Magnesium: supplementation, absorption and effect on blood pressure and exercise.

Submitted by Lindsy Kass to the University of Exeter
as a thesis for the degree of
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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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Abstract

Introduction

Magnesium is required by the human body in modest amounts for the maintenance of health and optimal functioning.

Objectives

This portfolio of work sets out to investigate whether magnesium supplementation has hypotensive effects and to determine if habitual dietary magnesium intake or loading strategies modulate the effects of magnesium supplementation. The habitual dietary magnesium intake of hypertensive patients was also examined to ascertain adequacy of dietary magnesium in this cohort. A meta-analysis was performed on the effect of magnesium supplementation on blood pressure. Other variables such as dosage, duration and study design were considered and findings from the meta-analysis used to influence future work.

A further objective was to examine the effect of supplementation on aerobic and resistance exercise and subsequent recovery. Finally, the efficacy of an alternative means of magnesium delivery in the form of a transdermal magnesium cream was investigated.

Methods

A 300 $mg.day^{-1}$ elemental magnesium aspartate or magnesium citrate was used as a supplementation in studies 1,2,4 and 5.

Participants were instructed to continue with their normal diet and for study 6 participants were required to eat the same foods for the 24 hours prior to both laboratory blood taking sessions. With the exception of the meta-analysis, food diaries were kept for various lengths of time, detailed in the publications. Aerobic and resistance exercise protocols were carried out in studies 1,2 and 4, with both performance and cardiovascular parameters investigated for any effect from supplementation.

Where supplementation was in the form of a transdermal cream, this was applied to the torso and absorption of the cream was determined by investigating changes in serum and urinary magnesium levels.

Summary of results

Blood pressure decreased with magnesium supplementation of 300 mg.day^{-1} for 7 days with greater reductions in systolic versus diastolic blood pressure consistently evident.

Magnesium supplementation of 300 mg.day^{-1} for 7 and 14 days increased power during resistance exercise but no changes in aerobic exercise performance were observed.

A high habitual dietary magnesium intake attenuated the hypotensive effect derived from magnesium supplementation when compared to those on a low habitual dietary intake. The meta-analysis supported these results.

A habitually low dietary magnesium intake was observed in a cohort of clinically diagnosed primary hypertensives.

Conclusion

These studies show that there is a link between low habitual dietary magnesium intake and elevated blood pressure and that magnesium supplementation appears to be associated with blood pressure. An improvement in resistance exercise performance with magnesium supplementation was also observed. Finally, a transdermal magnesium cream was shown to increase serum magnesium levels and may provide an alternative to oral supplementation.

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Author's declaration

Magnesium: supplementation, absorption and effect on blood pressure and exercise.

Submitted by Lindsy Kass, to the University of Exeter as a thesis for the degree of *Doctor of Philosophy by Publication* in The effect of magnesium supplementation on blood pressure with physical activity August 2017

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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Definitions and abbreviations

Hypermagnesemia	High levels of magnesium in the blood
Hypomagnesemia	Low levels of magnesium in the blood
NAOEL	No observable adverse effect level hypomagnesia.
RDA	Recommended Dietary Allowance
RNI	Reference nutrient intake
Transdermal	Route of administration whereby active ingredients are delivered across the skin
TRPM6 and TRPM7	Transient receptor potential melastatin 6 and 7
WAnT	Wingate anaerobic test to measure mean and peak power

Section 1 Introduction

Literature Review

1.1 Background to magnesium

Minerals, such as magnesium (in the form 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 and it is the fourth most abundant cation in the human body after calcium, potassium and sodium and the second most abundant in cellular systems after potassium. Magnesium is a cofactor to over 325 enzymatic reactions in the body (Newhouse and Finstad, 2000).

Around 99% of total body Mg^{2+} is located in the bone, muscles and non-muscular soft tissue with extracellular magnesium accounting for 1% of the body's total stores and which is identifiable in serum and red blood cells (Elin, 2010). The mean serum Mg^{2+} concentration in humans is roughly 0.85 $mmol.l^{-1}$, with a referenced interval of 0.7-1.0 $mmol.l^{-1}$ (Wacker, 1980). In serum, approximately one third of Mg^{2+} is bound to protein, 25% is bound to albumin and 8% to globulins (Maguire, 2006). 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).

The stores of Mg^{2+} in the body equate to approximately 24 g (1 mole). Total Mg^{2+} in an average adult (70 kg with 20% body fat) equates to ~20 mmol/kg of fat free mass (~1000-1200 mmol) (Fox, Ramsoomair and Carter, 2001).

Magnesium has a variety of biological functions, including structural roles by complexing negatively charged groups such as phosphates in nucleic acid, a controlling role in the inhibition or activation of enzymes and regulatory roles by modulating cell proliferation, cell cycle progress and differentiation (Fox, Ramsoomair and Carter, 2001)

1.2 Magnesium absorption, excretion and markers of flux and homeostasis

Mg^{2+} homeostasis is maintained by the intestine, kidneys and bones (Jahnen-Dechent and Ketteler, 2012). Mg^{2+} is absorbed in the intestine, stored in the bone and any excess Mg^{2+} that has been consumed will be filtered by the kidneys, then excreted from the body in urine. Absorption of Mg^{2+} mainly takes place in the small intestine (Fox, Ramsoomair and Carter,

2001; Yogi *et al.*, 2011) although some 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 (de Baaij, Hoenderop and Bindels, 2012) using transport proteins. 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 the most permeable for ions because of the relatively low expression of tightening claudins 1, 3, 4, 5 and 8 (de Baaij, Hoenderop and Bindels, 2012). As such para-cellular Mg^{2+} transport seems mainly restricted to these areas. Claudins 16 and 19, linked with Mg^{2+} permeability (Schweigel and Martens, 2000), are not expressed in the small intestine. Therefore, the exact mechanism involved in the facilitation of Mg^{2+} absorption remains unknown.

The non-saturable paracellular pathway accounts for the majority of Mg^{2+} absorption (90%) which occurs passively between the enterocytes consisting of tight junctions and intracellular space; this is mediated by the transepithelial electrochemical gradient, transepithelial concentration gradient and solvent drag, allowing the movement of ions across cell membranes by bulk transport following the movement of water (Touyz, 2003).

The saturable pathway mechanism may act via a carrier-mediated process or by diffusion across the intestinal mucosa, each of which is functionally associated with low and high Mg^{2+} intakes, respectively (Bohl & Volpe 2002), therefore consumption of adequate magnesium intake may affect the absorption of Mg^{2+} via the TRPM 6 and 7 pathways. Mg^{2+} transcellular absorption is via the brush border epithelium across the luminal membrane and extrusion out of the cell across basolateral membranes, both of which exhibit passive and active transport (Schweigel and Martens, 2000). Whether this saturable transcellular active transport system is key to the regulation of Mg^{2+} absorption is unknown. It would be of interest to determine the effect of high and low dietary intake on the transporters to determine a saturation point of the transporters with a high Mg^{2+} diet and further to determine whether there is a downregulation of the transporters with a high Mg^{2+} intake.

In Addition to the carrier mediated transport mechanisms discussed above, other mechanisms have also been suggested to facilitate the influx of Mg^{2+} for example solute

transport carriers (SLC41A1/A2) and other protein carriers (ACDP1, MagT1, MMgT1 and MMgT2) (Romani and Scarpa, 1992; Quamme, 2010). Recent publications have shown active transcellular transportation of Mg^{2+} in relation to specific Mg^{2+} transport proteins TRPM6 and TRPM7 (transient receptor potential melastatin 6 and 7), which are situated within the length of the intestine (TRPM6) and among various tissues (TRPM7). TRPM6 is associated principally with Mg^{2+} transport within the epithelium in both the intestine and distal convoluted tubule (DCT) of the kidney (Quamme, 2010). TRPM6 has been shown to fundamentally influence Mg^{2+} homeostasis via (re)absorption and excretion within the DCT.

This Mg^{2+} absorption function of TRPM6, which is expressed in the kidney may be related to additional cellular mechanisms of Mg^{2+} absorption evident via paracellin-1 (Rondón *et al.*, 2008). Paracellin-1(PCLN-1) is a newly identified protein also known as claudin 16. This protein is expressed in humans in the thick ascending Loop of Henle in the tight junctions and is thought to play a critical role in the control of paracellular permeability for magnesium and calcium (Blanchard *et al.*, 2001).

However, the exact mechanistic action by which paracellin-1 effects the paracellular fluxes of Mg^{2+} remains inconclusive (Wolf, 2004). Schlingmann et al (2007) suggest that when intraluminal Mg^{2+} concentrations are low, Mg^{2+} absorption occurs primarily via the active transcellular pathway, with any further intraluminal Mg^{2+} concentration increases re-establishing Mg^{2+} absorption via paracellular pathways.

Previous research indicates that a diet deficient Mg^{2+} induces an increased TRPM6 expression thus enhancing Mg^{2+} absorption and reabsorption (Rondón *et al.*, 2008). A review by Schlingmann and Gudermann (2007) concluded that the novel identification of TRPM6+7 provides insight into the cellular mechanism and multifunctional role of TRPM7 and specific role of TRPM6 in relation to epithelial Mg^{2+} uptake. These mechanisms, in particular TRPM7, are modulated by coupling of TRPM7's channel-kinase domain and phosphotransferase activity (Schlingmann *et al.*, 2007) and it could therefore be suggested that TRPM7 may be the biological modulator of Mg^{2+} homeostasis (Wolf, 2004). Again, no research examined this to determine whether diet affects TRPM7.

Finally it has been shown that Mg^{2+} nucleotide complexes i.e. MgATP, G-proteins and phosphoinositide turnover suppress/inhibit the active ion channel and function expressed by TRPM7 (Wolf, 2004; Schlingmann *et al.*, 2007). It has been suggested that this inhibitory action and the potential association with suboptimal plasma Mg^{2+} may affect cellular processes requiring Mg^{2+} as a co-factor for enzymatic activity such as ATP production and therefore may affect critical cellular metabolic processes (Wolf, 2004)

The average dietary intake of Mg^{2+} is approximately 300 mg.day^{-1}, all derived primarily from green vegetables, cereal grains and meat. An individual consuming this diet absorbs around 40% of the Mg^{2+} consumed, which is absorbed primarily in the small intestine (Elin, 2010). The absorption process commences around 1 hour after consumption and continues at a uniform rate for 2-8 hours. Twelve hours after 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 (Nicar and Pak, 1982). When a diet low in Mg^{2+} is consumed up to 75% of ingested Mg^{2+} may be absorbed, whilst when consuming a diet rich in Mg^{2+} as little as 25% may be absorbed (Elin, 2010).

The major excretory pathway for absorbed Mg^{2+} is via the kidneys. The capacity for renal excretion of Mg^{2+} is thought to be between 120 to 140 mg/24 h, for a person consuming a diet adequate in Mg^{2+} (Wacker, 1980). 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, and as such the kidney is the major organ responsible for serum magnesium homeostasis. 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 (Wacker, 1980).

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, 2010). Roughly 20% to 30% of the filtered Mg^{2+} is absorbed along the proximal tubule (Quamme, 2010). 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. The distal tubules and collecting ducts therefore absorb very little Mg^{2+} (Quamme, 2010).

The excretion of Mg^{2+} follows a circadian rhythm, with most Mg^{2+} being excreted in the evening/night (Fox, Ramsoomair and Carter, 2001). Under normal physiological conditions approximately 2400 mg 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 (Fox, Ramsoomair and Carter, 2001). 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. Conversely, the kidneys can also increase excretion when the body is in a state of hypermagnesemia (Jahnen-Dechent and Ketteler, 2012).

A growing list of hormones and other factors have also been reported to influence TRPM6 and TRPM7 protein expression and, in some cases, TRPM6- and TRPM7-mediated Mg^{2+}

transport. Angiotensin II, aldosterone, bradykinin, epidermal growth factor, thrombin, oestrogen, metabolic acidosis/alkalosis, thiazide diuretics, certain immune-suppressants and mechano- or osmo-induced stretch have been implicated in affecting TRPM7 and/or TRPM6 in a variety of cells. These changes may be mediated by covalent modification, or through controls at the level of gene and protein expression (Quamme, 2010)

1.3 Methods of Assessment of Magnesium absorption and concentration

In order to clarify human requirements and assess Mg^{2+} status, it is essential to identify specific biomarkers of Mg^{2+} status (Witkowski, Hubert and Mazur, 2011). A variety of methods have been used to measure magnesium in the body including serum magnesium, plasma magnesium, red blood cell (RBC) magnesium, and 24h urinary magnesium excretion

1.3.1 Serum Magnesium

It has been postulated that serum Mg^{2+} correlates poorly with total body stores. When serum magnesium level is low, intra- cellular magnesium may also be low. However, when serum magnesium levels are normal, intra-cellular magnesium may be depleted. Therefore, if the serum magnesium level is low, a person is deficient; however, if it is normal, the person may still be magnesium deficient at the cellular level (Fox, Ramsoomair and Carter, 2001) However, it is still amongst the most widely used method of Mg^{2+} assessment (Shils and Rude, 1996). There is preferential use of serum over plasma as the anticoagulant used for plasma separation tubes is often magnesium salts and or may be contaminated with Mg^{2+} (Swaminathan, 2003). As intracellular methods of Mg^{2+} assessment, including RBC and muscle biopsies, are often expensive as their assays include nuclear magnetic resonance (NMR) spectroscopy and ion-specific electrode measures, therefore, due to the cost implications, serum magnesium is often deemed acceptable in assessing changes in Mg^{2+} status (Fox, Ramsoomair and Carter, 2001). It was thought that the analysis of serum Mg^{2+}, despite being the "entry level" test, was a good determinant of assessing possible disorders or deficiency as a low Mg serum concentration is concomitant with low intracellular Mg^{2+} levels (Elin, 1991). However, more recent research has found that serum Mg^{2+} does not reflect total body Mg^{2+}, neither does it correlate with tissue stores, excluding interstitial fluid and bone (de Baaij, Hoenderop and Bindels, 2012)**.** The point being that it is possible to have serum levels ≥0.7 $mmol.l^{-1}$ but be intracellularly depleted. It is thought that severely depleted serum Mg^{2+} (<0.4 $mmol.l^{-1}$) is a marker of intracellular depletion (McLean, 1994).

Fawcett, Haxby & Male (1999) reported that only 1% of Mg^{2+} in the body is located in extracellular fluids, furthermore only 0.3% 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%). It was 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.

1.3.2 24-hour Urinary Mg^{2+} Excretion

Although time consuming and cumbersome, 24h urinary excretion of Mg^{2+} has been used to measure magnesium intake. Mg^{2+} excretion has been shown to increase in response to an elevated level of Mg^{2+} ingestion whether this be through dietary manipulation or supplementation (Fatemi *et al.*, 1991; Feillet-Coudray *et al.*, 2002; Lukaski and Nielsen, 2002). The circadian rhythm plays a role in the renal excretion of Mg^{2+}, with the maximum excretion occurring at night. Therefore it is important for a full 24h sample to be collected for accurate assessment of Mg^{2+} excretion and absorption (Jahnen-Dechent and Ketteler, 2012). The results of a 24h urinary test after ingestion or an intravenous load of Mg^{2+} can provide information on intake and absorption with high urinary excretion indicating a renal elimination of Mg^{2+} due to sufficient Mg^{2+} and greater Mg^{2+} retention suggesting insufficient intake or high absorption rates (Elin, 1988). Trauninger (2002) suggested an estimation of normal 24 hour urinary Mg^{2+} loss to be 3.6 mmol (range 2.7-4.4 mmol) for females and 4.8 mmol (range 3.2-5.2 mmol) for males.

1.4 Magnesium and blood pressure

Mg^{2+} supplementation has been shown to significantly improve blood pressure (Cunha et al. 2012; Bain et al. 2015) and modify vascular tone (Cunha *et al.*, 2012) and is considered an important regulatory factor for the pathophysiology of hypertension. This may be attributed both to the intra and extracellular concentrations of Mg^{2+} present in cardiac and vascular cells; influencing cardiac excitability, vascular reactivity, contractility and tone (Laurant and Touyz, 2000), all of which support the underlying aetiology of hypertension as an increase in peripheral resistance (Touyz, 2003). Mg^{2+} has been shown to be a mediatory cation for an induced vasodilatory action in addition to acting as a Ca^{2+} antagonist (Laires, 2004). The exact cellular basis for the molecular contractile action of Mg^{2+} is unknown, but it has been suggested that Mg^{2+} influences Ca^{2+} handling, which has been shown to increase contractility of vascular smooth muscle cells (Jahnen-Dechent and Ketteler, 2012). Inside the vascular smooth muscle cells, Mg^{2+} acts by inhibiting transmembrane calcium transport and calcium entry into the cell thus decreasing contractile actions of vasoactive agents relying on calcium signalling. By acting as a calcium antagonist Mg^{2+} modulates the vasoconstrictor actions of increased Ca^{2+} in smooth muscle cells. Elevated levels of Mg^{2+} compete with the influx of Ca^{2+} 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).

Conversely, low intra/extracellular Mg^{2+} concentration results in an ionic imbalance causing an influx of Ca^{2+} and an increased Ca^{2+}:Mg^{2+} ratio (Kesteloot *et al.*, 2011), causing arterial stiffness (Resnick et al., 1997). Low Mg^{2+} concentration may also potentially prevent the dephosphorylation processes associated with inositol-1,4,5-triphosphate (PI_3) that may stimulate PI_3 mediated Ca^{2+} mobilisation, resulting in an increased intracellular Ca^{2+} and increased vascular tone (Laurant and Touyz, 2000). These effects will subsequently result in a yet to be determined increase in blood pressure.

1.5 Magnesium and energy metabolism

With regards to energy metabolism, Maguire (2006) states that the binding of Mg to ATP (MgATP complex) influences fundamental cellular phosphorylation processes, which are crucial for physical performance. The role of Mg^{2+} in the formation of the MgATP complex, is it facilitates the resynthesising of ATP via a conformational change which results in improved adenosine diphosphate (ADP) and inorganic phosphate (Pi) alignment, in addition to aiding the removal of a water molecule while bound to Pi so as to complete the formation of ATP (Schlingmann *et al.*, 2007). This conformational change lowers the activation energy required for the formation of the MgATP complex and subsequently improves the bioenergetics involved (Gout *et al.*, 2014). Mitochondrial Mg^{2+} also enhances energy metabolism by improving enzyme activation e.g. enzymes such as dehydrogenases and cytochrome c oxidase impact directly on the mitochondrial respiratory rate by increasing the release of adenine nucleotides and also affecting mitochondrial membrane potential, and hence the mitochondrial volume, (Wolf and Trapani, 2008). In light of the potential of magnesium to enhance ATP generation, Mg^{2+} also has an impact on muscle contraction, a process that requires crossbridge cycling between actin and myosin, which is ATP dependant (Schlingmann *et al.*, 2007). Mg^{2+} alters myosin motor activity by altering the steps associated with actin attachment and detachment. The mechanism of altering detachment may be due to Mg^{2+} exchange at the active site, this has still to be fully elucidated. Further free Mg^{2+}concentrations increase in skeletal muscle during exercise and initial recovery, which suggests that Mg^{2+}could also play a role in muscle fatigue (Swenson *et al.*, 2014).

1.6 Magnesium and exercise

As well as blood pressure, magnesium has also been investigated with regards to its potential to enhance exercise performance, in particular ATP re-phosphorylation and muscle

contraction. There is limited research on this and to date there are no systematic reviews or meta-analyses to provide an overview of this area.

Performance improvements have been limited when supplementing with Mg^{2+} and the mechanisms responsible for the limited performance increases, are likely multifactorial (Pokan *et al.*, 2006).

Lukaski (2004) suggests the relevance of magnesium supplementation in relation to physically active individuals based on two principles determined from their previous review on micronutrient intake and athletes (Lukaski, 1995); 1) active individuals consume a dietary intake consisting of less vitamins and minerals in relation to their inactive counterparts, and 2) physical activity increases the rate of turnover of micronutrients resulting from excessive micronutrient loss via increased catabolism and excretion (sweat and urine). Consequently, the distortion of neuromuscular function due to Mg^{2+} deficiency may occur and causes the decline of overall physical performance and impair exercise capacity (Finstad and Newhouse, 2001; Bohl C and Volpe, 2002; Nielsen and Lukaski, 2006) and impair cardiovascular parameters in the general population (Chakraborti *et al.*, 2002; Khan *et al.*, 2010). Laires et al (2014) have highlighted the importance of evaluating magnesium status in athletes not only because the practice of exercise with a magnesium deficit may compromise performance, but also because the practice of exercise with a magnesium deficit may render the athlete more susceptible to cellular damage (Laires *et al.*, 2014). Therefore, the consumption of dietary Mg^{2+} along with supplementation is a consideration for the athlete.

Higher intracellular Mg^{2+} levels may improve intracellular adenosine triphosphate production as Mg^{2+} is a cofactor for several ATP synthases (Laurant and Touyz, 2000). Mg^{2+} has also been reported as an ubiquitous physiological calcium blocker (Shechter, 2010; Lutsey *et al.*, 2014) reducing the release of calcium from and the re-uptake into the sarcoplasmic reticulum whilst protecting the cells from calcium overload under conditions of ischaemia. As mentioned above, Mg^{2+} reduces systemic and pulmonary vascular resistance with a concomitant decrease in blood pressure resulting in a slight increase in the cardiac index (Wilborn *et al.*, 2004; Cunha *et al.*, 2012). Cardiac index is defined as cardiac output/body surface area (Jefferson *et al.*, 2015) and therefore relates heart performance to the size of the individual. Investigations into magnesium's effect on muscular contraction and strength are limited (Bohl C and Volpe, 2002; Carvil and Cronin, 2010; Matias *et al.*, 2010). The rationale for investigating this is that muscle contraction and relaxation are dependently regulated by intra/extracellular Ca^{2+} and Mg^{2+} concentrations. During a state of Mg^{2+} depletion it has been suggested that there is insufficient relaxation time for crossbridge formation due to the antagonistic role of Mg^{2+} as a Ca^{2+} channel-blocker (Lukaski and

Nielsen, 2002). An elevated extracellular Mg^{2+} concentration influenced by supplementation has been seen to induce an increased intracellular Mg^{2+} concentration, changing the Mg^{2+} to Ca^{2+} ratio and therefore optimising vascular smooth and skeletal muscle cell bioenergetics and contraction (Zhang, Altura and Altura, 1997).

The research in regard to strength is considerably weaker and restricted to just 4 published articles, three of which were conducted in athletic populations (Brilla and Haley, 1992; Matias *et al.*, 2010; Santos *et al.*, 2011) and the other in an elderly male and female population (Dominguez *et al.*, 2006b). In regard to the only study that looked at strength and Mg^{2+} supplementation, Brilla and Haley (1992) illustrated significantly greater absolute and relative peak torque quadriceps strength for the Mg^{2+} supplemented group versus control group. Mechanisms implicated in the augmented strength due to Mg^{2+} intake and supplementation include: 1) MgATP complex formation optimising energy metabolism and muscular contraction, 2) increased protein synthesis rate, and 3) protection against cellular damage. Further findings have shown that high intracellular Mg^{2+} concentrations and subsequent formation of the MgATP complex, influence protein synthesis and cell proliferation by stimulating the activation of mammalian target of rapamyacin (mTOR), therefore accelerating translation towards DNA synthesis (Rubin, 2005); Mg^{2+} regulatory action on insulin and the activation of specific enzymes (i.e. phosphotidylinositol-3 kinase (PI3K)) within the mTOR cascade further account for cellular functions augmenting protein synthesis (Zhang, Altura and Altura, 1997; Rubin, 2005).

1.6.1 Magnesium, exercise and inflammation

It has been postulated that low serum and dietary Mg^{2+} levels are correlated with low grade systemic inflammation. Magnesium plays an essential role in a wide range of fundamental cellular reactions with a focus being on the association with markers of inflammation being associated with Mg^{2+} deficiency (Mazur *et al.*, 2007)

Kruse & Orent (1932) were the first to observe clinical symptoms of inflammation in magnesium deficient rats. Characteristics commonly seen in reaction to allergies including erythema (redness of the skin or mucous membranes), hyperemia, and oedema, occur spontaneously in the hypomagnesemic hypercalcemic rats (Classen *et al.*, 1993). The most prominent change identified in blood is an increase in leukocytes, predominantly neutrophils and eosinophils (Belin and He, 2007). The increase in leukocytes is also accompanied by an increase in pro-inflammatory cytokines such as Interleukin 6 (IL6) which also has transcriptional control of C-reactive protein (CRP) (Pepys and Hirschfield, 2003). In most cases the circulating value of CRP reflects ongoing inflammation whether in

response to tissue damage or to a systemic inflammatory state (Pepys and Hirschfield, 2003). Acute or unaccustomed exercise can induce inflammation due to damage to the contracting muscle which activates signalling via the NFKB pathway, resulting in release of TNF-alpha and IL6, stimulators of the release of CRP from hepatocytes (Beavers, Brinkley and Nicklas, 2013). However, chronic exercise training has been shown to have an anti-inflammatory effect. The exact mechanisms by which physical activity may reduce inflammation are not entirely understood, however there are some data pointing to factors that may contribute to an effect of repeated bouts of muscle contraction leading to improvements in inflammatory status over time. These factors include, shifts in monocyte phenotype, specifically reductions in immune cell production of inflammatory mediators, with exercise training. Immune function adaptations that occur locally in exercised skeletal muscle and exercise-induced adaptations in intracellular generation of reactive oxygen species (ROS) (Beavers, Brinkley and Nicklas, 2013)

With regards to dietary supplementation, a study by Phillips *et al* (2003) investigating the effect of a dietary supplement containing tocopherols, flavonoids and docosahexaenoate concluded that exercise induced inflammation, evaluated by changes in IL6 and CRP were significantly reduced by this supplement (Phillips *et al.*, 2003)

A more recent study by Welch et al (2016) investigated association between circulating CRP, muscle mass and leg explosive power to determine whether dietary magnesium may impact on this association. This study specifically looked at females and age related skeletal muscle loss in order to identify indirect and direct impact of dietary magnesium on chronic low-grade inflammation which is a risk factor for loss of skeletal muscle mass and strength. They concluded that higher CRP was negatively associated with skeletal muscle mass and that a higher dietary Mg^{2+} intake attenuated this negative relationship by 6.5% with greatest effects in women older than 50y (Welch *et al.*, 2016). This association between magnesium intake and inflammation may impact magnesium studies investigating performance and recovery from performance as well as muscle power and sarcopenia.

1.7 Magnesium consumption

To maintain an adequate physiological levels of Mg^{2+}, humans must consume Mg^{2+} at regular intervals (Jahnen-Dechent and Ketteler, 2012). The daily recommendation for Mg^{2+} is controversial, as the literature is conflicting and varies between countries, although values of ≥ 300 $mg.day^{-1}$ are usually reported for healthy adults with adjustment for age, sex and nutritional status (Jahnen-Dechent and Ketteler, 2012). The Committee On Medical Aspects

(COMA) calculated a reference nutrient intake (RNI) of 300 mg.day^{-1} for adult males and 270 mg.day^{-1} for adult females (Public Health England, 2014b).

The richest dietary sources of Mg^{2+} include whole seeds such as un-milled grains, vegetables, legumes and nuts. Green leafy vegetables are also a key source of Mg^{2+} as the mineral plays a key role in plants as well as biological systems as it is the central ion of chlorophyll (Black, Yin and Casey, 2006). It is difficult to absorb Mg^{2+} from refined foods as the refining process almost completely leaches the Mg^{+} content from the unprocessed form of the food. Dietary Mg^{2+} in the western diet is declining due to the ever increasing consumption of processed foods (Ford and Mokdad, 2003). The nutritional guidelines in the UK for Mg^{2+} consumption may be inappropriate as research now suggests, that the Committee on Medical Aspects (COMA) calculation of an RNI of 300 mg.day^{-1} for adult males and 270 mg.day^{-1} for adult females (Ashwell, 1991) is below the recommended values in the literature and less than the US recommendation. After adjustment for age, sex and nutritional status values of ≥300mg are usually reported within the literature (Jahnen-Dechent and Ketteler, 2012) and The institute of Medicine recommends 320 mg.day^{-1} and 420 mg.day^{-1} for adult females and males respectively (Institute of Medicine (US), 2005). This is the minimum consumption as it is based on daily recommended intakes, the lowest continuing intake level of a nutrient that, for a specified indicator of adequacy, will maintain a defined level of adequate nutrition in an individual (Institute of Medicine (US), 2005). Additional Mg^{2+} may be consumed via drinking water with ~10% of total intake coming naturally from this source (Marx and Neutra, 1997). The total amount of Mg^{2+} received from water will differ depending on geographical location as water in different parts of the country will contain varying concentrations of Mg^{2+}.

The main dietary constituent that interacts with Mg^{+} absorption is dietary fibre. A high fibre diet can reduce Mg^{2+} absorption through the binding of magnesium to phytate (Bohn *et al.*, 2004). As well as phytate, intestinal absorption can be attenuated by alcohol or an excess of phosphate and Ca^{2+} by lowering the concentrations in the lumen (Fine *et al.*, 1991). Although there are interactions between magnesium and calcium, at the cellular level, Fine et al. (1991) detected no significant effect of Ca^{2+} supplementation on magnesium absorption.

1.8 Magnesium supplementation – dosage and type

Magnesium supplementation regimes applied to elicit improvements in exercise performance have differed considerably across the research literature in terms of duration, dose and the type of Mg^{2+} salt administered. For instance duration of Mg^{2+} administration has ranged between 1-3 months, with a dose between 116-500 mg/d provided as an organic,

inorganic or amino acid chelate form (Newhouse and Finstad, 2000; Walker *et al.*, 2003). In regard to the latter, the characteristics highlighted by each Mg^{2+} supplement are influenced by the specific anion attachment with Mg^{2+}, thus influencing supplement solubility, elemental Mg^{2+} bioavailability and supplement effectiveness (Ranade and Somberg, 2001). Research has shown that organic forms of Mg^{2+} supplementation i.e. attached to aspartate, citrate, lactate, pidolate, fumurate, acetate, ascorbate and gluconate to demonstrate greater solubility and bioavailability in comparison to inorganic forms i.e. oxide, sulphate, chloride and carbonate (Newhouse and Finstad, 2000). Despite such factors influencing the efficacy of Mg^{2+} supplementation, the inorganic form of Mg^{2+} oxide due to its low cost has been extensively used in research. However, although Mg^{2+} oxide has been reported to sustain high elemental Mg^{2+} quantities (61%) (Guerrero-Romero and Rodríguez-Morán, 2002) the administration of this supplement may be questioned due to findings showing that Mg^{2+} oxide has a consistently poor bioavailability of only approximately 43% (Lindberg *et al.*, 1990), in comparison to organic forms of Mg^{2+} (55-70%) (Mühlbauer *et al.*, 1991; Ranade and Somberg, 2001; Walker *et al.*, 2003; Coudray *et al.*, 2005). Previous findings suggests that the poor bioavailability of Mg^{2+} oxide induces an osmotic effect in the bowel, resulting in an increase in stool frequency and intestinal motility (Walker *et al.*, 2003), therefore potentially hindering the complete absorption of elemental Mg^{2+}.

