ).

By

Luke William Pitkin

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## Abstract

### **Abstract**

**Objective:** The objectives of this study were to determine if there is a significant effect on blood pressure, serum levels of magnesium and sports performance including 1RM and 10 kilometre running time trial following Magnesium ( $Mg^{2+}$ ) supplementation over a five week intervention period in recreationally active athletes when compared to a 5 week placebo intervention.

**Methods:** Subjects participated in a 14 week protocol which employed a randomized blind cross over controlled design. Fifteen subjects were initially screened and accepted for participation. During the protocol two subjects dropped out, failing to complete. This left nine male and four female subjects who were successful in completing the protocol (Age:  $24.85 \pm 6.49$  years, Height:  $175\text{cm} \pm 10.34$  cm, Weight:  $71.9$  Kg  $\pm 11.46$  Kg).

**Results:** Statistically significant differences were observed ( $P < 0.05$ ) in the running time trial, blood pressure readings pre and post 10 kilometre running trial (systolic and diastolic) and in heart rate (HR) recorded at 10 minute intervals during the running trial following  $Mg^{2+}$  supplementation. Following the  $Mg^{2+}$  intervention there was an average decrease in 10K completion time of exactly 1 minute (1.77%) ( $P=0.001$ ). A significant decrease in HR by 2.58 BPM (2.58%) ( $P=0.03$ ). Diastolic blood pressure was significantly reduced both pre and post completion of the 10 kilometre run by 10.23 mmHg (13.85%) ( $P=0.03$ ) and 5.38 mmHg (7.38%) ( $P=0.008$ ) respectively. Whilst a significant reduction in systolic blood pressure was only seen following the 10 kilometre run 8.23 mmHg (6.17%) ( $P=0.05$ ).

As  $Mg^{2+}$  is a co-factor in over 325 enzymatic reactions (Newhouse & Finstad, 2000), it's importance as a mineral is clear and warrants research to improve scientific knowledge into its role within health and sports performance, as  $Mg^{2+}$  deficiency can also be detrimental to health.

**Conclusion:** The results of the current study show that 500mg/day of  $Mg^{2+}$  supplementation will significantly decrease time taken to complete a 10 kilometre run, reduce systolic and diastolic blood pressure and significantly reduce HR.

**Keywords:** Magnesium ( $Mg^{2+}$ ); Blood Pressure; Hear Rate; Serum Magnesium.

## Introduction

Achieving optimal functioning and performance is highly sought after by many individuals. It is common knowledge in the current day that nutrition is a key factor in achieving this. An optimal diet must contain adequate amounts of macro and micro nutrients, aiming to fulfil the body's daily requirements (Gibney, Macdonald & Roche, 2003).

Previous research into Magnesium ( $Mg^{2+}$ ) supplementations impact on sporting performance has been conflicting. Some studies have reported that  $Mg^{2+}$  may have a positive effect on sporting performance (Lukaski, Bolonchuk, Klevay, Milne & Sandstead, 1983; Brilla & Haley, 1992; Brilla & Gunter, 1995) whilst conflicting literature suggests that  $Mg^{2+}$  supplementation will have no effect or a negative effect on performance (DeHann et al., 1985; Weight et al., 1988; Ruddell et al., 1990; Terblanche et al., 1992; & Weller et al., 1998.). The goal of the current paper was to determine the effects of a 5 week 500mg  $Mg^{2+}$  supplementation intervention on subject's performance in a 10 kilometre run, bench press till fatigue trial, blood pressure and levels of  $Mg^{2+}$  found in blood serum compared to a placebo intervention period.

### Hypothesis

Alternative Hypothesis:  $Mg^{2+}$  supplementation will significantly reduce time taken to complete a 10 kilometre running time trial.

Null Hypothesis:  $Mg^{2+}$  supplementation will have no impact on time taken to complete running time trial.

## Literature Review

Varying results have been reported around  $Mg^{2+}$  supplementation and the impact that it has on blood pressure, blood serum levels and athletic performance. This review will look to analyse the previous literature surrounding  $Mg^{2+}$  supplementation and its subsequent impact on blood pressure, blood serum levels and athletic performance.

### Magnesium within the body

Mineral elements, such as magnesium ( $Mg^{2+}$ ), are required by the human body in modest amounts for the maintenance of health and the development of optimal functioning (Lukaski, 1995).  $Mg^{2+}$  is an important mineral element and it is the fourth most abundant cation in living organisms resulting in  $Mg^{2+}$  being a cofactor to over 325 enzymatic reactions occurring within the body (Newhouse & Finstad, 2000). This suggests that deficiency of the mineral may have various physiological and exercise performance implications (Newhouse & Finstad, 2000; Wolf & Cittadini, 2003).

Elin (2010) stated that about 99% of total body  $Mg^{2+}$  is located in the bone, muscles and non-muscular soft tissue (table 1). Fifty to sixty percent of the stored  $Mg^{2+}$  is located in the bone, whilst the remaining is located in soft tissues and skeletal muscle (Aikawa, 1980). The  $Mg^{2+}$  content of the bones has been said to decrease with age, resulting in less bioavailability when the body's stores of  $Mg^{2+}$  are depleted (Maguire & Cowan, 2002). However, this will not prevent the bone from exchanging  $Mg^{2+}$  as a buffer when serum  $Mg^{2+}$  levels begin to deplete, as one third of skeletal  $Mg^{2+}$  may be exchanged to maintain physiological extracellular  $Mg^{2+}$  levels (Alfrey & Miller, 1973).

Approximately 1% of the total body  $Mg^{2+}$  is present in the serum and intestinal bodily fluids (Elin, 1987). The mean serum  $Mg^{2+}$  concentration in humans is roughly 0.85 mmol/L, with a referenced interval of 0.7-1.mmol/L (Wacker, 1980). In serum, approximately one third of  $Mg^{2+}$  is bound to protein, 25% is bound to albumin and 8% to globulins (Kroll, 1985). For the two thirds of the plasma  $Mg^{2+}$  that is ultrafiltrable, approximately 80% is in the form of free ion and approximately 20% is complexed to phosphate, citrate and other compounds (Elin, 1987).

Table 1. Distribution of  $Mg^{2+}$  in the adult human being (Elin, R. J. 1988).

Tissue	Body weight (kg wet weight)	Concentration (mmol/kg wet weight)	Content (mmol)	% of total body magnesium
Serum	3.0	0.85	2.6	0.3
Red blood cells	2.0	2.5	5.0	0.5
Soft tissue	22.7	8.5	193.0	19.3
Muscle	30.0	9.0	270.0	27.0
Bone	12.3	43.2	530.1	52.9
Total	70.0	64.05	1000.7	100.0

Within serum and red blood cells  $Mg^{2+}$  is said to act as a counter ion for adenosine triphosphate (ATP) molecules (Jahnen-Dechent & Ketteler, 2012) Wherever there is ATP there is a need for  $Mg^{2+}$  in the biological process (Aikawa, 1980). Protein synthesis requires the involvement of ribonucleic acid (RNA) and the genetic mechanism involving deoxyribonucleic acid (DNA) also have a need for  $Mg^{2+}$  (Aikawa, 1980). Cunningham,

Rodríguez, & Messa (2012) also suggested that  $Mg^{2+}$  contributes to the regulation of heart rhythm, bone formation, vascular tone and platelet activated thrombosis.

$Mg^{2+}$  and calcium compete with one another for the same binding sites on plasma protein molecules (Walser, 1967; Hunter, Haworth & Southard 1976). It was reported that  $Mg^{2+}$  antagonises calcium dependant release of acetylcholine at motor endplates; this may suggest that  $Mg^{2+}$  is a natural calcium antagonist (Wacker, 1980). Calcium is seen as a powerful 'death trigger' (Orrenius, Zhivotovsky, & Nicotera 2003). The role of  $Ca^{2+}$  as a death trigger was first suggested by Fleckenstein et al., (1977) who proposed that the influx of excess  $Ca^{2+}$  into the myocytes may be the mechanism underlying the cardiac pathology that occurs after ischemia. Later studies have emphasised the general importance of this observation, as both receptor overstimulation (Leonard, & Salpeter, 1979) and cytotoxic agents (Schanne, Kane, Young, & Farber 1979; Trump, & Berezsky, 1995) were found to cause an excessive influx of  $Ca^{2+}$  into cells (Orrenius, Zhivotovsky, & Nicotera 2003).

Recommendations for the suggested daily intake of  $Mg^{2+}$  differ in the literature. Seelig (1964) concluded that at least 6 mg/kg per day were required for males and females to maintain an adequate balance of  $Mg^{2+}$ . Furthermore in 1980, the food and nutrition board of the National Academy of Sciences and the National Research Council recommended a daily intake of 300mg and 350mg for adult females and males respectively (National Academy of Sciences, 1980). If you were to assume a mass of 70kg for males and 60kg for females this suggested intake would equate to 5 mg/kg per day. The  $Mg^{2+}$  intake for the average individual is very closely correlated with the total number of calories consumed (Flink, 1980), this is assuming that a large percentage of the calories are not derived from alcohol or refined sugars, which essentially have no mineral content. Nuts, green vegetables, soy beans, chocolate and whole grain cereals are foods all of which are rich in  $Mg^{2+}$  content (Elin, 1987). In addition to this drinking water, especially if it's "hard" water may be a major source of  $Mg^{2+}$ .  $Mg^{2+}$  content is reduced during the water filtration process, resulting in "soft" water. This reduction in  $Mg^{2+}$  content may be a contributing factor in the increasing numbers of people suffering from hypomagnesemia in the population (Elin, 1987).

To maintain an adequate  $Mg^{2+}$  status it is said that humans must consume  $Mg^{2+}$  at regular intervals (Jahnen-Dechent & Ketteler, 2012). The daily allowance for  $Mg^{2+}$  is controversial as the literature is conflicting and varied, although values of  $\geq 300$  mg are usually reported with adjustment dosages for age, sex and nutritional status (Jahnen-Dechent & Ketteler, 2012). The Committee On Medical Aspects (COMA) has calculated a RNI of 300mg/day for adult males and 270mg/day for adult females (COMA, 1991). The RNI for infants and children ranges from 55 to 280 mg/day. An RNI for  $Mg^{2+}$  during pregnancy was not

calculated by COMA. The suggested RNI for adult males and females is supported by other literature, which suggests a similar consumption of 350mg for males and 280-300mg for females respectively (Weast, 1987; Elin, 1988; Rude, 1996; Saris, Mervaala & Karppanen, 2000).  $Mg^{2+}$  can also be acquired through drinking water, it is said that around 10% of daily  $Mg^{2+}$  intake will come from drinking water (Marx & Neutra, 1997). Chlorophyll is the main source of  $Mg^{2+}$  therefore suggesting that green vegetables would contain a high  $Mg^{2+}$  content. Furthermore, nuts, unprocessed cereals and seeds will also contain high levels of  $Mg^{2+}$  (Fox, Ramsomair & Carter, 2001). Low  $Mg^{2+}$  levels are found in most available dairy products (Jahnen-Dechent & Ketteler, 2012). The literature suggests that when foods are processed a large percentage of  $Mg^{2+}$  is lost (Elin, 1988), this may be the cause of the decrease in consumption of  $Mg^{2+}$  seen in the Western society (Ford & Mokdad, 2003).

It is suggested that  $Mg^{2+}$  actually inhibits calcium induced cell death (Reynolds, Joannides, Skepper, McNair, Schurgers, Proudfoot, & Shanahan 2004). Apoptosis is a genetically controlled and evolutionary conserved form of cell death that is of importance for normal embryonic development and for maintenance of tissue homeostasis in the adult organism (Orrenius, Zhivotovsky, & Nicotera 2003). Apoptotic cell death is triggered by extrinsic receptor-mediated, or intrinsic mitochondria mediated signalling pathways that may induce death-associated proteolytic activities. Apoptosis occurs in an organised sequence of events (Kerr, Wyllie, & Currie, 1972). The cell primarily undergoes nuclear and cytoplasmic condensation with blebbing of the plasma membrane. It then breaks up into fragments, known as apoptotic bodies, which are recognised and engulfed rapidly by neighbouring cells or macrophages. The intracellular ATP concentration and the status of mitochondrial function are crucial factors that influence the mode of death (Eguchi, Shimizu, & Tsujimoto, 1997; Leist, Single, Castoldi, Kühnle, & Nicotera, 1997). A link that has been made between  $Ca^{2+}$  and apoptosis was the finding that  $Ca^{2+}$  induced a typical apoptotic ladder-like DNA fragmentation pattern in isolated thymocyte nuclei through the activation of  $Ca^{2+}$  and  $Mg^{2+}$  dependant endonuclease (Wyllie, 1980; Cohen, & Duke 1984). Subsequent dissection of the mechanism of glucocorticoid-induced apoptosis in thymocytes showed that extracellular  $Ca^{2+}$  was necessary for cell death to occur. For all these reasons it can be assumed that  $Mg^{2+}$  deficiency can result in poor health and living.

When the human body is in a state of hypomagnesaemia it will aim to utilise some of the stores from the various organs and bones. There is said to be a considerable variation in the plasma/tissue exchange of  $Mg^{2+}$  in various organs (Maguire & Cowan, 2002). This observation indicates that each cell type will handle  $Mg^{2+}$  differently, again different to  $Ca^{2+}$  (Saris, Mervaala, Karppanen, Khawaja, & Lewenstam, 2000). Fat tissue, brain tissue, skeletal muscle, kidney parenchyma, myocardium, and lymphocytes all exchange

intracellular and extracellular  $Mg^{2+}$  at different rates. However, the equilibrium for  $Mg^{2+}$  among most tissue compartments is achieved at a much slower rate (Elin, 2010). It has been reported that  $Mg^{2+}$  exchanges extremely slowly or is non-exchangeable, furthermore one third of  $Mg^{2+}$  stored in the bones is transferrable with serum during a state of hypomagnesaemia (Jahnen-Dechent & Ketteler, 2012). Alfrey & Miller (1973) reported  $Mg^{2+}$  exchange to be varied with maximum bone uptake occurring within the first five minutes. Exchange of  $Mg^{2+}$  was virtually complete by four hours, with very little additional bone uptake occurring between 4 and 48 hours (Alfrey and Miller, 1973).

### Effects of high $Mg^{2+}$ consumption

High  $Mg^{2+}$  consumption through food has not been linked with any adverse health effects and has not been shown to pose any harm (Scientific Committee on Food, 2001).  $Mg^{2+}$  supplementation may be used by those failing to attain significant amounts through the diet and is considered to be safe. McDonald and Keen (1988) have claimed that excessive  $Mg^{2+}$  consumption will not cause any serious issues, although Al-Ghamdi et al (1994) showed that anything over 500 mg/day of  $Mg^{2+}$  supplementation may cause gastrointestinal difficulties.

### $Mg^{2+}$ absorption and excretion

$Mg^{2+}$  homeostasis is maintained by the intestine, kidneys and bones (Jahnen-Dechent & Ketteler, 2012).  $Mg^{2+}$  is absorbed in the intestine and stored in the bone. Any excess  $Mg^{2+}$  that has been consumed will be excreted by the kidneys, then removed from the body in urine. Absorption of  $Mg^{2+}$  mainly takes place in the small intestine (Graham, Caesar & Burgen, 1960; Fox, 2001; Touyz, 2004), although  $Mg^{2+}$  is also absorbed in the large intestine. Both the duodenum and jejunum have a high fractional absorption of  $Mg^{2+}$ . These segments of intestine are relatively short however, and the transit time is rapid. Therefore their relative contribution to total  $Mg^{2+}$  absorption is less than that of the ileum. Two  $Mg^{2+}$  absorbing pathways have been identified in the mammalian intestine. Para-cellular transport is a passive mechanism which involves absorbing  $Mg^{2+}$  through small spaces between the epithelial cells. The second system involves the active transport of  $Mg^{2+}$  to the blood via the interior of the epithelial cell. During transport  $Mg^{2+}$  must pass through two cell membranes (Baaij, Hoenderop & Bindels 2012). Para cellular  $Mg^{2+}$  absorption is responsible for roughly 80-90% of intestinal  $Mg^{2+}$  uptake.

It has been suggested that the ileum and distal parts of the jejunum are known to be the most permeable for ions because of the relatively low expression of tightening claudins 1, 3, 4, 5 and 8 (Amasheh, Fromm, & Günzel, 2011). As such paracellular  $Mg^{2+}$  transport seems mainly restricted to these areas. Claudins 16 and 19, linked with  $Mg^{2+}$  permeability (Hou, et

al., 2009), are not expressed in the small intestine (Amasheh, Fromm & Gunzel, 2011). Therefore, the exact mechanism involved in the facilitation of  $Mg^{2+}$  absorption remains unknown.

The average dietary intake of  $Mg^{2+}$  is roughly 300 mg/day, all derived primarily from green vegetables, cereal grains and meat. An individual consuming this diet absorbs around 40% of the  $Mg^{2+}$  consumed, most of which is absorbed primarily in the small intestine (Elin, 1987). The absorption process is said to commence roughly 1 hour after consumption and continues at a uniform rate for 2-8 hours. After 12 hours of ingestion the material will normally be in the large bowel, which absorbs very little  $Mg^{2+}$ . The absorption of  $Mg^{2+}$  in the small intestine is inversely related to consumption levels. When a diet low in  $Mg^{2+}$  is consumed up to 75% of that ingested  $Mg^{2+}$  may be absorbed, whilst when consuming a diet rich in  $Mg^{2+}$  as little as 25% may be absorbed (Elin, 1987).

The major excretory pathway for absorbed  $Mg^{2+}$  is via the kidneys (Elin, 1987). The renal excretion of  $Mg^{2+}$  is said to lie between 120 to 140 mg/24 h, for a person consuming a diet adequate in  $Mg^{2+}$  (Wacker, 1980; Aikawa, 1981). Therefore, the amount of  $Mg^{2+}$  that is absorbed in the small intestine is similar to the amounts excreted by the kidneys. This process allows one to maintain  $Mg^{2+}$  balance (Elin, 1987). The kidney is also the major organ that is involved in the concentrations of  $Mg^{2+}$  in the serum. The excretion rate of  $Mg^{2+}$  from the kidney can range from 10 to 500 mg/24 hour, depending on the  $Mg^{2+}$  levels in the plasma.

It has been estimated that 70% to 80% of the plasma  $Mg^{2+}$  is filtered through the glomerular membrane; in a person consuming an adequate diet, the  $Mg^{2+}$  which is bound to the protein does not pass through the glomerular membrane (Elin, 1987). Roughly 20% to 30% of the filtered  $Mg^{2+}$  is absorbed along the proximal tubule (Quamme, 1986). The primary site for the absorption of  $Mg^{2+}$  is the thick ascending limb of the loop of Henle, where more than 50% of the filtered  $Mg^{2+}$  is reabsorbed (Quamme, 1986). The distal tubules and collecting ducts therefore absorb next to no  $Mg^{2+}$ .

The excretion of  $Mg^{2+}$  follows a circadian rhythm, with most  $Mg^{2+}$  being excreted in the evening/night (Fox, Ramsoomair, & Carter, 2001). Under normal physiological conditions approximately 2400mg of  $Mg^{2+}$  located in blood plasma is filtered by the glomeruli in 24 hours. Of this filtered  $Mg^{2+}$ , 95% is re-absorbed instantaneously, leaving 3-5% which is excreted in urine (Massry & Seelig, 1977; Saris, Mervaala & Karppanen, 2000). However, the kidneys can manipulate the  $Mg^{2+}$  excretion by increasing or lowering the amount removed from the serum. It is believed that the excretion and reabsorption rates can fluctuate immensely from 0.5% to 70% respectively. This means that the kidneys are able to

conserve  $Mg^{2+}$  when the body is falling into a state of hypomagnesemia; by reducing the excretion amount in urine. On the other hand, the kidneys can also increase excretion when the body is in a state of hypermagnesemia (Jahnen-Dechent & Ketteler, 2012).

### Assessing $Mg^{2+}$ status

Assessing  $Mg^{2+}$  within the body is a complex task; the most common method of assessing  $Mg^{2+}$  status is via evaluation of serum  $Mg^{2+}$  concentrations (Huijgen, Sanders & Van Olden, 1998; Touyz, 2004). Although serum  $Mg^{2+}$  concentration is not comparable with  $Mg^{2+}$  stored within tissues, therefore, it is not an accurate account of overall body  $Mg^{2+}$  (Elin, 2010; Spiegel, 2011). Fawcett, Haxby & Male (1999) reported that only 1% of bodily  $Mg^{2+}$  is located in extracellular fluids, furthermore only 0.3% of bodily  $Mg^{2+}$  was found in the serum, where it is present in three altered states, ionised (62%), protein bound (33%) and phosphate (5%) with most  $Mg^{2+}$  being stored in bone (53%), intracellular compartments of muscle (27%) and soft tissues (19%). Fawcett, Haxby & Male (1999) concluded therefore, that serum  $Mg^{2+}$  is a poor indicator of  $Mg^{2+}$  status and can only be used as a predictor or an indicator of  $Mg^{2+}$ , not total body  $Mg^{2+}$  content. Red blood cell and muscle  $Mg^{2+}$  concentrations have also been reviewed, although the relationship between these and total body  $Mg^{2+}$  is still unresolved (Fawcett, Haxby & Male 1999).

A second approach to assess  $Mg^{2+}$  status is via urinary  $Mg^{2+}$  excretion. Duley & Johanson (1994) suggested an estimation of normal renal  $Mg^{2+}$  in 24 hours. This estimated 24 hour renal excretion was a urinary loss of 3.6 mmol for females and 4.8 mmol for males. A further refinement is the Retention of  $Mg^{2+}$  Test. After an initial 24 hour baseline urine sample is attained, a  $Mg^{2+}$  load is provided to the patient. Following supplementation another urine sample is collected 24 hours later, if excretion of  $Mg^{2+}$  is 60-70% or greater this would suggest that  $Mg^{2+}$  depletion is unlikely. This test aims to quantify the exchangeable pool of  $Mg^{2+}$  which is commonly located in bone (Ryan, 1991). This approach to assessing  $Mg^{2+}$  status will not be adopted in the current trial due to the added time commitment it will place on the subjects.