Nevertheless, analysis of Mg^{2+} bioavailability literature from differing supplement forms does little to clarify the most effective type of Mg^{2+} supplementation in humans (Coudray *et al.*, 2005). Mühlbauer et al. (1991) compared the bioavailability of Mg-L-aspartate hydrochloride (HCL) and Mg^{2+} oxide in healthy males and females for a duration of 28 days supplementation. Results showed that Mg-L-aspartate HCL at both 60 and 90 mEq daily doses for 7 days gave a greater total absorption compared to the same dose of Mg^{2+} oxide. Additional research supports findings from both an acute (24h) and chronic (60 days) supplementation design, whereby a comparable 300 mg/d dosage of elemental Mg^{2+} from Mg^{2+} citrate facilitated a greater absorption when compared to amino acid chelate and oxide forms (Walker *et al.*, 2003). A more recent publication evaluated the comparative bioavailability of ten organic and inorganic Mg^{2+} salts (oxide, chloride, sulphate, carbonate, acetate, pidolate, citrate, gluconate, lactate and aspartate) using a methodological approach inducing an initial dietary Mg^{2+} depletion, followed by 2 weeks of Mg^{2+} repletion via 550 mg/d supplementation (Coudray *et al.*, 2005). Findings corroborated previous research indicating that organic Mg^{2+} forms had a greater bioavailability than inorganic forms. However, solely basing supplementation on bioavailability may be flawed as the amount of substance needed to consume the 300 mg elemental dosage varies greatly between the different magnesium intake requirements (table 1).

Table 1. Supplemental Mg^{2+} quantity relative to the production of elemental Mg^{2+} corresponding to the male RNI of 300 $mg.d^{-1}$.

Type of supplemental Mg^{2+}	**Quantity mg**
Mg L-Aspartate HCL	3750
Mg Citrate	2670
Mg Lactate	2530
Mg Gluconate	6000

This thesis will consider the effect of magnesium supplementation on blood pressure, exercise performance and recovery. It will also consider the habitual dietary habits of a cohort of hypertensives and the absorption of a magnesium cream. Dietary magnesium intake and magnesium loading strategies will also be presented.

Section 2. Aims, objectives, methodology and overview of findings

2.1 List of contribution, by publication, from Lindsy Kass:-

Study 1 Kass contributed 40% of the work, to included data analysis, interpretation of results and writing of abstract.

Study 2 As Principal Investigator and first author, Kass contributed 70% of the work, to include initial idea, project design, research methodology, data analysis and interpretation and writing of manuscript.

Study 3 As Principal Investigator and first author, Kass contributed 70% of the work, to include initial idea, data collection, data analysis and interpretation and writing of manuscript.

Study 4 As Principal Investigator and first author, Kass contributed 70% of the work, to include initial idea, project design, research methodology, data analysis and interpretation and writing of manuscript.

Study 5 As Principal Investigator and first author, Kass contributed 90% of the work, to include initial idea, project design, data collection, research methodology, data analysis and interpretation and writing of manuscript

Study 6 As Principal Investigator and first author, Kass contributed 80% of the work, to include initial idea, project design, data collection, research methodology, data analysis and writing of manuscript.

2.2 Aims and objectives

2.2.1 General aim

The overarching aim of this PhD by publication was to investigate effect of dietary and supplemental magnesium on blood pressure and exercise.

2.2.2 Specific aims

1. To determine if magnesium supplementation effects blood pressure in healthy males.
2. To determine if magnesium supplementation effects aerobic and resistance exercise in recreationally active people.
3. To establish the habitual dietary magnesium intake in a population group of with primary hypertension.
4. To determine the effect of loading strategies of magnesium supplementation on recovery from exercise and blood pressure in a recreationally active group of people.
5. To review previous research on magnesium supplementation and blood pressure, evaluating overall results via a meta-analysis
6. To determine via serum and urinary magnesium analysis the absorption of a transdermal magnesium cream on a healthy male and female population group.

2.3 General Methods

2.3.1 Ethical approval, consent forms and health and safety considerations

Prior to data collection, ethical approval was granted. With the exception of Study 5 this was granted from the University of Hertfordshire School of Life and Medical Science Ethics Committee. For study 5 ethical approval was granted by the North and East Herts Local Research Ethics Committee.

Before any testing started, participants were informed orally and in writing of the aim, risks and benefits of taking part and were given the opportunity to ask any questions. A health screen was then completed along with a consent form. Risk assessments were undertaken for all studies and formed part of the ethics procedure.

2.3.2 Participants

For all studies participants were volunteers and did not receive any financial or other incentives to complete the study. However, upon completion of each study each participant could receive their results as appropriate to the investigations undertaken. Common inclusion criteria were that the participants were in good health, with the exception of study 5 where the intervention group were hypertensives. For those studies that included resistance or aerobic exercise participants needed to be injury free and "recreationally trained". This was defined as training 2-3 times a week for the past year in the study specific exercise protocols as described and justified in the submitted papers.

2.3.3 Measurements

Common to all studies was the recording of food diaries. These varied between 3-8 days. Dietplan 6 software (Forestfield software Ltd, West Sussex, UK) was used for all dietary analysis. Study 6 required participants to repeat their dietary intake on the days when serum magnesium was to be collected in order that an acute high dietary magnesium intake on one day would not skew results. All other studies did not have any specific dietary requirements. Participants were instructed on how to complete a food diary and provided with pictures of sample food sizes adapted from The Medical Research Council Collaboration Centre for Human Nutrition Research, (Public Health England, 2014b).

Both serum and urinary magnesium were analysed by colorimetric assay (RX MONZA, Randox Laboratories Ltd, UK). All samples were frozen at -80^{o}C for between 4-12 weeks and then analysed in one batch. Blood samples were collected by venepuncture from the median cubital, basillic or cephalic vein. Serum separator vacutainers were inverted 10 times before being left to rest for 30 minutes. Subsequently samples were centrifuged at 3000rom for 10 minutes. Serum was checked to be free from haemolysis and was immediately pipetted and frozen.

Urine was collected into 3 litre collection vessels over 24 hours, with the first collection being the second evacuation of the day and the final collection being the first evacuation the next day. Urine was then decanted into a measuring vessel and volume of urine was recorded. The measuring vessel was then placed on a magnetic stirrer at 100rpm. To re-suspend the magnesium, the pH was lowered to 3-3.25 by adding 5 M hydrochloric acid. Duplicate 1 mL aliquots were then frozen.

All cycle protocols were carried out on the Monark Ergomedic 874 E Cycle Ergometer (Monark, Sweden) and handle bar height and seat height were recorded at baseline and duplicated for each visit to the laboratory.

Blood pressure was taken from the right arm using the Omron MX3 (Omron Healthcare, Kyoto, Japan). Callibration is required every 5 years and equipment was less than 5 years old. However, at random sampling times blood pressure was taken twice, once with the habitual cuff and again with another cuff in order that validity could monitored.

Data were presented as mean values and standard deviation for numeric variables or as percentages for categorical variables. All investigated parameters were tested for normality using the Kolmogorov-Smirnov test. SPSS (Version 22, IBM New York, USA) or STATA (Version 11, StatCorp LP, College Station, TX, USA)) were used for all data analysis. The alpha value was set at 0.05 for all studies. Power analysis, were undertaken using GPower (Faul *et al.*, 2007) for studies 4 and 5. Where data in magnesium were limited, as was expected due to the novelty of this work, calcium results were used from previous research to determine sample size for the studies. Post Hoc power calculations, with a power level of 80%, have now been undertaken for pilot and main studies and are discussed in section 2.3.7 (Limitations to studies).

For study 3, the meta-analysis, the Critical Appraisal Skills Programme (CASP) (copyright Public Health England 2006, Appendix 1) was used to judge the quality of the research for inclusion into the study. This is a critical appraisal tool that uses 10 questions to help to consider, if the trial is valid, what are the results and will the results help locally. This information was not required for publication and was removed from the methodology.

2.4 Overview, findings and results

2.4.1 The effect of magnesium on blood pressure, power and peak torque with short term high intensity exercise

The first study undertaken as part of this portfolio investigated the effect of Mg^{2+} supplementation on three main outcomes: (i) peak and mean power during an all-out maximal intensity short term exercise protocol, (ii) systolic and diastolic blood pressure post maximal exercise and (iii) recovery of isokinetic knee extensor muscle function post exercise.

The reason for undertaking this study was due to there being limited previous research on the effect of Mg^{2+} on short term high intensity exercise and its subsequent effect on blood pressure and muscle recovery post exercise.

The study used a double blind, repeated measures design with participants supplemented with either placebo (cornflour) or 300 mg of Mg^{2+} aspartate for 2 days prior to the test day and 300 mg on the test day 60 minute prior to testing. Maximal isokinetic extensions of 5 repetitions by the right leg were performed by each participant 1 week prior to the testing to establish a baseline for peak torque (Nm). The test day consisted of participants performing a 30 second Wingate Anaerobic Test (WAnT) and resting for 30 minutes post-WAnT before performing 5 maximal isokinetic knee extensions at 60º/second on the right leg. A further 100mg Mg^{2+} aspartate or placebo was given to each participant immediately post-WAnT. Peak power and mean power (W) were recorded from each WAnT. Peak torque (Nm) was measured from the isokinetic knee extensions. Systolic and diastolic blood pressure was recorded at rest and every 5 minutes during the 30 minutes recovery after the WAnT

Mean peak power (W) was significantly ($p=<0.05$) increased during the WAnT between the placebo condition (851.1±119.5 W) and the Mg condition (911.9±107.7 W). Mean total power (W) was also significantly increased during the WAnT with the intervention compared to the placebo (704.5±97.1 W & 683.1±95.6 W respectively). A significant increase was also found in mean peak torque (N/m) between the placebo condition (195.7±47.4 N/m) and the Mg condition (222.2±33.2 N/m). Systolic blood pressure (SBP) was 5% lower during the 30 minutes post-WAnT in the Mg condition (119.7±6.8 mmHg) compared to the placebo (125±7.3 mmHg). However, this effect was not seen in the diastolic blood pressure.

These improvements in knee extension peak torque may be due to increased ATP synthase activity due to the magnesium supplementation along with an increase in tyrosine-kinase activity and auto-phosphorylation at the insulin receptor level (Guerrero-Romero *et al.*, 2004). This improvement may also be attributed to the role of Mg^{2+} at the ribosomal level in protein synthesis (Brilla and Haley, 1992) although no further studies have investigated this and the exact mechanism has, to date, not been demonstrated.

MgATPase activity and Mg-bound ATP present on the acto-myosin heads results in faster and higher rate of filament detachment and reattachment producing faster and more powerful muscle contraction. This also occurs due to the increased rate of ATP hydrolysis from higher ATPase activity on the myosin heads (Brilla and Haley, 1992).

The results from this study concur with previous research such as Brilla & Haley (1992) and Finstad & Newhouse (2001) and therefore efficacy of Mg supplementation for endurance exercise and recovery was investigated in subsequent papers submitted as part of this thesis.

This study is the first to investigate the effect of Mg^{2+} supplementation on blood pressure after exercise. The observed decrease in systolic blood pressure was in line with previous work in the resting state, but the effect on post-exercise blood pressure had not previously been investigated. The results from this small study raised many questions that led to further investigation forming part of the body of work. Mechanisms for this reduction in blood pressure have been postulated in the introduction above (p15-16) but only observational measurements were available in this first investigation.

For this study magnesium aspartate was used, but it was subsequently banned in the UK due to the concern that aspartate is a known excitatory neurotransmitter and that an overdose may occur.

From the positive results found from this first study, Mg^{2+}-L-Aspartate HCL would have been the primary choice of supplemental form for administration for further studies. However, European legislation as published by the European Food Safety Authority (Aguilar *et al.*, 2008) banned the consumption of supplemental aspartate due to the NAOEL (no observable adverse effect level) being low and an intake above this may induce an amino acid imbalance which has been shown to cause neural excitability (Aguilar *et al.*, 2008). It is, however, still available on prescription. Magnesium citrate or oxide were used for all subsequent studies.

The changes in blood pressure were greatest in this investigation compared to other work forming part of this thesis. It cannot necessarily be attributed to the form of magnesium used but is a consideration for future work.
Nevertheless, the literature available of Mg^{2+} bioavailability from differing supplement forms provides minimal indication as to elucidate and specify the most effective type of Mg^{2+} supplementation within humans (Coudray *et al.*, 2005).

Having used Mg^{2+} aspartate for this first study the results showed a significant increase in peak power and mean peak power during the WAnT and a significant increase in peak torque on the knee extension. There was also a significant decrease in SBP during the 30-minute recovery with the magnesium supplemented group. The results for this were presented at the Nutrition Society Annual Conference (2010) as a poster and interest in the

blood pressure results was much greater than had been anticipated. Previous research had already shown that there was an association between magnesium and blood pressure although the literature did not demonstrate consensus with respect to dose and length of intervention. There was also very little research examining this in combination with physical activity or exercise. It was also questioned whether the amount of habitual dietary magnesium intake impacts on the absorption and effect of magnesium supplementation. These therefore formed the research questions for the next investigation.

2.4.2 Dietary stratification of high and low magnesium intake and effect on blood pressure and performance

After demonstrating the benefits of magnesium supplementation on anaerobic power, peak power and blood pressure in the first study, the next study was designed to determine whether magnesium supplementation influenced aerobic and resistance exercise performance and post-exercise blood pressure. To date no research has analysed habitual dietary Mg^{2+} intake and any interaction with the effects of supplementation. As Mg^{2+} transporters TRPM6 and TRPM7 have upper saturation levels (Rondón *et al.*, 2008), it was postulated that those with adequate dietary intake of magnesium may not benefit from an additional intake via the supplement, although no harmful effect would be anticipated either.

Many studies have asked participants to record food diaries to establish what are habitual daily magnesium intake with the goal of determining whether the RNI for magnesium was achieved. However, the influence of habitual Mg intake on the efficacy of Mg supplementation has not previously been assessed.

The objective for this pilot intervention was to investigate the effect of magnesium supplementation on systolic blood pressure whilst resting and during recovery from aerobic and resistance exercise and on endurance and resistance exercise performance. The stratification of high and low dietary intake was not an *a-priori* to the study and was introduced after dietary magnesium intake had been analysed from the food diaries.

Sixteen male volunteers were randomly assigned to either a 300 mg/d magnesium oxide supplementation or a control group for 14 days with a parallel design. This was further stratified into high and low dietary Mg^{2+} intake with the cut off being at the RNI of 300mg/d. Resting blood pressure and heart rate was measured before a 3 minute warm-up after which participants performed a maximal 30 minute cycle time trial immediately followed by three isometric contractions held for five seconds each whilst performing a bench press, blood pressure measurement was repeated post exercise. The laboratory conditions were a

constant 21^0c on all days of testing and data was collected between November and January. A 3-minute warm-up had been considered standard up to this study but 5 minute warm-ups were undertaken for subsequent research as reference to other studies showed this to be the standardised time for a warm-up and to decrease risk of injury for a maximal protocol, although no injuries were experienced in the present study. A 3-day food diary was recorded for all participants. In the experimental group for all participants (n=8), mean resting systolic BP was reduced by 8.9 mmHg, p=0.01) and post exercise by 13 mmHg (p=0.01). Recovery BP was 11.9 mmHg lower in the experimental group compared to control (P=0.006). HR was 7 beats per minute lower in the experimental group (69.0 ± 11.6 bpm, P=0.02). Performance indicators were not different between the groups.

When blood pressure results were stratified by dietary Mg^{2+} intake there was no significant difference found for resting systolic BP in the control group for those above or below the RNI pre to post intervention. However, a significant change was seen in resting systolic BP in the experimental group from pre to post intervention in the low dietary magnesium intake cohort (n=3) (from 126.3±8.2 to 114.6 ± 11.6 mmHg, P=0.02) but with no change observed in those with a high dietary Mg intake after supplementation (n=5). Whilst the sample size of this pilot study were low, the results suggest that supplementation has a greater effect on those with a habitual low dietary intake than on those with a high dietary intake. No other studies have set out to determine this and further research is warranted in this area. Baseline SBP was also different between the high (121±6.1 mmHg) and low (127±8.3 mmHg) habitual dietary intake groups although a post hoc t-test showed that there was no significant difference between the groups (p=0.115). The differences in SBP found between a low and high magnesium dietary intake both at baseline and after supplementation also raises the question as to whether habitual dietary intake is low in those with clinically diagnosed hypertension and was investigated for Study 5 below.

The Honolulu heart study undertaken by Joffres, Reed and Yano (1987) determined that Mg^{2+} intake from diet and supplementation had a strong inverse relationship with both systolic (SBP) and diastolic blood pressure (DBP). Results from both habitual diet and supplementation showed that greater Mg^{2+} intake reduced SBP by 4.6 mmHg and DBP by 2.8 mmHg. Supporting these findings, Zhao (2004) also observed an inverse relationship between magnesium and hypertension when studying dietary differences and blood pressure between north and south China. It was identified that the population in the south of the country consumed significantly more Mg^{2+} than the north, and both SBP and DBP were lower in the south by 7.9mmHg and 6.9 mmHg respectively (Zhao *et al.*, 2004). Although there was a relationship between the two variables, the lower blood pressures could not be completely attributed to the intake of magnesium as other nutrients may have

also affected blood pressure and were not adjusted for, although these were not analysed as part of this study. Joffres et al (1987) observed 61 dietary variables in a group of 615 men who were free of cardiovascular disease. Their results were similar to those of Zhao et al., (2004) who, in finding an association between total Mg^{2+} intake and blood pressure, could not attribute the findings solely to Mg^{2+} as the trend was also associated with dietary potassium, phosphorous and fibre. Their results suggested that the high Mg^{2+} content of vegetables, whole grains and fruits may contribute to the low blood pressure seen in vegetarians. On the contrary a cross-sectional study using data from the National Health and Nutrition Examination Survey (Public Health England, 2014b) reported no significant correlation between magnesium intake and blood pressure.

Magnesium intake, in addition to other food sources, affects the bioavailability of Mg^{2+}, highlighting the significance of dietary analysis. The current recommendation for dietary Mg^{2+} intake in the UK is the reference nutrient intake (RNI) values of 300 $mg.day^{-1}$ and 270 $mg.day^{-1}$ for males and females, respectively, implemented by the Committee on Medical Aspects of Food and Nutrition policy (Department of Health, 1998). The National Diet and Nutrition Survey (Public Health England, 2014b) stated that the average consumption of the RNI in the UK is at 90% for males and 82% for females, representing a population consuming less than recommended. Such statistics corroborate the increased prevalence of hypertension in the UK. For example the UK prevalence of hypertension is at 42% , establishing UK as the fourth highest European country in terms of hypertension prevalence, which is also greater than for the United States (14.2%) and Canada (16.6%) (Wolf-Maier *et al.*, 2003). Reasons suggested for this prevalence include both dietary and lifestyle factors and better health checks where hypertension can be diagnosed and medication prescribed (Geleijnse, Grobbee and Kok, 2005). In a comparative study between 5 European countries Geleijnse et al (2005) found that mineral intake inadequacies i.e. suboptimal Mg^{2+} (<350mg/d) and K^{+} (<3.5g/d) or surplus Na^{+} (>2.4g/d) increased the prevalence of hypertension. In this study 80% of the UK participants were below the RNI, which was greater than in other countries; additionally, the study associated the low Mg^{2+} intake as a risk factor for hypertension with a population attributable risk percentage (PAR) of 7%. This realisation of the insufficient Mg^{2+} intake in the UK from these previous studies shaped future work and study 5 from this PhD investigates whether those with hypertension habitually ate a diet that was low in Mg^{2+}.

In addition to the established RNI for Mg^{2+} which applies to the general population, it has been shown that the amount required for an athletic population may be higher in order to avoid hypomagnesia. For example, for general population dietary Mg^{2+} intakes of <200-250

mg/d would be needed to cause hypomagnesia (<0.65 $mmol.l^{-1}$) but in an athletic population this would only need to be less than 300mg/d (Geiger and Wanner, 2012).

This study has been the first to investigate whether dietary intake influenced the effects of Mg supplementation. To date there are no studies that determine if higher habitual dietary intake affects absorption of Mg supplements or if supplementation affects absorption of dietary intake. There is no direct evidence to suggest a threshold effect but this has been proposed with the suggested mechanism of saturation of the TRMP transporters. This natural divide of the high and low dietary magnesium intake, although not an *a-priori* hypothesis in this study allowed a new area to be piloted for further work. Other questions that arose due to this study were that of length of supplementation. If intestinal Mg transporters were to become saturated, would continued supplementation add further benefit or would absorption efficiency be impaired as transporters became saturated? There were no definite guidelines on how long Mg^{2+} supplementation should be taken, how much should be ingested and little information on what type was best. There was also conflicting information with regards to the benefit of Mg^{2+} supplementation on hypertension. Many studies showed a reduction in blood pressure in particular systolic pressure, yet the NICE guidelines specifically stated that Mg^{2+} supplements should not be considered for hypertension. Therefore, for study 3 a meta-analysis was performed to pool data across all available studies to investigate effects of Mg^{2+} on BP.

2.4.3 Effect of magnesium supplementation on blood pressure – a meta-analysis

This study was a systematic review of existing literature to determine the overall effect of magnesium supplementation on blood pressure. This was a defining piece of work that was to shape future investigations on magnesium by synthesising the evidence and identifying gaps in the literature and following publication received much attention.

Previous research on magnesium supplementation and blood pressure was equivocal and this meta-analysis was undertaken to synthesise all available evidence as to the effect of Mg^{2+} on blood pressure and to establish the characteristics of trials showing the largest effect size. Primary outcome measures were systolic blood pressure and diastolic blood pressure at the end of a follow up period.

One hundred and forty-one papers were identified of which 22 trials with 23 sets of data (n=1173), with 3 to 24 weeks follow up, met the inclusion criteria with a supplemented elemental magnesium range 120mg to 720mg (mean dose 398mg). 95% confidence intervals were calculated using DerSimonian and Laird's (DerSimonian and Laird, 1986)

random effect model with effect size calculated using a Hedges G (Hedges and Olkin, 1985). The inclusion criteria were: 1) magnesium supplements as the only active Intervention 2) presence of a placebo or control group. 3) participants over the age of 18. 4) random allocation of participants to treatment conditions. 5) parallel or crossover trial design. All criteria had to be met for inclusion in the meta-analysis.

Combining all data, an overall effect size of 0.36 and 0.32 for DBP and SBP respectively was observed (95% CI 0.27 to 0.44 for DBP and 0.23 to 0.41 for SBP), with a greater effect for the intervention observed in crossover trials (DBP 0.47, SBP 0.51). Effect size increased in line with increased dosage. Although not all individual trials showed significance in BP reduction, combining all trials did show a decrease in systolic BP of 3-4mmHg and diastolic BP of 2-3 mmHg, this further increased with crossover designed trials and intake above 370 mg/d.

It was concluded that magnesium supplementation elicits a small but clinically significant reduction in BP, an effect worthy of future prospective large scale randomised trials using robust methodology.

Several studies have demonstrated that Mg^{2+} supplementation has a positive effect in reducing blood pressure in both hypertensive and normotensive individuals (Motoyama, Sano and Fukuzaki, 1989; Itoh, Kawasaka and Nakamura, 1997; Kawano *et al.*, 1998; Doyle, Flynn and Cashman, 1999). Ranges of between 120-1000 mg/d of Mg^{2+} have been used to reduce blood pressure as can be seen in meta-analyses carried out prior to and subsequent to the present meta-analysis (Jee *et al.*, 2002; Dickinson *et al.*, 2006; Zhang *et al.*, 2016). However several studies contradict this finding suggesting that Mg^{2+} supplementation will not aid in the reduction of blood pressure (Cappuccio, 1985; Yamamoto *et al.*, 1995; Sacks *et al.*, 1998). For these studies there was variation between the population groups and the dosage and duration of supplementation, but no single factor can be identified to have caused this variation in results. This contradictory literature adds to the doubt concerning the potential relationship between Mg^{2+} and blood pressure.

However, a previous meta-analysis by Dickinson et al. (2006) did not find an association between Mg^{2+} and blood pressure and despite the fact that this is the only meta-analysis not to have found an association, it was accepted by the Cochrane Library and therefore that single review influences healthcare decision makers in the UK and policy makers such as the National Institute of Clinical Excellence (NICE) and guides the decision that magnesium should not be used as an aid to lower high blood pressure. Moreover, even the authors of the Dickinson study (2006) have made the following conclusion '*In view of the*

poor quality of included trials and the heterogeneity between trials, the evidence in favour of a causal association between magnesium supplementation and blood pressure reduction is weak and is probably due to bias. This is because poor quality studies generally tend to over-estimate the effects of treatment. Larger, longer duration and better quality double-blind placebo controlled trials are needed to assess the effect of magnesium supplementation on blood pressure and cardiovascular outcomes'.

This meta-analysis by Dickinson et al., (2006) found that there was a small but significant reduction in DBP but no effect on SBP, a finding that contradicts every other study investigating this area. The report only reviewed 12 trials with a total of 545 people. However, the authors concluded that most of the trials were of poor quality, with the intervention not long enough or large enough to detect the possible consequences of magnesium supplementation on high blood pressure.

The inclusion criteria for Dickinson et al., (2006) were: 1) RCTs of a parallel or crossover design comparing oral magnesium supplementation with placebo, no treatment, or usual care; 2) treatment and follow-up ≥8 weeks; 3) participants over 18 years old, with raised systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥85 mmHg; 4) SBP and DBP reported at end of follow-up. The current meta-analysis' inclusion criteria were: 1) magnesium supplements as the only active intervention; 2) presence of a placebo or control group; 3) participants over the age of 18; 4) random allocation of participants to treatment conditions and 5) parallel or crossover trial design. All criteria had to be met for inclusion into the study. The inclusion criteria were very similar between the studies. Of the 12 studies included in the Dickinson et al (2002) study, 3 were not included in the current study, one because of participants being on a low sodium diet (Nowson and Morgan, 1989) , a second because the participants were on thiazide, a diuretic used to reduce blood pressure (Paolisso *et al.*, 1992) and the third due to hawthorn extract being used as an intervention alongside magnesium (Walker *et al.*, 2002). The inclusion of these studies appears to go against the inclusion criteria of 'no treatment and usual care' and also adds a second intervention into the studies. However, the current meta-analysis, which is the more recent, included 13 studies which were not in the Dickinson et al (2002) study of which only 3 were published since publication of that earlier study. The reason for the non-inclusion of the other 10 studies cannot be determined and reference to the Dickinson et al (2002) paper for more detail on this for the interested reader would be suggested.

The current meta-analysis concluded that Mg^{2+} supplementation lowers BP. Further it showed that a cross-over design affects response more than a parallel design. For crossover trials the effect size increased substantially for both DBP and SBP when compared to the

non-crossover trials, reinforcing the idea that paired data would have more robust results from the intervention than non-crossover and that the effect from the intervention would be augmented.

Dosage was found to have had a linear effect on response magnitude. A sub-analysis was carried out with results divided into those with supplementation <370 $mg.day^{-1}$ and those ≥ 370 mg Mg per day. Results for both SBP and DBP showed greater efficacy of magnesium supplementation at the higher dose. When the higher magnesium dosage was further analysed a much higher effect size (DBP = 0.66 and SBP = 0.70; 95% CI: 0.51 to 0.82 and 0.56 to 0.89 for DBP and SBP respectively) was found, and those using <370mg demonstrated high levels of variation.

There was substantial heterogeneity between the findings of the trials for both DSP and SBP (I^2 =82 and 88 respectively) which could be explained by random variation, the various population groups, the interventions or the methods used in the trials and length of intervention. A more homogenous sample of studies may increase effect size; although a random effects model was used, the difference between the studies was high.

Only one systematic review has previously looked at dietary magnesium and blood pressure (Mizushima *et al.*, 1998) and one since the publication of this meta-analysis (Han *et al.*, 2017). Mizushima (1998) concluded that there was a negative association between the two variables, although the authors indicated that inconsistencies between design and analysis of the studies complicated data interpretation. Han et al (2017) included 10 studies, of which only one was published after this meta-analysis by Huitron-Bravo (2015). This study had data collected in 2006 with a 7 year follow-up period and found only modest inverse relationship between dietary intake and BP in a Mexican population. In agreement with our work, Hans et al (2017) concluded that the current evidence supports the inverse dose-response relationship between dietary magnesium intake and the risk of hypertension.

In summary major limitations evident in most of the studies included in the meta-analysis were: -

- lack of data on background dietary intake, which may have had major implications for all studies (see study 2).
- lack of pre- and post-supplementation serum magnesium concentration, which would have enhanced understanding of absorption over a range of magnesium intakes.

Magnesium supplementation produced a greater effect size in studies employing a cross-over rather than non-crossover design. This may be because the non-crossover design will be confounded by inter-individual variation in habitual magnesium intake whereby those who habitually consume more magnesium may show less of a response to the supplement than those who habitually consume less magnesium. This meta-analysis highlighted gaps in the literature with regards to dietary magnesium intake and blood pressure and the influence of dietary magnesium intake on magnesium supplementation effects, both of which form part of this body of work. Another unanswered question was the duration of supplementation required for effects to be observed.

The next study investigated the duration of supplementation. As mentioned above, magnesium transporters may become saturated once optimum magnesium is ingested. The research looks at whether continuing to supplement with magnesium after the initial dosage has been given will further enhance the effect of magnesium. This investigation therefore sets out to determine whether the response to the first acute dose is the same as that of the Nth dose i.e. is chronic dosing necessary?

2.4.4 Further effects of magnesium on blood pressure with acute and chronic dosing strategies

The meta-analysis showed that there are many aspects in relation to the effects of Mg^{2+} supplementation and the most efficacious dosing regime that were still unknown. One of these being the on-going question as to whether there is a cumulative effect of Mg^{2+} supplementation or whether favourable effects are due to repeated acute responses to each individual dose. From a mechanistic perspective this would be very difficult to determine at the cellular level. We therefore investigated whether the effects of acute (A) (1 week) and chronic (Chr) (4 weeks) loading on exercise and blood pressure were comparable as well as augmentation index (Aix). This index is a parameter measured by pulse wave analysis (PWA) and is used as a surrogate measure of arterial stiffness. This allowed direct comparison of the response to one and four weeks of supplementation to isolate whether cumulative effects were evident. We hypothesised that the absence of a cumulative effect would indicate transporter saturation was achieved after one week. No previous studies had investigated loading strategies to determine differences in outcomes on exercise performance and blood pressure from acute and chronic supplementation.

This study set out to determine whether either acute or chronic magnesium supplementation would have an effect on performance (strength and cardiovascular) and blood pressure with exercise and/or on a second bout of exercise after a 24 hr recovery period. It was hypothesised that as acute Mg^{2+} supplementation has been demonstrated to have beneficial

effects on BP, CV parameters and augmentation index (Aix) a longer loading strategy (4 weeks) would amplify these results, giving a more beneficial and greater response.

A total of 13 participants (female = 6) were recruited from recreational running, cycling and triathlete clubs. Six participants were allocated randomly to the acute (1 week) intervention group (m = 3, f = 3) and 7 to the chronic (4 week) intervention group (m = 4, f = 3), receiving the same daily dose with post-supplementation measures taken at the same interval after the last supplement dose.

The study was a randomised, double-blind, crossover, placebo controlled, 2 day repeated measures protocol and was run across 2 consecutive days pre at baseline and again after either 1 or 4 weeks of intervention.

A 40 km cycling time trial was followed by as many bench press as possible at 80% 1RM to exhaustion for two consecutive days with distance, blood pressure and augmentation index (Aix) being measured for both an acute and chronic loading strategy.

Primary findings showed variance across treatment groups on exercise (strength and recovery) and cardiovascular responses. The chronic Mg^{2+} intervention showed no significant performance gains for bench press and strength. The acute Mg^{2+} intervention showed variation in results across all variables analysed with a trend shown for improvement in the bench press. However, the magnitude of the effects of acute and chronic loading of Mg^{2+} were similar.