### Hypomagnesemia

As accurate clinical tests of  $Mg^{2+}$  status are still lacking, it is hard to diagnose  $Mg^{2+}$  deficiency (Jahnen-Dechent & Ketteler, 2012). Evaluation of serum  $Mg^{2+}$  concentration and collection of urine samples are the most accurate assessments available at this present time, for the diagnosis of  $Mg^{2+}$  deficiency. Following this a  $Mg^{2+}$  retention test could also be performed (Elin, 1988). Previous literature has concluded serum urine of  $\leq 0.75$  mmol/L as deficient in  $Mg^{2+}$  (Chernow, Bamberger & Stoiko, 1983; Whang & Ryder, 1990; Mori, 1990).

Mg<sup>2+</sup> deficiency is highly common in hospitalised patients with a prevalence of 65%. This figure has been reported to increase in intensive care units (Rude, 1993). Prolonged Mg<sup>2+</sup> deficiency may result in poor health conditions such as cirrhosis, cerebrovascular disease and malignant tumours (Chernow, Bamberger & Stoiko, 1989). Mg<sup>2+</sup> also plays a role in muscle contraction. This suggests that Mg<sup>2+</sup> deficiency may lead to muscle cramping, ultimately resulting in a reduction of muscle performance (Touyz, 2003).

Bergeron (2003) claims that deficiency in a variety of minerals, mainly Mg<sup>2+</sup>, calcium and potassium can cause muscle cramps during exercise (Benda, 1989; Williamson, Johnson, Hudkins & Strate, 1993). Any athlete competing in excessive temperature conditions may subsequently become deficient in one or more of the discussed minerals could certainly experience muscle cramps or various other neuromotor issues (Bergeron, 2003). This may suggest that calcium; Mg<sup>2+</sup> and potassium supplementation may be warranted to aid prevention of muscular cramping. Alongside deficiency in essential minerals insufficient conditioning and fatigue may contribute to muscle cramping in an over worked muscle (Bentley, 1996; Schwellnus, Derman & Noakes, 1997). This cramping is often localised and can be resolved relatively quickly by stretching, massaging or icing. With heat cramps the affected areas become more widespread across many voluntary muscles, this usually inhibits performance. Stretching, massaging and icing are interventions that can be used to reduce heat cramps making them much less persistent (Bergeron, 2003).

As an individual becomes progressively dehydrated the extracellular fluid compartment becomes increasingly contracted (Nadel, Mack & Nose, 1993). A loss of interstitial fluid may result in a deformation of the nerve endings thus increasing the surrounding ionic and neurotransmitter concentrations (Bergeron, 2003). This can cause selected motor nerve terminals to become hyperexcitable and spontaneously begin to discharge (Jansen, Joosten & Vingerhoets, 1990; Layzer, 1994). An athlete's first indication of heat cramps usually begins with twitches in the voluntary muscles that are almost undetectable. These twitches are often more evident when an athlete is stationary, for example when a tennis player is sitting down during change over. If an athlete can identify the early twitching stages and deal with them accordingly, a full blown heat cramp which would occur 20-30 minutes following the initial onset can be prevented (Bergeron, 2003).

The onset of Mg<sup>2+</sup> deficiency can be initiated in many different ways. The most common onset is by reduced intake, usually caused by poor nutrition, resulting in decreased absorption (see figure 1). Deficiency may also be triggered by increased excretion of Mg<sup>2+</sup> in some medical conditions such as diabetes mellitus (Jahnen-Dechent & Ketteler, 2012).

Chronic  $Mg^{2+}$  deficiency is hard to diagnose this is because an individual may carry a sufficient level of  $Mg^{2+}$  in the serum, whilst this may have been sourced from  $Mg^{2+}$  stores located in the bone, ultimately depleting those stores resulting in total body  $Mg^{2+}$  depletion. It is near impossible to assess which tissue pools of the body are in equilibrium for  $Mg^{2+}$ . If the level of  $Mg^{2+}$  in the serum is attained, it may not provide any further information about the concentration of  $Mg^{2+}$  in other bodily tissues (Elin, 1987).

Three separate studies have shown no correlation amongst serum, erythrocyte and mononuclear blood cell (MBC) concentrations of  $Mg^{2+}$  in humans (Elin, 1982; Elin, 1985; Millart, 1985). Therefore, data on  $Mg^{2+}$  concentration for a particular tissue will be limited to that tissue. Serum is undoubtedly the most frequently used tissue for determining  $Mg^{2+}$  status (Arnaud, 2008). However, the concentration of  $Mg^{2+}$  in serum has not been shown to correlate with any other tissue pools of  $Mg^{2+}$  except from interstitial fluid. For assessing acute changes in  $Mg^{2+}$  status the assessment of the  $Mg^{2+}$  concentration found in the serum is valid. This investigation will aim to assess  $Mg^{2+}$  status by using the serum attained during collecting venepuncture samples.

Some literature suggests that healthy individuals will have a  $Mg^{2+}$  serum concentration that is closely maintained within the physiological range (Walser, 1967; Rude, 1996; Fox, Ramsomair & Carter 2001). This referenced range is 0.65-1.05 mmol/L for total  $Mg^{2+}$  concentrations in adult blood serum (Tietz, 1990) and 0.55-0.75 mmol/L for ionised  $Mg^{2+}$  (Maj-Zurawska, 1994). Arnaud, (2008) reported, serum  $Mg^{2+}$  levels <0.75 mmol/L as a useful measurement for deficiency. Different populations and methodologies for the determination of  $Mg^{2+}$  probably contribute to the width of this range (Wacker, 1980). This range will be used in this investigation and blood serum levels below 0.75 mmol/L will be classified as deficient.

Chronic  $Mg^{2+}$  deficiency has also been linked to hypertension, malignant tumours, kidney stones, and atherosclerosis.

### Hypermagnesemia

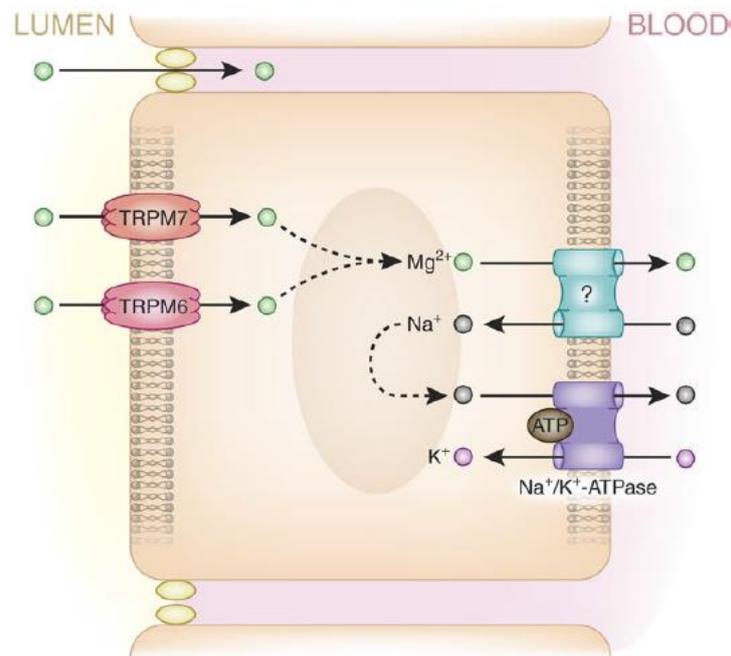
As previously discussed the kidneys play a vital role in the regulation of  $Mg^{2+}$  status within the body. In a state of chronic kidney disease the compensatory mechanisms may be affected, resulting in the development of hypermagnesemia (Cunningham, Rodriguez, & Messa 2012). Hypermagnesemia may also develop with excessive consumption of oral  $Mg^{2+}$  or drugs containing  $Mg^{2+}$  such as laxatives (Xing & Soffer, 2001). Prevalence rates of

hypermagnesemia are much lower than hypomagnesemia with only 9.3% of hospitalised individuals suffering with hypermagnesemia (Wong, Rude & Singer, 1983). These reported figures are disputed as Hashizume & Mori, (1990) reported a prevalence of only 7.9% within hospitalised individuals and Whang & Whang, (1990) reported an even lower prevalence of only 5.7% in hospitalised individuals. In intensive care the prevalence of hypermagnesemia increased, with reports indicating a prevalence rate of 13.5% (Escuela, Guerra & Anon, 2005).

### Signs of hypomagnesemia and hypermagnesemia

Clinical signs and symptoms of hypomagnesemia and hypermagnesemia are highly similar and often not specific. Signs of hypomagnesemia may include symptoms such as; muscle fasciculation, tremor, agitation, depression and hypokalaemia (Wacker, 1980; Hashizume & Mori, 1990; Saris et al., 2000). In the early stages of hypomagnesemia an individual may feel an overall sense of weakness alongside vomiting and tremors (Hashizume & Mori, 1990). If hypomagnesemia is prolonged then the symptoms will become worse. Examples of the symptoms include; muscle cramps, sudden changes in behaviour caused by excessive electrical activity, numbness, tingling and personality changes (Hashizume & Mori, 1990). In extreme cases of hypomagnesemia an individual may experience heart irregularities.

Hypermagnesemia may stay undetected for a long time; this is due to the lack of clinical symptoms and no drop in the serum concentrations due to the body's ability to maintain homeostasis by the quantitative influx and efflux of  $Mg^{2+}$  across intestine, bone and kidney. (Hashizume & Mori, 1990). If  $Mg^{2+}$  intake reaches an extreme an individual may suffer neuromuscular dysfunction, this can vary from drowsiness to respiratory depression.



**Fig. 1. A schematic overview of  $Mg^{2+}$  absorption pathways in the intestine, showing proteins associated with  $Mg^{2+}$  transport in enterocytes. In the intestinal epithelia, paracellular  $Mg^{2+}$  transport occurs via unidentified claudins, occurring concurrently with transcellular  $Mg^{2+}$  transport via transient receptor potential channel melastatin member 6 (TRPM6) and TRPM7 to facilitate  $Mg^{2+}$  absorption. (Baaij, Hoenderop & René 2012).**

In otherwise healthy individuals,  $Mg^{2+}$  supplementation is usually successful in treating individuals suffering mild hypomagnesemia (Guerrero-Romero et al., 2004). Fuentes, Salmon & Silver (2006); Mathers & Beckstrand (2009) have reported that both acute and chronic  $Mg^{2+}$  supplementation is tolerated and safe for individuals suffering from hypomagnesemia. Discontinuation of  $Mg^{2+}$  supplementation is suggested for treating mild cases of hypermagnesemia. In extreme cases of hypermagnesemia haemodialysis may be required.

Too much  $Mg^{2+}$  consumed in the diet through food ingestion does not pose a health risk in healthy individuals because the kidneys are able to eliminate excess amounts via urine (Muso, 2009). However, high doses of  $Mg^{2+}$  from dietary supplements or medication can often result in diarrhoea that may be accompanied by abdominal cramping (Ross et al., 2011). Forms of  $Mg^{2+}$  most commonly reported to induce laxative effects include  $Mg^{2+}$  sulphate carbonate, chloride gluconate and oxide (Ranade & Somberg, 2001). The laxative effects of  $Mg^{2+}$  salts are reportedly due to osmotic activity of unabsorbed salts in the intestine and colon and the stimulation of gastric motility.

Extreme dosages of  $Mg^{2+}$  containing laxatives and antacids typically provided more than 5,000 mg/day of  $Mg^{2+}$  and have been associated with  $Mg^{2+}$  toxicity (Kutsal et al., 2007), including fatal hypermagnesemia in a 28 month old boy (McGuire, Kulkarni & Baden, 2000) and elderly man (Onishi & Yoshino 2006). Symptoms of  $Mg^{2+}$  toxicity usually start to develop after serum concentrations reach an excess of 1.74 – 2.61 mmol/L. common symptoms can include hypotension, nausea, vomiting, facial flushing, retention of urine, depression and lethargy before progressing to muscle weakness, breathing difficulties, irregular heartbeat and cardiac arrest (Musso, 2009). The risk of  $Mg^{2+}$  toxicity increases with impaired renal functioning or kidney failure because the ability to remove excess  $Mg^{2+}$  is reduced or completely lost (Musso, 2009).

Previous literature has reported that ingestion of  $Mg^{2+}$  may affect the body in different ways encouraging different responses. One of the most commonly researched effects of  $Mg^{2+}$  ingestion is the possibility of a reduction in blood pressure (Jee, et al., 2002). A reduction in blood pressure can be a highly beneficial adaptation for the body as the vasodilation effect may help to improve performance in cardiovascular activities and improve the health of the population suffering from hypertension.

### Blood pressure

Blood pressure (BP) is a measure of the pressure that is exerted by the blood against the wall of the vessels, most commonly the arteries. During one cardiac cycle the blood pressure will vary between a maximum (systolic) and a minimum (diastolic). The systolic and diastolic blood pressures will both be caused by the contraction and relaxation of the heart; during each cardiac cycle (Caro, Pedley, Schroter, & Seed; 1978). The systolic blood pressure in normotensive individuals has been said to average around 120 mm Hg during the systole phase of the cardiac rhythm. During the hearts relaxation phase (diastole) the blood pressure will lower, producing the diastolic blood pressure reading. The diastolic blood pressure in normotensive individuals has been said to average at around 60 - 80 mmHg.

For over three decades the National Heart, Lung, and Blood Institute (NHLBI) has administered the National High Blood Education Programme (NHBPEP) coordinating committee (Chobanian, Bakris, Black, Cushman, Green, Izzo & Roccella 2003). The committee consists of a coalition of 39 major professional, public and voluntary organisations. One important function of the committee is to issue guidelines and advisories to increase awareness, prevention, treatment and control of hypertension (Chobanian, Bakris, Black, Cushman, Green, Izzo & Roccella 2003).

The guidelines which can be seen in Table 2 provide the classification of blood pressure for adults aged 18 years and older. The classification should be based on the average of two or more properly measured, seated blood pressure readings on each or at least two visits. These classification guidelines were attained from the European Society of Hypertension and are the classification guidelines referred to in the United Kingdom.

Table 2. Definitions and classification of blood pressure for adults (European Society of Hypertension, 2013).

Category	Systolic		Diastolic
Optimal	<120	and	<80
Normal	120–129	and/or	80–84
High normal	130–139	and/or	85–89
Grade 1 hypertension	140–159	and/or	90–99
Grade 2 hypertension	160–179	and/or	100–109
Grade 3 hypertension	≥180	and/or	≥110
Isolated systolic hypertension	≥140	and	<90

Pesola, Pesola, Lin, Nelson, & Westfal (2000) conducted a study, which supports the normotensive blood pressure values of 120mmHg over 80mmHg. The trial recorded bilateral indirect blood pressures from 100 subjects with no history of hypertension. All blood pressures were taken in a seated position by two observers, one of which recorded the systolic and diastolic blood pressures announced by the fellow observer that was taking the blood pressure, blinded to the values. This blinding measure would have prevented the observer taking the blood pressure, from subconsciously recording falsified results. Therefore this increases the validity of results collected in the trial.

The obtained results for average left and right systolic blood pressures were 112.1mmHg ± 16.5 and 112.7mmHg ± 16.3, whilst the average left and right diastolic pressures were 64.4mmHg ± 11.6 and 63.5mmHg ± 9.9. Statistical analysis reported that there was no

significant difference in systolic or diastolic blood pressures between different genders of subjects with right or left hand dominance (Pesola et al., 2000). Results of this study support the normotensive values presented by the joint national committee on prevention, detection, evaluation and treatment of high blood pressure which are illustrated in table 2. However, this does not present an average blood pressure reading as each individual will be different and there are several determinants such as height, weight and age that can influence blood pressure at any given moment.

Historically diastolic blood pressure was seen as the most important pressure in predicting coronary heart disease (Mancia, Rosei, Cifkova, DeBacker, Erdine, Fagard, & Zannad., 2003). This is present in the design of major randomised control studies that were conducted in the 1990's (Collins, Peto, MacMahon, Godwin, Qizilbash, Hebert & Fiebach., 1990). However, since the 1990's there has been confirmation via controlled trials that both systolic and diastolic blood pressures are related with cardiovascular disease (Vasan, Larson, Leip, Evans, O'Donnell, Kannel, & Levy, 2001).

### Hypertension

Hypertension has been reported as being a major risk factor in inducing cardiovascular disease (CVD). Examples of various types of CVD are strokes, ischemic heart disease, cardiac failure and end stage renal disease (Touyz, 2003). Hypertension is an abnormal alteration in the stiffness of blood vessel (arterial) walls within the body. Major vascular changes associated with hypertension include reduced vessel lumen diameter and media thickening which may be due to an increase in peripheral resistance (hardening of the arteries) ultimately impairing the body's vasodilatation ability (Touyz et al., 2003).  $Mg^{2+}$  has been reported to have a positive effect on reducing peripheral resistance allowing vasodilatation to occur within the arteries.

Hypertension is caused predominantly by a haemodynamic abnormality which involves a reduction in the size of the lumen in the arteries of the human body this is commonly known as peripheral resistance. As the lumen in the arteries begins to shrink the resistance in the artery begins to increase, resulting in an overall increase in hypertension.

The onset of hypertension may also be related to functional, structural and mechanical alterations in the body (Korner et al., 1989; Folkow, 1990). It is reported that 43 million people the United States are currently suffering with hypertension and are taking antihypertensive medications. Essential hypertension accounts for around 95% of hypertension cases (Carretero & Oparil., 2000) and it is a worldwide complication accounting

for 6% of adult deaths (Burt, Whelton, Roccella, Brown, Cutler, Higgins, Horan & Labarthe, 1995).

Diseases of the heart and circulatory system (cardiovascular disease or CVD) occurring due to hypertension are the main cause of deaths in the United Kingdom and accounted for almost 180,000 deaths in 2010. The most common forms of CVD are coronary heart disease and stroke. Around 45% of all CVD deaths are from coronary heart disease and over a quarter, roughly 28% are a result of strokes (British Heart Foundation, 2012).

Coronary heart disease alone is the most common cause of death in the United Kingdom. In 2010, just under one in five male deaths and one in ten female deaths were from the disease, this is approximately a grand total of 80,000 deaths in one year (British Heart Foundation, 2012). Strokes caused a total of 50,000 deaths whilst another 49,000 deaths occurred from various other circulatory diseases. Acute myocardial infarction (heart attack) is also another significant cause of death in the United Kingdom, with the majority of deaths occurring in individuals over the age of 85 (British Heart Foundation 2012).

The exact form of the underlying genetic mechanism of hypertension still remains unclear. The identification of variant genes that contribute to the development of hypertension is complicated by the fact that the two entities that determine BP (Cardiac output and total peripheral resistance) are controlled by other intermediary phenotypes, including the autonomic nervous system, vasopressor/vasodepressor hormones, the structure of the cardiovascular system, body fluid volume renal function and many others (Gong & Hubner, 2006). Identification of genes underlying BP variation has the capacity to define primary physiological mechanisms causing this trait, thereby clarifying disease pathogenesis, establishing molecular diagnostics and developing a novel therapy (Lifton, Gharavi & Geller; 2001). Over the last decade progress has been made towards the detection of genes underpinning several Mendelian forms of hypertension traits, which may present early in life with distinct phenotypes (Lifton, Gharavi & Geller; 2001). Some variables which may lead to an increase in blood pressure, resulting in hypertension are: weight, age, gender, sedentary lifestyle, high alcohol consumption, high salt intake, low calcium intake and low potassium intake (Sever & Poulter., 1989; INTERSALT Co-operative Research Group., 1988).

Lewington et al (2002) supported the theory that there is a linear relationship between blood pressure and CVD. Lewington, conducted a meta-analysis which concluded that between the ages of 40-69 there is an increase in mortality rates from ischemic heart disease. The findings suggested that with each increase in systolic blood pressure of 20mmHg and in

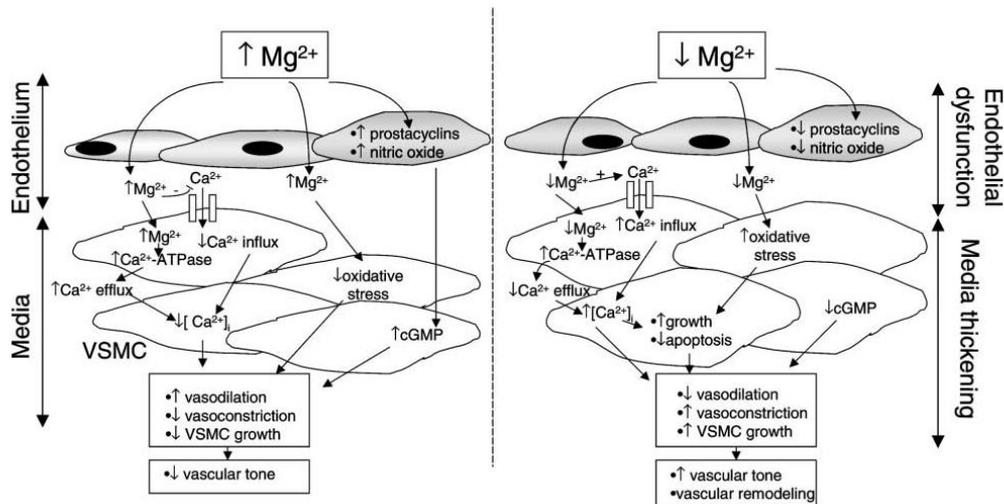
diastolic blood pressure of 10mmHg there would be a twofold increase in the risk of stroke mortality. These findings help to link the increase seen in blood pressure with the subsequent increase in mortality rates.

### Mg<sup>2+</sup> effect on blood pressure

Several studies have demonstrated that Mg<sup>2+</sup> supplementation will have a positive effect in reducing blood pressure in both hypotensive and normotensive individuals (Dyckner & Wester, 1983; Motoyama et al, 1989; Witterman et al, 1994; Kazue et al, 1997; Kawano, 1998 & Doyle, 1999.) whilst the results of several studies also contrast these findings suggesting that Mg<sup>2+</sup> supplementation will fail to aid in the reduction of blood pressure (Cappuccio et al, 1985; Henderson et al, 1986; Sacks et al, 1995; Yamamoto et al, 1995 & Sacks et al, 1998).

Mg<sup>2+</sup> has been linked to various physiological functions occurring within the human body, including cardiovascular regulation as it may play an important role in the control of neural activities, cardiac excitability, neuromuscular transmission, muscular contraction, vascular tone, blood pressure and peripheral blood flow (Altura, 1995). Therefore, it may be assumed that deficiency of this micronutrient may result in abnormalities occurring such as heart disease, congestive heart failure, diabetes, insulin resistance and as discussed previously high blood pressure resulting in hypertension (Altura, 1995).

Increased concentrations of extracellular Mg<sup>2+</sup> cause vasodilation, whereas decreased concentrations cause contraction and potentiate agonist evoked vasoconstriction. The exact cellular basis for the molecular contractile action of Mg<sup>2+</sup> is unknown, but it has been suggested that Mg<sup>2+</sup> influences Ca<sup>2+</sup> which has been shown to increase contractility of vascular smooth muscle cells (Jahnen-Dechent & Kettele, 2012). Inside the vascular smooth muscle cells, Mg<sup>2+</sup> acts by inhibiting transmembrane calcium transport and calcium entry into the cell thus decreasing contractile actions of vasoactive agents acting as a calcium antagonist thereby modulating the vasoconstrictor actions of increased Ca<sup>2+</sup> in smooth muscle cells. This can be seen in figure 2 which displays the role which Mg<sup>2+</sup> plays in reducing smooth muscle cell contractility. Elevated levels of Mg<sup>2+</sup> compete with the influx of Ca<sup>2+</sup> and stimulate a mediated calcium efflux resulting in decreased intracellular free calcium which in turn may reduce vascular contractility reducing blood pressure by vasodilatation (Touyz, 2003)



**Fig. 2. Displays the mechanism of action by which intracellular Mg<sup>2+</sup> can reduce the levels of Ca<sup>2+</sup> to aid a reduction in blood pressure (Touyz et al, 2003).**

Kawano (1998) conducted a study looking into the effects of Mg<sup>2+</sup> supplementation in hypertensive patients. This study was of a double blind randomised cross over design involving 62 subjects of both genders between the ages of 35-74y with baseline blood pressure levels of > 140mmHg (systolic) and > 90mmHg (diastolic). The protocol involved a randomised crossover design where subjects were supplemented with either 8 weeks of Mg<sup>2+</sup> followed by an 8 week control period or an 8 week control period followed by an 8 week Mg<sup>2+</sup> period. Twenty mmol/day (480mg) of Mg<sup>2+</sup> supplement was given to the subjects for ingestion. Blood pressure measurements were recorded in the home environment twice a day throughout the duration of the intervention period and the control period, whilst casual office (work environment) and 24 hour ambulatory blood pressures were measured at the end of the control period and at the end of the intervention period.

Table 3. Office, Home and 24-hour ambulatory BP during control supplementation and Mg<sup>2+</sup> supplementation periods (Kawano et al., 1998).

Parameter, mm Hg	Control Period	Mg Period	Difference
Office			
SBP	148.6±1.6	144.9±1.7	3.7±1.3†
DBP	90.0±0.9	88.3±0.9	1.7±0.7*
Home			
SBP	136.4±1.3	134.4±1.4	2.0±0.8*
DBP	86.8±0.9	85.4±0.8	1.4±0.6*
24-h			
SBP	133.7±1.3	131.2±1.1	2.5±0.8†
DBP	81.0±0.8	79.6±0.8	1.4±0.6*
Day			
SBP	137.7±1.2	135.2±1.3	2.5±1.1*
DBP	84.0±0.8	82.5±0.9	1.5±0.7*
Night			
SBP	125.9±1.9	123.4±1.5	2.5±1.3
DBP	74.8±1.1	73.6±0.9	1.2±0.7

Day indicates 6:30 AM to 10 PM; Night, 10:30 PM to 6 AM.  
\*P<0.05, †P<0.01.

Table 3 displays the findings that were recorded by Kawano (1998), clearly showing a reduction of blood pressure in the office of 3.7±1.3 (systolic) and 1.7±0.7 (diastolic), a blood pressure reduction in the home of 2.0±0.8 (systolic) and 1.4±0.6 (diastolic) and a reduction in the 24 hour assessment of 2.5±1.1 (systolic) and 1.5±0.7 (diastolic) in the day and 2.5±1.1 (systolic) and 1.2±0.7 (diastolic) in the night.

The resulting drop in blood pressure was concluded to be significantly different in the office, at home and in the 24 hour readings in male populations. Female populations also recorded a lower blood pressure post intervention in the office, at home and 24 hour trails, although these findings were not concluded as having a significant difference to baseline recordings. These findings may be evidence of how blood pressure can be reduced successfully in the male population by Mg<sup>2+</sup> supplementation although this cannot be concluded for both genders as female's reductions were not significantly different.

As well as reporting lower blood pressure readings when comparing males to females Kawano (1998) also reported that Mg<sup>2+</sup> supplementation tended to have a greater effect on the older populations when compared with the younger populations. Gender, age, height, and weight all are determining factors in hypertension. Kawano (1998) suggested that there could be several mechanisms responsible for the significant decrease in blood pressure.

Mg<sup>2+</sup> ions lower resting levels of intracellular Ca<sup>2+</sup> by competing with Ca<sup>2+</sup> for binding sites and modulating Ca<sup>2+</sup> binding and release from the sarcoplasmic reticulum thus inducing vasodilation as an intracellular Ca<sup>2+</sup> blocker (Kawano, 1998). At the cell membrane, Mg<sup>2+</sup> regulates ion flux through voltage-gated, acetylcholine-activated, Ca<sup>2+</sup>-activated, and ATP-activated K<sup>+</sup> channels. These action may also be involved in the cardiovascular effects of Mg<sup>2+</sup> (Kawano, 1998).

Doyle et al, (1999) presented academic research also suggesting that Mg<sup>2+</sup> has an important role in the regulation of blood pressure. Doyle (1999) also highlighted that Mg<sup>2+</sup> supplementation may have a beneficial role in bone growth and metabolism. Doyle (1999) conducted a double blind placebo controlled, randomised crossover epidemiological study, involving 26 young and healthy females with an average age of 23± 2, height of 1.64m ± 0.05, weight of 60.3kg ±7.6 and a body mass index (BMI) of 22.2 ± 2.

The testing consisted of two different periods each lasting for a duration of 28 days, during which Mg<sup>2+</sup> supplementation was given to the subjects, the Mg<sup>2+</sup> intakes were 11.3mmol or 21.6mmol of Mg<sup>2+</sup> per day. Subjects were assigned to one of the Mg<sup>2+</sup> interventions or the placebo intervention for a period of 28 days after which a cross-over occurred. Doyle (1999) found no significant difference in systolic or diastolic blood pressures for either supplementation groups, conflicting the findings of Kawano (1998). These findings contradict the theory that Mg<sup>2+</sup> supplementation may reduce blood pressure, although these findings may have been due to the short duration of the intervention. Subjects were only asked to consume Mg<sup>2+</sup> supplementation for a period of 28 days which may be a contributing factor as to why blood pressure was not seen to fall significantly.

Although there are many different studies reporting a reduction in blood pressure with Mg<sup>2+</sup> supplementation it cannot be concluded that supplementation will have the same effect if administered to a subject already attaining the recommended daily allowance of Mg<sup>2+</sup> in their diet as many studies have failed to assess the dietary intake of subjects. This provides a rationale for this research as the methodology proposed aims to assess the dietary intakes of subjects partaking in the testing procedure and analyse if Mg<sup>2+</sup> supplementation will have a significant effect on performance, blood pressure and serum levels when RNI of Mg<sup>2+</sup> is already acquired through the diet.

## Effect of exercise on blood pressure

Alongside Mg<sup>2+</sup> supplementation exercise has also been reported to have an effect on blood pressure. Exercise is commonly known to have favourable effects on blood pressure, within individuals (Fagard, 2006). This would support the theory that fitter individuals will have lower systolic and diastolic resting blood pressure readings

Fagard (2006) stated that exercise can be divided into two broad categories dynamic, aerobic endurance training and resistance training. Aerobic endurance training is generally conducted to increase aerobic stamina; this type of exercise usually involves recruiting large muscle groups in an aerobically active manner.

Cornelissen and Fagard (2005) performed a comprehensive meta-analysis to assess the effect that exercise will have on blood pressure. The meta analysis involved a total of 72 trails, consisting of 105 study groups, n=3936. Cornelissen and Fagard (2005) concluded that aerobic exercise will have a positive effect on reducing blood pressure in both normotensive and hypertensive individuals and their findings are displayed below in Table 4. It was also concluded that lowering of blood pressure as a result of exercise will be greater in hypertensive individuals rather than normotensive individuals (Cornelissen & Fagard, 2005).

Table 4. Baseline data for all groups and weighted net changes in response to aerobic endurance training (Cornelissen & Fagard, 2005).

Variable	Subgroup	n	No. of Subjects	Baseline	Net Change	P Value Within Groups	P Value Among Groups
VO <sub>2</sub> max (mL · kg <sup>-1</sup> · min <sup>-1</sup> )	Normal pressure	25	554	31.6 (28.9; 34.3)	3.5 (2.5; 4.4)	<0.001	
	Prehypertension	39	852	31.5 (29.5; 33.4)	3.9 (3.1; 4.6)	<0.001	NS
	Hypertension	17	279	29.2 (25.6; 32.8)	4.4 (3.7; 5.1)	<0.001	
HR (bpm <sup>-1</sup> )	Normal pressure	18	306	71.0 (67.1; 74.9)	-7.1 (-9.3; -5.0)	<0.001	
	Prehypertension	23	404	71.9 (67.9; 75.8)	-4.4 (-5.6; -3.2)	<0.001	NS
	Hypertension	23	340	74.4 (72.4; 76.3)	-4.5 (-6.5; -2.6)	0.001	
BP (mm Hg)							
	SBP						
DBP	Normal pressure	28	599	114.3 (112.8; 115.9)	-2.4 (-4.2; -0.6)	<0.01	
	Prehypertension	46	1087	127.2 (125.9; 128.5)	-1.7 (-3.1; -0.29)	<0.05	<0.001
	Hypertension	30	492	145.4 (142.4; 148.4)	-6.9 (-9.1; -4.6)	<0.001	
DBP	Normal pressure	28	599	73.0 (71.8; 74.1)	-1.6 (-2.4; -0.74)	<0.001	
	Prehypertension	44	1063	80.3 (79; 81.6)	-1.7 (-2.6; -0.75)	<0.001	<0.001
	Hypertension	30	492	92.3 (89.5; 95.1)	-4.9 (-6.5; -3.3)	<0.001	

These results may indicate that aerobic endurance training can have a positive effect in the reduction of blood pressure. This may be due to a reduction in systemic vascular resistance, in which the sympathetic nervous system and the renin-angiotensin system appear to be involved (Fagard, 2006).

The second category of exercise described was resistance training, which refers to exercise involving strength, weight, static or isometric exercises incorporated specifically to encourage an increase in muscular strength, endurance and power (Fagard, 2006). It has been suggested that resistance exercise will also reduce blood pressure by reducing the peripheral resistance at rest (Kelley & Kelley, 2000).

Kelley & Kelley, (2000) conducted a meta-analysis aiming to examine the effect that resistance exercise will have on blood pressure. The analysis conducted involved 11 studies, containing a total of 320 subjects. The overall result of the meta-analysis suggested that progressive resistance exercise results in small reductions in resting, systolic and diastolic blood pressures concluding that progressive resistance exercise will be effective in reducing resting systolic and diastolic blood pressures in adults.

#### Mg<sup>2+</sup>'s effect on sports performance

It has also been shown that Mg<sup>2+</sup> may have an effect on athletic ability and performance. Some studies have reported that Mg<sup>2+</sup> may have a positive effect on sporting performance (Lukaski, Bolonchuk, Klevay, Milne & Sandstead, 1983; Brilla & Haley, 1992; Brilla & Gunter, 1995) whilst conflicting literature suggests that Mg<sup>2+</sup> supplementation will have no effect or a negative effect on performance (DeHann et al., 1985; Weight et al., 1988; Ruddell et al., 1990; Terblanche et al., 1992; & Weller et al., 1998.)

Mg<sup>2+</sup> has been deemed essential in during a wide variety of cellular activities and is necessary for maintaining optimal muscle performance and muscle contraction (Dominguez et al., 1992). Mg<sup>2+</sup> has been shown to significantly increase muscle strength in young subjects (Brilla & Haley, 1992). Rodrigues et al (2003) stated that studying the performance for different intensities (5 of 1RM) may help understand the behaviour of different muscle groups and different fitness levels.

Brilla and Gunter (1995) conducted a double blind four week cross-over design study on 20 females and 12 males (recreationally active). After consumption of either placebo or Mg<sup>2+</sup> supplementation (314mg/day), subjects completed an exercise trial which involved performing contractions on an isometric leg dynamometer until exhaustion. After another four weeks supplementation subjects returned for a second isometric leg trial to exhaustion.

They reported that there was a significant increase in time to fatigue when  $Mg^{2+}$  was compared to placebo. This may suggest that  $Mg^{2+}$  is effective in increasing the time to fatigue on a leg dynamometer. Brilla and Gunter (1995) failed to provide subjects with a washout period between interventions which may have had a negative effect on recorded results as levels of  $Mg^{2+}$  may not have returned to baseline level for the group experiencing placebo as their second intervention.

Furthermore, it has also been suggested that endurance athletes may experience a state of  $Mg^{2+}$  deficiency when competing in long distance endurance events. It has been proposed that this state of  $Mg^{2+}$  deficiency may be caused by the increased  $Mg^{2+}$  losses catabolised by an increased sweat rate or as a result of increased  $Mg^{2+}$  requirements due to high habitual daily rates of energy expenditure (Terblanche et al., 1992). Therefore  $Mg^{2+}$  supplementation would be beneficial to prevent deficiency from occurring during ultra-endurance sporting events.

Terblanche (1992) assessed the effects that  $Mg^{2+}$  supplementation may have on performance in a marathon footrace. Twenty athletes were divided equally into two matched groups and were assessed four weeks prior to the event and six weeks post event. The trial was double blind with the experimental group receiving 365mg of  $Mg^{2+}$  daily. It was reported that  $Mg^{2+}$  supplementation did not increase either muscle or serum concentrations, following blood samples and muscle biopsy's consequently resulting in no positive effect on marathon performance. The lack of increase may be related to the level of  $Mg^{2+}$  provided to subjects as 365mg/daily may have not been adequate enough to promote a significant response.

Research has proposed that substantial redistributions within the body may occur during bouts of exercise, resulting in losses of  $Mg^{2+}$  (Lukaski, 2000). When compared to pre-exercise conditions a shift can be seen from the plasma into the red blood cells (Deuster, Dolev, Kyle, Anderson & Schoomaker, 1987). Deuster (1987) conducted a study and concluded that the direction and magnitude of  $Mg^{2+}$  redistribution in the circulation was influenced by the intensity of the exercise. Deuster (1987) stated that the greater the energy requirement from anaerobic or glycolytic metabolism, the greater the translocation would be of  $Mg^{2+}$  from the serum to the red blood cells.

Another route of  $Mg^{2+}$  loss during exercise is via sweat and cellular exfoliation (Lukaski, 2000). A trial conducted on men performing controlled work for 8 hours on cycle ergometers in temperatures of 40°C proceeded to lose 15.2-17.8mg  $Mg^{2+}$ /d in sweat (Condolazio, 1983). This loss of  $Mg^{2+}$  equated to 4-5% of the individuals daily  $Mg^{2+}$  supporting the suggested theory that  $Mg^{2+}$  loss is at an increase during exercise.

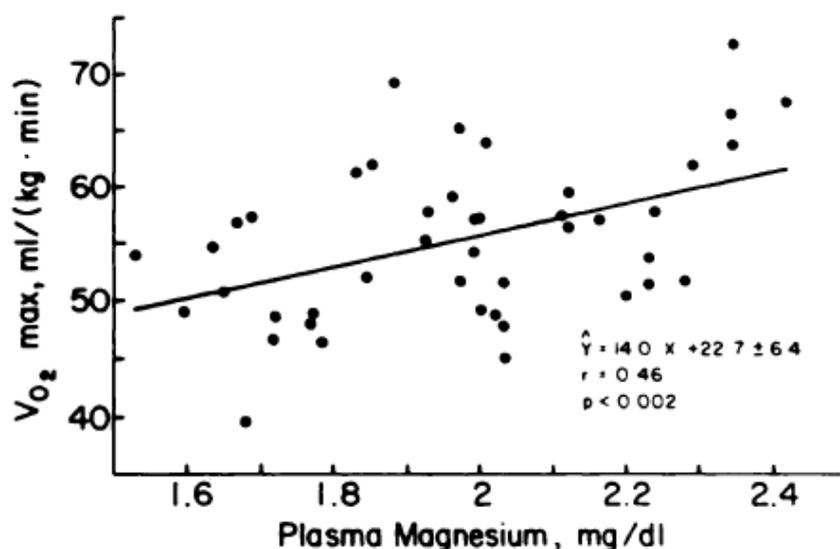
As previously discussed  $Mg^{2+}$  may also have a positive effect on sports performance as it plays a vital role in muscle contraction. In a state of hypomagnesemia an individual may experience muscle cramping as it has been proposed that  $Mg^{2+}$  deficiency may cause cramps during exercise (Stamford, 1993). Bergeron (2003) reported that tennis players competing in hot and humid conditions may be susceptible to muscle cramping. Bergeron (2003) went on to say that extreme sweat loss in hot and humid conditions will lead to an increased loss in  $Mg^{2+}$  ultimately resulting in muscle cramping.  $Mg^{2+}$  supplementation should therefore aid in the prevention of such events occurring by increasing overall  $Mg^{2+}$  status (Bergeron, 2003).

Golf et al (1993) conducted a study on female rowers, during which they were supplemented with either 360mg of  $Mg^{2+}$  aspartate or a placebo for a 3 week intervention. It was concluded that the athletes consuming  $Mg^{2+}$  had lower activities of total serum creatine kinase and creatine kinase isoenzyme from skeletal muscle after training in comparison to the placebo group. Furthermore it was also reported that the  $Mg^{2+}$  supplementation group had lower serum lactate concentrations and 10% lower oxygen uptake when performing a submaximal performance trial, concluding a positive impact on sports performance. Ripari (1989) conducted a similar trial, during which subjects were presented with either  $Mg^{2+}$  (250mg  $Mg^{2+}$ /day) or placebo supplementation. Improved cardiorespiratory function during a 30 minute submaximal exercise test was reported. These findings suggest that  $Mg^{2+}$  supplementation could provide a beneficial effect on work efficiency and muscle metabolism.

$Mg^{2+}$  supplementation has also been shown to improve muscle functioning. Brilla and Haley (1992) provided  $Mg^{2+}$  ( $Mg^{2+}$  oxide 8mg/kg day) or placebo to two groups of young men. They then assigned the participants with a seven week training programme which was to be followed alongside the supplementation plan. Total  $Mg^{2+}$  intakes for the  $Mg^{2+}$  intervention group were estimated to be 250/mg per day. Following the 7 week training programme peak knee-extension torque was assessed and it was reported that the  $Mg^{2+}$  intervention group had a greater increase when compared to the placebo group. This also suggests that  $Mg^{2+}$  may play a role in performance activities that require a predominant contribution of glycolytic metabolism.

Lukaski (1983) recruited 44 healthy male university athletes and 20 untrained males for a study to assess maximal oxygen consumption, during a maximal treadmill test, in relation to  $Mg^{2+}$ . Before conduction of the maximal treadmill test subjects were required to fast for a twelve hour period and provide an antecubital blood sample. Recorded weak, positive trend ( $r = +0.46$ ,  $p < 0.002$ ) between maximal oxygen uptake expressed per kg body weight and plasma  $Mg^{2+}$  in athletes as can be seen in figure 3.

## Mechanisms of Magnesium



**Fig. 3. displays the relationship found between maximal oxygen consumption ( $VO_{2\ max}$ ) and fasting plasma  $Mg^{2+}$  concentration in 44 male athletes (Lukaski, Bolonchuk, Klevay, Milne & Sandstead. 1983)**

The relationship reported between plasma  $Mg^{2+}$  and maximal oxygen consumption in the 44 male athletes suggests the possible metabolic role of  $Mg^{2+}$  during exercise, other than its already acknowledged role as a co-factor in neuromuscular functioning (Shils & Shike, 2006). 2,3-BPG is formed from 1,3-BPG by the enzyme BPG mutase. 1,3-BPG is created during the normal glycolytic pathway, which may be dephosphorylated by phosphoglycerate kinase. Magnesium may facilitate the delivery of oxygen to the working muscles by its involvement in the production of 2,3-diphosphoglycerate (2,3-DPG) in the erythrocyte athletes (Lukaski, Bolonchuk, Klevay, Milne & Sandstead. 1983). Scientific evidence has shown  $Mg^{2+}$  status to decrease during and shortly after long duration exercise in marathon runners (Rose, Carroll, Lowe, Peterson & Cooper, 1970; Noakes, Dennis, Marais & Eckert, 1992) and cross country skiers (Refsum, Tveit, Meen, & Strömme, 1973). Serum  $Mg^{2+}$  levels were reported to have dropped by up to 10% after each race.

In vitro studies have also shown correlations between erythrocyte 2,3-DPG and ATP and  $Mg^{2+}$  content of whole blood (Lukaski, Bolonchuk, Klevay, Milne & Sandstead. 1983). The

production of 2,3-diphosphoglycerate (2,3-DPG) in the erythrocyte will increase the delivery of oxygen to the working muscles, thus increasing sports performance. The addition of  $Mg^{2+}$  ions to anticoagulated whole blood has been shown to increase the levels of 2,3-DPG content (Darley, 1979). Oken (1971) reported that there was a decreased level of erythrocytic  $Mg^{2+}$ , a decreased glucose utilisation with reduced ATP and 2,3-DPG contents in a group of hypomagnesemic rats where spherocytic haemolytic anemia was described. In the circulation when Hb is deoxygenated there is a net increase in the amount of free  $Mg^{2+}$  ions within the erythrocyte which in turn causes the inhibition of the enzyme hexokinase to be relieved, thereby stimulating glycolysis and further production of 2,3-DPG (Oken, Lichtman, Miller & Leblond, 1971).