2.4.4.1 Strength performance:

For the acute group improvements were seen in 1-RM bench press. In the published article this is presented as 17.7±1.3% (p=0.031) after 1 week of supplementation and no decline in recovery (day 2) performance, whereas the correct figure should be 7.7±1.3% (p=0.031). An erratum has been submitted to the journal. The chronic group showed no improvement in 1-RM bench press (p=0.281) and there was a performance decrement of 32.1±8.2% on the recovery day (day 2). Previous research has shown that high dietary Mg^{2+} intake and high serum Mg^{2+} concentration significantly enhanced bench press (Matias *et al.*, 2010) and strength performance (Dominguez *et al.*, 2006a; Santos *et al.*, 2011). In the present study acute but not chronic Mg^{2+} loading showed a significant strength increase, and where a decrease in force would be typically be expected on day 2 (recovery) a small improvement was seen.

These performance enhancements for the strength associated tests are suggestive of physiological-regulatory functions of Mg^{2+} in muscle contraction and relaxation; i.e. regulating troponin expression via Ca^{2+} concentration gradients, MgATP complex formation optimising energy metabolism/muscular contraction, increasing protein synthesis rate, protection against cellular damage and, greater amount of actin-myosin crossbridges (Brilla and Haley, 1992; Lukaski, 2004; Barbagallo, Dominquez and Resnick, 2007; Carvil and Cronin, 2010) all of which contribute to increased strength and force production. Consideration must be given as to why such a contrasting difference between Chr and A groups occur specifically when regarding strength performance measures. The Mg^{2+} supplementation dose in the current research was 300mg/d, therefore equating the Chr and A group mean daily intake for Mg^{2+} to 675 mg/d and 668 mg/d, respectively, when combined with dietary Mg^{2+} intake as analysed from food diaries. This adds a sense of greater ambiguity when considering the Mg^{2+} - strength performance relationship, and comparing to research highlighting observations that intakes of 500 mg/d or greater result in further increases in strength (Brilla and Haley, 1992; Lukaski, 2004).

Although the RNI was exceeded, the expected effect was not seen. It could be suggested that the acute effect observed after 1 week was attenuated after 4 weeks daily supplementation due to saturation of Mg^{2+} transporters. It could be suggested that participants within the Chr loading group might be more susceptible to Mg transporter downregulation or cell tolerance for Mg^{2+} absorption based upon the understanding that high Mg^{2+} intakes result in a lower Mg^{2+} absorption (Jahnen-Dechent and Ketteler, 2012). This is a repeated theme in this body of work and is something that will continue to form future research questions. There is no evidence to support this as, to date, there is no published work investigating upper thresholds or tolerance levels. Additionally, Mg^{2+} homeostasis may be postulated to exhibit no greater benefit from the chronic perspective due to the kidney function for Mg^{2+} excretion as to maintain a balanced concentration of Mg^{2+} (de Baaij, Hoenderop and Bindels, 2012; Jahnen-Dechent and Ketteler, 2012); for example, could the principle of a higher Mg^{2+} dose, longer supplemental duration and associated proportional increase of Mg^{2+} excretion highlight the body's efficiency in maintaining a state of homeostasis? Alternatively, chronic loading through providing a regular high Mg^{2+} intake may influence extracellular Mg^{2+} concentrations which coincide with manipulation of Mg^{2+} transporter TRPM6 function, resulting in a potential decrease in TRPM6 expression in conjunction to increasing the urinary excretion of Mg^{2+} (Alexander, Hoenderop and Bindels, 2008). Thus, an acute ingestion protocol may prove more effective for enhancing exercise performance than a chronic supplementation approach.

2.4.2. Blood pressure

Significant reductions in SBP and DBP were seen in post-exercise measurements in the chronic group and rest and post-exercise in the acute group across day 1 and 2 compared to both baseline and placebo groups. Resting SBP declined to a greater extent after A Mg^{2+} (2±0.6 mmHg), in comparison to 0.7±0.4 mmHg with the Chr Mg^{2+} treatment. In addition, both resting and post-exercise DBP showed reductions with a greater day-to-day DBP reduction in the A Mg^{2+} in comparison to Chr Mg^{2+} as shown by a 69.2% and 50% (9 mmHg and 3 mmHg difference) at rest and post exercise for A and Chr groups respectively. These findings are in agreement with previous research (Motoyama, Sano and Fukuzaki, 1989; Itoh, Kawasaka and Nakamura, 1997) showing the importance of Mg^{2+} and its influence on blood pressure regulation. This supports the findings discussed previously in the meta-analysis which forms part of this body of work (study 3).

Such reductions in blood pressure could be speculated as being an outcome influenced by increases in the extracellular concentration of Mg^{2+}, an effect that has been associated with reductions in arterial tension and tone. These reductions in arterial tension and tone correspond to typical Mg^{2+} induced vasodilatory actions, which potentiate effects of endogenous vasodilators such as adenosine, K^{+}, nitric oxide and cyclo-oxygenase-dependent mechanisms via production of PGI2 (Pokan *et al.*, 2006). In combination, Mg^{2+} acts as an antagonist to blocking Ca^{2+} channels (O'Rourke, Backx and Marban, 1992; Laires, 2004; Santos *et al.*, 2011) and further enzymatic mobilisation of Ca^{2+} (Laurant and Touyz, 2000). Thus, data in the current study concur with previous research on the efficacy of Mg^{2+} supplementation in reducing blood pressure (Itoh, Kawasaka and Nakamura, 1997; Guerrero-Romero and Rodríguez-Morán, 2002) and its capacity to suppress agonist vasoconstriction (Touyz, 2003). Many of these models have been shown in rats, with very few in vitro studies in humans. This is another area for future research.

For the augmentation index no changes were seen at rest for both cohorts. However, there was a significant lowering post exercise on day 1 for the acute treatment group and day 2 for the chronic treatment group which does not correspond to the SBP changes but does correspond to changes in DBP for the acute post exercise measurements.

Average dietary Mg^{2+} intakes within the A and Chr groups corresponded to 368 mg/d and 375 mg/d, respectively which are above the RNI. Greater effects may have been seen in a population with sub-optimal Mg^{2+} intake. Therefore, it could be considered that the blood pressure reduction in Chr and A loading strategies, may be attributed to the Mg^{2+} supplementation and that a higher daily intake may be beneficial in reducing blood pressure. Research by Geleijnse et al.(2005) in a comparative study between 5 European countries

which included the UK, corroborates with this study, recommending a potential increase in Mg^{2+} intake due to the hypotensive and further suggesting that <350mg/d of Mg^{2+} may be suboptimal, increasing the prevalence of hypertension.

The initial focus of this body of work was on the application of Mg supplementation to exercise performance and physiology. However, despite the absence of ergogenic effects of chronic Mg^{2+} for performance, there appeared to be the potential for health benefits associated with the potential for reduced BP. Further, when investigating the effects of dietary intake and its effect upon magnesium supplementation and blood pressure it was observed that there was a gap in the literature regarding the habitual dietary intake of magnesium in those who had been diagnosed with hypertension

2.4.5 Habitual dietary magnesium intake in the hypertensive population; observations on general population dietary intake

This investigation set out to determine whether those with hypertension habitually ate a diet that was low in Mg^{2+}; a question that had not previously been addressed by research in the UK. Many studies have found a positive correlation between magnesium supplementation and reductions in hypertension (Kawano *et al.*, 1998; Townsend *et al.*, 2005; Rylander, 2012, Itoh, Kawasaka and Nakamura, 1997) Zhang *et al.*, 2016) but to date there have been no studies that have looked at those already diagnosed with primary hypertension and investigated their dietary magnesium intake. A recent study by Bain (2013) investigated magnesium intake and BP in relation to risk of stroke, with BP being causal for up to 70% of all stroke cases. This study was specific to the British population with participants completing a 7-D food diary. Multivariate adjustment was made for age, BMI, total energy intake, education level, smoking status, physical activity, baseline history of stroke, heart attack or diabetes mellitus, family history of stroke or heart attack, use of aspirin medication (>3 month), antihypertensive medication, dietary sodium, potassium, alcohol and total fat intake. Results showed that there was an inverse relationship between dietary magnesium and blood pressure, significant in men, but not in women. In men differences between top and bottom quintiles of - 7mmHg and - 4.3mmHg, for SBP and DBP respectively were observed. This decrease in BP could be associated with a reduction of stroke risk by up to 40%, a highly meaningful clinically significant effect that has been explored further in paper 5 (p.58).

A study on a Mediterranean population (Schroder, Schmelz and Marrugat, 2002), observed the relationship between diet and blood pressure. The study specifically looked at sodium, calcium and potassium in populations with different blood pressure status and found to be directly related to blood pressure. This relationship was also observed further seen with the sodium to potassium ratio, independently of hypertensive drug treatment. An inverse association was also observed between blood pressure and dietary calcium intake. Furthermore, moderate sodium in combination with a calcium intake reduced the risk of inadequate blood pressure control. Finally they observed that those with controlled hypertension have a significantly higher calcium intake than non-controlled participants (Schroder, Schmelz and Marrugat, 2002). This study found that nutrient intake was similar among groups of different blood pressure status after adjusting for sex, age and energy consumption. These adjustments were not made in the study carried out for this portfolio of work. This study set out to determine the habitual dietary magnesium intake in those with hypertension and for normotensives in the local area and then to cross reference this with the National Health and Nutrition Survey data (Public Health England, 2014b) to ensure that the population sample was representative . This project will form the preliminary work for a grant application to conduct a larger study in which the methodology from Schroder et al (2002) will be adopted to ensure a statistically robust approach to data analysis and interpretation. This will include adding age as a confounding factor along with energy intake. The mean age in the Schroder et al (2002) study were normotensive 45.1±12.5 y, non-medicated hypertensive 58.6±11.1 y and medicated hypertensive 62.0±9.6 y which are similar to the ages of the participants in this study and appear to be a normal representation of those with and without hypertension. This further supports the need for a larger study and to include adjustments for age.

The current RNI in the UK is 300mg and 270mg daily for males and females respectively from 18-75+ years (Public Health England, 2014b). For the USA the Recommended Dietary Allowance (RDA) is 400mg and 310 mg for males and females respectively from ages 19 – 30 years and 420-320 mg daily for those aged > 31 years old (Institute of Medicine. Food and Nutrition Board., 1997) The National Diet and Nutrition Survey (Public Health England, 2014b) states that the average consumption of the RNI in the UK is at 90% for males and 82% for females, representing a population consuming less than recommended. The study therefore was to determine whether those with primary hypertension habitually eat a diet that is both lower than the RNI and lower than the UK national average for age and sex. Further consideration was given to how this compares to the USA RDA with a substantially higher recommended intake. As the UK national average intake is already lower than the RNI these were compared to the higher RDA used in the USA to observe the greater

difference between intake in the UK and recommendation in the USA. Participants were recruited from the Lister Hospital. All participants were clinically diagnosed with primary hypertension by Prof Diana Gorog, who was kind enough to allow access to patients in the waiting rooms during clinics and would identify those patients who were suitable for the study.

Twenty-five participants (female n=11, male n=14, mean age 63.4±10.2y) were recruited from the East and North Herts NHS Trust.

A further 21 normotensive participants (female n=11, male n=10, mean age 46.7±10.6y) were recruited from the same geographical area to act as normotensive internal controls (NT). Participants were excluded if they suffered from hypertension (BP ≥ 140/90) or were being medicated for the condition, a pre-study blood pressure recording was undertaken to confirm this. All participants completed a 4-day food diary in week 1 including 3 mid-week days and 1 weekend day, this was repeated in week 4. The food diary was adapted from The Medical Research Council Collaborative Centre for Human Nutrition Research, (Medical Research Council.). Although classified as an estimated food diary, food could be weighed and food labels submitted, in addition to photos being provided to establish portion size. An external control of the general UK population (GP) aged 19y and over was established from data provided by the National Diet and Nutrition Survey (NDNS)(Public Health England, 2014b).

Average daily magnesium intake established from the 8-day food diaries were compared between the HT, NT and GP groups and these were compared to both the UK RNI and the USA RDA to establish differences amongst the hypertensives and the recommendations.

The observation from this study was that 80% of the UK participants analysed, habitually consumed below the RNI for Mg^{2+}, which was a greater proportion than in other countries. This concurred with the National Diet and Nutrition Survey (Public Health England, 2014b) that found 86% of the population were not meeting the RNI.

Public Health England (Public Health England, 2014a) stated that food source contributions from diet for Mg^{2+} intake were as follows:- cereals and cereal products 31%; dairy products 16%; meat and fish 13%; vegetables 11% and fruits 8%. Previous data indicate a considerable intake of Mg^{2+} from foods rich in dietary fibre i.e. cereals that are high in phytate (Food And Nutrition Board and Institue Of Medicine, 1997); the UK phytate daily intake has been recently published as 809 mg/d for adults (Amirabdollahian and Ash, 2010). It has been shown that phytates may induce inhibitory effects on Mg^{2+} absorption

decreasing the fractional absorption (Coudray *et al.*, 2005). Previous research, although limited in relation to Mg^{2+}, evaluated the effect of meals based on 200g of added phytic acid into acid-free white-wheat bread which equated it to a similar content to phytate products i.e. bran and whole-meal bread. The study revealed that the fractional Mg^{2+} absorption was significantly inhibited (60% lower) from white-wheat bread consumption when the phytic acid was added (Bohn *et al.*, 2004).

A further negative interaction is that of Ca^{2+} which has been shown to negate Mg^{2+} absorption when consumed in excess of 2000mg/d (Whiting and Wood, 1997)(Whiting and Wood, 1997) , or when combined with high Na^{+} which has been suggested to lead to a negative Mg^{2+} status due to enforced Mg^{2+} excretion (Burgess *et al.*, 1999). Kesteloot et al (Kesteloot *et al.*, 2011) suggested that an imbalance between the serum Ca^{2+}:Mg^{2+} ratio can be associated with elevated blood pressure levels. This reinforces the association between Mg^{2+} and blood pressure and will be considered for future research.

This current study further observed hypertensive cohorts for both male and females had a dietary Mg intake significantly lower than the RNI. Hypertensive males also had an intake that was significantly lower than the general population (which is already below the RNI) and that hypertensive female intake was significantly lower than the normotensive internal control. As age increased there was a general trend towards a decrease in daily Mg intake. The hypertensive group was also compared to the US RDA and found to be significantly lower for both sexes. Lastly, the UK general population were compared to the US RDA for both males and females and were found to represent 64% and 69% of the RDA respectively. It was concluded that daily Mg intake in hypertensives is lower than the general population, the UK RNI and the US RDA and that daily magnesium intake reduces with age.

With any dietary intake study in a clinical group of participants there is the concern of reverse causality. However, one of the drivers for investigation into magnesium and hypertension is that the general population tend not to know about the effect of magnesium on blood pressure and clinicians will follow the NICE guidelines which state not to recommend magnesium (Dickinson *et al.*, 2006). It is recommended by the Cochrane Library that salt is reduced in the diet to aid in the reduction of hypertension (Jürgens and Na, 2008) and this tends to be the main adjustment made by those with hypertension. However, some changes may be made by the individual to eat a diet that is perceived as healthier and this could affect the amount of green vegetables eaten and therefore increase the amount of dietary magnesium intake. However, the results of this current study do not point to this as the amount of magnesium in the hypertensives tended to be lower than the normotensives.

Water consumption will also affect the daily amount of magnesium ingested, however, in the UK magnesium is not a regulatory parameter and there is no requirement for the water authorities to check levels of magnesium (Drinking water inspectorate, 2017), therefore these data are not available. A review by Rosanoff et al (2013), stated that drinking water in the United States should contain a minimum of 25-50 ppm magnesium and at this concentration two litres a day would provide 15-25% of adult RDA. Yang and Chiu (2000) demonstrated a negative association between blood pressure and calcium and magnesium levels in drinking water in Taiwanese residents. Further when adjustment was made for magnesium levels in drinking water there was no difference between the groups with different levels of calcium showing that there is a significant protective effect of magnesium intake from drinking water on the risk of hypertension, independent of calcium. This important finding could be investigated in the UK if independent water analysis was taken on the local drinking water of participants and would make an interesting future investigation.

According to Long Term Health Conditions (Public Health England, 2014a) 1 in 4 adults is affected by high blood pressure in the UK. Figures show that by reducing the blood pressure of the nation as a whole, £850 million of NHS and social care costs could be avoided over 10 years. Further, if 15% more people currently being treated for high blood pressure could control it better, a further £120 million could be saved. (Public Health England, 2014a). This study was the first to show the association between low dietary Mg^{2+} intake in those with previously diagnosed hypertension, the first study in the UK to look at this and the first study on humans. It could prove significant in future work with dietary supplementation and it is proposed to use these results to apply for funding to carry out a larger study investigating dietary intake and hypertension. At present fortification of foods in the UK is voluntary for most foods, with some compulsory addition in certain foods. For example, white and brown bread must be fortified with thiamin, iron and niacin to replace those nutrients lost in processing. Magnesium is not compulsory in any foods (The British Nutrition Foundation, 2017). With the prevalence of hypertension in the UK, it is felt that additional research into dietary magnesium and hypertension.

All previous studies for this PhD have investigated either dietary or oral supplementation. However, transdermal creams and oils are now commercially available and offer an alternative way for people to supplement their micronutrient intake. The literature on this is varied and there have been no previous studies that have investigated the absorption of transdermal magnesium application on humans. The Institute of Magnesium Research has recently formulated such a cream and the author of this thesis was approached to investigate whether the cream absorbed systemically. This study forms the final publication for this PhD

and is novel in the area of transdermal creams. Further, this study observes an alternative source of magnesium supplementation which can be used in future methodology.

2.4.6 Transdermal magnesium absorption

The Institute of Magnesium Research undertakes many studies examining magnesium and in particular its effect on general wellbeing and on muscle cramps. After some correspondence with the institute's director a project was set up to determine whether magnesium is absorbed transdermally when suspended in a cream. There are various creams and oils sold commercially and a number of these contain magnesium. Current formulations include magnesium oils and trans-dermal creams, from which the magnesium may be absorbed by the skin and into the systemic circulation. However, in contrast to gastrointestinal epithelium, a primary function of the skin is to act as a barrier, which restricts the absorption of exogenous chemicals into the body.

Although less studied than organic molecules, metal ions are known to be able to cross the skin barrier, with the literature having focussed on metals that are known to cause irritant/toxic effects (Hostynek, 2003). The lower resistance to permeation of the skin appendages (skin associated structures such as hairs and sweat glands) and the ionised nature of metals means that their permeation across skin appendages is considered to be the most likely route of absorption (Hostynek, 2003; Staff *et al.*, 2011). However, the low surface area available for this in human skin means that metal ion absorption across skin is expected to be relatively low. Therefore, it has been questioned whether a transdermal route of administration could provide sufficient Mg^{2+} absorption to meet the systemic requirements and whether, in fact, any absorption is evidenced by an increase in plasma magnesium or urinary magnesium excretion.

Commercially available topical applications range from 75mg to 400mg of Mg^{2+} depending on the dosage recommended by manufacturers. This ranges from 5-30 sprays of magnesium oil and 2-4 teaspoons of magnesium cream, which can be applied in one application or throughout the day on any area of skin. Many commercial topical creams and oils do not state the concentration of magnesium in the product and to date no study has investigated the absorption of transdermal magnesium cream by measurement of either serum concentration or urinary excretion in humans. Since less than 1% of total body magnesium is contained in the blood, assessment by serum status may be problematic (Elin, 2010). It was therefore elected to measure both markers of magnesium status.

This study was designed as a pilot study to ascertain whether such a topical Mg^{2+} preparation might affect urinary excretion or serum Mg^{2+}. The purpose of this pilot study was to investigate whether a 56 $mg.day^{-1}$ dose of magnesium in a cream, applied transdermally to humans, would affect either serum magnesium levels or urinary excretion over a two-week period. Twenty-five healthy adults (female=13 male=12) aged 34.3 ±14.8y, height 171.5±11cm, weight 75.9 ±14 kg, were recruited and randomly assigned into either a magnesium cream or placebo cream group by random allocation.

Four participants were considered “athletes” as they engaged in at least 2hrs of physical exercise at least 5 days per week, three who were assigned to the Mg^{2+} intervention group and one assigned to receive placebo. All other 20 participants who completed the study were considered “non-athletes”. High levels of physical exercise have been shown to deplete human Mg^{2+} status (Nielsen and Lukaski, 2006). No previous studies have used physical activity level as an exclusion criterion when investigating the effect of magnesium supplementation and so the main study comprised of both athletic and non-athletic participants. However, due to the fact that sweat affects magnesium status the data were also analysed without the “athletic” sub-group. Results were statistically analysed in two ways: 1. “all participants”, including both athletes and non-athletes (n=24 who completed the study) and 2 “non-athletes” (n=20) which excluded the 4 athletes. Dietary magnesium intake was below the RNI in one of the placebo participants and three of the magnesium participants. This was not considered a bar to inclusion into the study as any change in serum or urinary Mg^{2+} from the cream could still be shown, and indeed may be more likely in those with suboptimal intakes. Baseline serum and urine Mg^{2+} concentrations as well as dietary Mg^{2+} recorded in the randomised intervention and placebo groups did not differ significantly from each other in all groups. Baseline data consisted of a 24-hour urinary collection to assess baseline magnesium excretion and venous bloods to assess serum magnesium levels.

A 4-day food diary (3 midweek days and 1 weekend day), was recorded prior to the intervention with a second 4-day food diary recorded at the end of the 12-14 day period giving a total of 8 days dietary analysis over the period of the intervention. This was analysed for Mg^{2+} intake using Diet Plan 6 software (Forestfield Software Ltd., West Sussex, UK).

After baseline measurements were taken, participants were randomly assigned to either the Mg^{2+} Cream or a placebo control cream and were instructed to apply 2 x 5ml spoon of cream per day for two weeks. The resulting daily Mg^{2+} dose received by participants in the Mg^{2+} group consisted of 56mg of Mg^{2+}.

The placebo was a commercially available aqueous cream containing no magnesium (by analysis). After 12-14 days, final urine and blood samples were collected. The cream was applied up to and including the day of the final urine and blood collection.

After the Mg^{2+} cream intervention there was a clinically relevant increase of 0.07 $mmol.l^{-1}$ in serum magnesium from 0.82 to 0.89 $mmol.l^{-1}$, ($p=0.29$) that was not seen in the placebo group (0.77 to 0.79 $mmol.l^{-1}$ l), but was only statistically significant ($p<0.05$) in the subgroup of non-athletes (0.75 to 0.92 $mmol.l^{-1}$). A clinically relevant increase can be defined as an increase that has an impact in clinical magnesium deficiency. Gruber et al (2015) stated that in individuals with serum magnesium levels of 0.75 $mmol.l^{-1}$, 50% of the individuals had a clinical magnesium deficiency, but at a cut off of 0.80 $mmol.l^{-1}$ (a 0.05 $mmol.l^{-1}$ increase) only 10% of individuals had a clinical magnesium deficiency (Gröber, Schmidt and Kisters, 2015). Urinary excretion of magnesium increased slightly in the Mg^{2+} group but with no statistical significance ($p=0.48$). The Mg^{2+} group showed an 8.5 ± 0.72% increase in serum Mg^{2+} and a 9.1±1.3% increase in urinary Mg^{2+} while these figures for the placebo group were smaller, i.e. 2.6±0.36% for serum Mg^{2+} and -32±8.2% for urinary Mg^{2+}. In the placebo group, both serum and urine concentrations showed no statistically significant change after the application of the placebo cream. A recent meta-analysis by Zhang et al (Zhang *et al.*, 2016) found that an average supplementation of 368 mg $Mg.day^{-1}$ over a median of 3 months showed a mean increase of serum Mg of 0.05 $mmol.l^{-1}$, this is the same mean rise that was seen after the 2 weeks of transdermal Mg^{2+} cream application in this investigation reinforcing the positive results found from the transdermal cream as determined by the increase in serum magnesium.

No previous studies have looked at transdermal absorbency of Mg^{2+} in human participants. In this pilot study, transdermal delivery of 56 mg $Mg.day^{-1}$ (a low dose compared with commercial transdermal Mg^{2+} products available) showed a larger percentage rise in both serum and urinary markers from pre to post intervention compared with participants using the placebo cream, but statistical significance was achieved only for serum Mg^{2+} in a subgroup of non-athletes. This was believed to be due to the loss of magnesium through sweat in the exercising participants.

Upon analysis for all participants, as well as for only non-athletes, use of the transdermal Mg^{2+} cream showed no significant rise in urinary Mg^{2+}, a measurement that reflects short term intestinal absorption of Mg^{2+} when supplemented orally. However, participants in the Mg^{2+} group showed slight rises in urinary Mg^{2+} (+9 to 11%) while those in placebo group showed a substantial decrease in urinary Mg^{2+} (-32%). Possibly the decreased urinary Mg^{2+}

excretion in the placebo cream group represents more active physiological Mg^{2+} retention processes that are not apparent in the Mg^{2+} cream group (Trauninger *et al.*, 2002; Larsson, 2013). Food diaries did not show a decrease in dietary magnesium intake which was also considered. It has been suggested that 24-hour urine excretion of Mg^{2+} may be a better indicator of tissue status than the serum Mg^{2+} concentration, but it is highly variable and it is questionable whether it can be used to reliably assess a given individual's Mg^{2+} status. The transdermal cream contained 56 mg of Mg^{2+} administered daily. This is at the lower end of creams sold commercially. The recommended dose of the few commercially available creams range between 70 mg/d to 400 mg/d per day, therefore results of this study may represent an "underdose" of transdermal Mg^{2+}. A higher concentration may produce greater results and is something to be considered for the future.

2.4.7 Limitations to studies

The specific limitations surrounding each study have been discussed throughout the thesis and therefore will not be repeated here. However further methodological considerations and potential limitations of the overall thesis are discussed below.

A key consideration throughout is sample size and the associated statistical power. As would be expected, the pilot studies had small sample sizes (studies 2 and 6), but the data were used to generate hypotheses for subsequent studies. Study 1 was undertaken as a student project with low participant recruitment but was included in the portfolio of work as this was the initial project which formed the underlying area of interest for the further studies which formed this PhD. For studies 4 and 5, power calculations were performed to ensure sample size was adequate (Faul *et al*, 2007). Study 5 was based on calcium supplementation research since no data were available for magnesium supplementation due to the novelty of this area. A subsequent post hoc power calculation based on the actual effect size (0.64) demonstrated that 22 participants would need to be recruited in order to generate 80% power against the 300 mg RNI. This compares favourably with the 25 participants used in the current study.

For study 4, in which the effects of acute and chronic magnesium loading strategies were investigated, the main effect was seen in the acute SBP group. However, with the observation effect size of 0.877, 22 people are needed per group to generate 80% power. The current study used only 6 participants in one group and 7 in the other. This is a limitation to this study and may have resulted in a type 1 error occurring in the acute group. Future

work should ensure that a large enough cohort is recruited for the investigation to have sufficient statistical power.

Both studies 2 and 6 were pilot studies and power analysis were not undertaken in advance. When carrying out post hoc analysis on study 2, with an effect size of 1.037, there would need to be 22 participants in each group of the high and low dietary magnesium intake. In the current study only 16 people were recruited and these were split into high and low dietary magnesium groups. For study 6, based on the serum magnesium results (as nothing was found with the urinary magnesium results), 92 people would be need in each group for the study to have enough power to make firm conclusions. The pilot study had 14 participants in the intervention group and 10 in the placebo control group. This small number formed the pilot study and a larger study would give a more definitive answer and is something that is being prepared for.

A further limitation was that in study 2, stratification of dietary magnesium intake was not an *a priori* hypothesis to the study. This component was added after analysis of food diaries. The basis for this was opportunistic as the high and low intake was observed during analysis and created an interest that could form a further line of investigation to this pilot study. The intention was to determine whether a diet low in magnesium would affect results. To date, no studies have investigated supplementing those with an adequate dietary magnesium intake and comparing it to those with a low dietary intake, this would have consequences for those who take supplements whilst eating a nutritionally substantial diet as the supplement would most likely be excreted without being utilised.

Study 6 was the first human study to investigate the absorption of a magnesium cream. Limitations to this study were again the low statistical power, due to this being a pilot study, as well as the relatively low dose, since the cream provided only 56 mg per application, approximately 1/6th of the RNI. Since completion of this study and on the basis of this work, a cream that contains double the concentration has been produced, research will be carried out on this shortly.

Limitations to study 5 were the confounding variables that were not taken into account. For example, but not limited to, total energy intake, other micronutrients known to regulate blood pressure, C-reactive protein and glomerular filtration rate (Sabanayagam and Shankar, 2011; Lutsey *et al.*, 2014). Most research looks at singular to two variables and their effect on blood pressure as it would be too difficult to look at many micronutrients at the same time and may increase the chance of a type 2 error (Jones, B., Jaris, P., Lewis, J.A., Ebbutt, A.F., 1996). Compounded to this was the low participant numbers and a relatively short food

diary (8 days within a one-month period). The average age of the hypertensives was 63.4±10.2y whereas the control group had a mean age of 46.7±10.6y this results in unbalanced groups with a mean difference of 16.7y. It is particularly difficult to recruit hypertensives in the younger age bracket as they do not tend to have primary hypertension. Conversely it can be difficult to recruit normotensives in the higher age group as the prevalence of hypertension increases with age (Lloyd-Jones *et al.*, 2010).

For robustness, a larger study needs to be carried out with a greater number of participants and with a longer examination of dietary intake. An intervention study would allow for a conclusion to be drawn categorically between low Mg^{2+} intake and hypertension.

Further analysis of the data for the inclusion of confounding variables in the models could not be carried out for the data not included in the original publications as it was a requirement of the University of Hertfordshire School of Life Sciences Ethics Committee that raw data be destroyed on completion of each project.

2.4.8 Summary of findings, future work and contribution made by the papers in the context of the approved field of study.

The above body of work forms the basis of my PhD by Publication. The work has focussed on magnesium status and effects of supplementation on blood pressure, and exercise performance and how these effects might be modulated by habitual dietary intake and the mode and form of magnesium delivery.

Reflecting on these studies allows for critical analysis as well as direction for future work.

Two of the studies were pilot studies (studies 2 and 6), where novel hypotheses were tested on small cohorts to determine whether further research was warranted. The novel areas were that of transdermal magnesium absorption and low and high habitual dietary magnesium intake's effect on magnesium absorption. Both of these studies were concerned with absorption as there is little literature showing how habitual diet may affect the uptake of Mg^{2+} supplementation, either by ingestion or transdermally. Study 2 looked at low and high habitual dietary Mg^{2+} intake and showed differences between results on rest and recovery from aerobic and resistance exercise and systolic blood pressure with the different dietary intakes suggesting that magnesium transporters can reach a saturation point above which further increases in Mg^{2+} supplement dose are not effective. A limitation to this study was that the stratification of dietary magnesium intake as this was not an *a priori* hypothesis to the study having been added after analysis of food diaries. The basis for this was opportunistic as the high and low intake was observed during analysis and created an

interest that could form a further line of investigation to this pilot study. Future work could make this an *a priori* hypothesis along with increasing the participant numbers. This would lead to some complexities to the research design. To make this *a priori* participants would need to be given a strict dietary protocol to follow either a high or low Mg^{2+} diet for 1-2 weeks. This may be difficult for the participants to adhere to. Normally a 2-week period is the shortest time adopted in studies investigating the effects of magnesium supplementation, however this study has shown that an acute 1-week dose may be sufficient. Consideration would need to be given to this before a study could be carried out. Supplementation would then be given at a standardised dose to investigate whether habitual Mg^{2+} affects serum Mg^{2+} and BP, which are the two main variables analysed with Mg^{2+}. However, results from this would add to the literature and to the understanding of magnesium absorption and indirectly to the understanding of transporters. To improve scientific robustness, all future investigations should standardise the population background for Mg^{2+} intake, or at least quantify habitual intake and include this variable as a covariate in data analysis.