Brewer (1969) indicated that in vivo only about 5% of the erythrocytic  $Mg^{2+}$  is unbound, and it is this free  $Mg^{2+}$  which may regulate glycolysis and 2,3-DPG production. The correlation coefficient ( $r= 0.46$ ) calculated by Lukaski et al (1983) for the reported association of maximal oxygen consumption and plasma  $Mg^{2+}$  in the 44 male athletes indicates that 21% of the variance accounted for in the maximal oxygen uptake was due to the differences seen in plasma  $Mg^{2+}$ . Thus, other factors accounted for the variance. Although as only 5% of erythrocyte  $Mg^{2+}$  may regulate the production of 2,3-DPG the 21% of the variance may identify that  $Mg^{2+}$  has an important effect (Lukaski, Bolonchuk, Klevay, Milne & Sandstead. 1983).

The relationship observed between maximal oxygen consumption, which is dependent on oxygen delivery to the working muscles, and plasma  $Mg^{2+}$  in the athletes may represent a cellular adaptation of  $Mg^{2+}$  metabolism to physical training. Studies have reported significant increases in erythrocyte 2,3-DPG in athletes after a single bout of short duration maximal activity (Taunton, Taunton, & Banister, 1973; Böswart, Kuta, Lisý, & Kostiuk, 1980). Whether these increases in 2,3- DPG were related to  $Mg^{2+}$  ion redistribution in the blood is not known.

The influence that prolonged exercise will have on  $Mg^{2+}$  status must be taken into consideration. Prolonged exercise status will potentially facilitate a  $Mg^{2+}$  deficient state in the individual, due to an increased requirement notably promoted by a greater metabolic response to exercise and affiliated redistributions of  $Mg^{2+}$  within the body (Rayssiguier et al., 1990).

Hypomagnesemia and hypermagnesemia commonly occur as a result of  $Mg^{2+}$  redistribution catalysed by long duration, low intensity endurance exercise and short bouts of high intensity exercise (Bohl & Volpe, 2002). Hypermagnesemia is said to occur due to a decrease in plasma volume, subsequent cellular compartmental shifts increasing serum or plasma  $Mg^{2+}$  concentration due to acidosis and muscular contraction (Deuster, Dolev, Kyle, Anderson &

Schoemaker, 1987). Another suggested mechanism of hypermagnesemia is acidification, which is proposed to elicit an increased intracellular  $Mg^{2+}$  by influencing the dissociation of  $Mg^{2+}$  from its intracellular binding sites (Kim, Cho, Kang & Kim, 2006).

Hypomagnesemia on the other hand has been linked to prolonged intense endurance exercise (Casoni, et al., 1990; Mooren, Golf, Lechtermann & Völker, 2005). It has been reported that during the state of exercise there will be a shift of  $Mg^{2+}$  onto three primary sites. Nielsen & Lukaski (2006) reported these three sites to be the erythrocytes, the adipocytes and the myocytes. In regard to the erythrocytic  $Mg^{2+}$  shift that is supported and contradicted by academic research, acknowledgements have presented an emphasis that the increased concentration of ionised  $Mg^{2+}$  within the erythrocyte highlights exercise and erythrocytic  $Mg^{2+}$  to modulate cellular signalling and energy metabolism (Nielsen and Lukaski, 2006). These reported increases in erythrocyte  $Mg^{2+}$  may coincide with previous research linking performance increments to the production of 2, 3-DPG, which is said to facilitate an increased maximal oxygen consumption due to the 2, 3-DPG function of sustaining sufficient haemoglobin oxygen affinity and oxygen transport (Lukaski, 1983).

Despite the suggested enhancements and performance gains due to  $Mg^{2+}$ , other research has reported an inverse relationship between erythrocyte  $Mg^{2+}$  and 2, 3-DPG (Resina et al, 1994). Although, erythrocyte intracellular  $Mg^{2+}$  reported at 0.2mM in an oxygenated state has been shown to increase to 0.6mM due to haemoglobin dissociation Laughlin & Thompson, 1996). Such a deoxygenation induced increase of  $Mg^{2+}$  corresponds to alleviating previous inhibitory actions on hexokinase, which will in turn stimulate glycolysis and the production of 2, 3-DPG (Lukaski, 1983). This provides a greater potential for increased exercise performance, although this cannot be said to be definitive as there is still room for further investigation into the  $Mg^{2+}$  and 2, 3-DPG relationship.

Although there are many controlled trials that support  $Mg^{2+}$ 's impact on physical activity there is still literature that contradicts the findings. Manore et al (1995) recruited recreationally active men who agreed to partake in a 12 week exercise programme, which predominantly consisted of aerobic activities. Subjects also consumed either  $Mg^{2+}$  (250mg/d) or placebo supplementation. Results reported an increase in peak oxygen uptake in both intervention groups thus resulting in the  $Mg^{2+}$  supplementation providing no significant effect. Therefore, these findings indicate that  $Mg^{2+}$  supplementation does not exert an independent effect on performance gains.

These findings suggest that  $Mg^{2+}$  supplementation may not be effective in improving cardiovascular sporting performance although further investigations are needed into this area.

## 10 kilometre run

Testing performance is one of the most common and important measures tested in sports science and physiology research (Currell & Jeukendrup, 2008). Performance testing allows a controlled stimulation of sports performance for scientific purposes. There are three main contributing factors that define a good performance test validity, reliability and sensitivity (Currell & Jeukendrup, 2008). A valid protocol is the one that closely resembles the performance that is being stimulated. When testing for race type events there are two main methods that are used, these are time trial and time to exhaustion tests (Currell & Jeukendrup, 2008). Time trials are said to have greater validity than time to exhaustion trials due to the fact that they provided a good stimulation of actual performance. Games sports such as football and tennis are harder to simulate due to constant changes in intensity and direction (Currell & Jeukendrup, 2008).

Research has shown that time to exhaustion protocols have a coefficient of variation of >10%, whereas time trials are more reliable as they have been shown to have a coefficient of variation of <5% (Currell & Jeukendrup, 2008).

## Validity

There are three types of validity that can be applied to performance protocols logical validity, criterion validity and construct validity (Currell & Jeukendrup, 2008). Logical validity also known as face validity assesses whether a test measures achieves what it is intended to measure, although this can be difficult to assess (Thomas, Nelson & Silverman, 2011). Criterion validity allows for an objective measure of validity. There are two different types of criterion validity concurrent and predictive (Thomas & Nelson, 2001). Concurrent validity assess that the performance protocol is correlated with a criterion measure (Thomas & Nelson, 2001). Predictive validity involves using a performance protocol to subsequently predict performance (Thomas & Nelson, 2001). Construct validity refers to the degree in which a protocol measures a hypothetical construct, in this case performance. It can be measured by comparing two different groups of subjects with varying abilities (Thomas & Nelson, 2001).

The use of time trials as a measure of performance is very common in sports physiology research (Currell & Jeukendrup, 2008). For a performance protocol to be a logically valid measure of performance it must aim to measure the performance in question. Therefore, a time trial protocol will have a high level of validity when researching race events such as cycling and running (Currell & Jeukendrup, 2008). Foster et al. (1993) showed that there was no physiological difference in a 5-km time during a laboratory trial when compared to actual

performance. Laursen et al. (2002) investigated the relationship of exercise test variables to Ironman triathlon cycling performance. Laursen et al (2002) concluded that there was no significant correlation between the time to exhaustion trial and cycling performance in an Ironman triathlon.

Time trials have been shown to correlate well with actual performance in both cycling (Palmer, Dennis & Noakes, 1996) and running (Russel, Redmann & Ravussin, 2004). Palmer et al (1996) found a strong relationship between time to completion for a 40-Km cycling trial performed on a Kingclye ergometer compared with time to completion of two outdoor 40-Km time trials. Russel, Redmann & Ravussin (2004) reported that completion of a 10-Km running time trial after a 90 minute preload at 65%  $VO_{2max}$  was found to have a high correlation ( $r = 0.95$ ) with the time taken to complete a 10-Km road race. Although further research is needed to help establish these relationships, the absence of a relationship between time to exhaustion and performance (Laursen, Rhodes & Langill, 2002) and the present relationship between time trail and performance (Palmer, Dennis & Noakes, 1996; Russell, Redmann & Ravussin, 2004) would suggest that time trials are a valid measure of running performance.

There are two main types of time trail that could be used. The target could be set at distance or work (Currell & Jeukendrup, 2008). An advantage of using an at distance target is that it provides a more valid representation of actual performance. The use of an at work target, whilst not as valid as a distance target allows for a performance test with higher levels of control (Currell & Jeukendrup, 2008).

### Reliability

Reliability is another important measure as it gives an indication of the biological and technical variation of the protocol (Bagger, Petersen & Pendersen, 2003). Measure of reliability can be used to calculate the necessary sample size for a given effect size, therefore, reducing the risk of potential errors occurring (Atkinsin & Nevill, 1998). There are various ways in which reliability can be expressed. The common measure of reliability used, is the coefficient of variation (CV). This expresses the standard deviation (SD) of the measure as a percentage of the mean, making it a lot easier to compare the amount of variation between various protocols (Currell & Jeukendrup, 2008). Other measures that can also be used are Pearson's product moment correlation ( $r$ ) in which a high significant correlation can lead to the conclusion that a specific protocol is reliable. The intraclass correlation coefficient (ICC) is an additional form of correlation that can be used to assess reliability.

Most research in the field of performance protocols has investigated into one type of protocol. Jeukendrup et al (1996) compared the reliability of various performance testing protocols. They compared time to exhaustion and time trial protocols and concluded that the time to exhaustion has poor reliability when compared with time trials and constant duration protocols. The findings by Jeukendrup et al (1996) suggest that the time trial approach being adopted in the current trial would provide greater reliability than a time till exhaustion trial.

### Sensitivity

When choosing a protocol it is important that the smallest worthwhile effect can be detected. (Currell & Jeukendrup, 2008). Analysis of the finishing times for the 2004 Athens Olympics cycling time trial shows that the difference between first and second place was 0.52%, suggesting that the small gains in performance can lead to massive changes in the outcome of a race. Hopkins et al (1999) used mathematical models of finishing times of the 1997 international Amateur Athletics Federation series to look at the smallest worthwhile increase in performance. It was concluded that in order to increase the chance of winning by 10%, the intervention must increase the athlete's performance by 0.3 times the athlete's CV between events. For this to increase to 20% the smallest worthwhile enhancement is 0.7 X CV. Jeukendrup and Martin (2001) used a mathematical model to look at the improvements that different interventions can have upon cycling a time trial performance. It was suggested that even the largest training effects may improve time trial performance in elite cyclists by 2% with nutritional interventions producing smaller effects. Therefore, it could be deemed useful to be able to detect these small improvements that interventions can make (Currell & Jeukendrup, 2008).

Validity, reliability and sensitivity are all interlinking factors; they are not separate. A protocol can be reliable, but not valid where as a valid protocol must be reliable (Atkinson & Nevill, 1998). The relationship between reliability and validity is such that a reliability correlation coefficient is the square of the validity correlation coefficient (Thomas & Nelson, 2001). Even taking all this into consideration it must be remembered that any effect seen in a test of performance conducted in a laboratory may not transfer directly to an actual performance situation. Jeukendrup et al (1997) showed an increase in the time taken to complete a laboratory based 1 hour time trial of 2.3% due to carbohydrate feeding; however would the same subjects experience the same gains of performance in a real competition? The relationship between the size of the effect seen in laboratory situations and the size of the effect that occurs is still unknown, but could still be a very important relationship to understand (Currell & Jeukendrup, 2008).

## Factors to control in a protocol

### Familiarisation

When conducting research there are many different factors that need to be taken into consideration (Currell & Jeukendrup, 2008). Firstly subjects should be familiarised with the protocol and equipment being used. Jeukendrup (1996) did not show any learning effect over six trials for a time trial lasting approximately 40 minutes. This may have been due to the fact that participants were trained cyclists that may have been used to the laboratory environment and testing procedures. However Laursen et al (2003) showed a learning effect for a 40-Km time trial. It was seen that when the first trial was compared with the third trial the CV was 2.9% compared with 0.9% when the second trial was compared with the third trial. Therefore, it may be suggested that subjects are familiarised with the protocol and equipment prior to the conduction of the protocol to prevent this learning effect from occurring.

### Measurements

It has been previously reported that taking physiological measurements during a performance protocol will interfere with the personal performance of the subjects; this has also been linked to having an effect on concentration (Currell & Jeukendrup, 2008).

There have been many methods that have been adopted in an attempt to measure performance as accurately as possible. Traditionally, researchers have attempted to assess endurance performance with a debate about whether a time to exhaustion or time trail protocol is the most appropriate. Time trails appear to have a lower variation when compared to time to exhaustion suggesting that they would provide a more valid account of what could occur in sporting performance. This form of protocol will be adopted in this investigation as subjects will be required to complete a 10-Km time trial.

## **Method**

### **Pre experimental**

*Pilot Study* Before commencing with the trial pilot testing was conducted on 4 participants to assess whether the protocol design would be effective, after the pilot testing was conducted the protocol was reviewed and adjusted. The original protocol involved several physical trials and lasted approximately 150 minutes. This was deemed to be too long and taxing on participants so the isometric leg dynamometer trial was removed accordingly. Blood pressure recording during exercise was also removed from the protocol as it was deemed too hard to ascertain blood pressure recordings whilst a participant was running on a treadmill, although blood pressure was still recorded before and after the 10 kilometre run.

*G Power Calculations* Before subject recruitment was conducted a power test was performed using a sample power software (SPSS SamplePower 3, IBM, Portsmouth, United Kingdom) utilising previous data on blood pressure (Cappuccio, Markandu, Beynon, Shore, Sampson, & MacGregor. 1985; Jee, Miller, Guallar, Singh, Appel, & Klag. 2002). Results indicated that a sample size of fifteen participants per intervention group would be sufficient to achieve a power score of 0.80 (see appendix).

### **Participants**

#### *Participants.*

Fifteen subjects were initially screened and accepted for participation. During the protocol two subjects dropped out, failing to complete. This left nine male and four female subjects who were successful in completing the protocol, characteristics of all subjects can be seen below (Table 5). The two subjects that failed to complete the study did so due to injury and the arrival of a child.

Table. 6 Characteristics of the 13 studied participants

Age (Years)	24.85±6.49
Height (cm)	175±10.34
Weight (Kg)	71.9±11.46
VO <sub>2</sub> <sub>max</sub> (ml/Kg/min)	48.77±6.87
One Rep Max (Kg)	64.62±26.65
Mg Serum (mmol/L)	0.71±0.05

Once written informed consent was obtained from the school of Life and Medical Science Ethics Advisory Committee, University of Hertfordshire, fifteen apparently healthy, physically active participants (Age:  $24.85 \pm 6.49$  years, Height  $175 \pm 10.34$  cm, Weight:  $71.9 \text{ Kg} \pm 11.46 \text{ Kg}$ ) were recruited from the county of Hertfordshire. Participants were sourced via a recruitment letter and e-mail that was circulated to local running and athletics clubs. These were targeted in an attempt to recruit a selection of participants that would be successful in completing the 10 kilometre run.

Before the selection of participants occurred a screening process was conducted to ensure all participants met the inclusion criteria. Selection criteria for inclusion in the trial included, males and females, individuals between the ages of 18-40, to be recreationally active, have no clinical history of hypertension, the expectation and willingness to attend the University on five separate occasions, the expectation and willingness to record a four day food diary, the expectation and willingness to consume 500mg of both  $\text{Mg}^{2+}$  (80mg elemental) and cornflour daily. Exclusion criteria included participants that suffered from high blood pressure, participants that had any underlying heart problems, participant's that had experienced a cold two weeks prior to the trial. Participants eligibility was assessed using a health screen questionnaire approved by the ethical committee of Life Sciences at the University.

The participants were then familiarised with the equipment Participants returned later that week to undertake a  $\text{VO}_2 \text{ max}$  test and a one repetition max test. The  $\text{VO}_2 \text{ max}$  test was conducted on the treadmill, it commenced on 8 kilometres per hour, with no incline. The speed and incline were increased alternately on every minute by 1% or 1 kilometre per hour until the participant could no longer maintain a state of exercise. All participants were verbally encouraged to continue for as long as possible and their  $\text{VO}_2 \text{ max}$  was recorded at the end. The mean  $\text{VO}_2 \text{ max}$  scores achieved were  $49.9 \pm 7.54$  ml/min/Kg for the male participants and  $47.5 \pm 4.65$  ml/min/Kg for the female participants. McArdle, Katch and Katch (2012) report several fitness categories relating to  $\text{VO}_2 \text{ max}$  scores. The average  $\text{VO}_2 \text{ max}$  scores achieved in this trial by both males and females falls into the good category. This confirms that the sample of participants who undertook the trial were good recreational athletes of similar fitness levels.

The one rep max score was attained after the subjects had recovered from the  $\text{VO}_2 \text{ max}$  test. This trial involved subjects performing a maximal voluntary contraction, bench press lift. This trial was conducted in the lifting cage with the safety bars correctly positioned and two researchers were present for safety and to help spot the lift. Each participant was started on a light weight, with increments of 2.5kg each time. Participants were allowed 5 minutes rest between lifts to allow for ATP restoration. The mean one repetition max scores achieved

were  $64.62\text{Kg} \pm 26.65\text{Kg}$ . The one rep max trial was conducted so that 75% and 50% of the max score could be calculated for later use in the protocol. The two tests provided an indication as to the overall fitness level of the 13 participants and the maximum weight that could be lifted in one all out bench press exertion.

A week later participants were required to return to the University to commence with the protocol. The protocol design was as follows; on arrival subjects were asked to complete a health screen questionnaire to confirm that they were feeling fit and able to participate. They were then left in the seated position for 5 minutes before having a blood pressure reading, recorded using an electronic blood pressure monitor (Digital Automatic Blood Pressure Monitor, Omron, Milton Keynes, United Kingdom).

After the blood pressure reading had been conducted participants partook in the bench press trial. This trial involved participants performing as many bench press repetitions as possible until failure, commencing with 75% of 1RM followed directly after by 50%. Before commencing with the lift participants were provided with time to warm up by completing a set of repetitions on 40% of their one repetition max scores. The lifting cage was prepared before conduction of testing by assuring that the safety bars were correctly set preventing participants from dropping the weight on their chest. Two spotters were also present. Participants then started with 75% of their one rep max scores and performed that until failure. This was followed by 50% of their one rep max; this was also performed a bench press until failure. During the trial total repetition count was being recorded by the researcher whilst mean and peak displacement (M/sec), mean and peak speed (M/sec), mean and peak power (N) and mean and peak force (N) were all being measured by the Globus Ergo System (Ergo System, Globus, Codognè, Italy).

Following the bench press participants proceeded to run the 10 kilometre trial. The run was a time trial, thus participants were able to manipulate the pace that they were running at, aiming to complete the distance in as short a time as possible. Before commencing with the trial participants were required to put on a cortex gas mask and heart rate monitor (Polar, Warwick, England) for analysis to be conducted throughout the duration of the run. Respiratory analysis was recorded by a cardiopulmonary exercise testing system (Cortex Metalyzer, Cortex, Leipzig, Germany). A Borg scale rating of perceived exertion (RPE) was another variable measured throughout the 10 kilometre running trial (Borg, 1982).

During the 10 kilometre running trial respiratory readings were recorded at 10 minute intervals from the metalyser (Cortex, Leipzig, Germany). At every 10 minutes the researcher would record  $\dot{V}O_2$  (L/Min),  $\dot{V}O_2/\text{Kg}$  (ml/Min), breathing frequency,  $\dot{V}E/\dot{V}O_2$  equivalent for  $O_2$

uptake,  $\dot{V}E$  (L/min) minute ventilation & RER (respiratory exchange ratio). Distance achieved and RPE at each 10 minute interval was also recorded.

Following completion of the 10 kilometre trial, participants were provided with time to cool down adequately and re-hydrate before returning to the bench press to complete the trial for a second time. Once the bench press trial had been conducted again at 75% and 50% of ORM subjects had blood pressure recorded for a second and final time.

Finally after completion of all physical trials and blood pressure readings a venous blood sample was collected. Subjects were required to sit upright in the phlebotomy chair with their right arm resting on the required stand. A tourniquet was applied and tightened accordingly. After the correct point of insertion was located the sight was cleaned with a sterile wipe to ensure that the sight was clean. Blood samples were collected using a standard butterfly needle (Williams Medical, Butterfly Needle, United Kingdom) and collected into a 5ml gold vacutainer, containing a clot activator and gel for serum separation. Once the vacutainer had been successfully filled the needle was removed and appropriately disposed of in a regulated sharps bin. Cotton wool was placed on the insertion site and pressure was applied until bleeding stopped.

Five minutes after blood samples had been collected participants were able to leave and were provided with food diaries and supplementation before departure. Supplementation was provided in capsule form, with each capsule containing 500mg of  $Mg^{2+}$  citrate. After subject departure blood samples were spun at 10'000 rotations per minute for a period of 10 minutes in a blood centrifuge allowing separation of the blood due to the clot activator located in the vacutainer. Serum samples were then transferred into a plastic sample tube, successfully sealed, labelled and stored in a freezer at  $-73^{\circ}C$  for later analysis.

Following the completion of the testing the blood samples were transported from the University of Hertfordshire to Hemel Hempstead hospital for analysis of the  $Mg^{2+}$  content. Analysis was conducted using the Cobas 8000, modular analyser series (Roche Diagnostics, Rotkreuz, Switzerland). Four samples were deemed to be faulty and were returned without a  $Mg^{2+}$  level.

Food diaries were recorded to provide an indication of the level of  $Mg^{2+}$  consumed by each participant through their diet. Dietary analysis was conducted with the aim of comparing the effects  $Mg^{2+}$  supplementation had on participants achieving adequate  $Mg^{2+}$  status through their diets against participants achieving a deficient  $Mg^{2+}$  status through their diets, as it has been suggested that  $Mg^{2+}$  supplementation will provide no further benefit to those achieving optimal  $Mg^{2+}$  status through their diet.

The design of the study involved the participants completing the protocol a total of four times over a fourteen week period. Participants were tested initially as a baseline measurement. Subjects were then provided with supplementation, either 500mg of placebo or magnesium which was picked at random, resulting in 8 subjects initially consuming placebo and 5 subjects consuming Mg<sup>2+</sup>. Supplementation was presented in capsule form and subjects were asked to consume one daily for a period of four weeks. The capsules had been separated and mixed up then placed into bags labelled A and B, this ensured that the interventions were blinded to both researchers and participants. After the four week supplementation period participants returned to the University to have post intervention testing conducted. Following this, participants had a five week washout period aiming to reduce any potential performance gains attained from the intervention and bring subjects back to baseline levels. After the washout period participants returned for a third time to complete a second set of baseline testing, participants were then presented with their second intervention. As the study was of a crossover design it was ensured that participants experienced both the placebo and magnesium interventions. After the final supplement intervention had been completed, participants returned one last time to conduct the protocol. A summary of this fourteen week testing period can be seen below (see table 6).