Study 6 was the first human study to investigate the absorption of a magnesium cream. Limitations to this study were again the low statistical power, due to this being a pilot study, as well as the relatively low dose, since the cream provided only 56 mg per application, approximately $1/6^{th}$ of the RNI. Since completion of this study and on the basis of this work, a cream that contains double the concentration has been produced. The 56mg application did show clinically relevant increases in serum magnesium, but this was only shown on one small group of applicants. Urinary excretion showed no significant changes with the application of the cream. Further, those participants who exercised heavily showed a reduction in serum Mg levels from pre to post intervention, even when on the magnesium cream. This is suggestive of a greater need for Mg^{2+} supplementation for athletes or those who exercise intensely and regularly supporting the work of Matias et al. (2010). Previous research has examined the effect of magnesium supplementation on cramp in elite tennis players (Liu, Borowski and Rose, 1983) and has also suggested a higher requirement for those undertaking vigorous exercise.

This therefore gives two aspects for future research which to date have not been appropriately addressed in the current literature. One is the need to investigate the demands of athletes for magnesium and the changes in serum level pre and post exercise. Further to analyse the normal serum magnesium levels of athletes to determine whether they are all below the clinical cut off point for disease of 0.07 $mmol.l^{-1}$. Below this level athletes should consider supplementation as part of their lifestyle when exercising, or increase consumption of magnesium rich foods.

The second focus for future work is in relation to the currently poorly understood magnesium transporters (TRPM) that facilitate intestinal magnesium absorption. The body of work presented in this thesis showed that chronic supplementation does not have a greater affect than an acute dosage. This leaves the question as to whether magnesium supplementation effects are saturable. A possible explanation for may be that there is an upper limit of absorption and transportation of magnesium via intestinal transporters and/or downregulation of capacity with chronic supplementation. Such work would not be possible with in *vivo* research in human participants, but I have been approached to conduct some in *vitro* studies with rat tissue. Other consideration may be gut microbes and the effect on magnesium absorption which presently has equivocal results. The conclusions from these studies are harder to apply as a dosage when trying to determine an exact difference in nutrient absorption in order that a dose specific amount of magnesium may be ingested. Whisner et al (2013) investigated gut bacteria and calcium absorption and found that the increase in absorption was greatest in the urine collected after 24h, which is consistent with lower gut absorption. Jumpertz et al (2011), investigated energy-balance studies and nutrient absorption and concluded that it remains uncertain as to what extent gut microbiota are an important regulator of nutrient absorption in humans (Jumpertz *et al.*, 2011). Dr Gautam Bhave of the Vanderbilt University Medical Center, cares for patients with magnesium wasting disorders. In personal communication he has expressed the need for a cream as the gut absorption of Mg is very low (as discussed above) and gastrointestinal side effects can limit compliance with oral consumption. For this reason, a topical application may give a greater net result regardless of the benefit of gut microbes.

Adding to the theme of absorption was study 4 which investigated the effects of acute and chronic supplementation on resistance exercise performance and blood pressure. The performance results improved on day 1 of the acute loading strategy but not on day two or on either day on the chronic intervention. The blood pressure results were also different between the acute and chronic dosage, leading to the conclusion that different mechanisms may affect blood pressure with respect to length of magnesium intake and different mechanisms that may or may not become saturated after a longer ingestion period. As mentioned previously, participants within the Chr loading group might be more susceptible to a possible Mg^{-2+} transporter down-regulation or cell tolerance for Mg^{2+} absorption based upon the understanding that high Mg^{2+} intakes result in a lower Mg^{2+} absorption (Jahnen-Dechent and Ketteler, 2012). Alternatively, chronic loading through providing a regular high Mg^{2+} intake may influence extracellular Mg^{2+} concentrations which coincide with manipulation of Mg^{2+} transporter TRPM6 function, resulting in a potential decrease in TRPM6 expression in conjunction to increasing the urinary excretion of Mg^{2+} (Alexander,

Hoenderop and Bindels, 2008). Thus, an acute ingestion rate as opposed to chronic may result in a more efficient use for Mg^{2+}.

Although this study adds to the literature and to the theme of absorption, a more direct approach to assessing transport saturation needs to be considered. This has previously been carried out on rats but further research *in vivo* on humans would add to the literature.

A further addition to the literature was the finding that those with primary hypertension habitually eat a diet that is low in magnesium (study 5). This finding has implications for the cost of hypertension to the NHS. Although previous literature has examined the dietary intake of the UK with respect to Mg^{2+} and it has been shown that Mg^{2+} supplementation may help to reduce blood pressure, no studies have looked at the habitual diet of hypertensives. This finding of the deficiency in Mg^{2+} intake is exciting and provides much promise for future research. Limitations to study 5 include confounding variables that were not examined for example, but not limited to, total energy intake, other micronutrients known to regulate blood pressure for example calcium and phosphorus, C-reactive protein and glomerular filtration rate (Sabanayagam and Shankar, 2011; Lutsey *et al.*, 2014). For robustness, a larger study needs to be carried out with a greater number of participants and with a longer examination of dietary intake. Further an intervention study would allow for a conclusion to be drawn categorically between low Mg^{2+} intake and hypertension. It is intended that a funding application be submitted in order to continue with this novel approach to dietary intake of magnesium. If Mg supplementation proves to be a successful strategy for lowering blood pressure in hypertensives, it has the potential to generate considerable NHS cost savings.

Other studies comprising this PhD investigated blood pressure. With so much inconclusive literature on the relationship between magnesium intake and blood pressure, the meta-analysis carried out as part of this portfolio of work, had the greatest impact, already cited over 100 times only 5 years after publication. In the pooled cross-study population, blood pressure was lower in those with a higher magnesium intake, yet this was not a consistent finding of previous research. Across all of the studies for this body of work systolic blood pressure was shown to reduce with an average of 300 mg magnesium supplementation by between 3-8mmHg and this reduction was seen at rest and after exercise.

The body of work submitted for this PhD by publication suggests an important link between low magnesium intake and elevated blood pressure and would support continued research in this area through larger scale observational and interventional studies applying both oral and trans-dermal supplementation approaches.

A final line of investigation would be to determine whether athletes require a higher daily intake of dietary or supplementary magnesium as a recurrent finding amongst the athletic participants across these investigations was that serum magnesium levels appear to be low in that cohort.

Future work may choose to investigate the athletes further to determine whether magnesium has an effect on the inflammatory response to exercise, in particular CRP and IL-6.

Section 3 Publications

3.1 Pulford, R & Kass, L,. The effects of acute magnesium supplementation on maximal intensity short term exercise and subsequent effect on blood pressure and isokinetic knee extension during recovery. Proceedings of the Nutrition Society (2010), (Abstract)

Proceedings of the Nutrition Society (2010), 69 (OCE1), E60 doi:10.1017/S0029665109992485

Over- and undernutrition: challenges and approaches. 29 June–2 July 2009

The effects of acute magnesium supplementation on maximal intensity short-term exercise and subsequent effect on blood pressure and isokinetic knee extension during recovery

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Mg is an important mineral for >300 processes in the human body such as energy production, muscle anabolism and blood pressure regulation. However, there is limited research into Mg^{2+} performance-related effect on short-term high-intensity exercise and subsequent effect on blood pressure and muscle recovery post exercise. The aim of the present study was to investigate the effect of Mg supplementation on peak and mean power during all-out maximal-intensity short-term exercise in order to examine its effect on systolic (SBP) and diastolic blood pressure (DBP) post maximal exercise and to investigate whether muscular recovery, using post-exercise isokinetic knee extensions, could be improved.

Six healthy male subjects (mean age 20.8 years old, mean height 1.80m and mean mass 79kg) were required to fill out a 3 d diet sheet before testing for analysis of Mg intake. The study was a double-blind repeated-measures cross-over design with subjects supplemented with either placebo (cornflour) or 300mg magnesium aspartate for 2d before the test day and 300mg on the test day before testing. Maximal isokinetic extensions of five repetitions by the right leg were performed by each subject 1 week before the testing to produce a baseline result for peak torque (N/m). The test day consisted of subjects performing a 30s Wingate anaerobic test (WAnT)[1] and resting for 30min post WAnT before performing five maximal isokinetic knee extensions at 60/s on the right leg. A further 100mg magnesium aspartate or placebo was given to each subject immediately post WAnT. Peak power and mean power (W) were recorded from each WAnT. Peak torque (N/m) was measured from the isokinetic knee extensions. SBP and DBP were recorded at rest and every 5min during the 30min recovery after the WAnT.

Mean peak power (W) was significantly increased ($P<0.05$) during the WAnT between the placebo condition (851) and the Mg condition (912). Mean power (W) during the WAnT was also significantly increased (placebo 683, Mg 705; $P<0.05$). A significant increase ($P<0.05$) was also found in mean peak torque (N/m) between the placebo condition (196) and the Mg condition (222). There was also a significant decrease ($P<0.05$) in SBP during the 30min post WAnT in the Mg condition (120mmHg) compared with the placebo condition (125mmHg), which represents a 5% lower mean SBP over the 30min. However, there was no significant difference ($P>0.05$) in DBP between the placebo and the Mg condition during the 30min recovery post WAnT.

These improvements in WAnT and peak torque may be a result of Mg acting as a 'second messenger' for insulin, thus increasing the insulin sensitivity of cell membranes[2], allowing for a greater uptake of energy substrates such as creatine into the muscles[3], a consequence of which would be an improved intramuscular recovery of phosphocreatine and ATP. It has been known for some time that intramuscular Mg elicits ATPase activity[4] and that Mg-bound ATP present on the acto-myosin cross-bridges has been proved to speed up filament detachment[5]. This increased rate of ATP hydrolysis (from higher ATPase activity) on the myosin heads results in faster and stronger filament detachment and reattachment producing a faster and more powerful muscle contraction, as shown by the results of the present study. It is further suggested that the reduction in blood pressure can be attributed to increased vasodilation attributed to decreased Ca channel activity in the presence of Mg, resulting in decreased vascular resistance[6].

The present study demonstrates that Mg supplementation increases peak and mean power during a WAnT and can enhance recovery by reducing SBP and improving peak torque in an isokinetic knee extension after maximal short-term exercise. Further research on hypertensive recreational athletes is needed to build on these initial findings.

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3.2 Kass, L, Skinner, P & Poeira, F., A pilot study on the effects of magnesium supplementation with high and low habitual dietary magnesium intake on resting and recovery from aerobic and resistance exercise and systolic blood pressure. Journal of Sport Science and Medicine. (2013)

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Research article

A Pilot Study on the Effects of Magnesium Supplementation with High and Low Habitual Dietary Magnesium Intake on Resting and Recovery from Aerobic and Resistance Exercise and Systolic Blood Pressure

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Abstract
The effects of magnesium supplementation on blood pressure (BP) have been studied for over 25 years and results have been inconsistent. Blood pressure reductions in randomized studies have varied from 12 mmHg reductions to no reduction. The objective of this pilot intervention was to investigate the effect of magnesium supplementation on systolic blood pressure whilst resting and during recovery from aerobic and resistance exercise and on performance. A further objective was to see whether the effect of a high vs low habitual dietary magnesium intake affected these results. Sixteen male volunteers were randomly assigned to either a 300 mg·d^{-1} magnesium oxide supplementation (MO) or a control group (CG) for 14 days. Resting blood pressure (BP) and heart rate (HR) were measured before subjects performed a maximal 30 minute cycle, immediately followed by three x 5 second isometric bench press, both at baseline and after the intervention. Blood pressure and heart rate were recorded immediately post exercise and after five minutes recovery. A 3 day food diary was recorded for all subjects to measure dietary magnesium intake. At the end of the intervention, the supplemented group, had a reduction in mean resting systolic BP by 8.9 mmHg (115.125 ± 9.46 mmHg, p = 0.01) and post exercise by 13 mmHg (122.625 ± 9.88 mmHg, p = 0.01). Recovery BP was 11.9 mmHg lower in the intervention group compared to control (p = 0.006) and HR decreased by 7 beats per minute in the experimental group (69.0 ± 11.6 bpm, p = 0.02). Performance indicators did not change within and between the groups. Habitual dietary magnesium intake affected both resting and post exercise systolic BP and the subsequent effect of the magnesium supplementation. These results have an implication in a health setting and for health and exercise but not performance.

Key words: Blood pressure, magnesium supplementation, aerobic performance, isometric contraction, recovery, dietary magnesium.

Introduction

Magnesium, one of the most abundant minerals in the body, is essential for over 300 biochemical processes and plays important roles in activating cellular enzymatic activity, such as those needed to synthesize DNA and RNA, and also in metabolism (Musso, 2009). For athletes it is important because of its involvement in glycolysis, the citric acid cycle and creatine phosphate production. It has also been suggested that free magnesium levels can affect excitation contraction coupling of the myocardium (Michailova et al., 2004). Magnesium has been consistently linked with a reduction in blood pressure within both a clinically hypertensive population and a normotensive population (Itoh et al., 1997). Meta-analyses of randomized studies by Jee et al., (2002) and Kass et al. (2012) on the effects of magnesium on BP shows dose dependent reductions. A study by Itoh et al., (1997) on 33 normotensive patients showed that magnesium lowered BP after 4 weeks. The authors attributed the reduction to the excretion of sodium in urine which acts as a relaxant to the blood vessels. However, Doyle et al. (1999) performed a study on healthy females and found no reduction in BP with increased magnesium intake after 4 weeks.

Exercise causes magnesium levels in the body to be depleted through losses in sweat, urine and alterations in the blood magnesium levels (Rayssiguier et al., 1990). An athlete will therefore have a higher requirement for daily magnesium intake than the sedentary population. Athletes on calorie restricted diets may also be at risk of deficiency.

Evidence shows that exercise causes a redistribution of magnesium to the active sites in the body (Nielsen and Lukaski, 2006), and that deficiency of the mineral negatively affects performance (Newhouse and Finstad, 2000). However, there has been little evidence to show that magnesium supplementation in adults with adequate magnesium intake will increase performance (Laires and Monteiro, 2007).

There are two suggested mechanisms for the effect of magnesium on systolic BP at rest. One mechanism is that magnesium acts as a driving force of the sodium potassium pump in the cell membrane and mobilizes more sodium to be excreted (Bara et al., 1993) . Reduction of intracellular sodium may cause the smooth muscle cells in the vascular walls to relax and BP to reduce. Another suggested mechanism is that magnesium acts as a calcium channel blocker (Touyz, 2004), working as a relaxant of the smooth muscle in blood vessels, increasing arterial compliance and reducing BP.

There have been suggestions of an inverse relationship between daily dietary magnesium intake and BP (Kawano et al, 1998; Ma et al. 1995), however, dietary magnesium is rarely assessed in the studies reviewed and no large studies have looked at the impact of dietary magnesium on BP in hypertensive patients. Common foods which contain magnesium are grains such as buckwheat flour and oat bran, vegetables such as artichoke, spinach and black and white beans and nuts for example almonds and brazils. It should be considered however that the daily dietary intake of magnesium in

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Western society has been declining from about 500 mg·day^{-1} in the 1900's to a value closer to 175 mg·day^{-1} (Altura, 1994), increasing the likelihood of an individual being deficient in magnesium. This figure falls someway short of the current UK RNI outlined by the UK Food Standards Agency, (2003) of 300 mg·day^{-1} for men and 270 mg· day^{-1} for women (12.35 mmol and 11.10 mmol).

To the best of the authors' knowledge, no studies have addressed how magnesium impacts maximal aerobic exercise and performance in healthy individuals. Further there are no studies which look at the effect of magnesium supplementation in those with a high vs low habitual dietary magnesium intake.

Although reviews have shown a reduction in blood pressure with magnesium supplementation, (Jee et al., 2002; Kass et al., 2012) there is limited research on the effect of supplementation on BP during exercise or on performance. It is important for magnesium levels to stay high during recovery to affect cellular metabolism and protein synthesis (Groff and Gropper, 2000). Therefore low magnesium levels may impair muscle recovery and BP during and post exercise. Normal BP response post exercise is rapid hypotension (Orri et al., 2004). However, this may differ in response to magnesium supplementation and in the extent of hypotension.

The aims of this pilot investigation were to determine 1) the relationship between magnesium supplementation and systolic BP at rest and post exercise and its effect on performance, 2) to determine whether a high or low dietary magnesium intake impacts on these results. Further objectives were to investigate any changes in heart rate throughout.

Methods

Subjects

Sixteen male subjects were recruited. They were all undertaking aerobic exercise (>4 hours per week), apparently healthy and with no physical impairments. Exercise was defined as any physical activity perceived to be at 13 or above on The Borg Scaling of Perceived Exertion. Fifteen subjects were white Caucasian and 1 was Asian. Subjects volunteered to take part in the project and completed a consent form and health screen. Ethical approval was received from The University of Hertfordshire, Life Science Ethics Committee. Full inclusion and exclusion criteria can be seen in Table 1.

Design

Subjects were randomized to either the supplement or the control group. Subjects in both groups were asked to come in for familiarization and then twice for testing over a 4 week period, with only the experimental group taking the magnesium supplementation and the control group undertaking the same tests but with no supplementation. A total of 3 visits to the laboratory were required from each of the subjects.

Protocol

Familiarization and Test 1

Diet: Subjects were asked to complete a 3 day dietary recall to assess habitual magnesium dietary intake using dedicated software (Dietplan 6.50 Forestfield Software Ltd., West Sussex, UK). Dietary intake was categorized into high and low categories based on the Committee on Medical Aspects of Food and Nutrition Policy (COMA, 1991) calculated a Reference Nutrient Intake (RNI) of 300 mg/day for adult males (<300 mg·d^{-1} and ≥300 mg·d^{-1}).

Supplementation: Supplements consisted of a vegetarian capsules filled with 150mg of medical grade Magnesium Oxide Light 13138 (Sigma-Aldrich, Missouri, USA). Each capsule was individually weighed (Ohaus adventurer balance AR1530 New Jersey, USA), Subjects were instructed to take two tablets per day resulting in a total of 300mg per day per subject, one in the morning on waking, and at the end of the day for a total of 14 days.

Exercise familiarization: On the first visit to the laboratory subjects were shown the equipment and the full protocol was carried out as described below in order that the subjects were able to familiarize themselves with the process. They were then invited back to the laboratory in a time frame of 3-7 days after familiarization had taken place for the protocol to be repeated and baseline measurements recorded. Supplementation was distributed after baseline measurements were recorded. The protocol was repeated 14 days after baseline measurements had been taken.

Exercise protocol: Following 10 minutes of silent rest, a 3 minute warm up at self-selected pace was undertaken on the cycle ergometer (Monark Ergomedic 874 E Cycle Ergometer, Monark Sports and Medical, Sweden), with 1kg load, with heart rate kept below 140bpm. A Polar T31 transmitter (Polar Electro Oy, Kempele, Finland), measured heart rate throughout all exercise. Subjects then cycled on the cycle ergometer with a 1kg load for 30 minutes at their maximal capacity in order to achieve the greatest distance in the allocated time. After completion of the 30 minute maximal cycle a

Table 1. Inclusion and exclusion criteria for subjects.

Inclusion criteria	Exclusion criteria
Male	
Between 19-24 years old	
Normotensive blood pressure (110/70 mmHg - 135/85 mmHg)	Exercising for minimum of 4 hours per week
Female	
Under 19 and over 24 years of age	Hypertensive (>135/85) Hypotensive (<110/70 mmHg) Exercising less than 4 hours per week On any medication
Ingested any supplement 72 hours before baseline test	Suffering from any ailment or injury that affects performance Failed Health Screen

Table 2. Subject characteristics (n=16). Values are means (± Standard Deviation).

	Control (n = 8)	Experimental (n = 8)	All (n = 16)
Age (years)	21.38 (1.60)	20.38 (2.00)	20.88 (1.82)
Height (m)	1.83 (.04)	1.78 (.08)	1.80 (.07)
Weight (kg)	74.44 (10.30)	73.39 (15.01)	73.91 (12.49)
RHR (bpm)	74 (12)	76 (12)	75 (12)
RSBP (mmHg)	125.25 (5.06)	122.75 (7.09)	124.63 (6.05)
RDBP (mmHg)	70.75 (7.09)	71.13 (10.06)	71.00 (8.42)

RHR = Resting heart rate, RSBP = Resting systolic blood pressure, RDBP = Resting diastolic blood pressure.

two minutes rest was given with subjects being advised to continue to pedal for at least 60 seconds of the two minute interval at self-selected intensity that was lower than that undertaken during the 30 minute trial. After the two minute interval, 3 x 5 second maximal isometric bench press contractions were completed, each separated by a 30 seconds rest. The isometric contraction was undertaken with the subject supine on a bench and pushing maximally against a static metal bar on the Marcy Smith Machine Plus, (Marcy Fitness Products, Ontario, Canada) and measured by a Digital Analyser Isometric Transducer (MIE Medical Research Ltd., Yorkshire, UK). The 3 isometric bench press weights were averaged and percentage change calculated between baseline and week 2. As subjects differed in size, bench press output results could be skewed due to the different size of the levers. Therefore, the joint angle of the upper arm and shoulder was standardized to 40°.

Measurements pre and post-exercise from rest to recovery: After 10 minutes of silent rest, BP was measured from the right arm (Omron MX3, Omron Healthcare, Kyoto, Japan). At timepoint 15 minutes, during the 30 minute maximal cycle, gas was analyzed for O_2 and CO_2 using Cortex Biophysik Metalyser 3B (Leipzig, Germany) with Metasoft 3.9.2 software. Gas was collected again at the 30 minute time mark and respiratory exchange ratio (RER) calculated. After completing all three of the isometric contractions, BP was measured again. A 5 minute seated recovery period then took place with subjects having a final BP and heart rate measurement taken at this time.

Heart rate (HR) was measured pre and post-exercise and recorded 4 times throughout the protocol. Total HR recordings were at Pre-exercise at rest (RHR), after 15 minutes cycling (HR15), at the end of Exercise (HR30), and post-exercise, after 5 minutes recovery (RecHR).

Test 2

After 14 days both groups repeated the exercise protocol, with the same measurements being taken. Subjects came in on the same time of day, and the same tester was present during both tests for all subjects.

Data analysis

Food diaries were input into Dietplan 6 (Dietplan 6.50 Forestfield Software Ltd., West Sussex, UK) and subjects were stratified into high and low habitual dietary magnesium intake based on these results. Data were then analyzed for the cohort as a whole and again stratified for low and high habitual dietary intake. All data were analyzed using Excel and SPSS7 (PASW Statistics v17.0.2 IBM, New York, USA) and tested for normality using a Shapiro-Wilk test. As the data were normally distributed, independent t-tests were performed between groups, and dependent paired t-tests between tests to assess significance. The alpha value was set at 0.5.

Results

Sixteen male subjects were recruited (age 20.88 ± 1.82 yrs, height 1.80 ± 0.07 m, weight 73.91 ± 12.49 kg, resting systolic blood pressure 124.63 ± 6.05mmHg). Baseline characteristics were further broken down into the control and experimental group (Table 2). No statistical significance difference was found between the groups.

In the supplemental group three subjects were above the RDA (300 $mg \cdot d^{-1}$) and 5 were below. The control group had an even amount of subjects above and below the RDA.

Blood pressure

At baseline there were no significant differences between the groups in blood pressure at any of the time points (Table 3). Post intervention there was as a significant

Table 3. Summary of mean blood pressure values, at baseline and week 2 for both subject groups. Values are means (± Standard Deviation).

	Baseline		Follow-up	
Measurements	**Control**	**Supplement**	**Control**	**Supplement**
RSBP (mmHg)	125.3 (5.1)	124.0 (7.2)	124.5 (3.9)	115.1 (9.5) *†
Post SBP (mmHg)	136.3 (4.3)	135.6(15.3)	139.1 (4.5)	122.6 (9.9) *†
Rec SBP (mmHg)	117.5 (8.5)	118.1(16.2)	121.6 (6.1)	110.4 (9.2) †
RHR (bpm)	74 (12)	76 (15)	68 (12)	69 (12)
HR15 (bpm)	155 (20)	152(24)	163 (16)	171 (11)
HR30 (bpm)	180 (12)	187(13)	186 (13)	187 (12)
RecHR (bpm)	102 (15)	101(16)	100 (16)	106 (11)

* indicates significance at week 2 compared to baseline ($p < 0.05$). † indicates signifincance between control and experimental groups at week 2 ($p < 0.05$). RSBP – Resting systolic blood pressure. PostSBP – Post exercise systolic blood pressure. RecSBP – Systolic blood pressure after 5 minutes recovery. RHR – Resting heart rate. HR15 – Heart rate at 15 minutes cycling. HR30 – Heart rate at 30 minutes cycling. HR30 – Heart rate at 30 minutes cycling. RecHR – Heart rate after 5 minutes recovery

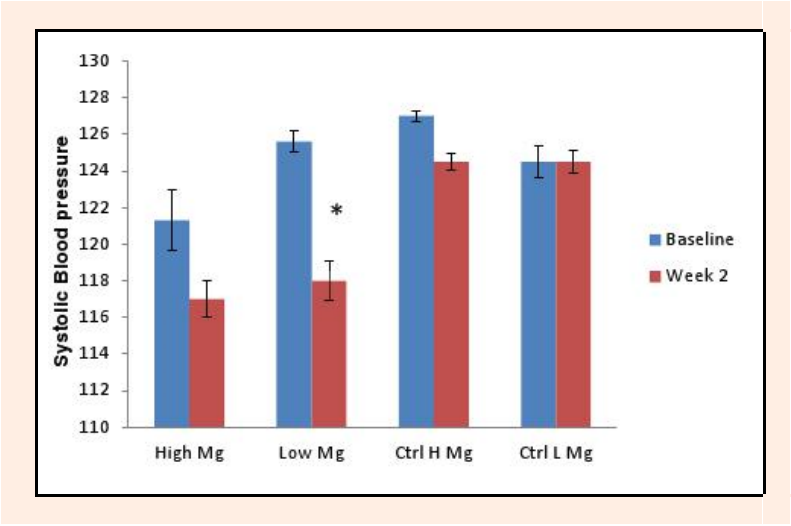

Figure 1. Mean resting systolic blood pressure change in the experimental and control groups split into high (≥300mg) and low (<300mg) magnesium intake subgroups between baseline and week 2.

difference between the control and the supplemented group for resting pre-exercise, post and recovery blood pressure. A significant difference from baseline was also seen in the supplemented group for resting pre-exercise and post exercise blood pressure after 2 weeks supplementation.

RSBP decreased by 7.7% post intervention in the experimental group compared to baseline ($p = 0.017$) and inter-group between experimental and control post intervention ($p = 0.014$). Post SBP decreased by 10.6% at follow-up in the experimental group compared to baseline ($p = 0.017$) and between experimental and control groups at follow up ($p = 0.000$) (Table 3).

Rec SBP decreased by 7.0% at follow-up in the experimental group compared to baseline ($p = 0.006$) although no significance was found for Rec SBP at follow-up between the baseline and experimental group (Table 3).

Habitual dietary magnesium intake

There was no significant difference found for habitual magnesium intake between control and experimental subjects ($p = 0.18$). When stratified by dietary magnesium intake, the supplemented group showed significance in resting systolic BP at week 2 compared to baseline for the low habitual dietary magensium intake (114.0 ± 11.6 mmHg, $p = 0.02$) (Figure 1) and in the high magnesium intake group post exercise (120.7 ± 1.5 mmHg, $p = 0.01$) (Figure 2). The control group showed no significant change in either resting or post exercise SBP at week 2 compared to baseline (Figures 1 and 2).

However, significance was found in resting systolic BP at week 2 compared to baseline in the low magensium intake group (114.00 ± 11.60 mmHg, $p = 0.02$) within the intervention group.

Performance

Cycling time trial distance increased from baseline to week 2 significantly in both groups, however this increased distance was lower at week 2 in the supplemented group compared to the control group. In the experimental group baseline distance was 20.5 ± 2.5 Km and after the intervention increased to 22.7 ± 2.4Km an increase of 10.6%. However, in the control group baseline

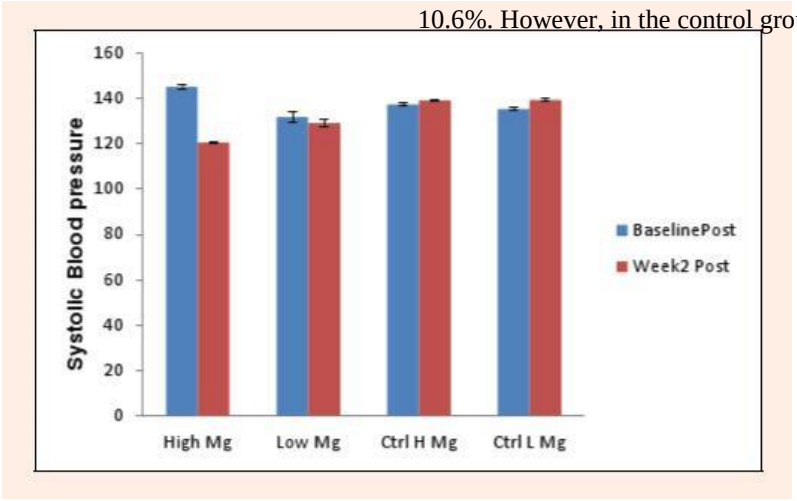

Figure 2. Mean post exercise systolic blood pressure change in the experimental and control groups split into high (≥300mg) and low (<300mg) magnesium intake subgroups between baseline and week 2.

distance was 19.4 ± 1.8 Km and at the end of the trial was 22.1± 2.3 Km, an increase of 14.1%.

The 3 isometric bench press weights were averaged and percentage change calculated between baseline and week 2. Average strength increased by 11.4% in the control group, from 24.2 ± 8.67 kg to 26.9 ± 8.08 kg and decreased by 18.0% in the experimental group from 35.50 ± 16.72 kg to 29.0 ± 10.88 kg. No significant changes were shown.

Heart rate and RER

No significant difference was found between the control group and the experimental group at any time point. For the RER at the 30 minute time point the intervention group changed from 1.14 ± 0.13 to 1.10 ± 0.07 from baseline to week 2 and the control group from 1.06 ± 0.03 to 1.11 ± 0.02 from baseline to week 2. No significance change was shown. For heart rate, as expected, significance was found timewise in the change from resting to recovery heart rate and throughout the 30 minute cycle in both the control and supplemented group (Table 3).

Discussion

In this pilot study resting, post exercise and recovery systolic BP significantly decreased from baseline to week 2 for the magnesium supplemented group when compared to the control group. This is consistent with other studies that have reported significant reductions of 4.1 mmHg and 5 mmHg respectively for systolic BP (Itoh et al.,1997; Kawano et al.,1998).

Different to most supplementation studies, this pilot investigation looked at whether habitual magnesium intake influenced the BP results at both resting and post exercise conditions. The data were used to stratify results for both the supplemented and control group into high and low magnesium intake. Although not the main focus for the study and the study is not powered to examine this, there is an indication that allowed consideration to be given as to whether supplementation showed the same changes in those who have a naturally high dietary intake of magnesium as to those who have a low dietary intake. In the control group for both the high and low habitual magnesium intake, resting systolic BP did not change at week 2 compared to baseline. However, a significant lowering was found in resting systolic BP at week 2 compared to baseline in the low magnesium intake group within the intervention group. This suggests that magnesium supplementation may have a greater effect on resting BP in subjects that have a low habitual dietary magnesium intake than those who have a naturally higher intake above the RNI.

Sanjuliani et al. (1996) found over 10 mmHg reduction in mean BP with 600 $mg \cdot d^{-1}$ oral magnesium oxide supplement in hypertensive patients. They attributed this significant reduction to decreased intracellular sodium levels and increased magnesium levels in the blood.