Table 6. An overview of the requirements of participants during the 14 week study.

<b>Week 0</b>	<b>Week 1-4</b>	<b>Week 5-10</b>
Familiarisation to machines, exercises and VO <sub>2 max</sub> test. Returned later in the week to complete VO <sub>2 max</sub> and one repetition max test, which was used for baseline data.  Pre-testing, maximal testing, baseline and initial venous bloods were collected.	Supplementation was ingested which lasted a duration of 4 weeks, whilst recording a three day food diary. Initial intervention testing to be conducted on sight at the University at the end of week 4 during which a second blood was taken.	Washout period, during which subjects consumed no supplementation
<b>Week 10</b>	<b>Weeks 11-14</b>	
Second baseline testing was conducted subjects were asked to return to conduct same procedure. Third venous bloods were taken.	Alternative supplementation to be consumed lasting a duration of 4 weeks. Final intervention testing to be conducted on sight at the University at the	

	end of week 14 during which the final blood will be taken.	
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### Data Analysis

Data analysis was conducted using SPSS (IMB, SPSS, United States) software. Before analysis could be conducted averages were calculated for each variable. Data was then analysed using a Repeated Measures Anova to conclude if recorded results contained significant differences. Standard deviation, Standard error and percentage change were also calculated alongside the significance levels and data was presented in graph and table format.

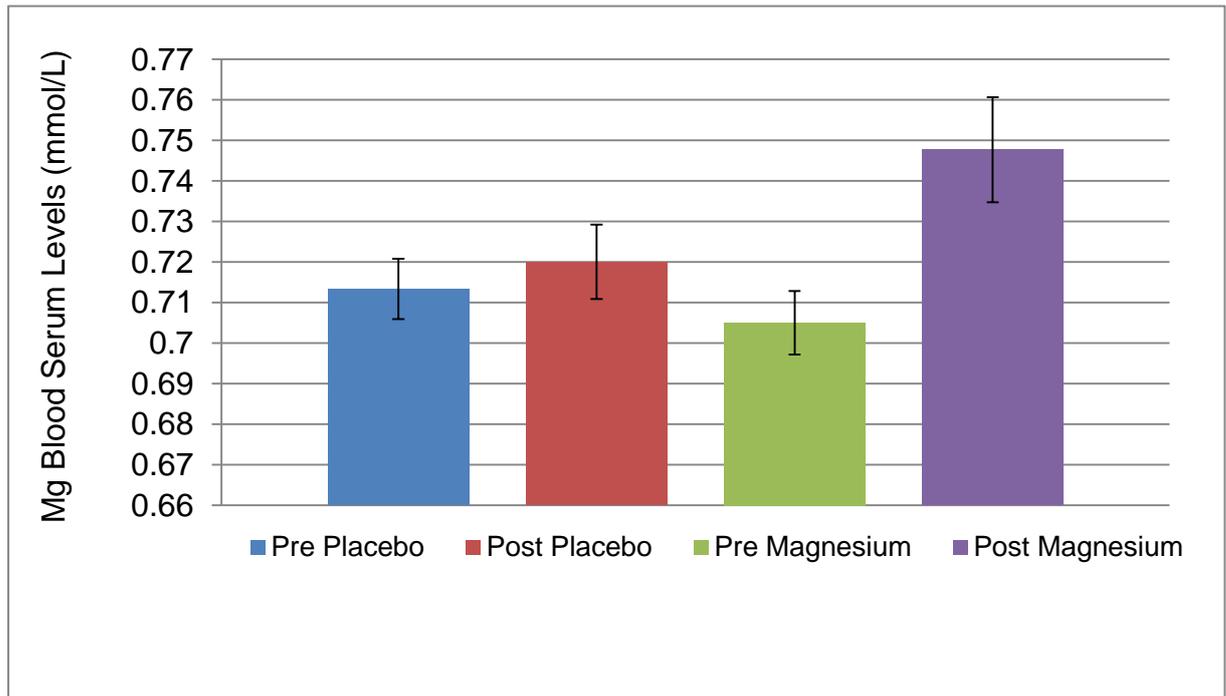
Dietary analysis was conducted using DietPlan 6 following the collection of the food diaries. Dietary analysis was conducted with the aim of assessing whether Mg<sup>2+</sup> supplementation would impact those achieving optimal Mg<sup>2+</sup> status when compared to those achieving a deficient Mg<sup>2+</sup> status through their diet.

## Results

The mean plasma  $Mg^{2+}$  level at baseline was 0.71 mmol/L, this is below the mean serum concentration in humans, which has been suggested to be roughly 0.85 mmol/L (Wacker, 1980).

### *Mg<sup>2+</sup> blood serum level*

Figure 4 displays the average pre and post  $Mg^{2+}$  blood serum level (mmol/L) for all participants in each intervention.



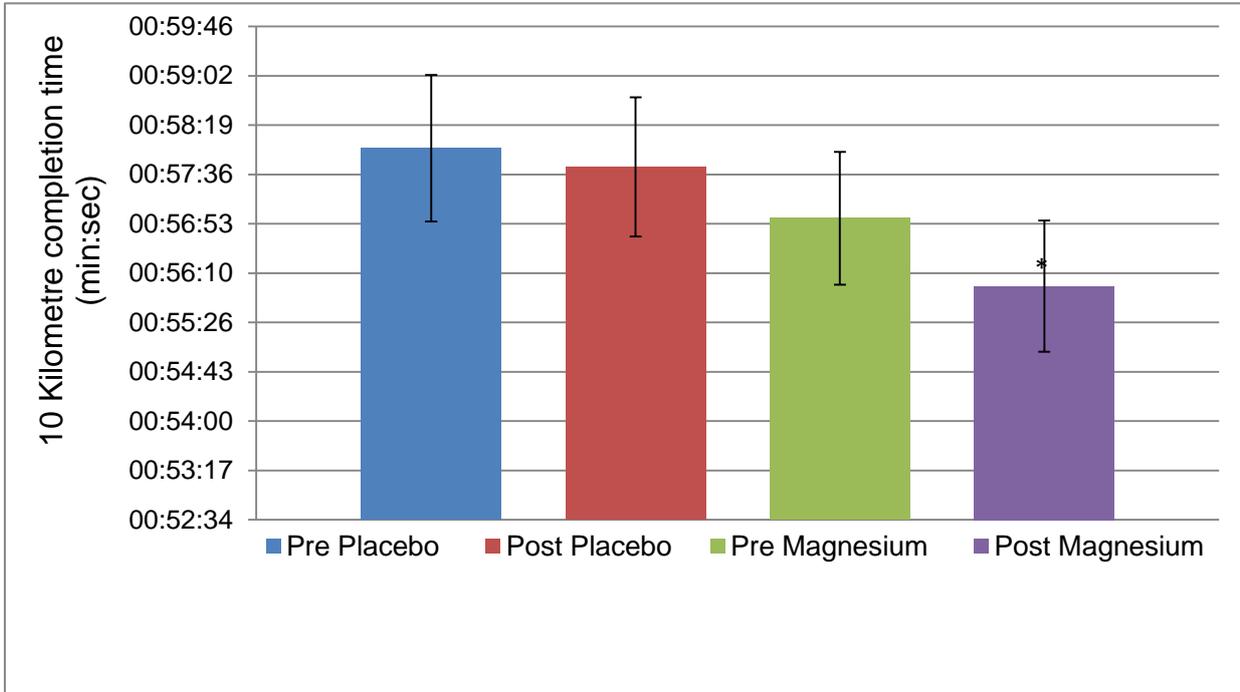
**Fig. 4. A comparison of average  $Mg^{2+}$  blood serum level (mmol/L) between both interventions at baseline and post intervention stage. Values are means  $\pm$  SEM.**

No significance was seen between any of the groups although there was a 5.7% (0.04mmol/l) increase from pre to post  $Mg^{2+}$  intervention but this did not show significance ( $P=0.741$ ).

This finding shows a trend as  $Mg^{2+}$  appears to increase after the  $Mg^{2+}$  supplementation intervention. An increase of 0.02 (3.7%) mmol/L was also seen when post  $Mg^{2+}$  stage was compared to the post placebo stage and an increase of 0.03 (4.6%) mmol/L when compared to the pre placebo stage.

10 kilometre time trial completion time

Figure 5 displays the average completion time recorded for all subjects' pre and post Mg<sup>2+</sup> and placebo interventions.



**Fig. 5. Comparisons of average completion time of a 10 kilometre run (min:sec). Values are means  $\pm$  SEM. \* = $P < 0.05$ , concluding that a significance was seen between the post Mg<sup>2+</sup> intervention and all other intervention stages.**

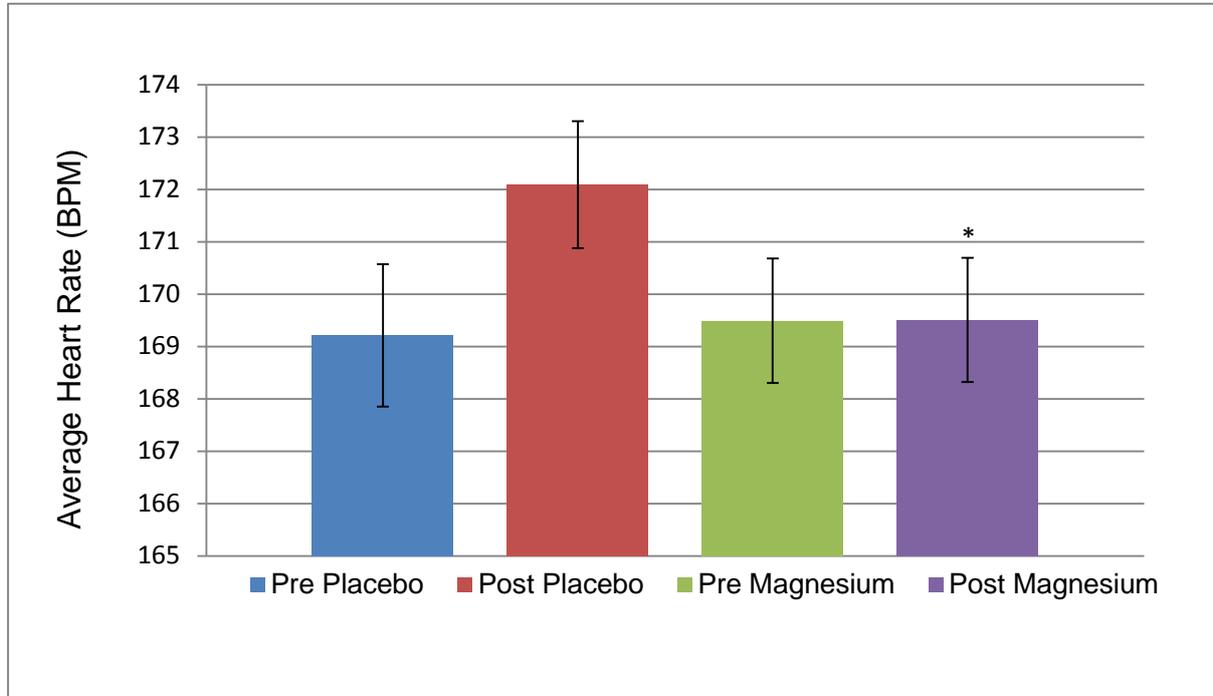
Pre Placebo completion time was recorded as 00:57:59  $\pm$  00:07:43 min:sec, post placebo completion time was recorded as 00:57:43  $\pm$  00:07:20 min:sec. Pre Mg<sup>2+</sup> completion time was recorded as 00:56:58  $\pm$  00:06:59 min:sec whilst post Mg<sup>2+</sup> completion time was recorded as 00:55:58  $\pm$  00:06:54 min:sec.

Following the Mg<sup>2+</sup> intervention there was an average decrease in 10K completion time of exactly 1 minute (1.77%) Following completion of a Repeated Measures Anova the decrease in completion time was deemed significant at  $P = 0.001$ .

When post placebo intervention was compared to pre placebo intervention a decrease of 16 seconds (0.27%) was seen in completion time, this did not show significance ( $P = 0.10$ ).

### Average heart rate

During the completion of the 10 K time trial subject's heart rate was recorded at 10 minute intervals. Figure 6 displays the average heart rate (BPM) calculated from the readings taken at each 10 minute interval that was recorded pre and post both  $Mg^{2+}$  and placebo interventions.



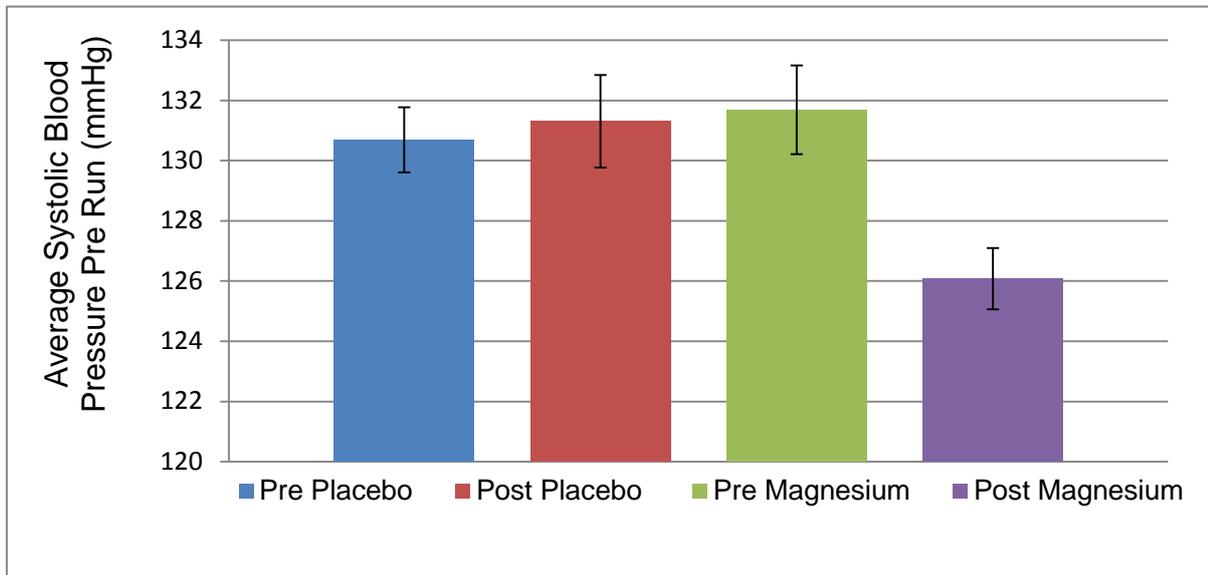
**Fig. 6. A comparison of average heart rate (BPM) during the running time trial at each intervention stage, both baseline and post intervention. Values are means  $\pm$  SEM. \* = $P < 0.05$ , concluding that a significant difference was seen between the post  $Mg^{2+}$  intervention and the post placebo intervention.**

Pre placebo average heart rate recorded was  $169.21 \pm 9.82$  BPM, post placebo average heart rate was recorded as  $172.09 \pm 8.74$  BPM, pre  $Mg^{2+}$  average heart rate was recorded at  $169.49 \pm 8.56$  BPM and post  $Mg^{2+}$  average heart rate was recorded at  $169.51 \pm 8.56$ .

Following the  $Mg^{2+}$  intervention period there was a slight decrease average heart rate by 0.01 BPM (0.007%) when post  $Mg^{2+}$  was compared to  $Mg^{2+}$  baseline. Although the difference between the pre and post  $Mg^{2+}$  interventions was not significant. A significant increase in heart rate would be expected alongside the significant decrease in 10 kilometre completion time. Significance was seen between post  $Mg^{2+}$  intervention and post placebo intervention ( $P = 0.03$ ), a decrease of 2.58 BPM (2.58%). This further supports an increase in performance and efficiency as participants achieved a significantly faster 10 kilometre completion time with a significantly lower heart rate when post  $Mg^{2+}$  and post placebo interventions are compared.

*Average systolic blood pressure pre run*

Figure 7 displays the average resting systolic blood pressure measurement (mmHg) calculated from each participant's systolic blood pressure score recorded prior to conduction of the time trial run.



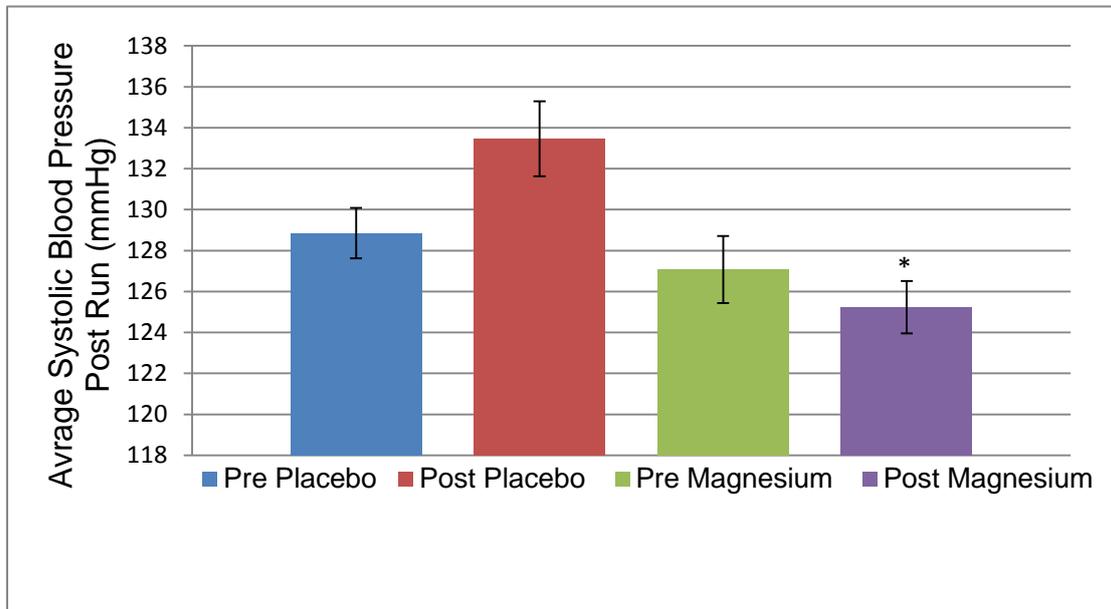
**Fig. 7. A comparison of average systolic blood pressure (mmHg) recorded before the conduction of the time trial for each intervention, both baseline and post intervention. Values are means  $\pm$  SEM.**

Average systolic blood pressure pre placebo intervention was recorded as  $130.69 \pm 7.81$  mmHg, post placebo average blood pressure was recorded as  $131.30 \pm 11.12$  mmHg. Pre  $Mg^{2+}$  average blood pressure was recorded as  $131.69 \pm 10.63$  mmHg and post  $Mg^{2+}$  average blood pressure was recorded as  $126.08 \pm 7.37$ .

Following the  $Mg^{2+}$  intervention period there was an average decrease in systolic blood pressure (mmHg) by 5.62 mmHg (4.26%) This difference was not deemed to be significant. ( $P = 0.262$ ).

*Average systolic blood pressure post run*

Figure 8 displays the average systolic blood pressure measurement (mmHg) calculated from each participant's systolic blood pressure score recorded after the completion of the time trial run.



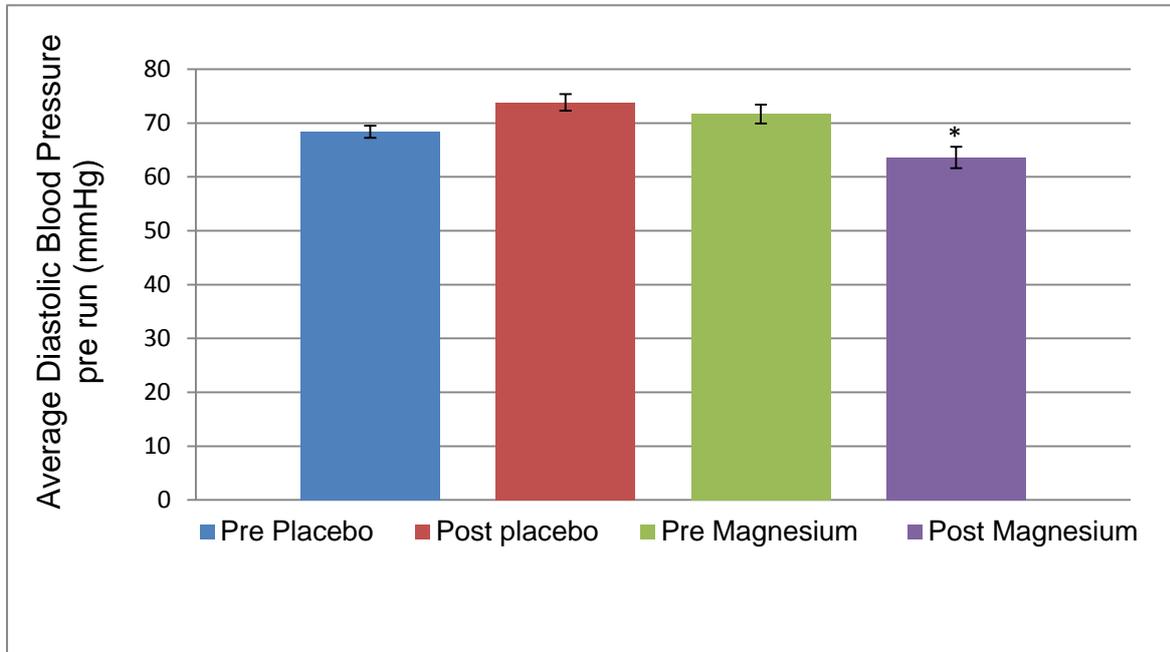
**Fig. 8.** A comparison of average systolic blood pressure (mmHg) recorded after completion of the time trial at pre and post, both  $Mg^{2+}$  and placebo interventions. Values are means  $\pm$  SEM. \*  $=P<0.05$ , concluding that a significant difference was seen between the post  $Mg^{2+}$  stage and the post placebo stage.

Average systolic blood pressure post run, recorded pre placebo intervention was  $128.85 \pm 8.88$  mmHg, post placebo average blood pressure was recorded as  $133.46 \pm 13.2$  mmHg, showing no significant change as would be expected. Pre  $Mg^{2+}$  average blood pressure was recorded as  $127.07 \pm 11.8$  mmHg, and post  $Mg^{2+}$  average blood pressure was recorded as  $125.23 \pm 9.22$  showing an average decrease in systolic blood pressure by 1.85 mmHg (1.45%) But this was still not large enough to show a significance difference.

When post  $Mg^{2+}$  was compared to the post placebo intervention there was a significant decrease of 8.23 mmHg (6.17%) ( $P=0.05$ ) The comparison made between the placebo and  $Mg^{2+}$  interventions is possible due to the similarity between the baseline readings.

*Average Diastolic blood pressure pre run*

Figure 9 displays the average diastolic blood pressure measurement (mmHg) calculated from each participant's diastolic blood pressure score recorded prior to the completion of the time trial.



**Fig. 9. A comparison of average diastolic blood pressure (mmHg) recorded prior to the completion of the time trial pre and post both  $Mg^{2+}$  and placebo interventions. Values are means  $\pm$  SEM.  $*=P<0.05$  vs. post placebo and baseline  $Mg^{2+}$  concluding a statistical significant difference.**

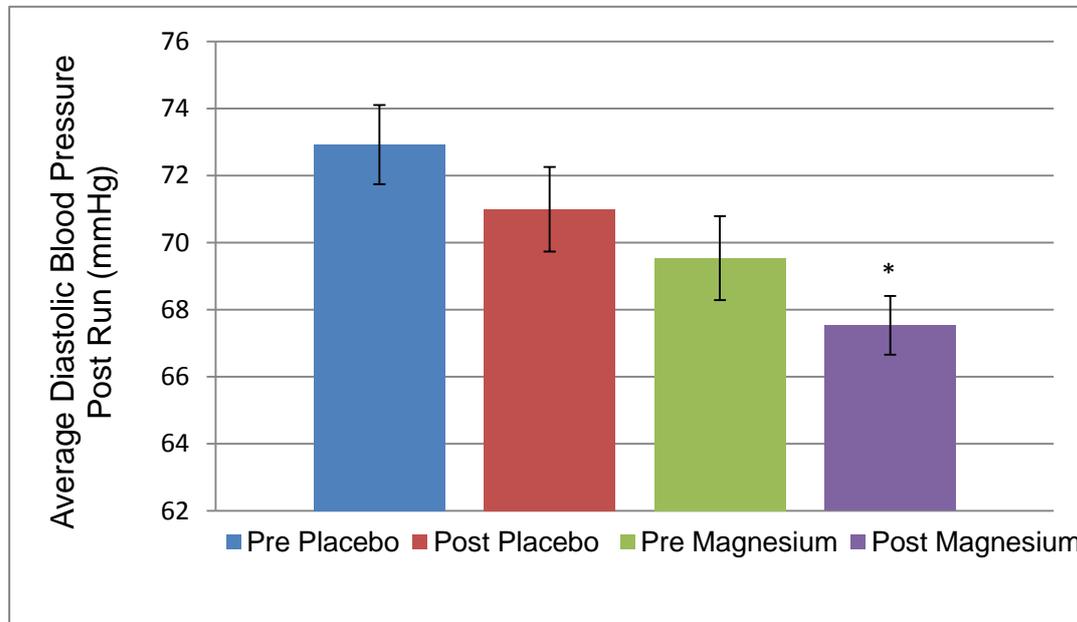
Average diastolic blood pressure pre placebo was recorded as  $68.38 \pm 7.99$  mmHg, post placebo average blood pressure was recorded as  $73.84 \pm 11.1$  mmHg, pre  $Mg^{2+}$  average blood pressure was recorded as  $71.69 \pm 12.65$  mmHg and post  $Mg^{2+}$  average blood pressure was recorded as  $63.61 \pm 14.22$  mmHg.

Following the  $Mg^{2+}$  intervention period there was an average decrease in diastolic blood pressure by 8.08 mmHg (11.26%) when post  $Mg^{2+}$  was compared to  $Mg^{2+}$  baseline. This decrease was deemed to be significant  $P=0.0004$

When pre placebo was compared to post placebo there was an increase in blood pressure 5.46 mmHg. This increase was not deemed to be significant  $P=0.19$ . In comparing post  $Mg^{2+}$  intervention to post placebo a decrease of 10.23 mmHg (13.85%) was recorded. This was the largest decrease seen between the interventions ( $P=0.03$ ).

*Average Diastolic blood pressure post run*

Figure 10 displays the average diastolic blood pressure measurement (mmHg) calculated from each participant's diastolic blood pressure score recorded after the completion of the time trial.



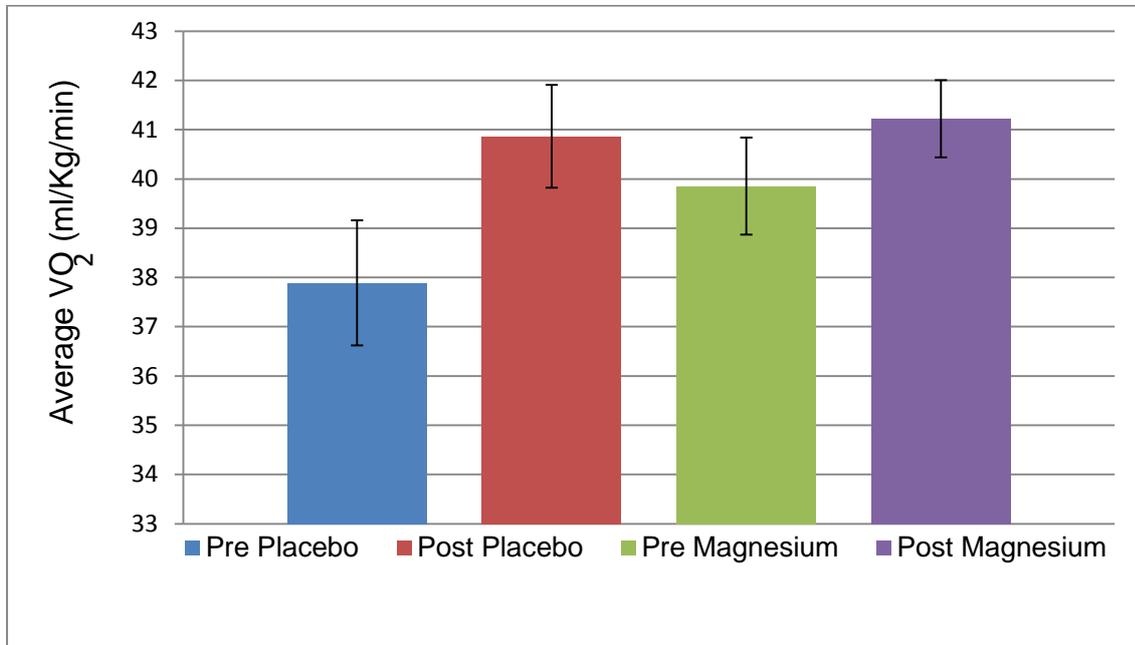
**Fig. 10.** A comparison of average diastolic blood pressure (mmHg) recorded after the completion of the running time trial at pre and post both  $Mg^{2+}$  and placebo interventions. Values are means  $\pm$  SEM.  $*=P<0.05$  when post  $Mg^{2+}$  intervention is compared pre placebo intervention concluding a statistical significant difference.

Average diastolic blood pressure post run, pre placebo was recorded as  $72.92 \pm 8.5$  mmHg, post placebo average blood pressure was recorded as  $71 \pm 9.09$  mmHg, pre  $Mg^{2+}$  average blood pressure was recorded as  $69.54 \pm 9.02$  mmHg and post  $Mg^{2+}$  average blood pressure was recorded as  $67.54 \pm 6.32$ .

Following the  $Mg^{2+}$  intervention period there was an average decrease in diastolic blood pressure (mmHg) by 2 mmHg (2.88%) when post  $Mg^{2+}$  was compared to pre  $Mg^{2+}$  intervention but no significance was found ( $P=0.41$ ). When post  $Mg^{2+}$  was compared to pre placebo intervention there was a recorded decrease of 5.38 mmHg (7.38%). This was the largest difference seen and after conduction of a repeated measure Anova it could be concluded that the reduction in diastolic blood pressure recorded at the post  $Mg^{2+}$  intervention was of significance  $P=0.008$  when compared to the pre placebo intervention stage.

### Average $VO_2$

During the time trial run  $VO_2$  (ml/Kg/min) was recorded at 10 minute intervals. Figure 11 displays the average  $VO_2$  readings recorded pre and post both  $Mg^{2+}$  and placebo interventions.

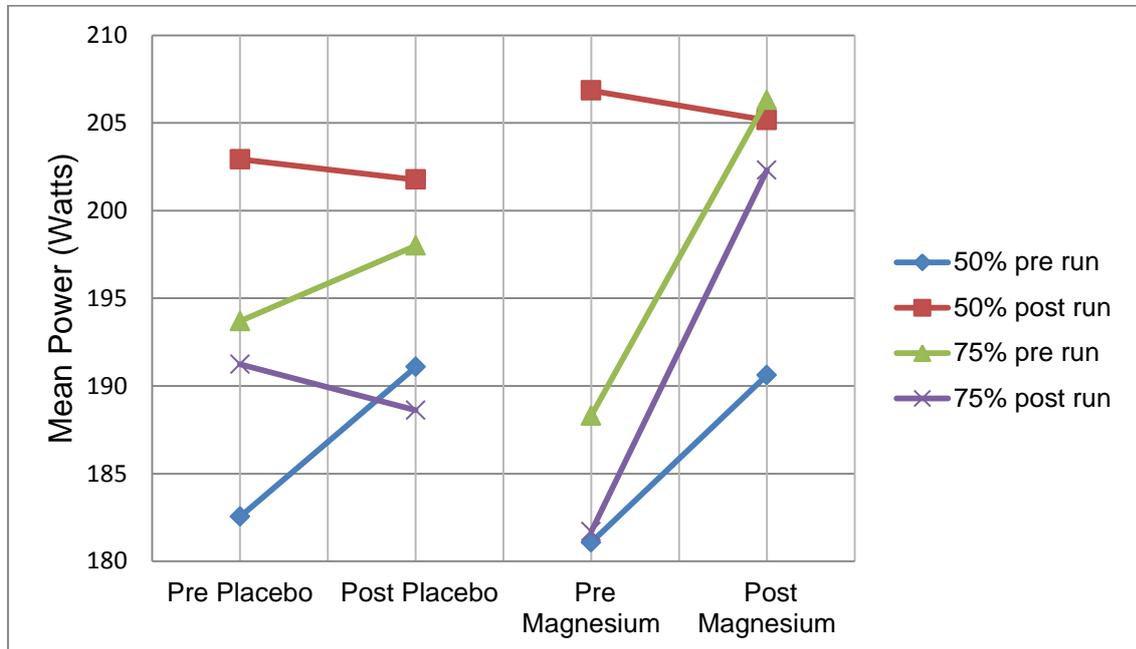


**Fig. 11.** A comparison of  $VO_2$  recorded during the running time trial (ml/Kg/min) at each intervention stage, both baseline and post intervention. Values are means  $\pm$  SEM.

Average  $VO_2$  pre placebo intervention was recorded as  $37.89 \pm 9.16$  ml/Kg/min, post placebo average  $VO_2$  was recorded as  $40.86 \pm 7.5$  ml/Kg/min showing no significant difference as would be expected. , Pre  $Mg^{2+}$  average  $VO_2$  was recorded as  $39.85 \pm 7.1$  ml/Kg/min and post  $Mg^{2+}$  average  $VO_2$  was recorded as  $41.22 \pm 5.66$  ml/Kg/min, this also did not show a significant change.

Mean power

Figure 12 displays the average mean power recorded at 75% and 50% of 1RM during the bench press trial, conducted pre and post completion of the 10K run for both Mg<sup>2+</sup> and placebo interventions.

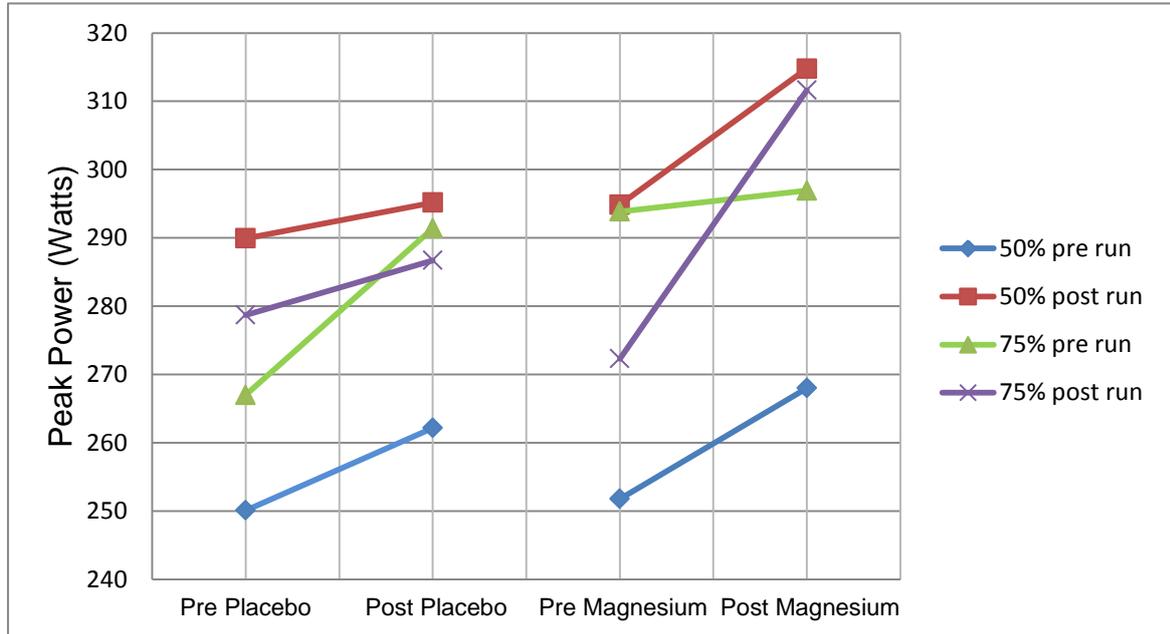


**Fig. 12. A comparison of mean power recorded pre and post the running time trial (Watts) at 75% and 50% of participants ORM at each intervention stage, both baseline and post intervention. Values are means  $\pm$  SEM.**

Following the Mg<sup>2+</sup> intervention there was increases in power in all trials except the mean power 50% post run. All results recorded had no significance.

Peak power

Figure 13 displays the average, peak power recorded at 75% and 50% of ORM during the bench press trial, recorded pre and post completion of the 10K run for both Mg<sup>2+</sup> and placebo interventions.



**Fig. 13. A comparison of peak power recorded pre and post the running time trial (Watts) at 75% and 50% of participants ORM at each intervention stage, both baseline and post intervention. Values are means ± SEM.**

Following the Mg<sup>2+</sup> intervention there was increases in power in all trials, when pre and post Mg<sup>2+</sup> interventions are compared although all results recorded had no significance. The lowest P value was seen between the pre and post Mg<sup>2+</sup> interventions at 75% of ORM after the completion of the time trial P=0.07. This shows a trend between the increases in power following Mg<sup>2+</sup> supplementation.

Throughout the protocol different variables were recorded in both the bench press trial and running time trial. Table 7 displays an overview of the mean results recorded in the bench press and running time trials.

Table 7. Average results recorded during the bench press trials and running time trials and the lowest P values seen after conduction of a Repeated Measures Anova \* = Significance found.

Variable Investigated	Pre Placebo	Post Placebo	Pre Mg	Post Mg
Breathing Frequency (RR)	54.83±7.31	56.29±8.02	55.03±8.36	57.78±6.9
Mean Force Pre Run 75% (N)	521.85±199.5	534.54±181.36	515.77±199.58	525.77±199.24
Mean Force Pre Run 50% (N)	331.69±135.63	345.84±119.64	335.69±138.26	338±138.91
Mean Force Post Run 75% (N)	514.54±199.65	540.92±181.19	504.53±203.75	519±194.67
Mean Force Post Run 50% (N)	383.46±150.5	415.69±162.19	370.76±144.25	381.07±153
Peak Force Pre Run 75% (N)	594.15±227.96	598.84±192.98	600.61±239.19	612.15±239.27
Peak Force Pre Run 50% (N)	417±169.98	434.07±155.45	426.23±174.16	412.69±170.64
Peak Force Post Run 75% (N)	602.76±241	631.76±218.41	595.84±249	621.61±263.3
Peak Force Post Run 50% (N)	473±196.5	496.61±180.33	465±194.22	472.76±193.92
Reps Pre Run 75% *	9.46±3.53	8.846±3.46	9.92±3.98	10.76±3.72
Reps Pre Run 50%	17.61±5.18	18±4.56	16.3±5	16.84±5.77
Reps Post Run 75%	7.69±3.98	7.38±3.81	8.15±2.67	8.3±2.98
Reps Post Run 50%	14.92±5.7	14.3±4.83	14±4.52	14.84±4.72
V'E (L Per Min) *	82.87±27.88	89.05±27.73	89.62±24.66	94.39±21.69
V'E (VO <sub>2</sub> ) *	27.54±3.51	28.35±3.85	28.46±3.21	29.85±2.64

Blood serum samples were collected pre and post both Mg<sup>2+</sup> and placebo interventions (mmol/l). Table 8 displays the Mg<sup>2+</sup> serum levels that were recorded both pre and post both Mg<sup>2+</sup> and placebo interventions.

Table 8. Average Mg<sup>2+</sup> serum levels recorded pre and post both Mg<sup>2+</sup> and placebo interventions (mmol/l).

Participant	Pre Placebo	Post Placebo	Pre Mg	Post Mg
	0.71 mmol/l	0.72 mmol/l	0.7mmol/l	0.74 mmol/l

Table 9. Average Mg<sup>2+</sup> consumption achieved by each participant, recoded during the four day food diary. Analysis was conducted using Dietplan6.

Subject	Gender	
1	Male	275mg
2	Male	156mg
3	Male	477mg
4	Female	264mg
6	Male	296mg
7	Male	254mg
8	Female	306mg
9	Male	219mg
12	Male	311mg
14	Male	384mg
15	Female	192mg
18	Female	243mg
19	Male	101mg

## Discussion

The current study aimed to examine the hypothesis that  $Mg^{2+}$  supplementation may have a significant performance effect on recreationally active males and females running a 10 kilometre time trial and conducting a bench press trial until fatigue when ingesting 500mg  $Mg^{2+}$  daily for a 4 week period. Alongside performance improvements this current study also looked to assess  $Mg^{2+}$  supplementations impact on blood pressure and  $Mg^{2+}$  levels in blood serum. The protocol design was based around the rationale that  $Mg^{2+}$  may facilitate the delivery of oxygen to the working muscles by the production of 2,3-diphosphoglycerate (2,3-DPG) in the erythrocyte (Lukaski, Bolonchuk, Klevay, Milne & Sandstead. 1983), and that  $Mg^{2+}$  may play a pivotal role in reducing blood pressure by its impact on vascular smooth muscle cell tone, reactivity and contractility (Laurant, & Touyz, 2000; Touyz, 2003; Barbagallo, Dominguez, Galioto, Cani, Malfa, & Paolisso, 2003).

Mineral elements, such as  $Mg^{2+}$ , are required by the human body in modest amounts for the maintenance of health and the development of optimal functioning (Lukaski, 1995).  $Mg^{2+}$  supplementation has become increasingly popular as  $Mg^{2+}$  is a cofactor to over 325 enzymatic reactions occurring within the body (Newhouse & Finstad, 2000). This suggests that deficiency of the mineral may have various physiological and exercise performance implications (Newhouse & Finstad, 2000; Wolf & Cittadini, 2003).

Results showed that a four week  $Mg^{2+}$  intervention was successful in decreasing the time taken to complete a 10 kilometre run. The significant decrease in performance time may be due to the  $Mg^{2+}$  supplementation period and various pathways, although at this point the source of the physiological adaptation is still unknown. A lot of control was put into place regarding previous exercise as most subjects were competitive runners, partaking in regular activity although less control was put into place regarding the participant's diets including alcohol consumption. The mean dietary intakes of  $Mg^{2+}$  seen in males was  $274.77mg \pm 113.15mg$ , whilst the female food diaries reported an average  $Mg^{2+}$  intake of  $251.25mg \pm 47.39mg$ . The  $Mg^{2+}$  intake recorded in the food diaries fell below the recommend daily intake of 300mg/day for adult males and 270mg/day for adult females (COMA, 1991). Altura (1994) reported that inactive females ingest approximately 175-225mg/day of  $Mg^{2+}$ , whilst Lukaski (1995) reported that female athletes would consume around 168-182mg/day of  $Mg^{2+}$ . Therefore it would seem that participants taking part in the current study were consuming intakes that were higher than the normal, but still below the recommended daily allowance. Apart from being recreationally active and between the ages of 18-39 the participants appeared to be diverse when it came to the dietary analysis. Some participants reported very healthy diets, whilst others tended to consume highly processed foods that have a lack

of  $Mg^{2+}$  content. In addition, as there was no correlation between  $Mg^{2+}$  in the diet and  $Mg^{2+}$  Serum levels, it can be assumed that there is a wide variety and variability in the way which  $Mg^{2+}$  is metabolised. This would also support the idea that  $Mg^{2+}$  supplementation would have a greater effect on some participants and less on the others. There was a significant decrease seen in the time taken to complete the 10 kilometre running time trial. Previous literature also supports this finding as studies also showed the benefits of  $Mg^{2+}$  supplementation and  $O_2$  consumption at submaximal workloads (Rose, Carroll, Lowe, Peterson & Cooper, 1970; Brilla & Haley, 1992), total workout (Vecchiet, Pieralisi, D'Ovidio, Dragani, Felzani, Mincarini, & Piovanelli, 1995) and time until exhaustion (Brilla & Gunter, 1995).  $Mg^{2+}$  supplementation has also been shown to increase submaximal performance ultimately by decreasing submaximal  $VO_2$ ,  $V_E$  and HR (Ripari, Pieralisi, Giamberardino & Vecchiet, 1989; Brilla & Gunter, 1995). Although the findings of the studies could be due to the fact that subjects were required to ingest higher amounts of  $Mg^{2+}$  or it may also be due to the fact that subjects already had a lower mean intake of  $Mg^{2+}$ . Some of the studies failed to report the baseline  $Mg^{2+}$  intakes of participants. This could also be linked to the current study as  $Mg^{2+}$  intakes were high and the participants all had low dietary intakes of  $Mg^{2+}$ . Although the current study utilised a highly controlled crossover experimental design, which may have contributed to the significant increase in performance reported. This has also been seen in other reports that also adopted a crossover design study and concluded significant results of  $Mg^{2+}$  supplementation (Vecchiet, Pieralisi, D'Ovidio, Dragani, Felzani, Mincarini, & Piovanelli, 1995). As some studies failed to assess  $Mg^{2+}$  status it is hard to conclude the validity of the reported findings, (Ripari, Pieralisi, Giamberardino & Vecchiet, 1989; Brilla and Gunter, 1995) as these studies failed to assess  $Mg^{2+}$  status prior to the supplementation intervention.