Conversely, two studies found no evidence for the role of increased dietary magnesium intake in the reduction of high BP. One study was in women only (Doyle et al. 1999) and magnesium was increased through diet alone but was still below the US RDA.

Mean post exercise BP showed the opposite. The results showed mean post exercise systolic BP significantly decreased by 24.3 mmHg at week 2 when compared to baseline, in the high magnesium intake subgroup of the experimental group (20.2%, $p = 0.017$). This shows that magnesium supplementation has a bigger effect on post exercise BP in subjects that have a high magnesium intake. The mechanism for this is not explained in the literature. It may be due to the increased demands of magnesium for a physically active person during exercise as it is bound to ATP or that a higher plasma concentration may result in more magnesium being used as a relaxant of the smooth muscle cells in the vascular walls. This has implications in health. Patients suffering from hypertension could gain larger BP reductions from a combined dietary change and oral supplementation program.

Secondary to the main aim, this investigation set out to determine whether supplementation had an effect on aerobic and resistance performance. For the 30 minute cycling time trial the results showed a significant increase of 2.74 kilometers and 2.19 kilometers for mean cycling time trial distance in both control and experimental groups. However, there was no significance found between groups. A review by Newhouse and Finstad (2000) supports this. They reviewed 33 years of research and concluded that most evidence shows no enhancement of performance. However, a study by Cinar et al. (2007) found that magnesium supplementation did improve performance of subjects who were exercising for 90-120 minutes per day for 5 days a week, rather than 2 separate bouts of exercise 14 days apart. The suggested mechanism for the increase is that magnesium increases the red blood cell count and hemoglobin levels, allowing greater oxygen distribution.

For the isometric bench presses the results showed the average strength increased in the control group by 11.4% and decreased by 18.0% in the experimental group, showing a 29.4% difference between the groups at week 2, although baseline and week 2 data were higher in the experimental group compared to control group throughout. None of the data were significant at either baseline or week 2 although a non-significant difference could be seen partly due to the fact that the experimental group had a higher baseline value than the control group. Much of the literature shows the contrary (Brilla and Haley, 1992; Domiguez et al., 2006). Reasons for this may be due to higher dosages of supplements ($8\ mg \cdot kg^{-1}$) over a longer period of supplementation (7 weeks) and untrained individuals rather than active subjects being used. For resistance exercise and isometric contractions magnesium supplementation has shown to produce mixed results on performance. A study by Brilla and Haley (1992) found a 20% strength increase of peak knee extension torque which they attributed to magnesium's role at ribosomal level. However, a review of

supplemental effects by Clarkson (1991) identified no relationship between magnesium supplementation and performance at any dosage. Magnesium also activates amino acids and aids the attachment of mRNA to the ribosomes in protein biosynthesis (Groff and Gropper, 2000). This is important in increasing strength and protein synthesis during resistance exercise and recovery. Magnesium has been shown to enhance insulin-like growth factor 1 (IGF-1) which may elevate testosterone (Dominguez et al., 1998). Both being anabolic hormones, supplementation of magnesium may increase strength when given alongside an exercise training program.

Limitations to this pilot study included the short supplementation period of only 2 weeks and also small sample size. Although the cohort were matched for weekly physical activity levels a more robust test of fitness, such as VO_{2max} testing was not carried out. Dietary intake was recorded for 3 days to assess habitual dietary magnesium intake. A cross-over design would also enhance the effect of the supplementation and allow for the trial to be double blinded.

Conclusion

In this pilot study, oral magnesium supplementation significantly reduces resting and post exercise BP. Furthermore, in those with a low background dietary magnesium intake there was a greater effect of supplementation on resting BP than those with a high dietary magnesium intake. Conversely, post exercise BP was reduced more in those with a high dietary magnesium intake. This pilot suggests that the effect of magnesium supplementation is relevant mainly in a health context rather than performance as no enhancements in athletic performance were found. Further research could investigate the effects of higher and longer dosages of magnesium supplementation on both aerobic and resistance exercise performance. An analysis of dietary intake looking at habitual magnesium intake in those showing resting hypertension would also aid in the understanding of magnesium and blood pressure

Acknowledgment

The authors declare that they have no conflict of interest.

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Key points

- Magnesium supplementation will have an effect on resting and recovery systolic blood pressure with aerobic exercise.
- Magnesium supplementation will have an effect on resting and recovery systolic blood pressure with resistance exercise.
- Magnesium supplementation did not have an effect on performance indicators.
- A low habitual dietary magnesium intake will negatively affect blood pressure.
- A high habitual dietary magnesium intake will impact on the effect of magnesium supplementation.

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SYSTEMATIC REVIEW

Effect of magnesium supplementation on blood pressure: a meta-analysis

L Kass[1], J Weekes[1] and L Carpenter[2]

To date, there has been inconclusive evidence regarding the effect of magnesium supplements on blood pressure (BP). This meta-analysis was conducted to assess the effect of magnesium supplementation on BP and to establish the characteristics of trials showing the largest effect size. Primary outcome measures were systolic blood pressure (SBP) and diastolic blood pressure (DBP) at the end of the follow-up period. One hundred and forty-one papers were identified, of which 22 trials with 23 sets of data (n ¼ 1173), with 3 to 24 weeks of follow-up met the inclusion criteria, with a supplemented elemental magnesium range of 120--973 mg (mean dose 410 mg). 95% confidence intervals (CI) were calculated using DerSimonian and Laird's randomeffects model, with effect size calculated using Hedges G. Combining all data, an overall effect of 0.36 and 0.32 for DBP and SBP, respectively, was observed (95% CI 0.27--0.44 for DBP and 0.23--0.41 for SBP), with a greater effect being seen for the intervention in crossover trials (DBP 0.47, SBP 0.51). Effect size increased in line with increased dosage. Although not all individual trials showed significance in BP reduction, combining all trials did show a decrease in SBP of 3--4 mm Hg and DBP of 2--3 mm Hg, which further increased with crossover designed trials and intake 4370 mg/day. To conclude, magnesium supplementation appears to achieve a small but clinically significant reduction in BP, an effect worthy of future prospective large randomised trials using solid methodology.

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Keywords: hypertension; meta-analysis; supplementation; magnesium

INTRODUCTION

Elevated blood pressure (BP) or hypertension is a major risk factor for mortality from cardiovascular and renal disease. Causes of essential hypertension include, but are not limited to, smoking, sedentary lifestyle, a diet high in sodium, and an inadequate intake of other minerals such as potassium, calcium and magnesium.[1]

It has been suggested that magnesium supplementation may decrease BP, as it acts as a calcium antagonist on smooth muscle tone, thus causing vasorelaxation.[2] There have been suggestions of an inverse relationship between daily dietary magnesium intake and BP.[3,4] There is also the possibility that individuals with dietary magnesium consumption in the higher quantiles are generally more health conscious and may take other steps to control BP. It should be considered, however, that the daily dietary intake of magnesium in the Western society has been declining from about 500 mg/day in the 1900s to a value closer to 175 mg/day,[5] increasing the likelihood of an individual being deficient in magnesium. This figure falls someway short of the current UK RNI outlined by the Department of Health[6] of 300 mg/day for men and 270 mg/day for women (12.35 and 11.1 mmol, respectively).

As hypertensives are usually advised to increase physical activity to improve BP and exercise causes increased excretion of magnesium in sweat and urine,[7] the authors set out to review the effect of magnesium on BP during exercise. There are no publications looking at the effect of magnesium intake on BP while undertaking physical activity or during the recovery period. Although regular exercise is advocated for hypertensive individuals, there can be concern that exercise may cause a transient increase in BP, negatively impacting an individual's BP. Both aerobic and resistance exercise can cause transient increases in BP, with resistance exercise often being perceived as having greater risk.[8] The introduction of magnesium through a supplement may enable the hypertensive to undertake an exercise programme at a greater intensity if BP is reduced through magnesium supplementation. Magnesium as a supplement has been shown to decrease BP in normotensives, yet is rarely considered as a supplement for elevated BP. Further, habitual dietary-magnesium intake is rarely assessed and no large randomised crossover studies have looked at baseline serum magnesium levels together with the impact of dietary or supplementary magnesium on BP. However, there is a focus in research among small trials on the effect of BP in various non-exercising individuals and this meta-analysis therefore sets out to review the general effect of magnesium on BP to enable future research into the effect of Mg on BP during exercise. Further, observation of the study designs used will help to influence the design of future research, allowing for more robust methodologies.

Some individual studies have shown significant reduction in both systolic (SBP) and diastolic blood pressure (DBP) with a magnesium intervention, although previous systematic reviews and meta-analyses have been less conclusive.[9] [11] Burgess et al.[9] found no significant benefit of magnesium supplementation in hypertensive patients from a review of 12 treatment studies, and did not recommend magnesium as an antihypertensive agent. Dickinson et al.[10] reviewed 12 treatment studies of hypertensive patients and reported a small non-significant decrease in SBP of 1.3 mm Hg and a significant reduction of DBP of 2.2 mm Hg. Jee et al.[11] reported a small, non-significant reduction of 0.6 mm Hg SBP and 0.8 mm Hg DBP by analysing 20 treatment studies of hypertensive and normotensive individuals. However, a dose-dependent effect was also reported, with a reduction of 4.3 mm Hg SBP and 2.3 mm Hg DBP for every 240 mg/day (10 mmol) increase in supplemental magnesium. The variations in the dose-dependent relationships found in the above studies helped to rationalise the subgroup analysis of high and low magnesium dosage included in this meta-analysis.

The aim of this meta-analysis was to assess the effect of oral magnesium supplementation on the BP of both hypertensive and normotensive individuals and to establish the characteristics of trials associated with the greatest BP reductions.

MATERIALS AND METHODS

Article selection

Treatment studies published before July 2010 relating to the effects of magnesium supplementation on human BP were identified through a comprehensive search of MEDLINE and the Cochrane Library, using the keywords 'magnesium', 'supplementation', 'BP' and 'human'. Reference lists from the returned articles and previous systematic reviews were also searched. Relevant data were extracted by one investigator (JW); any articles containing data of an unclear or ambiguous nature were submitted to another investigator (LK); consensus was reached over whether to include these articles.

Twenty-two trials were included in the analysis, resulting in 23 sets of data, as a result of one trial producing two data sets. The dosage of magnesium ranged from 120 to 973 mg/day, with an average of 410 mg/day±179. Elemental magnesium was stratified into dosage o370 mg or X370 mg/day. The majority of studies specified elemental magnesium dosages. In studies where elemental magnesium was not shown, the percentage of magnesium salt in the compound was used for the elemental magnesium. For magnesium oxide (MgO) 60% was used to calculate the elemental magnesium and 8.33% for magnesium chloride ($MgCl_2$).

The inclusion criteria were: (1) magnesium supplements as the only active intervention; (2) presence of a placebo or control group; (3) subjects over the age of 18; (4) random allocation of subjects to treatment conditions; and (5) parallel or crossover trial design. All criteria had to be met for inclusion into the study.

The primary outcome measures were SBP and DBP at the end of follow-up. Secondary outcome measures were total withdrawals from treatment and any adverse effects of treatment.

Statistical analysis

For each trial, effect size was calculated using the Hedges G method[12] ($m^2m^1/s.d.^2$). For randomised controlled trials, net change in BP was calculated as the mean difference (magnesium supplementation minus control) of the change in BP. For crossover trials, net change was calculated as the mean difference between the end of the magnesium supplementation and control or placebo periods. Standard error of the effect size was adjusted for crossover trials, as advised by Morris.[13] Repeated-measures designs can lead to errors in the exact variance, which underestimate the sampling variance. Where this correction can result in reduced accuracy of the meta-analysis, the degree of error is generally small and is therefore tolerated. Overall effect size estimates and 95% confidence intervals (CIs) were calculated using the DerSimonian and Laird's[14] random-effects model. The meta-analysis, Forest plots and Biggs funnel plots for both SBP and DBP were generated using STATA, version 11 (StataCorp LP, College Station, TX, USA).

RESULTS

In total 141 potentially relevant articles were identified by the search strategy. Of these, 108 articles were excluded and 33 were electronically retrieved for further analysis. Exclusion criteria were: (1) combination of magnesium with other vitamins or minerals that can affect BP; (2) lack of a placebo or control group; (3) inadequate data being available to calculate the difference in BP change between groups; (4) subjects being o18 years of age; and (5) non-randomised allocation of subjects to treatment conditions.

From these, a further 11 articles were eliminated, leaving a total of 23 sets of data from 22 intervention trials. These trials included a total of 1173 individuals, with a sample size ranging from 13 to 155 participants. Every trial reported the sex of the subjects, with two only including males,[15,16] three only including females,[17] [19] and the remaining 17 trials including both males and females. The total gender split of the subjects was 47% male and 53% female. The subjects were from 12 different countries (Brazil, Denmark, England, Finland, Holland, Ireland, Italy, Japan, Korea, Mexico, Sweden and USA). Thirteen trials were of parallel design, while 10 sets of data from nine trials were of crossover design. The mean age of individuals taking magnesium supplements in the trials was 50.1 years, while those on placebo treatment in the parallel trials had a mean age of 52 years. Subjects in three trials had non-insulin-dependent diabetes mellitus[20] [22] and one trial observed individuals with insulin resistance.[15] Some or all of the subjects in six trials were receiving antihypertensive medication such as beta-blockers and diuretics. The other trials enrolled normotensive subjects, untreated hypertensive subjects or hypertensive subjects abstinent from treatment for a period of at least 1 month.

The duration of treatment with magnesium ranged from 3 to 24 weeks, with a mean duration of 11.3 weeks. The dose of elemental magnesium in the observed studies ranged from 120 mg/day (5 mmol) to 973 mg/day (40 mmol), with a mean dose of 410 mg/day (16.9 mmol). A total of seven different supplemental magnesium compounds were used (MgO, Mg aspartate, $MgCl_2$, $MgOH_2$, Mg lactate, Mg citrate, Mg pidolate). For 21 out of the 23 sets of data, trials were under double-blind conditions; the trial by Hattori et al.[23] was single blind, while no blinding was used by Kawano et al.[4] All studies used a placebo, aside from Kawano et al.,[4] which used baseline data and compared this with post-intervention results. Mean SBP on entry ranged from 110 to 173 mm Hg. Initial DBP ranged from 73 to 106.5 mm Hg for the subjects assigned to receive magnesium supplements.

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Bias in the form of publication is difficult to avoid, but trials with a negative as well as a positive outcome were included in the meta-analysis to avoid this bias, as it is known that publication tends to favour positive outcomes. The quality of many of the included trials was poor and this may bias the results by way of an overestimation of the effect of treatment. Conversely, in this metaanalysis, it was seen that poorly designed trials may also bias the results against the supplement and its effect, as in the case of parallel designed trials.

Analysis of the overall effect

Effect size for the meta-analysis for DBP was 0.36 and for SBP was 0.32, showing a similar effect for both measures (Figures 1 and 2). CIs at 95% were 0.23--0.41 for SBP and 0.27--0.44 for DBP. Heterogeneity for both cohorts was high (I^2 ¼ 82% for DBP and 88% for SBP).

Sub analysis---crossover vs non-crossover design

Stratification by crossover and non-crossover design was carried out.

When stratified by design of the trial, the effect estimates for SBP were 0.51 (CI 0.39--0.64) for crossover trials and 0.13 (CI 0.00--0.26) for non-crossover trials (Figure 3), and for DBP 0.47 (CI 0.35--0.59) for crossover trials and 0.23 (CI 0.10--0.36) for noncrossover trials (Figure 4).

In the DBP funnel plot (Figure 5) it can be seen that overall estimates of effect sizes in larger sample studies is quite diverse and spread quite widely at the top of the funnel, suggesting that only small studies showing negative results are more likely to be

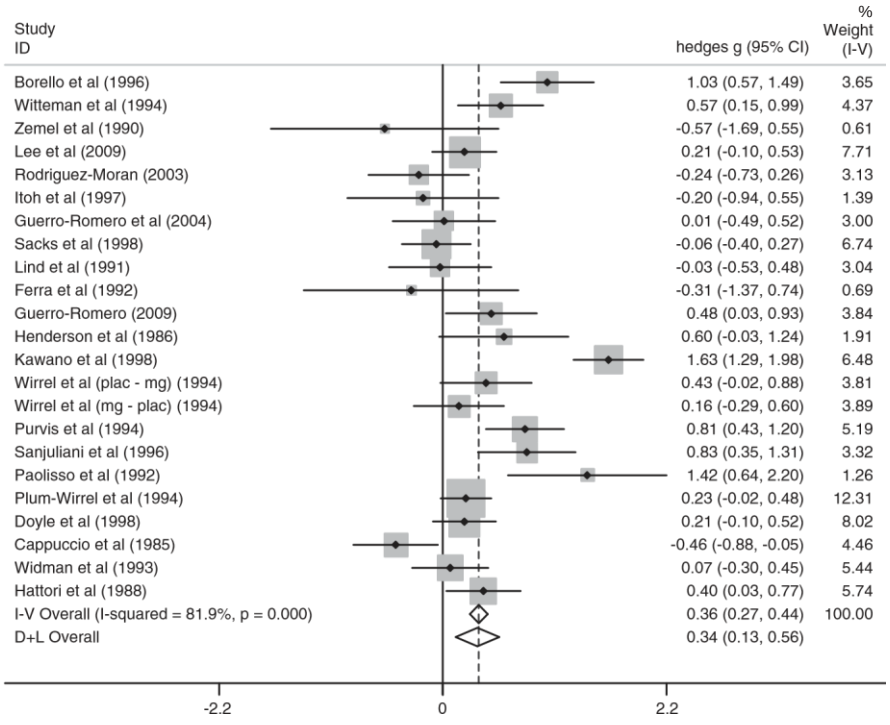

Figure 1. Forest plot for DBP.

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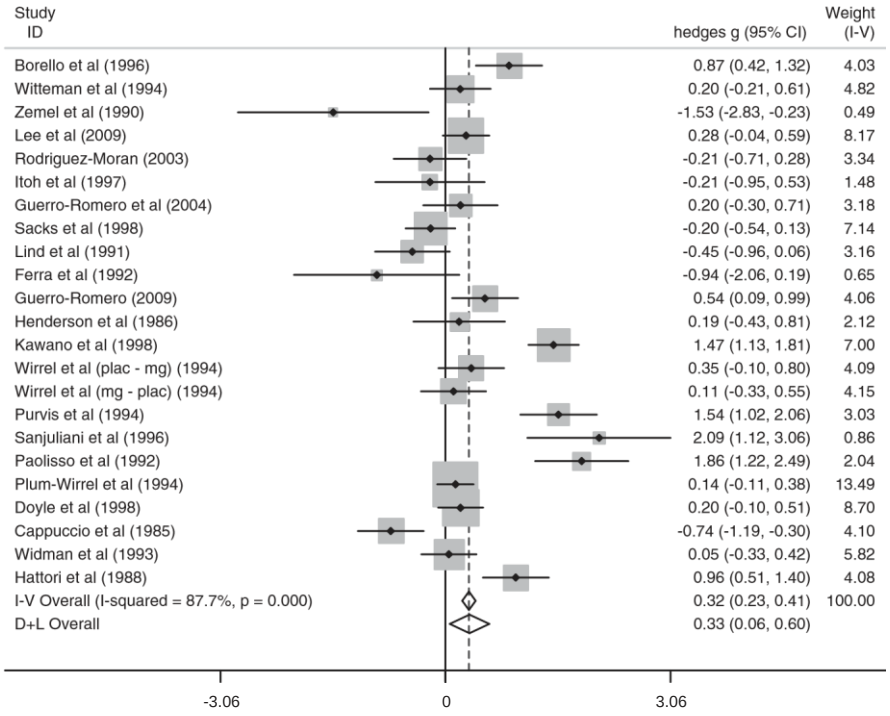

Figure 2. Forest plot for SBP.

published. For SBP (Figure 6) there seems to be a similar trend, but it can also be seen that there is a tendency for larger trials to fall within the left side of the funnel, around the 0 mark, suggesting that larger trials that show no effect are more likely to be published. From Figures 5 and 6 it can generally be seen that crossover trials show a more positive effect from the intervention.

Subanalysis---dosage

The Committee on Medical Aspects of Food and Nutrition Policy[6] calculated a Reference Nutrient Intake (RNI) of 300 mg/day for adult males and 270 mg/day for adult females. None of the studies included in this meta-analysis had intakes between 300 and 370 mg and cohorts were all male; therefore two groups were

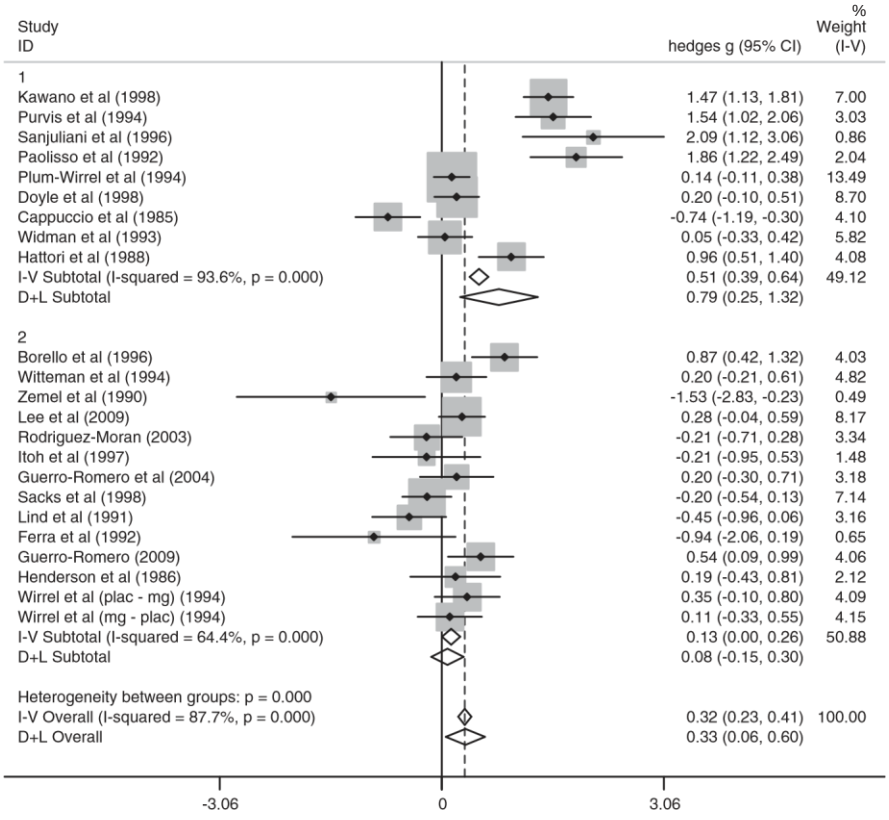

Figure 3. Forest plot for SBP by design. The top half (1) of the figure showing crossover designed studies; the lower half (2) shows non-crossover designed studies.

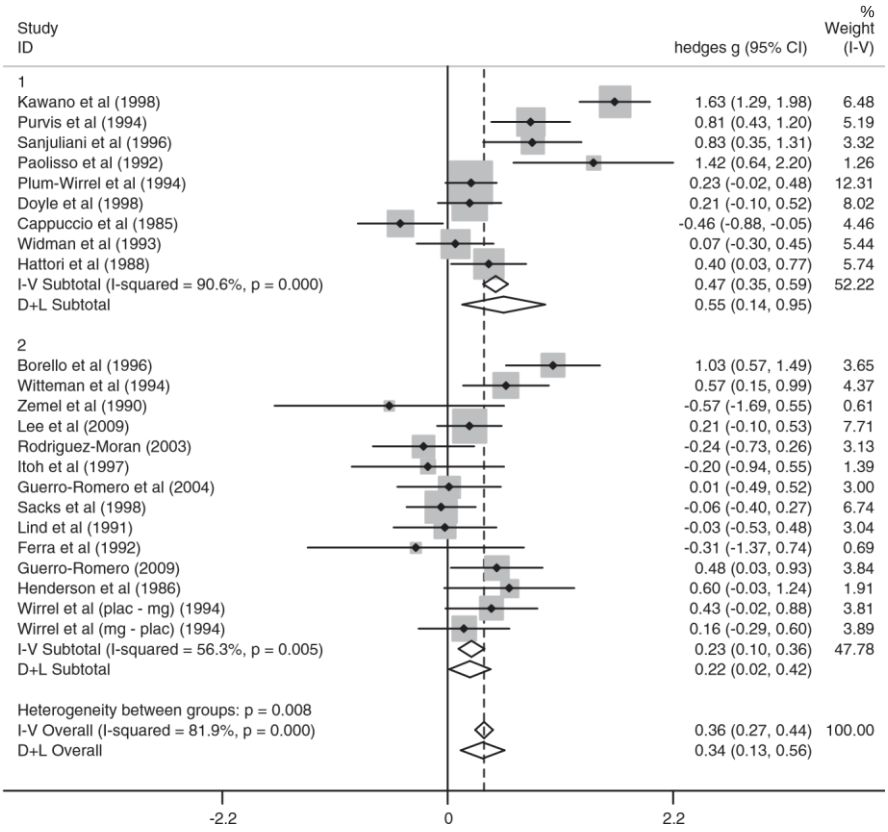

Figure 4. Forest plot for DBP by design. The top half (1) of the figure shows crossover designed studies; the lower half (2) shows non-crossover designed studies.

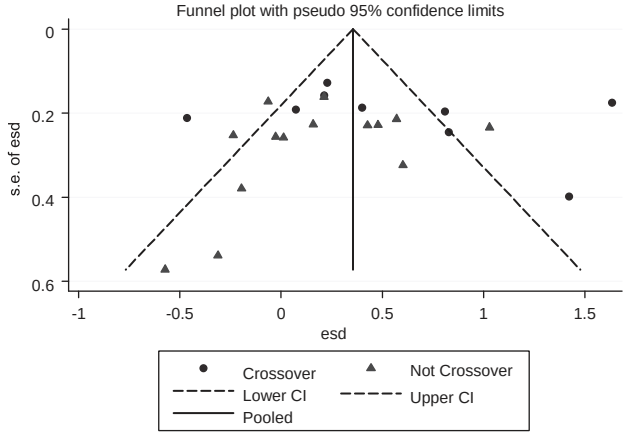

Figure 5. Funnel plot for DBP for all studies, coded for crossover and non-crossover design.

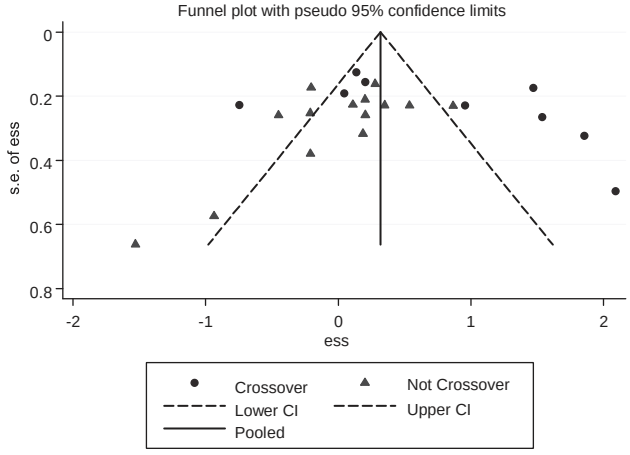

Figure 6. Funnel plot for SBP for all studies, coded for crossover and non-crossover design.

formed: one below and one above the RNI, with the division starting at the study with the nearest dosage above the RNI (370 mg). A subanalysis was then carried out dividing the dosage into o370 mg Mg/day and X370 mg Mg/day, with results showing greater efficacy of magnesium supplementation at the higher dose (Figures 7 and 8). The o370 mg trials for SBP had an effect estimate of 0.14 (CI 0.03 to 0.25) and for DBP had an effect estimate of 0.21 (CI 0.10 to 0.31).

The X370 mg trials for SBP had an effect estimate of 0.72 (CI 0.56--0.89) and for DBP had an effect estimate of 0.66 (CI 0.51--0.82).

Subanalysis---stratification by country

From the data analysed, no association could be found between country and effect size. Given the wide variation in publication origin and lack of information on the country of origin of subjects, no clinically relevant stratification by area could be made.

DISCUSSION

This review of interventional epidemiological studies is suggestive of a negative association between magnesium supplementation and DBP and SBP, with a greater reduction being seen in SBP. The average reduction in BP based on an effect size of 0.36 for DBP and 0.32 for SBP translate to an actual reduction of 2--3 mm Hg for DBP and 3--4 mm Hg for SBP. At the lower dosage[24] the effect sizes for SBP and DBP were 0.87 and 1.03, respectively, whereas at the higher dosage[25] effect sizes were seen to be 1.53 and 0.57, although Zemel et al.'s[25] work was anomalous to other higher-dosage studies. The overall effect size for DBP was slightly higher than that for SBP. The majority of trials showed a reduction in BP, although significance was not always shown.

The Antihypertensive And Lipid-Lowering Treatment To Prevent Heart Attack Trial (ALLHAT)[26] found, when comparing antihypertensive treatments, that a SBP reduction of between 0.8 and 2 mm Hg, depending on drug intervention, was clinically significant in reducing the incidence of coronary heart disease, heart failure and stroke. The clinical significance in the reductions found from this meta-analysis is potentially very important. The subanalysis discussed below allow for future research to realise the full potential of magnesium in lowering BP with appropriately designed trials.

Dosage

When a subanalysis for dosage was carried out (o370 mg Mg and X370 mg Mg/day), results for both SBP and DBP showed greater efficacy of magnesium supplementation at the higher dose. When the higher magnesium dosage was analysed a much higher effect size (DBP ¼ 0.66 and SBP ¼ 0.70; 95% CI 0.51--0.82 and 0.56--0.89 for DBP and SBP, respectively) was found. Those using o370 mg demonstrated high levels of variation. One anomaly for this was the study by Zemel et al.,[25] which used the highest dosage of 973 mg Mg/day but showed wide CI limits (DBP 95% CI 1.69 to 0.55, SBP 2.83 to 0.23) and a low effect size of 0.57 and 1.53 for DBP and SBP, respectively. This may be attributed to the small cohort size (n ¼ 13) or to study design. However, dosage was not related to habitual dietary magnesium intake, which would affect the effect of supplementation, and no baseline measures of habitual magnesium intake were recorded in any of the studies. Further, no observations were made on the social--economic status of the cohorts, which would influence dietary intake and may bias the overall results. Only one study recorded serum magnesium levels, which the authors suggest would affect magnesium absorption from supplementation.

There was substantial heterogeneity between the findings of the trials for both DSP and SBP (I^2 ¼ 82 and 88, respectively), which could be explained by random variation, the various population groups, the interventions or the methods used in the trials, and length of intervention. A more homogenous sample of studies may increase the effect size; although a random effects model was used, the difference between the studies was high.

Crossover vs non-crossover design

Further subgroup analysis was carried out for study design (crossover vs non-crossover). For crossover trials the effect size increased substantially for both DBP and SBP when compared with the non-crossover trials, reinforcing the idea that paired data would have more robust results from the intervention than non-crossover, and that the effect from the intervention would be augmented.

Interestingly, the majority of crossover studies fell outside of the 95% confidence limits for SBP, which may be due to the different population groups between the studies or may be attributed to publication bias; however, effect size was larger in the crossover designed trials (SBP crossover 0.51, non-crossover 0.13; DBP crossover 0.47, non-crossover 0.23).

Trials were chosen to be as homogenous as possible, although some of the studies were of a lesser quality, with one failing to conceal allocation[4] and intra-subject variation for age, weight, clinical status and nationality.

A major limitation in most of the studies was lack of data on dietary intake, which would have had major implications for all studies. It would also have been beneficial if trials had looked at pre-trial serum magnesium levels and then looked at this again

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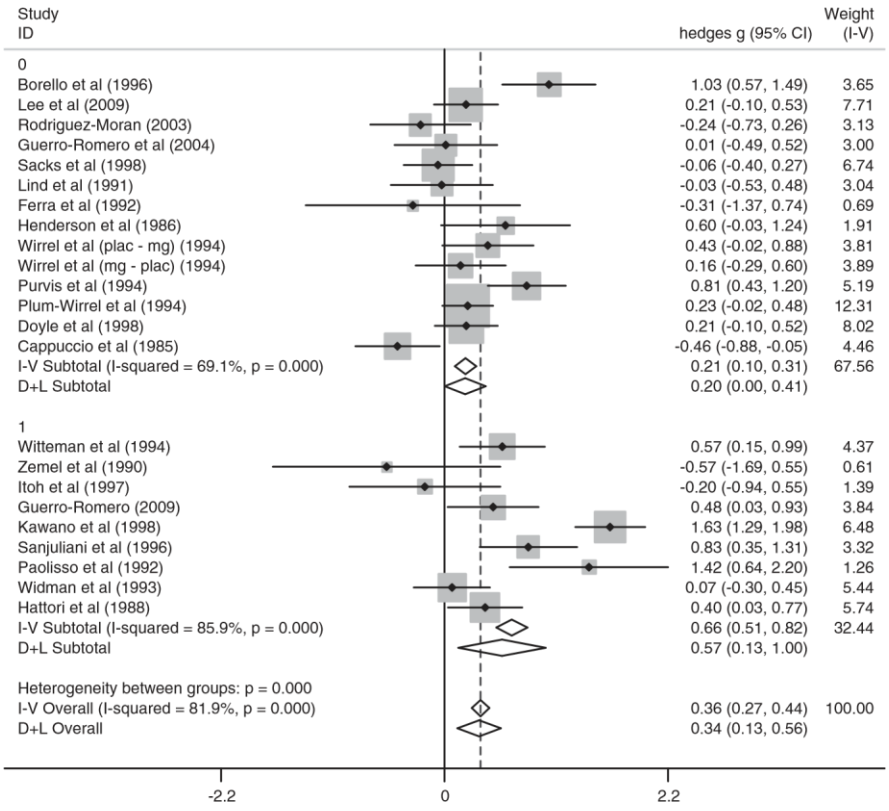

Figure 7. Forest Plot for DBP by dosage. The top half (0) of the figure shows o370 mg dosage magnesium; the lower half (1) shows X370 mg dosage magnesium.

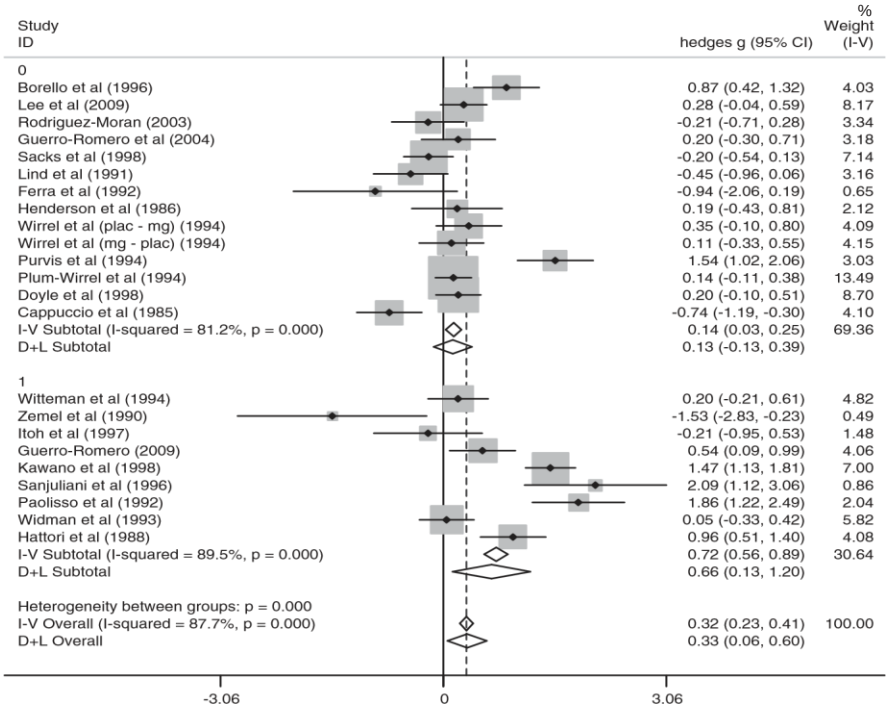

Figure 8. Forest plot for SBP by dosage. The top half (0) of the figure shows o370 mg dosage magnesium; the lower half (1) shows X370 mg dosage magnesium.

after supplementation; this would also have given a better understanding of absorption over a range of magnesium intakes. Differences were seen between cross-over and non-crossover design and this would exaggerate inter-individual dietary habits in both design groups. Those who had the higher-magnesium diet may show less of a response to the supplement than those who had a lower intake, with a non-crossover design being more greatly affected by diet than a crossover design. To the best of the investigators' knowledge, only one systematic review has looked at dietary magnesium and BP,[27] and this concluded that there was a negative association between the two variables, although the authors attributed methodological problems of dietary data collection to this.

Of the 22 studies in this meta-analysis, 13 reported adverse effects from the intervention and placebo treatments,[4,15,16,18 22,28 32] six reported no adverse effects[24,33 38] and three did not report any information relating to adverse effects.[17,23,25] Of these studies, the adverse effects were largely either diarrhoea, or unspecific mild abdominal or bone pain. Only three studies reported serious adverse effects from the treatments that led to withdrawal from the investigations: Lind et al.[29] reported one case of visual impairment of a subject on magnesium treatment; Wirrel et al.[31] reported a myocardial infarction of a subject but did not specify the treatment that the subject was receiving; Plum-Wirrell et al.[32] reported a blood coagulation defect of one subject but also did not specify the treatment arm. Three subjects of Ferrara et al.[30] were also unable to complete the study due to an increase in BP.

All the trials analysed for this meta-analysis used magnesium supplements, and although the data had a high level of heterogeneity there was a reduction in BP, being more evident in the higher-dosage trials. Future research could potentially observe the effect of increased dietary magnesium intake to see if the results were correlated to similar amounts given as a supplement. Some studies have looked at magnesium and exercise in respect to performance and recovery parameters;[7,38] future research may investigate BP response alongside performance parameters.

In summary, this meta-analysis showed an overall reduction in SBP and DBP from magnesium supplementation. Studies had high heterogeneity, but the effect of treatment could still be seen. The average reduction in BP based on an effect size of 0.36 for DBP and 0.32 for SBP translates to an actual reduction of 2--3 mm Hg for DBP and 3--4 mm Hg for SBP. This could be strengthened by both crossover design and dosage 4370 mg. Subanalysis for dosage showed greater efficacy of magnesium supplementation at the higher dose, with a higher effect size being seen. Those using o370 mg also demonstrated high levels of variation. Although these reductions are small, if optimised by the above suggestions, they could have a significant effect on BP, particularly on the pre-hypertensive population group. Further investigation may look at this effect during exercise and on pre-hypertensives who are encouraged to change their lifestyle and increase their physical activity level in order to maintain a normalised BP.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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RESEARCH ARTICLE Open Access

The effect of acute vs chronic magnesium supplementation on exercise and recovery on resistance exercise, blood pressure and total peripheral resistance on normotensive adults

Lindsy S Kass* and Filipe Poeira

Abstract

Background: Magnesium supplementation has previously shown reductions in blood pressure of up to 12 mmHg. A positive relationship between magnesium supplementation and performance gains in resistance exercise has also been seen. However, no previous studies have investigated loading strategies to optimise response. The aim of this study was to assess the effect of oral magnesium supplementation on resistance exercise and vascular response after intense exercise for an acute and chronic loading strategy on a 2-day repeat protocol.

Methods: The study was a randomised, double-blind, cross-over design, placebo controlled 2 day repeat measure protocol (n = 13). Intense exercise (40 km time trial) was followed by bench press at 80% 1RM to exhaustion, with blood pressure and total peripheral resistance (TPR) recorded. 300 mg/d elemental magnesium was supplemented for either a 1 (A) or 4 (Chr) week loading strategy. Food diaries were recorded.

Results: Dietary magnesium intake was above the Reference Nutrient Intake (RNI) for all groups. Bench press showed a significant increase of 17.7% (p = 0.031) for A on day 1. On day 2 A showed no decrease in performance whilst Chr showed a 32.1% decrease. On day 2 post-exercise systolic blood pressure (SBP) was significantly lower in both A (p = 0.0.47) and Chr (p = 0.016) groups. Diastolic blood pressure (DBP) showed significant decreases on day 2 solely for A (p = 0.047) with no changes in the Chr. TPR reduced for A on days 1 and 2 (p = 0.031) with Chr showing an increase on day 1 (p = 0.008) and no change on day 2.

Conclusion: There was no cumulative effect of Chr supplementation compared to A. A group showed improvement for bench press concurring with previous research which was not seen in Chr. On day 2 A showed a small non-significant increase but not a decrement as expected with Chr showing a decrease. DBP showed reductions in both Chr and A loading, agreeing with previous literature. This is suggestive of a different mechanism for BP reduction than for muscular strength. TPR showed greater reductions with A than Chr, which would not be expected as both interventions had reductions in BP, which is associated with TPR.

Keywords: Magnesium supplementation, Blood pressure, Bench press, Acute and chronic loading

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Background

Magnesium (Mg^{2+}) elicits significant enzymatic cellular involvement and physical regulation such as energy metabolism/production through formation of the Mg-ATP complex [1] and physiological regulation and control of neuromuscular cardiac activity, muscular contraction, vascular tone and blood pressure [2,3]. Its effect on muscular contraction and vascular tone have been shown to reduce blood pressure and subsequently vascular resistance [4].

Nutritional supplementation is a well-established method for enhancing performance in conjunction to training. Micronutrient intake has been highlighted to have gained greater prominence with athletes in relation to the importance of an adequate nutritional status [5], However, previous research highlights nutritional inadequacies and thus an impaired nutritional status (i.e. marginal nutrient defi-ciency) from both an athletic [5] and general population perspective [6]. This identifies physical activity as increasing the rate at which micronutrients are utilised, promoting excessive micronutrient loss via increased catabolism and excretion (sweat and urine). Magnesium is a mineral required at rest and during exercise [7]. This increase in Mg^{2+} turnover during exercise may lead to a state of in-sufficiency acting as a contributory factor towards an in-crease in blood pressure and a state of hypertension [8]. This, together with a decline in dietary intake below the RNI may have a negative impact on both performance and blood pressure.

Magnesium supplementation in relation to exercise has differed considerably in research opinion as to the dose and type of Mg2+ salt administered. It is influenced by the specific anion attachment with Mg2+, thus influencing supplemental solubility, elemental Mg2+ bioavailability and supplemental effectiveness [9]. Research has illustrated organic forms of Mg2+ supplementation i.e. Aspartate, cit-rate, lactate, pidolate, fumurate, acetate, ascorbate and glu-conate to exemplify a greater solubility and bioavailability in comparison to inorganic forms i.e. oxide, sulphate, chloride and carbonate [10] When considered relative to the quantity needed to be ingested to release 300 mg of elemental Mg2+ along with the fact that certain magnesiums are unavailable in the UK, magnesium citrate was considered to be the best option for this protocol.

Research to date consists of both positive [11-13] and negative [14,15] findings. The research appears to agree that Mg^{2+} supplementation has no effect on physical performance when serum concentrations are within the normal range (serum Mg^{2+} 0.8-1.2 $mmol \cdot L^{-1}$) [12,16]. However, manipulating intakes of Mg^{2+} by diet or supple-mentation has been shown to have performance [11,17] and blood pressure enhancements [13,18]. Limitations to many of these studies is the lack of information regard-ing either serum magnesium or dietary intake [19]. The general consensus appears to be that Mg^{2+} supplementation has a greater effect when habitual dietary intake or serum levels are low.

Further, to the best of the authors' knowledge research to date lacks analysis of Mg^{2+} from an acute (A) and chronic (Chr) viewpoint within the same study. Therefore, the aim of the current study was to assess the effect of oral Mg^{2+} supplementation on strength performance and vascular responses from both an A and Chr loading strategy as to establish potential differences in supplemental duration and influences of dietary status and supplemental dose on performance and vascular responses.

Methods

Subjects

= total of 13 subjects (males (m) = 7 females (f) = 6) were recruited from recreational running, cycling and triathlete clubs. Six subjects were allocated randomly to the acute intervention group (m = 3, f = 3) and 7 to the chronic intervention group (m = 4, f = 3). Subjects were recruited in accordance to meeting the inclusion/exclusion criteria, (Table 1). Informed consent and health screen were completed and ethical approval was granted by the University of Hertfordshire School of Life Sciences Ethics Committee.

Experimental design

The study was a randomised, cross-over, double-blind, placebo controlled, 2 day repeated measure protocol. Subjects were assigned to either the acute or chronic intervention and the two trials ran parallel. Within each trial subjects undertook both the magnesium interven-tion and a placebo intervention with a one week wash-out period in a randomised order. The two interventions were a chronic (Chr) (4 weeks) and acute (A) (1 week) loading strategy, sub-divided into a supplemental and a placebo control group with a 1 week washout period. A maximal graded exercise test for determination of VO_{2max} was conducted to ensure participant homogeneity with a cut off of 45 ml/kg^{-1} and 35 ml/kg^{-1} oxygen for males and females respectively. The study was tested across 2 consecutive days at each treatment time-point i.e. baseline

Table 1 Subject characteristics; including group sample size (n), age, height, weight, VO_{2max}, HR_{max}

	Chronic		Acute
N	7		6
Age (years)	40.8	± 4.4	35.8 ± 6.2
Height (cm)	176.2 ± 11		174.6 ± 12
Weight (kg)	73.2	± 13.2	72.1 ± 13
VO_{2max} (ml/kg)	51.8	± 9.1	53 ± 4.8
HRmax (bpm)	176.4 ± 3.8		180.8 ± 7.7

Values are mean ± SD.

and again after either 1 or 4 weeks intervention. A one week washout was then given and then a further intervention of the opposite treatment was given (placebo or magnesium) with the same loading phase.

Protocol

After familiarisation, subjects were tested for baseline measurements Anthropometric measures (height (cm), weight (kg)) and age (y)) were recorded. All subjects attended a familiarisation session on all equipment and testing protocols prior to testing. On both day 1 and recovery day 2 participants completed a 40 km time trial on bicycles owned by the subjects and set onto a rig. A set 40 km flat course with no wind setting was used on a Computrainer Pro ergometer (Computrainer, Seattle). All on-screen course data information was blinded, verbal encouragement was not given during the exercise testing. The time trial was carried out to elicit physiological stress as normally determined by training and competition. After a 30 minute rest participants completed the following tests to determine the effect of magnesium on strength and cardiovascular parameters.

Blood pressure, and augmentation index (Aix) were recorded at rest immediately and before the bench press. Subjects then performed a bench press corresponding to a 5 repetition maximum (5-RM) protocol [20]) for determination of their 1-RM. Upon completion, a 5-minutes rest period was given. Subsequently, a bench press at 80% 1-RM was performed to exhaustion. A measure of force (Newtons) was recorded during the bench press, with additional measures of blood pressure and Aix immediately upon completion of the bench press.

Supplementation

Magnesium citrate and placebo (cornflour) were capsulated into large vegetarian capsules. Capsules consisted of a total of 75 mg of elemental Mg^{2+} citrate, (Pioneer analytical balance. OHAUS, UK), 4 capsules per day were taken orally, equating supplemental Mg^{2+} to a total daily dose of 300 mg/d elemental Mg^{2+}. Supplements were ingested evenly throughout the day on a non-testing day, or ingested 3 hours before exercise testing. Finally, the supplementation period for both placebo and Mg^{2+} accounted for a total ingestion period of 1 week or 4 weeks within the A and Chr groups, respectively.

Diet

A 4-day weighed food and beverage diary was recorded in relation to 3 weekdays and 1 weekend day, which was used for analysis of habitual dietary magnesium intake through use of dietary analysis software (Dietplan 6.70 Forestfield Software, UK).

Statistical analysis

Data were analysed using SPSS version 20 (IBM limited, UK) and Microsoft excel 2007 for Windows. Box-whisker plots measured normality/data distribution and showed that the data were not normally distributed. Therefore non-parametric Wilcoxon 2 related samples tests were carried out on all results to look for differences. Alpha value was set at 0.05.

Results

There were no statistically significant difference found between anthropometric data, VO_{2max} and HR determining a homogeneous cohort (Table 1).

Table 2 shows averaged dietary data for both the Chr and A groups. Both the Chr and A control groups showed no significant difference between macronutrient and magnesium ingestion.

Performance

Bench press

Net strength gains as determined by 1-RM showed significant increase of 17.7% with the acute Mg^{2+} loading strategy compared to baseline ($p = 0.031$) (Figure 1). No significant strength gains were seen in the Chr intervention group ($p = 0.281$).

Furthermore, A Mg^{2+} showed no decline in recovery (day 2) performance for force (N) resulting in a small day 2 (recovery day) force increase of 2.7%, showing a trend but no significance (Figure 2). On the contrary Chr Mg^{2+} showed a day-to-day 32.1% performance decrement (Figure 3).

Resting SBP measures from day 1 and 2 show a significant decrease within A Mg^{2+} treatment ($P = 0.031$), conversely placebo showed a significant increase in SBP ($P = 0.047$) (Table 3). Further, significant day 2 reductions in SBP were noted between A treatments of Mg^{2+}-placebo ($P = 0.016$). On the contrary, Chr Mg^{2+} shows no significant reductions in resting SBP on day 1 or day 2.

In relation to post SBP responses, both Chr and A Mg^{2+} treatment resulted in significant SBP reductions; however, such reductions can be noted on day 1 ($P = 0.016$) and day 2 ($P = 0.016$) for a Chr Mg^{2+} induced SBP

Table 2 Dietary intake, values are mean ± SD

	Chronic intervention	Acute intervention	Chronic placebo control	Acute placebo control
Kcal	2513 ± 1201	2686 ± 938	3985 ± 519	3785 ± 734
CHO (g)	274 ± 170	296 ± 118	397 ± 209	343 ± 79
Fat (g)	96 ± 58	115 ± 49	114 ± 63	105 ± 48
Pro (g)	119 ± 38	114 ± 37	136 ± 66	129 ± 16
Mg^{2+} (mg)	375 ± 104	368 ± 173	551 ± 347	378 ± 79

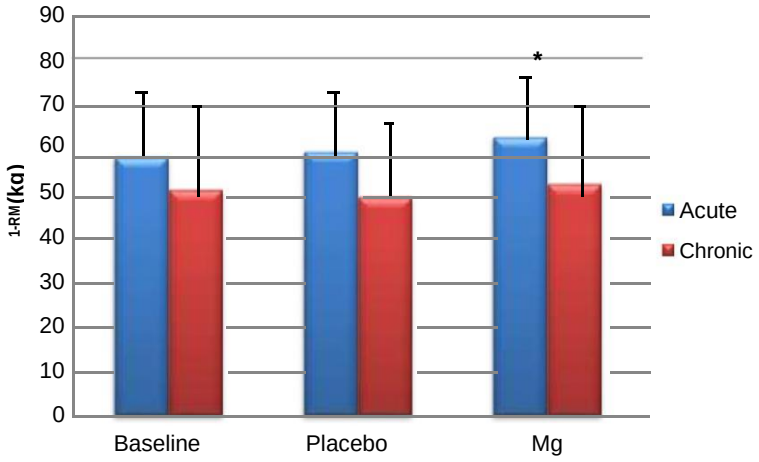

Figure 1 Acute and chronic bench press 1-RM scores on day 1 ± SD. * denotes significance.

reduction in comparison to placebo, whereas an A Mg^{2+} reduction can be accounted for on day 2 (P = 0.047) in comparison to placebo.

Resting DBP showed no difference for day 1 to day 2 between the placebo or Mg^{2+}. Post DBP showed no differences between Day 1 to Day 2 for acute supplementation group (Table 4). However, chronic intervention showed a decrease in DBP for post bench press on the recovery day 2.

Although no significance was seen for Aix at rest for both A and Chr loading strategies, a significant lowering post bench press was found as highlighted in Table 5 on day 1 for A treatment and day 2 for the Chr treatment group. Day 1 Aix reductions correspond to a significant Mg^{2+} lowering effect compared to baseline (P = 0.016) and placebo (P = 0.031), respectively. Whereas, similar Aix reductions for the Chr Mg^{2+} group is noted on day 2 post bench press resulting in significant values of P = 0.039, when compared to baseline and placebo, respectively.

Discussion

This study set out to determine whether either acute or chronic magnesium supplementation would have an effect on performance (strength and cardiovascular) and blood pressure with exercise and/or on a second bout of exercise after a 24 hr recovery period. As has been shown previously [13,21] acute magnesium supplementation has a positive effect on BP, plyometric parameters and torque, however its effect on resistance exercise has not been evident to date. Further, chronic loading strategies have not been investigated in respect to exercise as well as the effect of Mg supplementation on a second bout of exercise. It was hypothesised that as acute Mg^{2+} supplementation has been seen to have beneficial effects

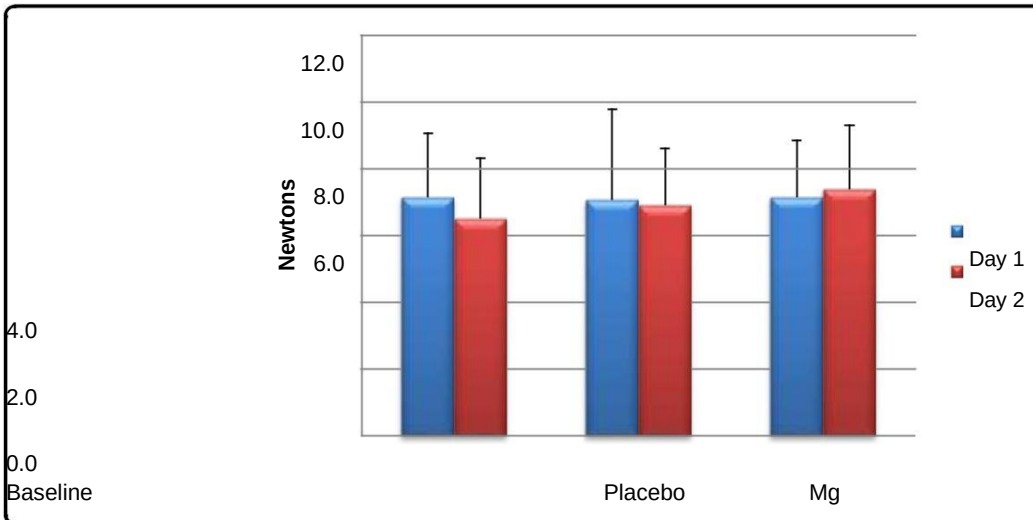

Figure 2 Acute force (newtons) output on day 1 and 2 (recovery) during repetitions to fatigue ± SD.

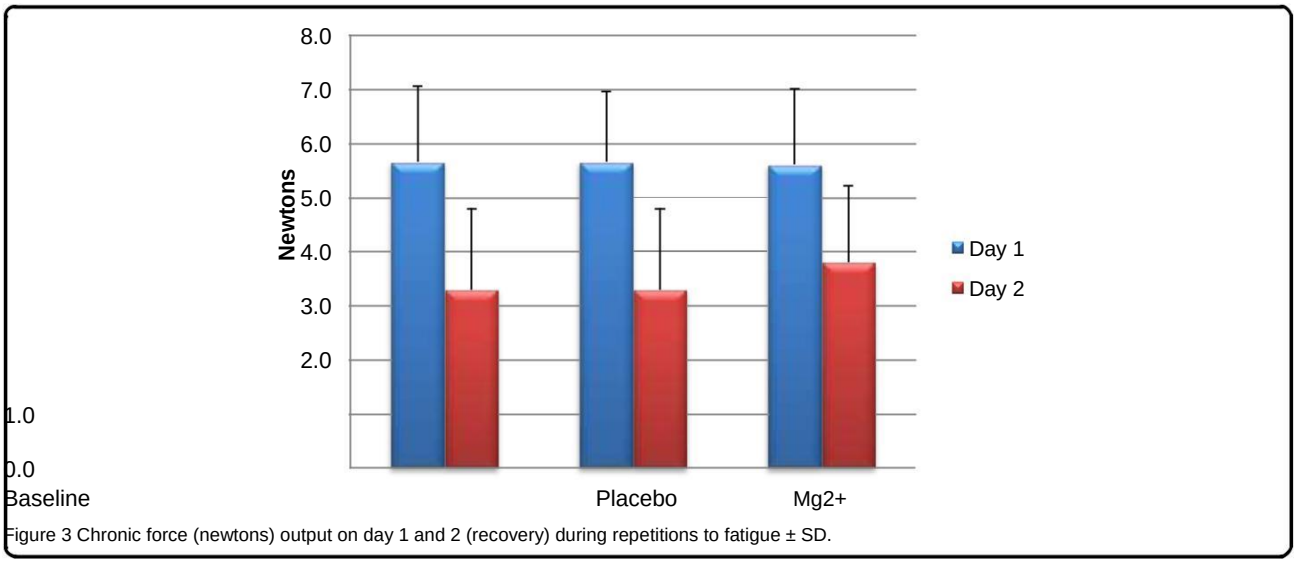

Figure 3 Chronic force (newtons) output on day 1 and 2 (recovery) during repetitions to fatigue ± SD.

on BP, CV parameters and peak torque a longer loading strategy (4 weeks) would amplify these results, giving a more beneficial and greater response. However, this study did not find that chronic loading of Mg^{2+} has a cumulative effect on the effect of supplementation, perhaps due to saturation of Mg^{2+} within the blood or limitations to transporters.

Primary findings showed variance across treatment Mg^{2+} supplementation compared to the Chr where a 2.0 groups on exercise (strength and recovery) and cardio- Newtons (32.1%) decrement was seen.

vascular responses. The Chr Mg^{2+} intervention showed no significance in performance gains for bench press net strength and force output (Figures 1, 2 and 3). The A Mg^{2+} intervention showed variance in results across all variable analysed with some improvements being seen in resting HR and blood pressure for both Chr and A treatment groups regarding strength related performance (Figures 1, 2, 3 and Tables 3, 4, 5).

These performance enhancements for the strength asso-

Strength performance

Strength related performance within the bench press showed statistical significant improvements (P = 0.031) within the A group and Chr group. Previous research has shown that Mg^{2+} significantly enhances bench press [22] and strength performance [11,23]. Acute Mg^{2+} loading showed a significant net strength increase of 5.5 kg between baseline and supplemental Mg^{2+} trials. Other strength related measurements of force (Newtons) illustrated A Mg^{2+} induced improvements. Typically, where a decrease in force would be expected on day 2 (recovery) of training as a normal physiological response to training, an A group improvement of 0.25 Newtons (2.7%) was seen with

When examining net strength of Chr compared to A groups a notable difference between baseline scores is evident implying that subjects within the A group might well be stronger due to a 7.3 kg 1-RM difference at baseline (Figure 1). Therefore, when considering the 10.5 kg difference between Chr and A group 1-RM trials after intervention of Mg^{2+}, inter-subject lifting capacity/ability could be a factor of concern for validating such a difference.

ciated tests are suggestive of physiological-regulatory functions of Mg^{2+} within muscle contraction and relaxation; i.e. regulating troponin expression via Ca^{2+} concentration gradients, Ca^{2+} transport, MgATP complex formation optimising energy metabolism/muscular contraction, increasing protein synthetic rate, protection against cellular

Table 3 Acute and chronic group mean SBP values at rest and post bench press at 80% 1-RM to fatigue on day 1 and 2 ±SD

Physiological variable	Treatment	Group			Physiological variable	Treatment	Group			
		C Chr	A				Chr		A	
Resting SBP (mmHg) day 1	Placebo	119 ± 7	120	± 5*[1]	Post SBP (mmHg) day 1	Placebo	143	± 7*[3]	136	± 5
	Mg2+	118 ± 6	122	± 4*[2]		Mg2+	136	± 9*[3]	137	± 6
Resting SBP (mmHg) day 2	Placebo	121 ± 8	125	± 2*[1, 3]	Post SBP (mmHg) day 2	Placebo	144	± 9*[4]	144	± 7*[5]
	Mg2+	118 ± 7	117	± 7*[2, 3]		Mg2+	137	± 10*[4]	134	± 5*[5]

*Denotes significance as paired by numbers.

Table 4 Acute and chronic group mean DBP values at rest and post bench press at 80% 1-RM to fatigue on day 1 and 2 ± SD

Physiological variable	Treatment	Group			Physiological variable	Treatment	Group			
		Chr		A			Chr		A	
Resting DBP (mmHg) day 1	Placebo	85	± 7	75 ± 7	Post DBP (mmHg) day 1	Placebo	92	± 8	85	± 12
	Mg2+	79	± 6	75 ± 4		Mg2+	87	± 7	82	± 5
Resting DBP (mmHg) day 2	Placebo	78	± 8	79 ± 6	Post DBP (mmHg) day 2	Placebo	91	± 5*[1, 2]	86	± 13
	Mg2+	75	± 7*[1]	74 ± 5*[2]		Mg2+	84	± 8*[2]	76	± 8*[3]

*Denotes significance as paired by numbers.

damage and, greater amount of actin-mysoin crossbridges [23-26] all of which contribute to the result of increased strength and force production. Consideration must be given as to why such a contrasting difference between Chr and A groups occur specifically when regarding strength performance measures. The A Mg^{2+} supplemented group showed day-to-day performance improvements across 3 trials, as opposed to 3 day-to-day non-performance im-proved trials exhibited within the Chr Mg^{2+} supplemented group which may be attributed to the different loading strategies within the current study. The Mg^{2+} supplemen-tation within the current research was 300 mg/d, therefore equating the Chr and A group mean daily intake for Mg^{2+} to 675 mg/d and 700 mg/d, respectively, when combined with dietary Mg^{2+} intake as analysed from food diaries. This adds a sense of greater ambiguity when considering the Mg^{2+} - strength performance relationship, and comparing to research highlighting observations that intakes of 500 mg/d or greater result in further increases in strength [24,25]. It could be suggested that subjects within the Chr loading group might be more susceptible to a possible reduction threshold or cell tolerance for Mg^{2+} absorption based upon the understanding that high Mg^{2+} intakes result in a lower Mg^{2+} absorption [27]. Additionally, Mg^{2+} homeostasis may be postulated to exhibit no greater benefit from the chronic perspective due to the kidney function for Mg^{2+} excretion as to maintain a balanced concentration of Mg^{2+} [27,28]; for example, could the principle of a higher Mg^{2+} dose, longer supplemental duration and associated proportional increase of Mg^{2+} excretion highlight the body's effi-ciency in maintaining a state of homeostasis? Alterna-tively, chronic loading through providing a regular high Mg^{2+} intake may influence extracellular Mg^{2+} concentra-tions which coincide with manipulation of Mg^{2+} trans-porter TRPM6 function, resulting in a potential decrease in TRPM6 expression in conjunction to increasing the urinary excretion of Mg^{2+} [29]. Thus, an acute ingestion rate as opposed to chronic could result in a more efficient use for Mg^{2+}.