These findings are contrasted by the reported results of studies that suggest  $Mg^{2+}$  will have no impact on exercise performance (Weight, Noakes, Labadarios, Graves, Jacobs, & Berman, 1988; Ruddel, Werner, & Ising, 1990; & Weller, Bachert, Meinck, Friedmann, Bärtsch, & Mairbäurl, 1998). Weight et al (1988) also adopted a cross over design similar to the current study although they found no significant effects on performance. This may be due to the fact that they only provided participants with 116mg/day of  $Mg^{2+}$  supplementation, which may not be an adequate amount to evoke a performance increase. The current study provided subjects with 500mg/day of  $Mg^{2+}$  supplementation and did report a significant increase in performance. Furthermore Weight et al (1988) recruited subjects with baseline  $Mg^{2+}$  levels of  $372mg \pm 122mg$ , which is reported to be an adequate  $Mg^{2+}$  daily intake. This may provide evidence to the theory that  $Mg^{2+}$  supplementation will only have a significant effect on performance if the individual is deficient in  $Mg^{2+}$ , as subjects in the current study were below the RNI and did show significant improvements in performance after an  $Mg^{2+}$

supplementation period, whilst the subjects partaking in Weight et al, (1988) were above the RNI and failed to show performance improvements.

Even though subjects in the current study were consuming less  $Mg^{2+}$  than the recommended daily intake it cannot be concluded that they had a deficient  $Mg^{2+}$  status as there is still no simple, rapid and accurate laboratory test to determine total body Mg status in humans (Arnaud, 2008). However, serum  $Mg^{2+}$   $<0.75$  mmol/L is a useful measurement for deficiency (Arnaud, 2008). The current study did require participants to provide a venous blood sample so that the  $Mg^{2+}$  status of participants could be assessed. Average  $Mg^{2+}$  serum was recorded at  $0.71 \pm 0.05$  mmol/L at baseline stage and increased to  $0.74 \pm 0.09$  mmol/L post  $Mg^{2+}$  supplementation stage. Even though an increase was seen after  $Mg^{2+}$  supplementation the average serum level was still below 0.75 mmol which would suggest participants were still deficient in  $Mg^{2+}$  after five weeks of 500mg/day  $Mg^{2+}$  supplementation, although this may also support the theory that  $Mg^{2+}$  will only have a significant increase on performance in deficient subjects as significant improvements were seen in the current study and after acquiring and analysing blood samples and food diaries, the majority of participants appear to be deficient.

### 10 kilometre run

The significant decrease seen in completion times of the 10 kilometre time trials support the hypothesis that 500mg/day of  $Mg^{2+}$  supplementation will reduce the time taken to complete a 10 kilometre run. This is demonstrated by the fact that results recorded post  $Mg^{2+}$  supplementation period were significantly faster when compared to the  $Mg^{2+}$  baseline stage, the post placebo stage and the baseline placebo stage. These findings conflict previous research that has been conducted into the effects of  $Mg^{2+}$  supplementation on running performance. Terblanche et al (1992) conducted a double blind placebo controlled study over a 10 week period. They recruited 20 experienced marathon runners and analysed the effects of  $Mg^{2+}$  supplementation on completion of a marathon, muscle damage incurred and the rate of recovery of muscle function following the marathon race. Terblanche et al, (1992) concluded that the consumption of 300-400mg<sup>2+</sup>/day supplementation for four weeks prior to the run had no effect on any of the variables. This confliction to the current study may be due to several different variables. Terblanche et al, (1992) failed to ensure that participants completed a marathon at baseline stage, instead they asked participants to provide their fastest marathon completion time which may have resulted in invalid data as the data provided will be subject to participant bias. Although the research protocol did require subjects to complete two five kilometre time trial runs, one prior to the marathon and one post completion of the marathon. The two five kilometre trials were conducted outside, which

may also affect the validity of the results as there would have been reduced control over external variables of the trial which could have been controlled if the run was conducted in a laboratory environment.

Weight, Kathryn & Noakes (1988) also support the null hypothesis suggesting that  $Mg^{2+}$  will have no impact on sports performance. Weight, Kathryn and Noakes (1988) recruited thirty competitive male athletes to complete a treadmill test to exhaustion after 0, 3, 6, and 9 months of  $Mg^{2+}$  supplementation. It was concluded that  $Mg^{2+}$  supplementation had no effect on performance in the treadmill trial.

There is a lack of evidence that suggests that  $Mg^{2+}$  supplementation will improve the performance of individuals that have an established magnesium-adequate status (Nielsen & Lukaski, 2006). This may provide an explanation as to why  $Mg^{2+}$  supplementation has not induced performance increases in previous research. The differences in  $Mg^{2+}$  status may be the reason for the numerous conflicting reports about the effect of  $Mg^{2+}$  supplementation on exercise performance (Nielsen & Lukaski, 2006). Because many studies have failed to attempt to assess the  $Mg^{2+}$  status of participants or used insensitive methods to assess, the  $Mg^{2+}$  status of individuals before commencing with supplementation. This may have resulted in positive effects being seen in only the individuals that had a deficient  $Mg^{2+}$  status before the supplementation period started.

Golf, Bohmer and Nowacki, (1993) concluded that a  $Mg^{2+}$  supplementation of  $360mg^{2+}/day$  when compared to a placebo supplement decreased creatine kinase levels in the blood serum of competitive female athletes with baseline  $Mg^{2+}$  concentrations at the low end of the range of normal values. Furthermore in a study of young men participating for seven weeks in a strength training programme and consuming  $250mg/day$  of  $Mg^{2+}$  (most likely inadequate), showed a greater increase in peak knee extension torque when supplemented with an additional  $250mg/d$  than those fed a placebo supplement (Brilla & Haley, 1992). Similar types of intakes by moderately trained adults resulted in those receiving a supplemental  $250mg/day$  (instead of placebo) having improved cardiorespiratory function during a 30-minute submaximal exercise test (Ripari, Giamberardino, Resina & Vecchiet, 1989).

The current study aimed to assess the  $Mg^{2+}$  status of participants prior to supplementation through the use of food diaries and venous blood samples. This was in an attempt to understand the connection between  $Mg^{2+}$  status and the effectiveness of extra supplementation if  $Mg^{2+}$  intake is already adequate. The food diaries concluded that four of the participants achieved adequate  $Mg^{2+}$  statuses through their diets resulting in the other nine participants having deficient  $Mg^{2+}$  statuses. The sample sizes of four adequate

participants and nine deficient participants are too small to be a statistically valid sample. As nine of the thirteen participants had a deficient  $Mg^{2+}$  status the decrease in completion of the 10 kilometre timed run may support the theory that  $Mg^{2+}$  supplementation will be effective if the individual is deficient. Although as the sample sizes are too small to provide validity, statistical analysis was not conducted on participants relevant to their  $Mg^{2+}$  status. So it cannot be concluded that the  $Mg^{2+}$  supplementation increased the performance of the deficient subjects, whilst having no effect on the participants with an adequate  $Mg^{2+}$  status. This may provide a research question as more research is needed into the effects of  $Mg^{2+}$  supplementation in relation to dietary status.

Contrary to the above there is also literature to support the reduction in 10 kilometre run completion time. Previous literature has reported that  $Mg^{2+}$  deficiency will impair sports performance by increasing oxygen requirements to conduct submaximal exercise (Rodriguez, DiMarco & Langley; 2009). Golf et al, (1993) reported that elite male rowers with serum  $Mg^{2+}$  concentrations at the low end of the range of normal serum values used significantly less oxygen during a submaximal rowing ergometer test after  $Mg^{2+}$  supplementation when compared to placebo supplementation. Lukaski and Nielsen (2001) also support this finding as they reported that during a submaximal ergocycle, postmenopausal women showed reduced oxygen use, decreased heart rate and improved ventilatory functioning after consuming either 322 or 360 $Mg^{2+}$ /day for a 35 day period when compared to 153 $Mg^{2+}$ /day pre intervention.

Pokan et al, (2006) further provide evidence to support the hypothesis that  $Mg^{2+}$  supplementation will have a positive impact on physical performance. Pokan et al, (2006) studied the effects of a six month oral  $Mg^{2+}$  therapy on exercise dependant heart rate as related to exercise tolerance and resting myocardial functioning. In a double blind controlled trial fifty three male participants were randomised to either oral  $Mg^{2+}$  or placebo supplementation. The six months of  $Mg^{2+}$  supplementation significantly increased the power output achieved in an incremental exercise test on a cycle ergometer, in an upright position to the limit of tolerance, demonstrating that the  $Mg^{2+}$  therapy induced an improvement in aerobic power. The exercise test started at an initial workload of 20 W followed by 10 W increments every minute until exhaustion.

The mechanisms responsible for the performance increases, seen due to  $Mg^{2+}$  supplementation are likely to be a result of multifactorial reasons (Shechter et al., 2000). Higher intracellular  $Mg^{2+}$  levels may improve intracellular adenosine triphosphate production and glucose utilisation because  $Mg^{2+}$  is a cofactor of adenosine triphosphate (Shechter et al., 2000).  $Mg^{2+}$  has also been reported as nature's physiological calcium blocker (Iseri &

French, 1984; Shechter, Kaplinsky & Rabinowitz, 1992) reducing the release of calcium from and into the sarcoplasmic reticulum whilst protecting the cells from calcium overload under conditions of ischaemia.  $Mg^{2+}$  also reduces systemic and pulmonary vascular resistance with a concomitant decrease in blood pressure and a slight increase in the cardiac index (Oczek, Lee & Davidov, 1977; Iseri & French, 1984; Rasmussen, Larsen, meier & Larsen, 1988; Shechter et al, 2000). Elevation of extracellular  $Mg^{2+}$  levels reduces arteriolar tone and tension in a wide variety of arteries (Shechter, Kaplinsky & Rabinowitz, 1996). This then potentiates the dilating effects of some of the endogenous vasodilators (Shechter, Kaplinsky & Rabinowitz, 1996). Therefore  $Mg^{2+}$  can result in mildly reduced systolic blood pressure, thereby assisting in unloading of the ischaemic ventricle (Shechter, Kaplinsky & Rabinowitz, 1996; Whelton, & Klag, 1989; Pokan, Hofmann, Duvillard, Beaufort, Schumacher, Fruhwald, & Schmid, 1997; Shechter et al, 2000).

Furthermore the improved completion time of the 10 kilometre run may be due to the  $Mg^{2+}$  vasodilation effect. Teragawa (2001) also concluded that magnesium supplementation will dilate both the epicardial and resistance coronary arteries. The mechanism that is potentially responsible for magnesium induced coronary dilation involves the activation of the endothelium derived nitric oxide (ENDO) pathway (Altura & Altura, 1987; Kemp, Gardiner & Bennett, 1993; Pearson, Evora & Secombe, 1998; Fonseca, Paiva & Silva, 1998). It is unclear as to whether the EDNO pathway is involved in the human coronary responses to magnesium. The nitric oxide (NO) pathway has numerous functions in the body, including the regulation of blood flow, muscle contractility, myocytes differentiation, glucose and calcium homeostasis, mitochondrial respiration and biogenesis (Stamler & Meissner, 2001; Dejam, Hunter, Schechter & Gladwin, 2004; Cooper & Giulivi, 2007).

This finding provides further support to the alternative hypothesis concluding that  $Mg^{2+}$  supplementation will have a positive impact on aerobic exercise reducing the time taken to complete a 10 kilometre run. This supports the vasodilation theory as the vasodilation effect would allow an increased flow of blood to the muscles, increasing oxygen supply and muscular contraction, ultimately aiding in completion of the running time trial.

Heaton and Elie (1983) conducted experiments using rat liver mitochondria to show that  $Mg^{2+}$  depletion in rats lead to a partial uncoupling of the respiratory chain, as evidence by a reduced ADP/ $O_2$  quotient (Heaton & Elie, 1983). A disturbance in mitochondrial inner membrane function was responsible for this uncoupling effect. In a state of  $Mg^{2+}$  deficiency the permeability of mitochondrial membranes might increase, causing a disruption of the specific proton gradient required for successful ATP synthesis. Partial uncoupling of the respiratory chain and oxidative phosphorylation will be the result. Digiorgio et al (1962) have

also described similar effects, that  $Mg^{2+}$  deficiency will have on the mitochondrial respiratory chain, whilst Digiorgio et al, (1962) also described similar effects occurring in the heart muscle cells. If these effects of  $Mg^{2+}$  deficiency and its normalisation can be transferred to the human muscle cell, then a reduced  $O_2$  requirement should occur in the competitive athlete during physical stress after a period of  $Mg^{2+}$  supplementation. This finding brings to light the importance of adequate  $Mg^{2+}$  in the diet and could be associated with the reduction in completion time seen in the 10 kilometre run and other previous research that has reported performance improvement.

### $VO_2$ consumption

During the completion of the 10 kilometre run subjects were connected to a cortex analyzer so that gas analysis could be conducted during the run. Results concluded there to be an increase in average  $VO_2$  after four weeks of consumption of 500mg/day  $Mg^{2+}$ . The increase in average  $VO_2$  was seen when post  $Mg^{2+}$  was compared to the baseline  $Mg^{2+}$  stage, the post placebo stage and the pre placebo stage, although the increase recorded was not statistically significant. This finding would support the hypothesis that  $Mg^{2+}$  supplementation will increase  $VO_2$  consumption after four weeks of  $Mg^{2+}$  supplementation.

The reported increase in  $VO_2$  seen during the 10 kilometre run is conflicted by previous research that suggest  $Mg^{2+}$  supplementation will have no effect on  $VO_2$  consumption during aerobic exercise (Weight, Noakes, Labadarios, Graves, Jacobs, & Berman, 1988; Ruddell, Werner & Ising, 1990; Weight, Myburgh, & Noakes, 1988; Terblanche, Noakes, Dennis, Marais, & Eckert, 1992; Weller, Bachert, Meinck, Friedmann, Bärtsch, & Mairböurl, 1998). Weight et al (1988) conducted a nine month placebo controlled crossover design study to determine whether supplementation would have a significant increase on performance of thirty competitive male athletes. After three months of supplementation it was concluded that there was no significant effect of  $Mg^{2+}$  supplementation. Weight et al (1988) failed to assess  $Mg^{2+}$  status prior to the completion of the supplementation period; this may explain why there was no significant improvement in performance as the participants may have all had an adequate  $Mg^{2+}$  status prior to the supplementation intervention period. Ruddell et al, (1990) also completed a three month double blind controlled trial into the effects of  $Mg^{2+}$  supplementation on swimming performance. Ruddell et al (1990) also reported no significant increase in performance even when participants received 468 mg/day of  $Mg^{2+}$  aspartate. This finding may also be due to participants having an adequate  $Mg^{2+}$  status, although the studies design lacked control as the protocol failed to be of a crossover design. Weller (1998) also conflicts the finding of the current study as no significant improvements in performance were seen when participants received 500 mg/day of  $Mg^{2+}$  oxide for a three

week duration in a double blind placebo controlled study. Weller (1998) also failed to assess dietary status of participants prior to supplementation removing validity from the results. The supplementation period lasted a duration of three weeks which may not be enough time for adaptation to have an effect, hence why no significant improvements in performance were recorded.

This increase in average  $VO_2$  seen after the  $Mg^{2+}$  intervention period has been reported before by previous literature that has found a relationship between plasma magnesium and maximal oxygen consumption in trained athletes (Lukaski, Bolonchuk, Klevay, Milne & Standstead, 1983). Lukaski et al (1983) took forty-four healthy male university athletes and twenty untrained men and put them through maximal treadmill exercise testing aiming to determine the relationship between maximal oxygen consumption and  $Mg^{2+}$  status. Lukaski et al (1983) concluded that average maximal oxygen consumption was significantly higher post supplementation period and that plasma  $Mg^{2+}$  was significantly correlated with oxygen consumption. This may suggest the possibility of a metabolic role for  $Mg^{2+}$  during a state of strenuous exercise other than its already acknowledged role as a co-factor to over 325 enzymatic reactions and in neuromuscular functions (Wacker & Parisi, 1968; Shils, 1980; Newhouse & Finstad, 2000).

### Blood Pressure

The current study also aimed to research the hypothesis suggesting that  $Mg^{2+}$  supplementation will have a significant effect on blood pressure. Blood pressure readings were recorded during the clinical trial both before and after the 10 kilometre run. Previous research has both supported (Dyckner & Wester, 1983; Sanjuliani, Fagundes & Francischetti, 1996; Kawasaki, Itoh, & Kawasaki, 1998; Kawano et al 1998; Sacks et al, 1998; Hatzistavri, Sarafidis, Georgianos, Tziolas, Aroditis, Zebekakis, & Lasaridis, 2009) and conflicted (Cappuccio, Markandu, Beynon, Shore, Sampson, & MacGregor, 1985; Patki, Singh, Gokhale, Bulakh, Shrotri, & Patwardhan, 1990; Zemel, Zemel, Urberg, Douglas, Geiser, & Sowers, 1990; Ferrara, Iannuzzi, Castaldo, Iannuzzi, Russo, & Mancini, 1992) the effects that  $Mg^{2+}$  supplementation has on blood pressure.

Hatzistavri et al, (2009) concluded that  $Mg^{2+}$  supplementation has a significant impact on reducing blood pressure, which supports the findings of the current study that also found a significant reduction in blood pressure after a five week  $Mg^{2+}$  supplementation period. The significant decrease in diastolic blood pressure was recorded both before and after to the 10 kilometre running trial whereas the significant decrease seen in the systolic blood pressure was only seen following the run. As a result of this conclusion the alternative hypothesis may

be accepted suggesting that  $Mg^{2+}$  supplementation will invoke a significant decrease in blood pressure.

Zemel et al, (1990) conducted a clinical trial into the effects of  $Mg^{2+}$  supplementation on blood pressure, thirteen patients with mild hypertension were analysed for a three month period seven of which were supplemented with 40 mmol of  $Mg^{2+}$  aspartate whilst the remainder were supplemented with placebo. It was concluded after the supplementation period there was no significant decrease seen in blood pressure. Ferrara (1992) further supported this finding after conducting a double-blind parallel clinical trial for a period of six months. Fourteen mild to moderate hypertensives' were randomly given either  $Mg^{2+}$  or placebo supplementation. These studies may have failed to find positive results due to their methods lacking a crossover design. It has also been suggested that  $Mg^{2+}$  supplementation will only affect blood pressure if the individual is deficient in  $Mg^{2+}$ . As it is difficult to assess  $Mg^{2+}$  status and the studies failed to attempt this in their methods, it may be suggested that this variable may be responsible for a lack of reduction seen in blood pressure.

A meta-analysis conducted by Kass, Weekes and Carpenter (2012) conflicts the research that suggests  $Mg^{2+}$  supplementation will have no impact on blood pressure. Kass et al, (2012) conducted a meta-analysis to assess the effect of  $Mg^{2+}$  supplementation on blood pressure. The analysis included 22 different trials with a supplemented elemental  $Mg^{2+}$  range of 120-973mg. After statistical analysis was conducted it was concluded that  $Mg^{2+}$  supplementation had a dose dependant effect on blood pressure. Studies that provided subjects with  $>370\text{mg/day}$   $Mg^{2+}$  recorded greater reductions in blood pressure.

Hatzistavri et al, (2009) studied the association between blood pressure levels and serum  $Mg^{2+}$  analysing if there was a correlation between the levels of  $Mg^{2+}$  found in blood serum and a change in the blood pressure readings. They took measurements of intracellular  $Ca^{2+}$  and  $Mg^{2+}$  alongside the blood pressure via blood samples collected in a vein catheter.

It was concluded after correlation analysis that there was a weak-to-moderate non-significant association between the changes in blood pressure and the changes in the serum intracellular ion levels in the intervention group, which would suggest that the relationship between  $Mg^{2+}$  serum levels and blood pressure needs to be researched in greater depth. As the current study also required subjects to provide a venous blood sample, correlation analysis could be conducted to provide further assessment into the relationship between serum  $Mg^{2+}$  and blood pressure. Correlation analysis revealed a significant, moderate negative correlation between blood pressure and serum  $Mg^{2+}$  ( $r = -0.5$ ). This also supports the idea that there may be a connection between the two variables, because as  $Mg^{2+}$  serum levels increase within the blood, blood pressure levels appear to drop accordingly. As the

correlation between the two variables is only moderate further research is required to establish if there is a definitive connection between serum  $Mg^{2+}$  status and blood pressure, although this finding is supported by previous literature and supports the findings of the current trial (Hatzistavri, Sarafidis, Georgianos, Tziolas, Aroditis, Zebekakis, & Lasaridis, 2009).

Despite the clinical and experimental research on the field, several aspects concerning the exact mechanisms through which  $Mg^{2+}$  exerts its BP-lowering effects still remain unclear (Hatzistavri, Sarafidis, Georgianos, Tziolas, Aroditis, Zebekakis, & Lasaridis, 2009). Among the several different mechanisms proposed, a pivotal role could be due to the impact of  $Mg^{2+}$  on vascular smooth muscle cell tone, reactivity and contractility (Laurant, & Touyz, 2000; Touyz, 2003; Barbagallo, Dominguez, Galioto, Cani, Malfa, & Paolisso, 2003). Inside the vascular smooth muscle cells,  $Mg^{2+}$  is said to act extracellularly by inhibiting transmembrane  $Ca^{2+}$  transport and  $Ca^{2+}$  entry, or acting intracellularly as a  $Ca^{2+}$  antagonist, thereby modulating the vasoconstriction action of increased intracellular  $Ca^{2+}$  (Touyz, 2003; Mubagwa, Gwanyanya, Zakharov, & Macianskiene, 2007). Furthermore, the influence of  $Mg^{2+}$  on vascular tone and reactivity could also be mediated by its role on the Na/K ATPase activity, which regulates the transmembrane  $Na^+$  and  $K^+$  transport (Touyz 2003; Barbagallo, Dominguez, Galioto, Cani, Malfa, & Paolisso, 2003; Mubagwa, Gwanyanya, Zakharov, & Macianskiene, 2007).