Cardiovascular responses at rest and post bench press performance

Significant reductions in SBP and DBP are illustrated from post testing in the chronic group and rest and post testing in the acute group data across day 1 and 2 compared to baseline and placebo (Tables 3 and 4). Resting SBP was accounted for by a greater reduction in the A Mg^{2+} of 2 mmHg, in comparison to 0.7 mmHg with the Chr Mg^{2+} treatment. In addition, both resting and post DBP showed reductions with a greater day-to-day DBP reduction in the A Mg^{2+} in comparison to Chr Mg^{2+} as shown by a 69.2% and 50% (9 mmHg and 3 mmHg difference) at rest and post exercise for A and Chr groups respectively. These findings are in agreement with previous research [13,30] showing the importance of Mg^{2+} and its influence on blood pressure regulation. This is supported by findings within a recent meta-analysis [19] looking at Mg^{2+} supplementation which showed that SBP and DBP reductions of 2–3 mmHg and 3–4 mmHg, respectively. These observations oppose some previous findings which emphasise supplemental ineffectiveness of Mg^{2+} [31-33].

Such reductions in blood pressure could be speculated as being an outcome influenced by increases within the extracellular concentration of Mg^{2+}, an effect that has been associated with reductions in the arterial tension and tone. These reductions in arterial tension and tone corres-pond to typical Mg^{2+} induced vasodilatory actions which potentiate effects of endogenous vasodilators such as adenosine, K^+, nitric oxide and cyclo-oxygenase-dependent mechanisms via production of PGI2 [34]. In combination, Mg^{2+} acts as an antagonist to blocking Ca^{2+} channels

Table 5 Acute and chronic group mean Aix values post bench press at 80% of 1-RM to fatigue on day 1 and 2 ± SD

Physiological variable	Treatment	Group			
		Chr		A	
Post Aix day 1 (%)	Baseline	7	± 11	17	± 5*[3]
	Placebo	9	± 6	14	± 6*[4]
	Mg2+	7	± 5	10	± 5*[3] *[4]
Post Aix day 2 (%)	Baseline	14 ± 7*[1]		12	± 6
	Placebo	14 ± 8*[2]		16	± 4
	Mg2+	8	± 12*[1, 2]	11	± 6

*Denotes significance as paired by numbers.

[11,35,36] and further enzymatic mobilisation of Ca^{2+} 3. Thus, data within the current study concur with previous research on the efficacy of Mg^{2+} supplementation in reducing blood pressure [13,38] and its capacity to suppress agonist vasoconstriction [4]. The above mechanisms may also be attributed to Mg^{2+} induced specific alter-ations within the vasculature, for example, Mg^{2+}'s medi-atory role within the endothelium corresponds to increased nitric oxide, PGI2 and decreases platelet aggregation, in combination to stringent down-regulation of Ca^{2+} voltage operated channel activity and release from the sarcoplasmic reticulum [39].

Average dietary Mg^{2+} intakes within the A and Chr groups corresponded to 368 mg/d and 375 mg/d, respectively. However, it must be considered that the blood pressure reduction in Chr and A loading strategies, may be attributed to the Mg^{2+} supplementation. With this in mind, it could be suggested that despite average dietary intakes of Mg^{2+} meeting the UK RNI a higher requirement for Mg^{2+} may be beneficial in reducing blood pressure. Further recommendations within the U.S are 420 mg/d and 320 mg/d for males, and females, respectively, in addition to Mg^{2+} requirements within the UK being determined many years ago [40]; Research by Geleijnse et al. [41] in a comparative study between 5 European countries which included the UK, corroborates with this study suggesting a potential increase of Mg^{2+} based on supplemental blood pressure enhancements, whereby the researchers highlighted a <350 mg/d of Mg^{2+} as suboptimal, augmenting the prevalence of hypertension. The study further accounted for an 80% insufficiency corresponding to Mg^{2+} intake to be evident within the UK population analysed [41].

A principle limitation within the current study concerns lack of monitoring of the subjects' Mg^{2+} status via serum concentrations therefore this research is limited to infer indirect associations between Mg^{2+} supplementation and performance from dietary intake determined from food diaries. The study duration and the nature of a consecutive 2 day protocol both consisting of a 40 Km time trial can be seen as to limit the potential for subject recruitment and therefore final number of participants recruited. The use of males and females within groups must also be noted to account for occasional group data variance, on various parameters and a high level of standard deviation.

Conclusion

The current study showed a positive effect with A Mg^{2+} supplementation in relation to net strength and force gains with bench press, findings that support previous research [11,22,23,25]. Further, cardiovascular responses to the bench press were significantly enhanced by Mg^{2+} supplementation reducing resting SBP and DBP with the greatest effect seen with A Mg^{2+} supplementation for rest and post exercise. Similarly, SBP, DBP and Aix showed a significantly greater and more consistent reduction in response to the A Mg^{2+} loading strategy, as opposed to the minimalistic effect induced by Chr Mg^{2+} loading strategy.

In conclusion, it can be stated that improvements seen with the A loading strategy cannot to the same extent be observed with the Chr loading of Mg^{2+}, thus potentially suggesting a regulatory effect within the body influenced by the duration of Mg^{2+} supplementation intake.

To conclude, from this study there appears to be no benefit in long term magnesium supplementation for those who have adequate dietary intake, but there are some benefits for taking an acute dose, particularly before intense exercise.

Future work may focus on the above parameters for those with low dietary Mg^{2+} intake and also for the optimum time that supplementation should be given to induce these positive findings.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
LK conceived of the study, participated in its design and coordination, statistical analysis and writing of the manuscript. FP carried out the data collection, statistical analysis and writing of the manuscript. Both authors read and approved the final manuscript.

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Low Dietary Magnesium Intake and Hypertension

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Abstract

Purpose: Magnesium (Mg) is a key factor in blood pressure regulation. However, only in recent years, magnesium dietary intake has been studied in relation to hyper-tension, with equivocal conclusions. Further no comparisons have previously been made between the UK general population and primary hypertensives, the UK RNI and the USARDA. **Methods:** Twenty-five hypertensives (HT) (mean age 63.4 y) and twenty-one normotensives (mean age 46.7 y) were recruited from the same geo-graphical area. Food diaries were completed and analysed to determine average daily Mg intake. Mg intake was compared between the observed group (OB), normotensives (NT) and general population (GP) and both the UK RNI and the USA RDA. **Results:** Study participants had a significantly lower dietary Mg intake than the UK RNI ($p < 0.05$) and the US RDA ($p < 0.05$). Intake for HT males was significantly lower ($p < 0.0001$) than the external control (general population) and, for HT females, intake was significantly lower than the NT ($p = 0.006$). The findings also suggest that with ageing there is a reduction in daily dietary Mg intake. Finally, when UK external controls were compared to the USA RDA for both males and females they were found to be around 35% and 30% respectively below the recommended values. **Conclusions:** Daily Mg intake in hypertensives is lower than the general population, the UK RNI and the USA RDA. Daily magnesium intake reduces with age. These findings suggest that low Mg dietary intake increases the risk of hypertension.

Keywords

Magnesium, Hypertension, UK RNI, USA RDA, Blood Pressure, Dietary Intake

1. Introduction

Increasing evidence suggests a key role for magnesium in the regulation of blood pressure [1] [2] and a deficiency of it has been demonstrated to contribute to a number of disease states such as type 2 diabetes, metabolic syndrome, elevated C reactive protein

and hypertension [3]. Observational studies have shown an inverse association between dietary magnesium intake and blood pressure (BP) [2] [4] [5]; however, a number of meta analyses examining magnesium supplementation have produced conflicting results [1] [6] [7] [8] [9] [10]. Experimental studies have suggested that a deficiency in magnesium may have detrimental effects on blood pressure and the cardiovascular system, and previous studies have examined the effect of magnesium supplementation on hypertension [11] [12] [13] yet there are limited studies that look at long term dietary intake and association with HT. A study by Chidambaram (2014) [14] looked at the Indian population and divided participants into normotensive and hypertensive groups and found that a reduction in Mg consumption was associated with increased blood pressure. However, this study looked only at urinary biomarkers and not food intake. Choi and Bae (2015) [15] investigated Mg intake in Koreans. The participants were grouped into quartiles of magnesium intake and BP assessed. Women were found to have a negative relationship between magnesium intake and HBP. However, this study did not include those on HT tablets or those with previously diagnosed HT. This cur-rent study aims to look at the association between habitual dietary Mg intake and those with existing HT.

Magnesium is the fourth most abundant cation in the body, involved in more than 300 enzymatic systems [16]. Total body stores vary between 21 - 28 g in the average 70 kg adult with most of the magnesium being stored in the bone mass, the remainder is found in soft tissue with only 0.3% in extra-cellular stores [16], this makes magnesium measurement difficult and is the reason why dietary magnesium intake is often the best measure of hypomagnesia.

Elevated blood of Mg can cause vasodilation and reduce vasoconstriction of the vas-culature in common with other minerals such as Ca^{2+}, a key contributor to vascular smooth muscle constriction [17] [18]. Magnesium exhibits a pharmacological profile comparable to a synthetic calcium channel antagonist; therefore, it has been suggested to be nature's "physiological calcium blocker" [19].

Few intervention studies have examined the effect of habitual dietary magnesium in-take on blood pressure. Furthermore, there have been no assessments of habitual dieta-ry magnesium intake in those clinically diagnosed as hypertensive. Therefore, the aim of this investigation is to determine if there is an association between habitual dietary magnesium intake and hypertension.

A study by Ohira *et al*., (2009) [20] attempted to determine whether there was a cor-relation between dietary magnesium intake and serum magnesium concentrations in relation to CVD risk. They found that individuals with both dietary and serum mag-nesium greater than the median had an approximately 35% lower risk of ischemic stroke.

The Honolulu heart study undertaken by Joffres, Reed and Yano, (1987) [21] demonstrated an inverse relationship between dietary magnesium supplementation and both systolic (SBP) and diastolic blood pressure (DBP). When dietary Mg without addi-tional supplementation was studied, results were similar although the link with DBP

was reduced (r = 0.6). In contrast, a cross-sectional study using data from the National Health and Nutrition Examination Survey (NHANES) [22] reported no significant correlation between magnesium and blood pressure but a limitation of the study was the fact it did not allow for directional or causal influences. The absence of firm consensus despite a demonstrated mechanistic link between the blood pressure reducing action of Mg suggests that dietary Mg intake is worthy of further investigation.

The current RNI in the UK is 300mg and 270mg daily for males and females respectively from 18 - 75+ years [23]. For the USA the Recommended Dietary Allowance (RDA) is 400 mg and 310 mg for males and females respectively from ages 19 - 30 years and 420 - 320 mg daily for those aged >31 years old [24]. The National Diet and Nutrition Survey [23] states that the average consumption of the RNI in the UK is at 90% for males and 82% for females, representing a population consuming less than recommended. This study therefore sets out to determine whether those with primary hyper-tension habitually eat a diet that is both lower than the RNI and lower than the UK national average for age and sex. Further consideration will be given to how this compares to the USA RDA with a substantially higher recommended intake. As the UK national average intake is already lower than the RNI these will be compared to the higher RDA used in the USA to observe the greater difference between intake in the UK and recommendation in the USA.

According to Long Term Health Conditions [25] 1 in 4 adults is affected by high blood pressure in the UK. Figures show that by reducing the blood pressure of the nation as a whole, £850 million of NHS and social care costs could be avoided over 10 years. Further, if 15% more people currently being treated for high blood pressure could control it better a further £120 million could be saved [25]. To date there have been no studies investigating the dietary intake of those with hypertension with regard to magnesium intake from habitual dietary intake.

2. Materials and Methods

Twenty-five participants (female n = 11, male n = 14, mean age 63.4 ± 10.2 y) were re-cruited from the East and North Herts NHS Trust. Ethical approval was granted by North and East Herts Local Research Ethics Committee. Individuals already diagnosed with primary hypertension (and not hypertension caused by either other medical con-ditions or drug intervention) were identified by a Consultant Cardiologist and were re-cruited as subjects. Written informed consent was obtained during their appointments with the cardiologist. Thirty-three subjects were recruited with 8 excluded for non-completion.

A further 21 normotensive participants (female n = 11, male n = 10, mean age 46.7 ± 10.6y) were recruited from the same geographical area to act as internal controls (NT). Participants were excluded if they suffered from hypertension (BP ≥ 140/90) or were being medicated for the condition, a pre-study blood pressure recording was undertaken to confirm this. All participants completed a 4-day food diary in week 1 including 3 mid-week days and 1 weekend day, this was repeated in week 4. The food diary was

adapted from The Medical Research Council Collaborative Centre for Human Nutri-tion Research, National Diet and Nutrition Survey [26]. Although classified as an esti-mated food diary, food could be weighed and food labels submitted, in addition to photos being provided to establish portion size. All food diaries were recorded between January and March. An external control of the general UK population (GP) aged 19y and over was established from data provided by the National Diet and Nutrition Survey (NDNS) [23]. The two control groups were measured against each other to ensure ho-mogeneity between groups to validate the food diary analysis and no statistically signif-icant difference was found between the external observed and internal control groups for both the males ($p = 0.6$) and females ($p = 0.12$), except for the fact that the internal control group's mean intake of Mg was more than the RNI (See **Table 1**).

All diaries were analysed using Dietplan 6 (Forestfield software Ltd. West Sussex UK). Data were analysed for skewness and kurtosis and one way ANOVA used for in-terpretation of differences using SPSS (Version 22, IBM New York, USA).

Average daily magnesium intake established from the 8 day food diaries were com-pared between the HT, NT and GP groups and these were compared to both the UK RNI and the USA RDA to establish differences amongst the hypertensives and the re-commendations.

The magnitude of the differences between observed and control groups were ex-pressed as the effect size using Cohen's *d*, calculated as the absolute difference between means divided by the pooled *SD* [27]. Qualitative descriptors of the effect size were as follows: negligible ($d < 0.19$), small ($d = 0.20 - 0.49$), moderate ($d = 0.50 - 0.79$), or large ($d > 0.8$).

3. Results

Mean Mg intake of the hypertensive males (HT) was compared to that of the normo-tensive male controls (NT) and the general UK population (GP) (90% of the RNI) as well as the RNI (**Figure 1**). A significant difference in dietary magnesium intake was seen between the HT group and the GP group ($p < 0.0001$) and the RNI ($p < 0.0001$). The observed group did not show a significant difference in intake compared to the internal control group, although a trend could be seen ($p = 0.14$) with a moderate effect size of 0.6 shown between the groups.

Mean Mg intake of the hypertensive females was compared against that of normo-tensive females, the general population and the RNI (**Figure 2**). The HT group had a

Table 1. Mean Mg intakes (mean ± SD) for all participants (HT + NT) stratified by age group (n = number of participants).

Age range	All Mg (mg/d)
19 - 50y	244 ± 65 (n = 17)
51 - 64y	259 ± 79 (n = 16)
65 + y	200 ± 45 (n = 14)
All	236 ± 69 (n = 47)

significantly lower mean dietary Mg intake than the RNI (p = 0.006) and the NT group (p = 0.006). However, there was no significant difference between the HT group and the GP (p = 0.264). An effect size of 0.4 was found between the HT group and the GP control corroborating with the lack of significance between these two groups.

When total data (hypertensive and normotensive together) for both males and fe-males were analysed, a one way ANOVA showed that there was a significant difference between the age groups (p = 0.042) (**Table 1**).

In females there is a reduction of over 100 mg/day between all participants (both HT and NT) aged 51 - 64 y and those aged 65 y and above giving a 37% reduction in intake.

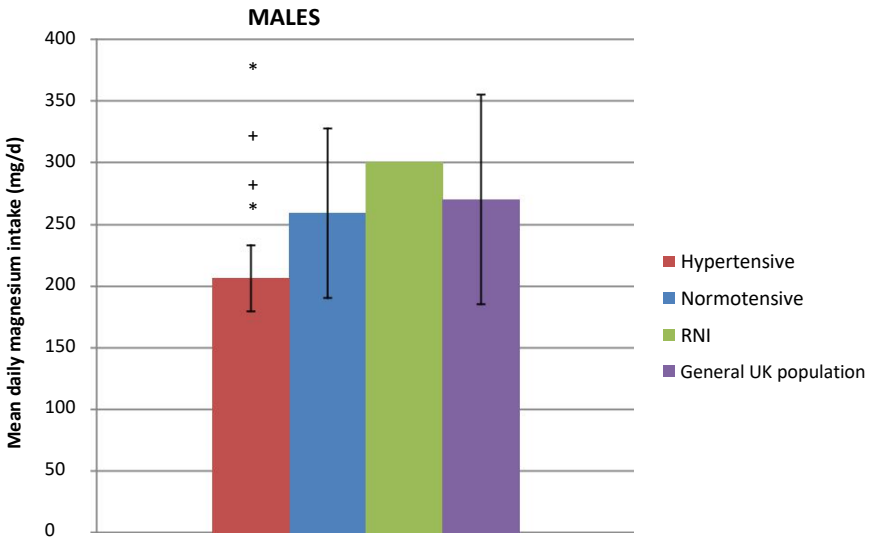

Figure 1. Mean +/− SD male daily magnesium intake comparing the hypertensive, normo-tensive, general UK population and the RNI (+denotes significant difference between ob-served and RNI, *denotes significant difference between HT and the GP with p ≤ 0.05).

FEMALES

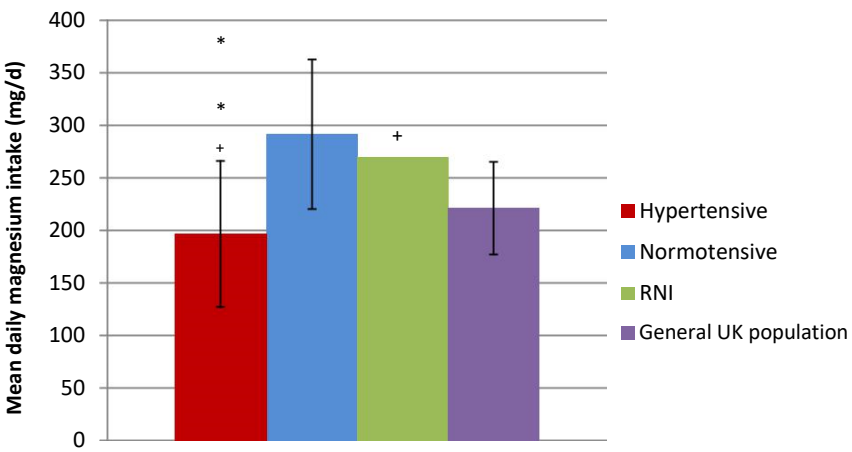

Figure 2. Mean (± SD) female daily magnesium intake comparing the observed, internal con-trol, external control groups with the RNI, (+denotes significant difference between observed and RNI, *denotes significant difference between HT and NT (p ≤ 0.05).

For males the greatest difference was 31 mg between the 18 - 50 yr and 65+ yrs population groups (**Figure 3**).

Male UK data for both the HT and NT was compared against the USA RDA (**Figure 4**). A significant difference in mean daily magnesium intake was shown when com-pared to the USA RDA (420 mg) for both groups ($p \leq 0.001$).

In females, there was a significant difference between the mean daily Mg intake of the hypertensive group and the USA RDA ($p \leq 0.001$); however, the normotensives did not show a significant difference to the USA recommendations ($p = 0.238$) (**Figure 5**).

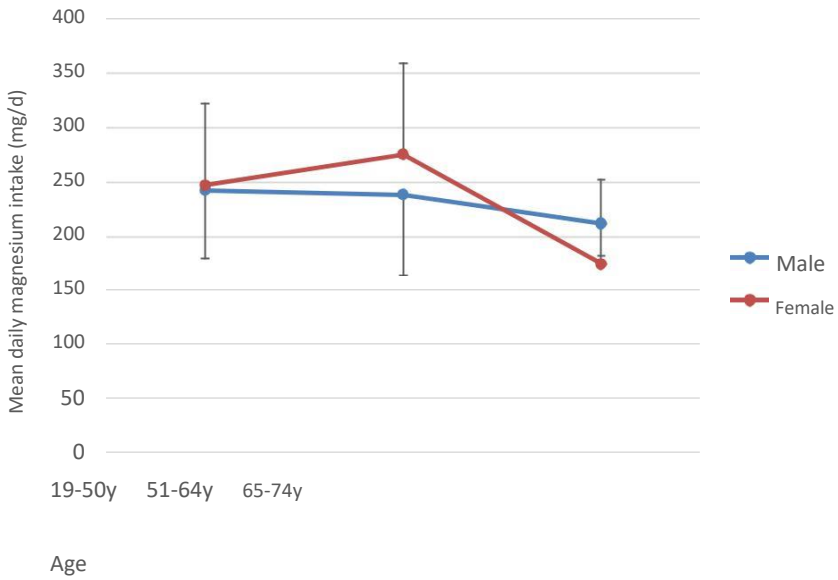

Figure 3. Mean daily dietary Mg intake for both males and females, (HT + NT) decreases with age. Error bars showing SEM.

MALES

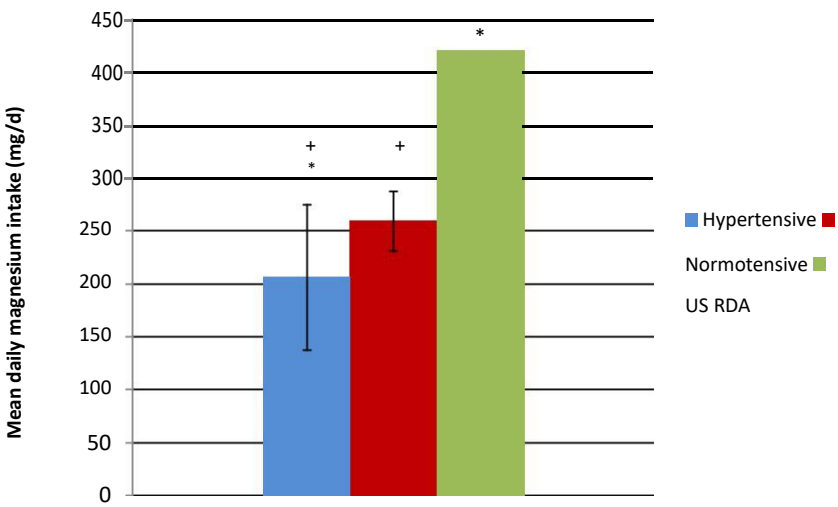

Figure 4. Male mean daily magnesium intakes (mean ± SD) for HT and NT groups com-pared with the USA RDA (+denotes significant difference between HT and USA RDA, *denotes significant difference between HT and NT of $p \leq 0.05$.

FEMALES

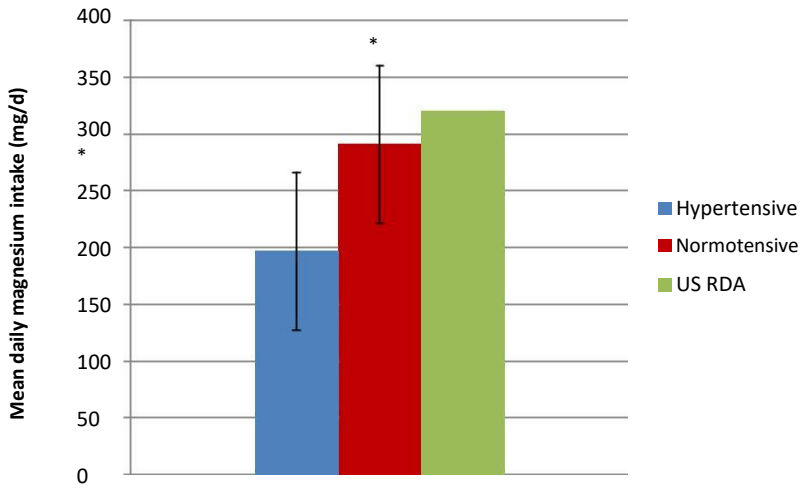

Figure 5. Female mean daily magnesium intake (mean ± SD) for HT and NT against USA RDA (*denotes significance of $p \leq 0.05$. This can be seen between the HT and the USA RDA).

The UK general adult population's mean daily Mg intakes of 270 mg and 221.4 mg (90% and 82% of the RNI) for males and females respectively, represented 64% and 69% of the USA RDA.

4. Discussion

The primary finding from the current study is that hypertensive females appear to have a significantly lower intake of dietary magnesium than normotensive females. Both male and female hypertensives appear to have low dietary Mg intake when compared to both the RNI and RDA.

Analysis based on individual sexes elicited different results with observed males de-monstrating a significantly lower daily dietary magnesium intake compared to both the GP and the RNI. The daily intake of the HT group was 94 mg/day less than the RNI and 54 mg/day less than the general population, considered to be a healthy population group. The normotensives demonstrated a trend (as evidenced by a moderate effect size of 0.8) towards a difference in daily intake although this was 11 mg/day less than the general population and therefore significance was expected. The large standard deviation may have affected the statistical results but in real terms both the NT and GP had a similar intake in males.

Females showed a significant difference between both the NT and the RNI although the GP did not show a significant difference compared with the HT group. The GP was only 82% of the RNI, a low value which was also seen in the HT group's daily intake. However, female NT's mean daily intake of magnesium was above the RNI, the only group to have met this recommendation. As the NT groups are considered normotensive, it can be suggested that low magnesium intake (82% of the RNI), does not have a negative effect on blood pressure; however, the reduction of a further 24 mg/day as

found in the HT group may be important in explaining hypertension. It would be of interest to see if the GP group developed hypertension over time due to their low mag-nesium intake and thought must also be given to the previously mentioned fact that 25% of the general population in the UK is thought to be hypertensive [25].

In this study, the difference between Mg intakes of the HT and NT groups was small, however, the GP is already below the RNI suggesting that when this further decrease in Mg intake is seen the long term consequence on BP could be even greater.

When mean male intake of 206 mg for the HT group across all ages is compared to the RNI the hypertensive patients have a significantly lower intake than recommended. Furthermore, the NDNS [26] states that generally males are at approximately 90% of the RNI, The NT group of healthy individuals were actually at 96%, supporting these findings. Although both the HT and the NT group were below the RNI, NDNS [26] da-ta used for the GP group showed that only 5% and 7% of the cohort (male and female respectively) had hypertension between the ages 19 - 64 y, increasing to 36% and 33% for the 65 y+ group. This results in mean hypertension of 21 and 20% for males and females in total, slightly below the established norm according to Public Health Eng-land [25]. This group (the GP) was at 90% of the RNI but below the expected norm for prevalence of hypertension. It can be suggested that Mg intake at 90% of the RNI has a small effect on blood pressure reinforcing previously published work showing a re-duced magnesium intake is correlated with increased blood pressure [6] [28] However, the HT male group consumed just 69% of the RNI implying that a lower intake may have an effect on blood pressure.

For females the HT group's mean daily Mg intake was at 196.6 mg/day equating to 72% of the RNI, while the NT group was above the RNI. Again this may suggest that a daily magnesium intake at 72% of the RNI may negatively affect blood pressure in adult women.

The NT group of healthy individuals for the North and East Herts NHS area were at 96% and 131% of the RNI compared to 90% and 82% for the general population show-ing that internal validity was higher in males than females. The high intake of dietary magnesium in the female NT group was unusual but the cohort was quite small and this over consumption only equated to 21 mg/day above the RNI. The Mg intake fig-ures are also means and therefore it is expected that about half of the subjects will fall above this mean value and half will fall below. However, the small female cohort and the high standard deviation could both be considered limitations of this work.

Previous research has shown that magnesium supplementation can have a positive effect on blood pressure [6] [28] [29], however the research in the UK is very limited. Bain *et al*. [29], using a UK population base found a greater association between males with low magnesium intake and risk of stroke, for which blood pressure is often a pre-cursor, than in females. However, there is very little research which has examined Mg intake in the UK with consequences for blood pressure. Furthermore, until now, there have been no studies observing the habitual dietary intake of those with high blood pressure in the UK. Choi and Bae (2015) [15] found that there were a higher percentage

of hypertensive males across all dietary magnesium intakes compared to females in the Korean population and Itoh (1997) [30] found a blood pressure decrease in 33 healthy Japanese who supplemented with magnesium. However Dickinson *et al*. (2009) [9] writing for the Cochrane Review used extensively for recommendations to the medical profession in the UK, did not find strong evidence for Mg reducing blood pressure. This study hopes to address this matter and move towards showing an association be-tween those with hypertension and habitual dietary intake low in magnesium.

In the USA the RDA, set at 320 and 420 mg/d for females and males respectively, is higher than the RNI of the UK. As a comparison, the data from this UK study was also compared with the USA RDA and the results showed a significantly lower intake than recommended by the USA for all population groups. The UK general population's mean intake of 270 mg and 221.4 mg (90% and 82% of the RNI) for males and females respectively, represented only 64% and 69% of the USA RDA.

Previous research has shown that 48% of the general population in the USA was be-low the RDA in 2005-2006, with rising calcium to magnesium ratios implicated in the rise of diabetes and stroke over the past 20 years [3]. Touyz [31], stated that magnesium may reduce arterial thickening and abnormal vascular tone as well as endothelial dys-function linked with cardiovascular disease and high blood pressure. Therefore, a magnesium deficiency may play a role in the pathophysiological processes underlying blood pressure elevation.

With the exception of females in the 51 - 65 y age group, all age groups consumed Mg below the RNI. The lowest intake could be seen in the >65 y age groups. In this age group, blood pressure increases become more prevalent due to decreases in arterial compliance and changes in the endothelial cells. Consideration should be given as to whether supplementation of this micronutrient might help reduce blood pressure in-creases normally seen in the older population group.

Although the USA has set the RDA at a higher level than the UK, the real issue lies with dietary intake and not with the recommendations. Initiatives to increase know-ledge and awareness of this micronutrient may help to reduce blood pressure in the UK, subsequently saving the NHS money on costly drug intervention. Although no conclusions can be drawn on the mechanistic role of magnesium in prevention of hy-pertension, the current study does provide evidence that further investigation is war-ranted on the role of dietary magnesium intake in this area.

Acknowledgements

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Ethical Approval

It was granted through the NHS Research Authority, NRES Committee South Central-Southampton A and the investigation was therefore performed in accordance with the

ethical standards laid down in the 1964 Declaration of Helsinki and its later amend-ments.

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3.6 Kass, L, Rosanoff, A, Tanner, A, Sullivan, K, McAuley, W., A pilot study on the effect of transdermal magnesium cream in humans on serum and urinary magnesium levels. Plos One (2017)

PLOS ONE

RESEARCH ARTICLE

Effect of transdermal magnesium cream on serum and urinary magnesium levels in humans: A pilot study

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Competing interests: In the future, Dr Andrea Rosanoff may receive royalties on a magnesium cream for which she has signed an agreement. The cream in which she will have an interest is different than the one used in this pilot study as the

Abstract

Background

Oral magnesium supplementation is commonly used to support a low magnesium diet. This investigation set out to determine whether magnesium in a cream could be absorbed trans-dermally in humans to improve magnesium status.

Methods and findings

In this single blind, parallel designed pilot study, n = 25 participants (aged 34.3+/-14.8y, height 171.5+/-11cm, weight 75.9 +/-14 Kg) were randomly assigned to either a 56mg/day magnesium cream or placebo cream group for two weeks. Magnesium serum and 24hour urinary excretion were measured at baseline and at 14 days intervention. Food diaries were recorded for 8 days during this period. Mg test and placebo groups' serum and urinary Mg did not differ at baseline. After the Mg^{2+} cream intervention there was a clinically relevant increase in serum magnesium (0.82 to 0.89 mmol/l,p = 0.29) that was not seen in the placebo group (0.77 to 0.79 mmol/L), but was only statistically significant (p = 0.02)) in a subgroup of non-athletes. Magnesium urinary excretion increased from baseline slightly in the Mg^{2+} group but with no statistical significance (p = 0.48). The Mg^{2+} group showed an 8.54% increase in serum Mg^{2+} and a 9.1% increase in urinary Mg^{2+} while these figures for the placebo group were smaller, i.e. +2.6% for serum Mg^{2+} and -32% for urinary Mg^{2+}. In the placebo group, both serum and urine concentrations showed no statistically significant change after the application of the placebo cream.