$Mg^{2+}$  has been said to play an important role in the regulation of various cation channels in the cardiovascular system. (Mubagwa, Gwanyanya, Zakharov, & Macianskiene, 2007) The proposed mechanisms underlying the effect of  $Mg^{2+}$  involve either a direct interaction with the channel, or an indirect modification of channel functioning via various proteins, such as enzymes and G proteins, which have been reported to covalently modify or interact with the channel (Mubagwa, Gwanyanya, Zakharov, & Macianskiene, 2007).

The theory that  $Mg^{2+}$  plays a vital role in the regulation of Na/K ATPase activity is supported by previous research (Fischer & Giroux, 1987). Fischer & Giroux (1987) fed Dawley rats a basal diet containing 200, 350, 500, or 650mg of  $Mg^{2+}$  per kilogram of bodyweight for a duration of 6 weeks. Fischer & Giroux reported ATPase activity to increase with increasing dietary intake, so the rats that were fed 500mg and 650mg of  $Mg^{2+}$ /Kg had significantly greater ATPase activity than those that were fed the diets containing 80mg and 200mg of  $Mg^{2+}$ /Kg.

This data provides direct evidence that  $Mg^{2+}$  deficiency decreases the activity of the sodium potassium pump in cardiac tissue (Fischer & Giroux, 1987). Although as the Fischer and Giroux (1987) study was conducted on animals it cannot be assumed the same results will

occur in humans. The current study may also provide further support to the theory that  $Mg^{2+}$  deficiency reduces the activity of the sodium potassium pump.

A state of  $Mg^{2+}$  deficiency may lead to abnormal functioning of the  $Na^+/K^+$ -ATPase pump, resulting in an abnormally low plasma potassium concentration. A state of abnormally low plasma is more commonly referred to as hypokalemia, which is most commonly caused by the use of some diuretics, some forms of kidney disease, or metabolic disturbances. These symptoms have been related to alterations in membrane potential and cellular metabolism, including fatigue, muscle weakness and cramps, which would ultimately lead to a reduction in sports performance (Sheng, 2000).

The current study shows that  $Mg^{2+}$  supplementation will have a lowering effect on diastolic blood pressure and systolic blood pressure, although as there is conflict within the literature further analysis is needed for  $Mg^{2+}$  supplementations effect on blood pressure to be conclusive.

### Heart Rate

During completion of the 10 kilometre run, participant's heart rate was recorded in 10 minute intervals. After completion of the study it was concluded that there was a significant variation in heart rate when the post  $Mg^{2+}$  results were compared to the post placebo results. Therefore the alternative hypothesis can be accepted, as  $Mg^{2+}$  supplementation appears to have a significant impact on heart rate.

Few previous clinical trials have analysed the effects that oral  $Mg^{2+}$  supplementation has on heart rate/myocardial functioning. The current study concluded there to be a significant variation in heart rate after completion of the  $Mg^{2+}$  supplementation period. This may provide evidence of an increased level of performance as there was a significant decrease in time taken to complete the 10 kilometre running trial. Participants of the study completed the 10 kilometre run significantly faster after the  $Mg^{2+}$  supplementation period. This increase in performance would have resulted in increased physical exertion, suggestively leading to an increased average heart rate. The significant decrease seen in average heart rate after the  $Mg^{2+}$  supplementation period may show an increased efficiency of the heart, resulting in participants being able to increase physical exertion, whilst the heart rate is reduced significantly.

Pokan et al, (2006) concluded there to be a reduction in heart rate during a maximal exercise test after an  $Mg^{2+}$  supplementation intervention. The investigators also concluded there to be a significant alteration in heart rate response in the  $Mg^{2+}$  group after the

intervention, with no change recorded in the placebo group. Finally Poken et al, (2006) also conducted an echocardiography on subjects and a significant change was seen in left ventricular end systolic diameter (LVSD) after the  $Mg^{2+}$  intervention period. The left ventricular, end systolic diameter reduced from  $36.1 \pm 0.5$  before intervention to  $31.5 \pm 0.4$  following the intervention period. These findings may provide a rationale as to why there was no significant increase in heart rate related to the significant decrease in completion time of the 10 kilometre run.

All participants that took part in the Poken et al, (2006) clinical trial had a deficient  $Mg^{2+}$  status at baseline observation. This may be responsible for the significant differences recorded after 6 months of oral  $Mg^{2+}$  supplementation. Again this increase in  $Mg^{2+}$  status may have also improved intracellular adenosine triphosphate production and glucose utilisation due to the fact that  $Mg^{2+}$  is a co-factor of adenosine triphosphate (Shechter, Paul-Labrador & Rabinowitz, 1998), ultimately resulting in an increase in performance.

Although these findings support the alternative hypothesis suggesting  $Mg^{2+}$  supplementation will have a positive effect on heart rate/myocardial functioning, a clinical trial conducted by Dyckner & Wester (1983) provides confliction. Dyckner and Wester (1983) also conducted analysis into the effects of  $Mg^{2+}$  supplementation on heart rate, after a six month  $Mg^{2+}$  intervention period, during which subjects were supplemented with 15 mmol/day of  $Mg^{2+}$  aspartate. Following the six month intervention period it was concluded that there was no significant difference in heart rate (Dyckner & Wester, 1983). Dyckner & Wester (1983) failed to assess the  $Mg^{2+}$  status of participants prior to the intervention period. This may be a limitation to the clinical trial, as it may have been that the participants had a deficient  $Mg^{2+}$  status prior to conduction of the study, resulting in no benefit from further  $Mg^{2+}$  supplementation. This may be why Dyckner and Wester (1983) recorded no positive effects from the  $Mg^{2+}$  intervention.

### Blood Serum

Assessing  $Mg^{2+}$  status accurately is difficult as clinical tests of  $Mg^{2+}$  status are still lacking, therefore it is hard to diagnose magnesium deficiency (Jahnen-Dechent & Ketteler, 2012). Evaluation of serum  $Mg^{2+}$  concentration and collection of urine samples are the most accurate assessments available at this present time, for the diagnosis of  $Mg^{2+}$  deficiency. Approximately 1% of the total body magnesium is present in the serum and intestinal bodily fluids (Elin, 1987). The mean serum magnesium concentration in humans is roughly 0.85 mmol/L, with a referenced interval of 0.7-1. mmol/L (Wacker, 1980). In serum, approximately one third of  $Mg^{2+}$  is bound to protein, 25% is bound to albumin and 8% to globulins (Kroll & Elin, 1985). For the two thirds of the plasma  $Mg^{2+}$  that is ultrafiltrable, approximately 80% is

in the form of free ion and approximately 20% is complexed to phosphate, citrate and other compounds (Walser, 1967). The current study aimed to test the hypothesis that  $Mg^{2+}$  supplementation will have a significant increase on  $Mg^{2+}$  serum levels following a four week intervention period. Previous research into the effects of  $Mg^{2+}$  on blood serum levels is contradictory, with some studies supporting (Cappuccio, Markandu, Beynon, Shore, Sampson & MacGregor, 1985; Itoh, Kawasaki & Nakamura, 1997; Hatzistavri et al, 2009) whilst other studies (Zemel et al, 1990; Geleijnse et al, 1994; Wirell et al, 1994 & Witteman et al, 1994) reject the idea that  $Mg^{2+}$  levels in the serum could be affected by  $Mg^{2+}$  supplementation. The current study also required participants to complete a four day food diary, to assess  $Mg^{2+}$  status prior to the  $Mg^{2+}$  intervention period. Although several studies have suggested that dietary  $Mg^{2+}$  will not correlate with serum magnesium levels because renal  $Mg^{2+}$  handling affects total body serum  $Mg^{2+}$  more than dietary consumption of  $Mg^{2+}$  (Chakraborti, Chakraborti, Mandal, Mandal, Das & Ghosh, 2002).

Itoh, Kawasaki & Nakamura, (1997) contradict this idea as the results of a double blind placebo controlled study indicated that, in middle aged subjects of both sexes, oral  $Mg^{2+}$  supplementation for a period of four weeks can produce significant increases in serum levels of  $Mg^{2+}$ . The significant increase recorded was seen in subjects that had an adequate baseline level of  $Mg^{2+}$  (0.85 mmol/l) and were consuming adequate intakes of  $Mg^{2+}$  through their diets (225 mg/d). These findings conflict the findings of the current study, although the daily  $Mg^{2+}$  dosage was higher, (548mg Mg/d) which may have been related to the significant increases seen in  $Mg^{2+}$  serum levels.

Hatzistavri et al (2009) also provided subjects with high dosages of  $Mg^{2+}$  supplementation (600mg mg/day), throughout the clinical trial. Hatzistavri et al (2009) also concluded there to be a significant increase in serum level  $Mg^{2+}$  after a twelve week supplementation period. This confliction to the current clinical trial may be due to the higher  $Mg^{2+}$  supplementation or longer duration of the supplementation intervention as the current trial only required 500mg  $Mg^{2+}$ /day for a four week duration.

As no significant difference in  $Mg^{2+}$  serum level was recorded in the current trial the null hypothesis was accepted suggesting that  $Mg^{2+}$  supplementation had no effect on serum levels of  $Mg^{2+}$ . This is contradictory, as trials that have reported a significant decrease in blood pressure, usually also report a significant increase in  $Mg^{2+}$  serum levels (Itoh, Kawasaki & Nakamura, 1997; Hatzistavri et al, 2009). The lack of significant increase in  $Mg^{2+}$  serum levels seen in the current study may be due to the body storing the supplemented  $Mg^{2+}$  in bone or muscle storage, as blood serum is known to contain only 1%

of total body  $Mg^{2+}$  (Elin, 1987) and participants showed signs of deficiency with baseline serum levels and food diaries, showing  $Mg^{2+}$  intakes of less than 300mg  $Mg^{2+}$ /day.

### Power

A bench press till fatigue trial was conducted both pre and post the 10 kilometre run. Participants were required to bench press (until fatigue) 75% of their ORM followed directly by 50% of their ORM. During the trial both peak and average power was recorded, the aim of the present study was to investigate whether an  $Mg^{2+}$  supplementation period would significantly increase average power in a bench press trial to fatigue.

The current study concluded there to be no significant difference in peak power or average power at 75% and 50% of ORM both pre and post the 10 kilometre run. Therefore the null hypothesis will be accepted due to a lack of significant change. It is important to note that whilst there is research that reports  $Mg^{2+}$  supplementation to have no effect on exercise performance there is still no research reporting negative effects of  $Mg^{2+}$  supplementation (Newhouse & Finstaad, 2000).

Previous literature that has investigated the effects of  $Mg^{2+}$  supplementation on power performance both support (Ripari, Perialisi, Giamberardino, Resina & Vecchiet, 1989; Brilla & Haley, 1992; Brilla & Gunter, 1995; Golf, Bender & Gruttner, 1998) and conflict (Weight, Myburgh & Noakes, 1988; Terblanche, Noakes, Dennis, Marais & Eckert, 1992; Ruddel, Werner & Ising, 1990; Weller, Bachert, Meinck, Friedmann, Bartsch & Mairbaur, 1998) its impact, so whether  $Mg^{2+}$  supplementation improves muscle functioning still remains to be shown (Dominguez, Barbagallo, Lauretani, Bandinelli, Bos, Corsi, Simonsick & Ferrucci, 2006).

Dominguez et al, (2006) support the hypothesis that  $Mg^{2+}$  supplementation will have a positive impact on sports performance, they sampled 1138 men and women ages  $66.7 \pm 15.2$  years who were not severely cognitively compromised and had no evidence of kidney disease or hypercalcemia. Muscle performance was evaluated by grip strength, lower leg muscle power, knee extension torque and ankle extension isometric strength. Dominguez et al, (2006) found a significant, independent and strong relation between circulating  $Mg^{2+}$  and muscle performance which was consistent across several muscle variables in both men and women. Dominguez et al, (2006) didn't provide subjects with  $Mg^{2+}$  supplementation, but assessed  $Mg^{2+}$  status and compared that to muscular performance in the older persons. This provides a study limitation as it cannot be concluded that the positive correlation seen between  $Mg^{2+}$  status and strength performance is solely caused by the  $Mg^{2+}$  level as there may be several other variables affecting this increase in muscular performance.

Brilla and Haley (1992) investigated the effects of dietary  $Mg^{2+}$  on strength development during a double blind 7 week strength training program in twenty six untrained subjects (14 = control and 12 =  $Mg^{2+}$  supplemented) between 18-30 years of age. Brilla and Haley provided supplementation of 8mg/kg of body weight/day. Strength was assessed with each subject performing three sets of ten repetitions, leg press and leg extension, three times a week. Brilla and Haley reported that both groups gained strength, however results reported a significant increase in strength for the  $Mg^{2+}$  group when compared to the control group in absolute and relative torque adjusted for body weight. This finding provides further support to the hypothesis that  $Mg^{2+}$  supplementation will improve strength. Brilla and Haley (1992) also conducted dietary assessments over a three day period to assure that subjects were adequate in  $Mg^{2+}$  status. The current study did not require participants to conduct maximal strength exertion, just exertion of strength till fatigue, which may be the limitation which resulted in no significant differences occurring.

Brilla, Giroux, Taylor & Knutzen (2003) followed up on the previous study (Brilla & Haley, 1992) by evaluating the effect of  $Mg^{2+}$ -creatine supplementation on body water and quadriceps torque. Participants were required to consume supplementation consisting of 800mg  $Mg^{2+}$ /day and 5g of creatine daily for a period of two weeks in a random assignment clinical trial. Brilla et al, (2003) concluded there to be a significant increase in quadriceps muscle torque when the  $Mg^{2+}$ -Creatine group was compared to the placebo intervention group. Although this study provides further evidence for the hypothesis that  $Mg^{2+}$  supplementation will have a significant increase on performance it cannot be determined whether performances increments were due to the 800mg of  $Mg^{2+}$ , the 5g of creatine or a combination of the two. This conflicts the finding of the current clinical trial which may also be due to the design limitation as participants were required to exercise to fatigue rather than a maximal output.

There are three potential mechanisms that may explain the performance improvements recorded in the previous clinical trials These are the role of  $Mg^{2+}$  in energetic metabolism, the increased relative oxygen species production in a state of  $Mg^{2+}$  deficiency and the proinflammatory effect of  $Mg^{2+}$  depletion. The previous studies discussed that were conducted on young participants, reported a significant increase in muscle performance, which may be due to  $Mg^{2+}$  key role in energetic metabolism, trans membrane transport, and muscle contraction and relaxation (Lukaski, 2004). A deficient  $Mg^{2+}$  status has been linked to structural damage to muscle cells through increased oxidative stress and impaired intracellular calcium homeostasis (Rock, Astier, Vignon, Gueux, Motta & Rayssiguier, 1995).

The majority of energy used in physiological functions in humans is produced in the mitochondria via the movement of electrons through the respiratory chain (Short, Bigelow, Kahl, Singh, Coenen-Schimke, Raghavakaimal, Nair, 2005).  $Mg^{2+}$  is known to be critical for basic mitochondrial functions, including ATP synthesis, electron transport chain complex subunits, and oxygen detoxification (Wolf & Cittadini, 2003). A deficient  $Mg^{2+}$  status may lead to a reduction in mitochondrial efficiency and increased production of relative oxygen species with consequent structural and functional impairment to proteins (Liu, Head, Gharib, Yuan, Ingersoll, Hagen, & Ames, 2002), DNA (Short, Bigelow, Kahl, Singh, Coenen-Schimke, Raghavakaimal, Nair, 2005), and other essential molecules, ultimately preventing strength gains and power improvements.

The level of  $Mg^{2+}$  present in the mitochondria can represent up to one third of total body  $Mg^{2+}$  and is present as a complex with ATP and as a component of membranes and nucleic acids (Wolf & Cittadini, 2003). Studies into the effects of  $Mg^{2+}$  deficiency in humans and animals has reported evidence of decreased antioxidant capacity (Dickens, Weglicki, & Mak, 1992; Freedman, Mak, Stafford, Dickens, Cassidy, Muesing, & Weglicki, 1992), with studies concluding a link between  $Mg^{2+}$  deficiency, mitochondrial swelling and altered ultra-structure in animal muscle (Rock, Astier, Vignon, Gueux, Motta & Rayssiguier, 1995), providing further explanation to the link between power and  $Mg^{2+}$  status.

Newhouse and Finstad (2000) conducted a meta-analysis into the effects of  $Mg^{2+}$  supplementation on sports performance. Out of twelve different studies analysed it was concluded that  $Mg^{2+}$  supplementation would have no increase in sports performance or power, with only three studies showing strength-related dependant variables. With only three studies to draw upon further research is needed to conclude the effects of  $Mg^{2+}$  supplementation on power. One consistency between pervious research is that improvements are seen in participants that have a lower state of fitness. The majority of previous research that reports a lack of effect has used highly trained athletes as subjects. Therefore supporting the theory that  $Mg^{2+}$  supplementation will be able to address a weak link in the physiology of untrained subjects whilst training has somehow strengthened this weak leak, although more research is needed to expand on this observation (Newhouse & Finstad, 2000).

This may provide a rational as to why in the current study  $Mg^{2+}$  supplementation failed to produce a significant increase in peak and average power, as all participants needed to be recreationally active and fit enough to ensure completion of a 10 kilometre run. The lack of results could also be linked to the study design, which required subjects to complete the trial to fatigue rather than maximal exertion.

## Limitations

This study aimed to be the first to assess participants  $Mg^{2+}$  status through the use of food diaries and blood samples and relate that status to performance. After analysis of the food diaries it was concluded that there was only three subjects with an adequate  $Mg^{2+}$  status. A sample size of four was not enough to provide a significant comparison. This provides limitation to the current study as it would have proved to be more effective to screen the participants for their  $Mg^{2+}$  status prior to the conduction of the protocol. This way subjects could have been excluded or included to the protocol depending on  $Mg^{2+}$  status, resulting in equal groups for comparison. Participants were not screened for  $Mg^{2+}$  status prior to the completion of the study due to time restraints placed on the research project. It would have been a time consuming process to create two groups based on  $Mg^{2+}$  status as the majority of individuals are deficient in  $Mg^{2+}$ , leaving less individuals with an adequate  $Mg^{2+}$  status. Although this does provide a topic for new research as greater depth is needed into the effects of  $Mg^{2+}$  supplementation in relation to  $Mg^{2+}$  status.

$Mg^{2+}$  supplementation presented to participants may also have proved to be a limitation, resulting in a lack of significant results. Previous studies have reported significant effects from  $Mg^{2+}$  supplementation of 800mg/day (Brilla, Giroux, Taylor & Knutzen 2003). The current study only required participants to consume 500mg/day of  $Mg^{2+}$  supplementation which may have resulted in some insignificant results, although previous literature has suggested that ingestion of 500mg/day of  $Mg^{2+}$  can result in gastrointestinal disturbances, particularly diarrhoea in some individuals, and often may exert a negative effect on phosphate balance (Lukaski, 1995). In future research an extra group could be added alongside the current groups, containing a higher dosage of  $Mg^{2+}$ .

The bench press till fatigue trial also contained limitations that may have affected the results of the study. The bench press trial required participants to complete as many repetitions as possible of their ORM until failure. If the study had required participants to exert as much force as possible (maximal) rather than until fatigue, there may have been an increase seen in the power readings recorded.

The participant sample size may also provide limitation to the current clinical. The final sample size for the clinical trial was 13 participants. Although this amount was seen as significant after a power analysis to provide validity a larger sample size would have provided an increased level of validity, furthermore the majority of subjects were male with only four participants being female. This is due to opportunity sampling, with many females not willing to partake in the study. This dominant male sample group may remove some validity from the trial and the effects of  $Mg^{2+}$  supplementation on the female population.

Cultural bias was also another limitation of the clinical trial as subjects recruited were all from a similar cultural background, again due to the opportunity sample. This will mean that the current findings cannot be generalised to all cultures.

Supplementation length may have also provided limitation to the current clinical trial, as previous research has contained supplementation periods of up to six months. The current study contained a five week supplementation period which may have not been long enough to affect  $Mg^{2+}$  status significantly. Although as the current clinical trial was of a crossover design and contained a washout period, of five weeks, an extension of supplementation period would have dramatically increased the studies duration. This may provide the rationale for a research project that assesses the optional length for supplementation.

### Conclusion

In conclusion the results of the current study show that 500mg/day of  $Mg^{2+}$  supplementation will significantly decrease time taken to complete a 10 kilometre run, reduce systolic and diastolic blood pressure and significantly reduce HR. As  $Mg^{2+}$  is a co-factor in over 325 enzymatic reactions (Newhouse & Finstad, 2000), it's importance as a mineral is clear and warrants research to improve scientific knowledge into its role within health and sports performance, as  $Mg^{2+}$  deficiency can also be detrimental to health.

Taking into consideration the findings of the current study, the positive results recorded suggest that  $Mg^{2+}$  supplementation will be beneficial for sports performance and certain health aspects. Therefore it can be concluded that it is worth taking  $Mg^{2+}$  supplementation to aid with health and performance. The current study provides conclusive evidence into the benefits of  $Mg^{2+}$  although if the study was to be conducted again, more attention would be paid to participant's baseline  $Mg^{2+}$  status to provide a conclusion to the question: does  $Mg^{2+}$  supplementation provide further benefit to individuals that achieve optimal  $Mg^{2+}$  status through their diets?

### Future Research

One future research questions can be drawn from the current clinical trial, which is as follows: Does  $Mg^{2+}$  supplementation improve performance in individuals with adequate dietary intake vs individuals with a deficient dietary intake? This further research would provide a greater depth of knowledge and may help to improve the understanding of how deficiency in  $Mg^{2+}$  can be detrimental to health and how  $Mg^{2+}$  supplementation can be beneficial to health and sports performance.

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