Conclusion

No previous studies have looked at transdermal absorbency of Mg^{2+} in human subjects. In this pilot study, transdermal delivery of 56 mg Mg/day (a low dose compared with commercial transdermal Mg^{2+} products available) showed a larger percentage rise in both serum and urinary markers from pre to post intervention compared with subjects using the placebo

magnesium concentration is more than double that used in the pilot study and it is a different formulation. The name of the cream is Natural Calm Cream. The U.S. Patent title is: Cream Transdermal Magnesium Supplement. Publication No. US-2016-0317576-A1. Publication Date: 11/ 03/2016. This does not alter our adherence to all the PLOS ONE policies on sharing data and materials.

cream, but statistical significance was achieved only for serum Mg^{2+} in a subgroup of non-athletes. Future studies should look at higher dosage of magnesium cream for longer durations.

Trial registration

ISRCTN registry ID No. ISRTN15136969

Introduction

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[1]). Mg^{2+} is an important mineral element and it is the fourth most abundant cation in living organisms, with Mg^{2+} being a cofactor to over 325 enzymatic reactions within the body [2]. Around 99% of total body Mg^{2+} is located in the bone, muscles and non-muscular soft tissue [3]. Mg^{2+} supplementation has been shown to significantly improve blood pressure [4±6] as well as modifying vascular tone by regulating endothelium and smooth muscle cell function [4].

To maintain an adequate Mg^{2+} status humans must consume Mg^{2+} at regular intervals [7]. The daily recommendation for Mg^{2+} is controversial, as the literature is conflicting and varies between countries, although values of 300 mg/day are usually reported for healthy adults with adjustment for age, sex and nutritional status [7].

Oral magnesium supplementation has been shown to affect various parameters, such as blood pressure [5,8], immune function [9], cardiovascular system [10] and metabolic syndrome [11]. A recent meta-analysis by Zhang et al (2016) in the Journal of Nutrition [12] found that in a healthy population group there was a dose dependent increase in both serum Mg concentration and urinary Mg excretion with supplemental oral magnesium intake ranging from 250±500mg/day. Kass, Weekes and Carpenter (2012) identified that a supplement of >370 mg/day of Mg^{+} shows greater efficacy than a lower dose in improving blood pressure and that magnesium supplementation gives a dose dependent response with regards to blood pressure [5].

An alternative method of perhaps attaining recommended magnesium intakes might be through topical application. Current formulations include magnesium oils and trans-dermal creams, from which the magnesium may be absorbed across the skin and into the systemic circulation. However, in contrast to gastrointestinal epithelium, a primary function of the skin is to act as a barrier, which restricts the absorption of exogenous chemicals into the body. The barrier function of the skin is thought to lie predominately in the outermost layer of the epi-dermis, the stratum corneum. The stratum corneum is thought to largely present a hydropho-bic barrier to the absorption of transdermal creams. The dermis, immediately below the epidermis, contains the blood vessels that are able to transport substances that have permeated the skin into the systemic circulation.

Although less studied than organic molecules, metal ions are known to be able to cross the skin, with the literature having focussed on metals that are known to cause irritant/toxic effects [13]. The lower resistance to permeation of the skin appendages, skin structures that serve a particular function including sensation, lubrication and heat loss, and the ionised nature of metals means that their permeation across these skin appendages is considered to be the most likely route of absorption [13,14]. However, the low surface area available for this in human skin means that metal ion absorption across skin is expected to be relatively low. Therefore, it

has been questioned that a transdermal route of administration might provide sufficient Mg^{2+} absorption to help meet systemic Mg^{2+} requirements. This investigation aims to determine if transdermal absorption of Mg^{2+} from a topical cream occurs ªin vivoº in humans. To date, no study has investigated the absorbency of transdermal magnesium cream in human subjects. Commercially available Mg^{2+} topical applications range from 75mg to 400mg depending on the dosage recommended by manufacturers. This ranges from 5±30 sprays of magnesium oil or 2±4 teaspoons of magnesium cream, which can be applied in one application or throughout the day. Disappointingly, many commercial topical creams and oils do not state the concentration of magnesium in the product.

This study was designed as a first time, pilot study to ascertain whether such a topical Mg^{2+} preparation might affect urinary or serum Mg^{2+}. Since less than 1% of magnesium is contained in the blood, assessment by serum status may be problematic [3]. It is often considered that 24-hr urine excretion of Mg^{2+} may be a better indicator of intestinal absorption than serum concentra-tion; however, urinary Mg^{2+} excretion is also highly variable and it is questionable whether it can be used reliably to assess an individual's Mg^{2+} status [15]. However, serum Mg^{2+} can reflect a lon-ger term dietary Mg^{2+} status over weeks or months whilst urinary Mg^{2+} can be a better marker of one's recent dietary intake [16]. These studies using serum and urinary Mg^{2+} markers in dietary Mg^{2+} research could not be assumed to be helpful in the design of this study. Djurhuus et al,[17], however, reported that although it is unlikely that a single determination of serum Mg^{2+} can be used in assessing whole-body Mg^{2+} status in an individual, serial determinations of serum Mg^{2+} might prove useful as an indicator of changes in whole body Mg^{2+} status. These authors also found that 24-hr urinary Mg^{2+} excretion is unlikely to be a reliable measure of whole body Mg^{2+} status and is not a good marker to measure changes in whole body Mg^{2+} status. Nonetheless, we decided to use 24-hr urinary Mg^{2+} as well as serum Mg^{2+} in this pilot study.

Therefore, the purpose of this pilot study was to investigate whether a 56 mg/day dose of magnesium in a cream, applied transdermally to humans, would affect either serum magne-sium levels or urinary excretion over a two-week period and to measure effect, if any, and vari-ance to inform a properly powered future study, if warranted.

Methods

Recruitment

Subject recruitment started April 2014 and data collection and follow-up was completed by February 2015. The study was not registered on a CT Database at the time of subject recruitment as trial registration in these kinds of studies is not commonly practiced. The trial was subsequently registered in order to comply with publication requirements according to the WHO guidelines. The authors confirm that all ongoing and related trials for this intervention are registered.

Participants

Twenty-five healthy adults (female = 13 male = 12) aged 34.3+/-14.8y, height 171.5+/-11cm, weight 75.9 +/-14 Kg, were recruited from the staff and student population of the University of Hertfordshire and word of mouth to local residents and randomly assigned into either a magnesium cream or placebo cream group by random allocation. Randomisation was determined by allocation to a group selected from a box with equal amounts of paper stating either placebo or magnesium and selected at time of recruitment. The trial was single blind with only the lead investigator being aware of the content of the cream. One participant dropped out of study before completion. Participants were excluded if they were taking magnesium supplementation in any form within a month of recruitment onto the trial, were under the age of 18y or above

the age of 60y.There were no height or weight restrictions, Written informed consent was com-pleted and ethical approval was granted by the University of Hertfordshire Health and Human Science Ethics Committee on 14th April 2014. Blood collection and blood and urine analysis was undertaken at the University of Hertfordshire Human Physiology Laboratory.

Dietary magnesium intake was below the RNI in one of the placebo participants and three of the magnesium participants. This was not considered a bar to inclusion into the study as any change in serum or urinary $Mg2^{+}$ from the cream could still be shown. High levels of physical exercise have been shown to deplete human Mg^{2+} status ([18]. Four participants were considered ªathletesº as they engaged in at least 2hrs of physical exercise at least 5 days per week during the study, three who were assigned to the Mg^{2+} intervention group and one assigned to receive placebo. All other 20 participants who completed the study were considered ªnon-athletesº, i.e. engaging in less than 2 hrs physical exercise per day for no more than 3 days per week during the study. Participants were instructed not to exercise 24h before blood draws, but ªathletesº may have disregarded this instruction, so results were statistically ana-lysed in two ways: 1. ªall subjectsº, including both athletes and non-athletes (n = 24 who completed the study) and 2. ªnon-athletesº (n = 20) which excluded the 4 athletes. Baseline serum and urine Mg^{2+} concentrations as well as dietary Mg^{2+} recorded in the randomised interven-tion and placebo groups did not differ significantly from each other (P<0.05) (Tables 1 & 2) in either the ªall subjectsº or ªnon-athletesº groupings.

Baseline measurements

Baseline data consisted of a 24 hour urinary collection to assess baseline magnesium excretion and venous bloods to assess serum magnesium levels. (Tables 1 and 2). Urine was collected from the second urinary passing of the day until and including the first urinary passing of the next day. Blood collection could take place at any time of the day to suit the participants but each participant had to return at that same timeslot for the second blood collection. No food was allowed for the 3 hours before blood collection although water was allowed.

Dietary analysis

Each participant recorded a 4-day food diary (3 midweek days and 1 weekend day) prior to the intervention plus a second 4-day food diary at the end of the 12±14 day period, giving a total of 8 days dietary analysis over the period of the intervention for each participant. This was analysed for Mg^{2+} intake using Diet Plan 6 software (Forestfield Software Ltd., West Sussex, UK). (Tables 1 and 2).

Intervention

After baseline measurements were taken, participants were randomly assigned to either the Mg^{2+} Cream or a placebo control cream and were instructed to apply 2 x 5ml spoonfuls of cream per day for two weeks. The resulting daily Mg^{2+} dose received by subjects in the Mg^{2+}

Table 1. Demographic and baseline serum, urine and dietary magnesium (mean +/- s.d. [range]) for all subjects, athletes plus non-athletes.

	Magnesium group N = 14	Placebo group N = 10
Age (years)	34.8±15.3	36.6±14.6
Height (cm)	171.2±8.9	173.8±12.4
Weight Kg)	77.5±15.9	75.6±11.5
Serum (mmo/l)	0.82±0.18 [0.62±1.24]	0.773±0.16 [0.56±1.16]
Urine (mmol/24h)	4.07±1.62 [1.30±7.00]	4.6±2.1 [2.7±9.0]
Dietary 9Mg)	294.9±93.8 [176±496]	329.1±91.9 [196±482]

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Table 2. Demographic and baseline serum, urine and dietary magnesium (mean +/- s.d. [range]) for non-athletes only.

	Magnesium group N = 11	Placebo group N = 9
Age (years)	34.6±13.7	38.5±14.6
Height (cm)	169.7±9.4	175.0±12.5
Weight (Kg)	75.6±17.1	75.8±12.1
Serum (mmol/l)	0.75±0.13 [0.62±1.07]	0.73±0.09 [0.56±0.85]
Urine (mnol/24h)	4.08±1.34 [2.02±6.11]	4.80±2.1 [2.9±9.0]
Dietary (mg)	285.8±82.2 [177±469]	331.0±97.3 [196±482]

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group consisted of 56mg of Mg^{2+}. This was manufactured, in the course of research and devel-opment, for the Center for Magnesium Education & Research by Urist Cosmetics of Vancou-ver, B.C. Canada. For full ingredients see S1 Text Ingredients list.

The placebo was a commercially available aqueous cream containing no magnesium (by analysis) and creams were packaged identically. Instructions to participants suggested that Mg^{2+} or placebo cream be applied to the stomach and torso in the first instance and then spread down to the legs. Time of day was not important, but no showering or washing could take place for a minimum of 3 hours after application. After 12±14 days, final urine and blood samples were collected. The cream was applied up to and including the day of the final urine and blood collection. Participants were instructed to stop use of the cream if there was any adverse reaction, of which there were none. At the end of the trial compliance was ensured from verbal communication as well of collection of the trans-dermal cream container to ensure full usage.

Sample collection

Serum blood samples were collected by venepuncture from the median cubital, basillic or cephalic vein. Serum separator vacutainers were inverted 10 times before being left to rest for 30 minutes. Subsequently, samples were centrifuged at 3000 rpm for 10 minutes. Serum was checked to be free from haemolysis and was immediately pipetted and frozen at -80ÊC for sub-sequent analysis

Urine was collected into 3 litre collection vessels over 24 hours. Urine was then decanted into a measuring vessel and volume of urine was recorded. The measuring vessel was then placed on a magnetic stirrer at 100rpm. To re-suspend the Mg^{2+}, the pH was lowered to 3±3.25 by adding 5 M hydrochloric acid. Duplicate 1mL aliquots were frozen at -80ÊC for batch analysis.

Analyses

Urine and serum samples were analysed by colorimetric assay for magnesium (RX MONZA, Randox Laboratories Limited, County Antrim, United Kingdom). The machine was calibrated according to the manufacturer's instructions and results were calculated from the standard concentration curve generated using the manufacturer's calibration standard. Low urinary and serum Mg^{2+} were determined to be below 3.0mmol/24h and 0.65mmol/l respectively [19].
All urine and serum samples were frozen and stored at -80ÊC for between 4±12 weeks.

Data analysis

Serum and urinary Mg^{2+} data for both pre and post intervention were analysed for skewness and normality prior to statistical analysis. All data passed the normality test so a standard two tailed, paired t-test was used to compare baseline to post-intervention values (serum and urinary Mg) for within group analysis.

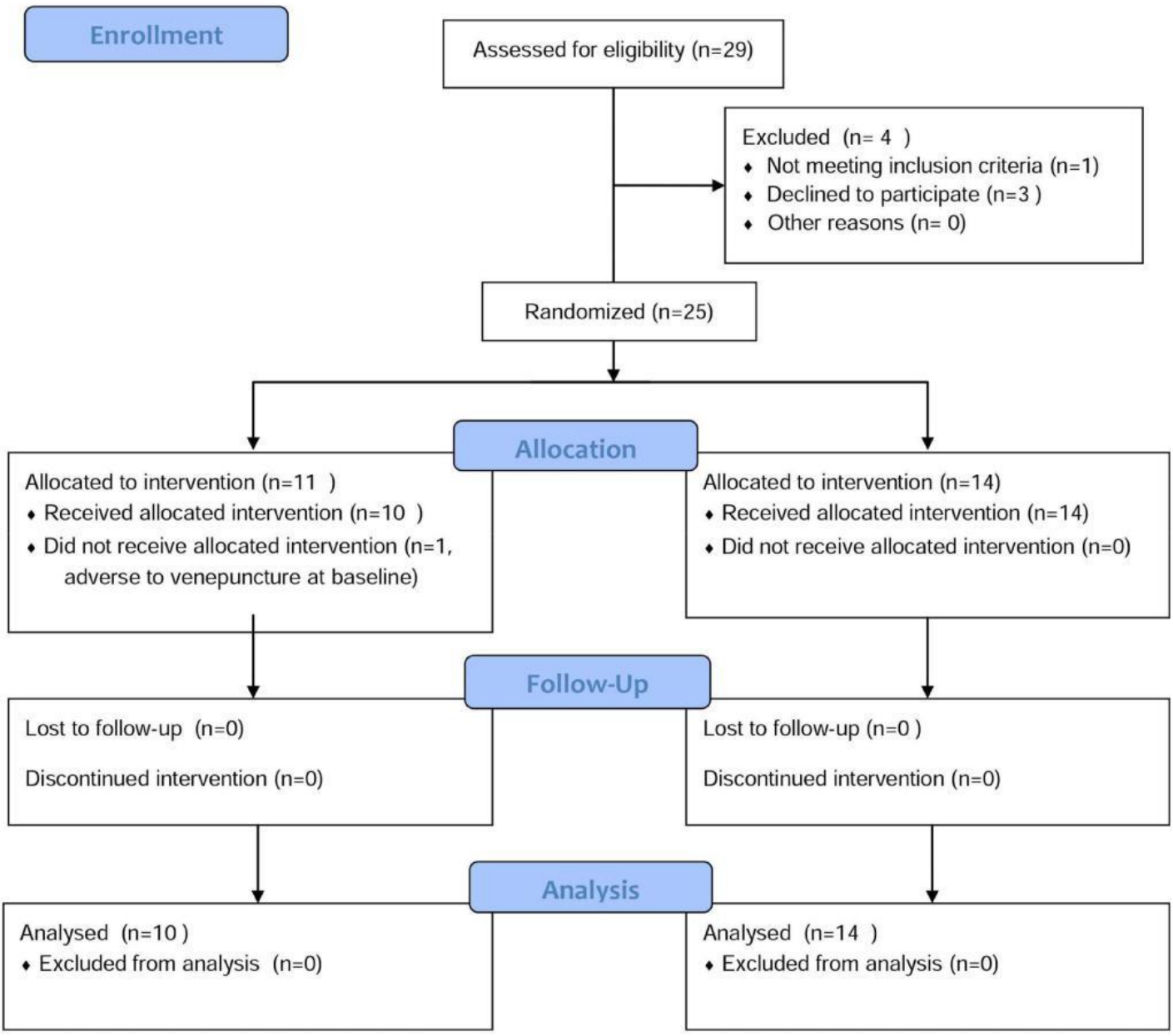

Fig 1. CONSORT flow diagram, showing inclusion and exclusion of participants in the study.

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Statistical analysis was conducted using SPSS V22 (IBM, New York, USA), with P value value of 0.05 accepted as statistically significant.

Results

Of the 29 subjects recruited, 1 was excluded for not meeting the criteria and 3 declined to par-ticipate after baseline data was collected. Fourteen participants were allocated to the magne-sium intervention group and completed the trial. Eleven were allocated to the placebo intervention from which one dropped out due to adverse reaction to the venepuncture at the

baseline blood collection before cream had been administered. Fig 1 depicts the inclusion and exclusion of the participants in the study.

Urinary and serum response to trans-dermal magnesium application

Mean serum and 24-hour urinary Mg^{2+} concentrations were obtained for all participants before and after a 2-week application period of the magnesium or placebo trans-dermal cream. Baseline (Tables 1 & 2) serum Mg^{2+} values were below normal reference values
(0.65mmol/l) in one magnesium intervention participant and one placebo participant (both non-athletes), whilst the baseline 24-hr urinary Mg^{2+} excretion was below normal (3.0mmol/ 24h) in two placebo participants (one non-athlete, one athlete) and three magnesium participants (two non-athletes, one athlete); these were different participants for both the serum and urine. Of the five participants with low urinary excretion, three had below the RNI for dietary Mg^{2+} intake however this was not the case for the low serum Mg^{2+} subjects.

All participants, Table 3

There was no statistically significant effect of Mg^{2+} cream on either serum or urinary Mg^{2+} status. However, after the Mg^{2+} cream intervention there was a clinically relevant increase [11, 19] in serum magnesium (0.82 to 0.89 mmol/l) that was not seen in the placebo group (0.77 to 0.79 mmol/L), but this was not statistically significant (p = 0.29). Similarly, there was a slight increase in magnesium urinary excretion in the Mg^{2+} group but again no statistical significance
(p = 0.48). A percentage increase of 8.54% for serum Mg^{2+} and 9.1% in urinary Mg^{2+} was seen in the Mg^{2+} group while for the placebo group these figures were smaller, i.e. +2.6% for serum Mg^{2+} and -32% for urinary Mg^{2+}. In the placebo group both serum and urine concentrations showed no statistically significant change after the application of the placebo cream.

Non-athletes, Table 4

For non-athlete participants (n = 11 in Mg group plus n = 9 in placebo group), there was a statistically significant rise (P = 0.02) in serum Mg^{2+} in the intervention group which was not seen in the placebo group. Serum Mg^{2+} in the intervention showed a % change of +22.7% while that in the placebo group showed a +4.11% rise. Urinary Mg^{2+} did not show any significant change in either Mg^{2+} or placebo group although a large non-significant negative change could be seen in the placebo group (-32.50%) when compared with the 11.3% increase in the intervention group.

Discussion

Transdermal Mg^{2+} effect on serum Mg^{2+}

Previous study of humans have shown that serial determinations of serum Mg^{2+} can prove useful as an indicator of changes in whole body Mg^{2+} status[17]. From this study, 56 mg/day

Table 3. Influence of Trans-dermal Magnesium vs placebo cream application on Serum and Urinary Magnesium Concentrations (mean +/- S.D.) for all subjects, athletes plus non-athletes.

	Serum Concentrations,	(mmol/L)	Urinary Excretion	(mmol/24h)
Intervention	**Magnesium (n = 14)**	**Placebo (n = 10)**	**Magnesium (n = 14)**	**Placebo (n = 10)**
Pre	0.82±0.18	0.77±0.16	4.07±1.62	4.6±2.1
Post	0.89±0.18	0.79±0.18	4.44±1.56	3.12±1.27
%Change	+8.54%	+2.6%	+9.1%	-32%

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Table 4. Influence of Trans-dermal Magnesium vs placebo cream application on Serum and Urinary Magnesium Concentrations (mean +/- S.D.) for non-athlete subjects.

	Serum Concentrations,	(mmol/L)	Urinary Excretion	(mmol/24h)
Intervention	**Magnesium (n = 11)**	**Placebo (n = 9)**	**Magnesium (n = 11)**	**Placebo (n = 9)**
Pre	0.75±0.13	0.73±0.09	4.08±1.34	4.80±2.10
Post	0.92±0.18*	0.76±0.17	4.54±1.75	3.24±1.28
%Change	+22.7%	+4.11%	+11.27%	-32.50%

21. P<0.02 compared with [a]pre-serum Mg^{2+} value

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Mg^{2+} applied as transdermal cream for 12±14 days had no statistically significant effect on serum concentration in this small (n = 25) human study when both athletes and non-athletes were included in the statistical analyses. However, a trend towards a rise in serum Mg^{2+} in the Mg^{2+} group could be seen with an increase of 0.07mmol/l, a clinically relevant rise in a mea-surement that is greater than many previous studies and a rise that would take months to show change with oral Mg^{2+} therapy [11, 19]. Zhang et al., [11] reported that in 41 trials, 941 partici-pants receiving a mean oral Mg supplement of 365 mg/day for a median of 12 weeks showed a mean rise of 0.05 mmol/L circulating Mg (0.78 to 0.83 mmol/L). A meta-analysis of 27 trials by Zhang et al [8] investigating oral Mg^{2+} supplementation, showed a significant ($p<0.001$) rise in serum Mg with 200 mg/day oral Mg supplement or one month supplement duration but that higher doses (300 mg/day) or durations of supplementation (2 months) were required to achieve a mean rise of 0.05 mmol/l in serum Mg^{2+}. Additionally, studies included in the Zhang et al [8] meta-analysis show a baseline mean C.V. for the 27 serum Mg^{2+} studies of 9.3% for Mg groups and 10.8% for placebo groups, i.e. as little as half of the variance for baseline serum Mg^{2+} measurements in this study (C.V. = 21.9% and 17.3% for Mg^{2+} groups, all subjects and non-athletes respectively; C.V. = 20.7% and 12.3% for placebo groups, all subjects and non-athletes respectively). This pilot study of all subjects, both athletes and non-athletes, shows that transdermal Mg^{2+} may possibly influence serum Mg^{2+} in a relatively short time frame (12±14 days), but a higher concentration of Mg^{2+} cream, a larger number of subjects given the serum Mg^{2+} variance, and perhaps a longer study is required to make any real conclusion.

Although participants were told to refrain from exercise for 24 hours before blood and uri-nary collection, 4 participants were undertaking regular high intensity training, the effects of which may affect Mg^{2+} parameters that can last longer than 24 hours [18]. When the data from these four subjects were removed from the statistical analysis a significant effect of the Mg^{2+} cream on serum Mg^{2+} concentrations could be seen.($p = 0.02$). Additionally, the %rise in serum Mg^{2+} in the non-athletes (+22.7%) was much larger than that shown in all subjects, i.e. both athletes and non-athletes (+8.54%). This is too small a sample size to reach a firm conclu-sion but this increase in percentage change in the non-athlete intervention group may be due to an additional uptake of Mg^{2+} from the cream which may have been utilised during exercise or for replenishment of Mg^{2+} stores rather than being transferred to serum in the athletic participants. This is an area of interest for further study.

Further, this analysis of the serum Mg data for the non-athletes in the intervention group showed a mean increase from 0.75 to 0.92 mmol/l which may have clinical relevance in particular with relation to cardiovascular disease. In a meta-analysis by Del Gobbo et al (2013)[20], it was found that a rise of 0.2 mmol/L circulating Mg was associated with a 30% lower risk of cardiovascular disease and fatal ischemic heart disease. In addition, Lutsey et al.(2014) [21] found that after 20+ yrs follow up serum Mg showed a linear inverse association with the risk

of incident heart failure. Relative to those in the highest category of serum Mg, those in the lowest category were at 2.58 times greater risk of incident heart failure after demographic adjustments. In these quintiles, the lowest serum Mg quintile was 0.7 mmol/L, the second was 0.75, the third was 0.8, the 4th was 0.85 and the highest was 0.9 mmol/L, i.e. each quintile was

0.05 mmol/l higher than the next lowest quintile (these results were converted from mEq/L by dividing by 2 to attain mmol/L). Our results show a mean rise of 0.05 mmol/L serum Mg^{2+} with daily application of transdermal Mg^{2+} for only two weeks, and when considering this Lut-sey[21] study and the Del Gobbo[20] results, our finding suggests a possibly significant favour-able impact of transdermal Mg on risk of heart failure that needs full study.

Transdermal Mg effect on urinary Mg^{2+}

Previous study of humans has suggested that 24-hr urinary Mg^{2+} excretion cannot be used as a measure of changes in whole body Mg^{2+} status [17].

Upon analysis for all subjects, as well as for only non-athletes, use of the transdermal Mg^{2+} cream showed no significant rise in urinary Mg^{2+}, a measurement that reflects short term intestinal absorption of Mg^{2+}. However, subjects in the Mg^{2+} group showed slight rises in uri-nary Mg^{2+} (+9 to 11%) while those in placebo group showed a substantial decrease in urinary Mg^{2+} (-32%). Possibly the decreased urinary Mg^{2+} excretion in the placebo cream group repre-sents more active physiological Mg^{2+} retention processes that are not apparent in the Mg cream group ([15,16]. It has been suggested that 24-hour urine excretion of Mg^{2+} may be a bet-ter indicator of tissue status than the serum Mg^{2+} concentration, but it is highly variable and it is questionable whether it can be used to reliably assess a given individual's Mg^{2+} status.

The trans-dermal cream contained 56 mg of Mg^{2+} administered daily. This is at the lower end of creams sold commercially. The recommended dose of the few commercially available creams range between 70mg/d to 400mg/d per day, therefore results of this study may repre-sent an ªunderdoseº of transdermal Mg^{2+}.

Conclusion

No previous studies have looked at effects of transdermal Mg^{2+} in human subjects. In this two-week pilot study, transdermal delivery of 56 mg Mg/day (a low dose compared with commer-cial transdermal Mg^{2+} products available) showed a larger percentage change in both serum and urinary markers from pre to post intervention compared with subjects using the placebo cream. In addition, the rise in mean serum Mg^{2+} seen in the Mg^{2+} group was clinically relevant although only statistically significant ($p<0.05$) when non-athletes were analysed separately.

Given the high variance in serum Mg^{2+} of these subjects, we suggest that future research focus on a larger number of human subjects given higher concentrations of Mg^{2+} cream appli-cation administered for longer durations to investigate whether transdermal application may show a significant contribution to improvement in magnesium status. It would also be of interest to look at the effect of transdermal Mg^{2+} supplementation on athletes as compared to a sedentary population group.

Supporting information

S1 Text. Ingredients list. Ingredients for magnesium and placebo creams **list**.
(DOCX)

S1 Fig. CONSORT checklist. Checklist of information to include when doing a randomised trial.
(DOC)

S1 Table. Dataset for repository. Subject data.
(XLSX)

S2 Text. Protocol
(DOCX)

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Dr. Andrea Rosanoff declares a beneficial interest in transdermal magnesium cream products.

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Investigation: LK.

Methodology: LK AR.

Project administration: LK.

Resources: LK AR.

Supervision: LK.

Validation: LK AR.

Visualization: LK AR.

Writing ± original draft: LK AR AT WM.

Writing ± review & editing: LK AR MP.

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Appendix 1

CASP
Critical Appraisal Skills Programme

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CASP Checklist: 10 questions to help you make sense of a Systematic Review

How to use this appraisal tool: Three broad issues need to be considered when appraising a systematic review study:

- Are the results of the study valid? (Section A)
- What are the results? (Section B)
- Will the results help locally? (Section C)

The 10 questions on the following pages are designed to help you think about these issues systematically. The first two questions are screening questions and can be answered quickly. If the answer to both is "yes", it is worth proceeding with the remaining questions. There is some degree of overlap between the questions, you are asked to record a "yes", "no" or "can't tell" to most of the questions. A number of italicised prompts are given after each question. These are designed to remind you why the question is important. Record your reasons for your answers in the spaces provided.

About: These checklists were designed to be used as educational pedagogic tools, as part of a workshop setting, therefore we do not suggest a scoring system. The core CASP checklists (randomised controlled trial & systematic review) were based on JAMA 'Users' guides to the medical literature 1994 (adapted from Guyatt GH, Sackett DL, and Cook DJ), and piloted with health care practitioners.

For each new checklist, a group of experts were assembled to develop and pilot the checklist and the workshop format with which it would be used. Over the years overall adjustments have been made to the format, but a recent survey of checklist users reiterated that the basic format continues to be useful and appropriate.

Referencing: we recommend using the Harvard style citation, i.e.: *Critical Appraisal Skills Programme (2018). CASP (insert name of checklist i.e. Systematic Review) Checklist. [online] Available at: URL. Accessed: Date Accessed.*

CASP
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Section A: Are the results of the review valid?

1. Did the review address a clearly focused question?

Yes ☐
Can't Tell ☐
No ☐

HINT: An issue can be 'focused' In terms of
- the population studied
- the intervention given
- the outcome considered

Comments:

2. Did the authors look for the right type of papers?

Yes ☐
Can't Tell ☐
No ☐

HINT: 'The best sort of studies' would
- address the review's question
- have an appropriate study design (usually RCTs for papers evaluating interventions)

Comments:

Is it worth continuing?

3. Do you think all the important, relevant studies were included?

Yes ☐
Can't Tell ☐
No ☐

HINT: Look for
- which bibliographic databases were used
- follow up from reference lists
- personal contact with experts
- unpublished as well as published studies
- non-English language studies

Comments:

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4. Did the review's authors do enough to assess quality of the included studies?

Yes ☐
Can't Tell ☐
No ☐

HINT: The authors need to consider the rigour of the studies they have identified. Lack of rigour may affect the studies' results ("All that glisters is not gold" Merchant of Venice – Act II Scene 7)

Comments:

5. If the results of the review have been combined, was it reasonable to do so?

Yes ☐
Can't Tell ☐
No ☐

HINT: Consider whether
- results were similar from study to study
- results of all the included studies are clearly displayed
- results of different studies are similar
- reasons for any variations in results are discussed

Comments:

Section B: What are the results?

6. What are the overall results of the review?

HINT: Consider
- If you are clear about the review's 'bottom line' results
- what these are (numerically if appropriate)
- how were the results expressed (NNT, odds ratio etc.)

Comments:

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7. How precise are the results?

HINT: Look at the confidence intervals, if given

Comments:

Section C: Will the results help locally?

8. Can the results be applied to the local population?

Yes ☐
Can't Tell ☐
No ☐

HINT: Consider whether
- the patients covered by the review could be sufficiently different to your population to cause concern
- your local setting is likely to differ much from that of the review

Comments:

9. Were all important outcomes considered?

Yes ☐
Can't Tell ☐
No ☐

HINT: Consider whether
- there is other information you would like to have seen

Comments:

10. Are the benefits worth the harms and costs?

Yes ☐
Can't Tell ☐
No ☐

HINT: Consider
- even if this is not addressed by the review, what do **you** think?

Comments: