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THE EFFECTS OF MAGNESIUM SUPPLEMENTATION ON EXERCISE PERFORMANCE AND RECOVERY INDICES IN PHYSICALLY ACTIVE FEMALES

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When the river flows it rushes and it rushes by

It carries all our hopes, all our dreams and all our cries

And when it looks as if it's overflowing and we're all going to die

We've got to let it take its course and go along for a wild ride

Many thanks: To Ian Newhouse, my advisor and mentor who taught me to overcome adversity and thrive on it, and with whom I spent one of the greatest days of his life when Joseph was born and we were eight hours away in Grand Forks - this led to another story . . . and another . . . (The novel will be out soon.) To Hank Lukaski and the USDA Human Nutrition Research Centre in North Dakota for the input - our hearts go out to you after the great flood of '97. To Jim McAuliffe and Barry Berringer for showing the way through the statistical and chemical mazes, respectively. To Teresa Socha, Moira McPherson and the Lakehead University Sports Institute for the assistance and equipment. To the phlebotomists who gave their time and great spirit so willingly - Gay Hornak, Donna Newhouse and Lena Saunders. To fellow Grad Students Cameron Stewart as testing partner, and Kerri Tolen as unyielding guinea pig. To Joseph, Heike and Grete for bringing us added joy. To the Gatorade Sport Science Institute for the support. To C.E. Jamieson and Company for the supplements and placebos. To Robert Harvey and Nova Biomedical Canada for the use of their time and equipment. To the participants who gave all but their right arm (actually they did that too for the blood sampling). To Kim for being the greatest of friends throughout. And to my parents, John and Lois, for absolutely everything that I have in this life including their support through thick and thin. I dedicate this to the memory of my grandmother, Bessie Gerry. We will always cherish the wonderful memories she gave us.

ABSTRACT

The effects of magnesium (Mg^{2+}) supplementation on performance and recovery were examined. Initial Mg^{2+} status as well as effects of Mg^{2+} supplementation on plasma Mg^{2+} were determined using the sensitive and recently advanced measure of ionic Mg^{2+} (iMg). The effects of initial Mg^{2+} status on these parameters were also studied. Physically active females (n = 121) were screened for [iMg] in plasma, and according to the criteria set by Altura, Shirey et al. (1994), 36.4% were marginally deficient in the mineral. Thirty-two subjects (21 $\pm$ 3 years) representing a broad range of [iMg] (0.54 $\pm$ 0.04 mmol/L) completed the main 14-week study. At baseline, participants submitted to a resting blood pressure measurement, and they completed both an anaerobic treadmill test and an incremental (aerobic) treadmill test. For the latter, values for workload, oxygen uptake and heart rate were obtained for anaerobic threshold and maximal effort. Blood samples for iMg, iCa, K^+, Na^+, Hb, Hct, lactate, and glucose were also collected pre-incremental treadmill test, and 4, 10, 30 min, and 24 hr post-test. Subjects received 212 mg/day Mg oxide supplement or matching placebo for four weeks in a double-blind fashion and were then retested. Following a 6-week washout period, the testing was repeated with a treatment crossover. Magnesium treatment significantly improved resting [iMg] levels in plasma but not performance or recovery from exercise. Initial Mg^{2+} status had no effect on these parameters. Higher than normal dietary intakes of Mg^{2+} and the relatively low dosage of the mineral may have contributed to the absence of treatment effect. In addition, it appears that the [iMg] measure is more sensitive than [TMg] or [EMg] warranting the use of this assay to assess the effects of Mg^{2+} treatment in athletic situations. Although not the focus of the study, it was indicated that [iMg] values were somewhat variable within individuals. This effect must be

studied further before these values can be clearly interpreted. This study was supported by the Gatorade Sports Science Institute (Barrington, IL), Nova Biomedical Canada Limited (Mississauga, ON), the United States Department of Agriculture Human Nutrition Research Centre (Grand Forks, ND), and C.E. Jamieson and Company Limited (Windsor, ON).

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CHAPTER ONE: INTRODUCTION

1.1 PURPOSE

The purpose of this investigation was to examine the following with regards to physically active females: (1) The effects of magnesium (Mg^{2+}) supplementation on Mg^{2+} levels, (2) the effects of Mg^{2+} supplementation on performance and recovery, and (3) the degree to which initial Mg^{2+} status affects the above. This was achieved by utilizing the highly specific, sensitive and recently advanced measure of ionic Mg^{2+} (iMg) assay, and by employing a randomized, double-blind, placebo-controlled crossover experimental design.

1.2 IMPORTANCE OF THE STUDY

Many North Americans fail to consume dietary Mg^{2+} in amounts consistent with recommended intakes (Altura, 1994; National Research Council of Canada [NRCC], 1979).[1] Increased use of Mg^{2+}-lacking fertilizers in the soil as well as increased refinement of foods has steadily reduced the amount of Mg^{2+} available in many foods grown or processed in North America (NRCC). Dietary intakes of normal populations (McDonald and Keen, 1988) as well as athletes (Seelig, 1994) have shown that up to half consume diets containing less than the United States Recommended Dietary Allowance (RDA) estimated for sedentary adults. Athletes in particular tend to have increased needs for Mg^{2+} most likely due to greater urinary and surface losses during periods of exercise training (Bohmer, 1995; Deuster, Dolev, Kyle, Anderson and

[1] Recommended intakes are 200 and 250 mg/day for adult females and males, respectively, in Canada (Health and Welfare Canada, 1990), and 280 and 350 mg/day, respectively, in the United States (Food and Nutrition Board, National Research Council, 1989).

Schoomaker, 1987; Resina et al., 1995; Rose, Carrol, Lowe, Peterson and Cooper, 1970). In addition, lower Mg^{2+} intakes and, thus, marginal Mg^{2+} deficiencies are more likely to be found in females rather than males (Bohmer, 1994; Faber and Spinnler-Benade, 1991; Lukaski, 1995a).

While it may be assumed that a suboptimal intake of Mg^{2+} could result in physiological impairments, research to date has been equivocal (Lukaski, 1995a) and has relied on insensitive indicators of Mg^{2+} status (Elin, 1991-92). Blood biochemical indices in the serum (consisting of protein-bound, complexed, and ionic Mg^{2+}) and erythrocytes are not negatively impacted until a very severe state of Mg^{2+} deprivation or if a preexisting pathology exists (Elin; Holm, Jepsen, Sjogaard and Hessov, 1987; Lukaski). A more sensitive, specific and physiologically meaningful indicator of Mg^{2+} status is now available with determinations of ionized (or free) Mg^{2+} (iMg) (Altura, 1994; Altura and Altura, 1994; Altura, Bertschat, Jeremias, Ising and Altura, 1994; Altura, Burack et al., 1994; Altura, Shirey et al., 1994; Fogh-Andersen and Siggaard-Andersen, 1994; Lewenstam (1994); Lewenstam, Blomqvist and Ost, 1994; Maj-Zurawska, 1994; Marsoner et al., 1994; Shirey, 1995). With this new technology one can assess whether a subclinical Mg^{2+} deficiency does exist and thereby allow a clearer examination of the effects of Mg^{2+} supplementation.

This study is important in providing evidence of whether Mg^{2+} supplementation is of value for physically active women. Athletes, coaches, health professionals (doctors, exercise physiologists and nutritionists), food production industries, and nutritional supplement companies would all have a vested interest in the findings. Not only is performance enhancement advantageous but adequate recovery is also important for athletes so that they are less susceptible to overtraining and thus are more likely to improve their performance and

health. In sport, it is increasingly necessary to find out every way possible to improve performance as well as to remain healthy in the process. This study also adds to the limited data on reference values for [iMg] in the physically active female population.

1.3 DEFINITIONS

(1) Overtraining - condition in which there is an imbalance between exercise and recovery resulting in severe and prolonged fatigue (Kuipers and Keizer, 1988)

(2) Physically Active - performing at least three workouts per week for at least two hours total at an intensity of at least 75% of maximum effort

(3) Recovery - the process of reestablishing homeostasis within the muscle

(4) Subclinical Magnesium Deficiency - [iMg] of less than 0.53 mmol/L with no apparent clinical symptoms (Altura, Shirey et al., 1994)

1.4 ABBREVIATIONS

ADP	- Adenosine Diphosphate
ANOVA	- Analysis of Variance
ATP	- Adenosine Triphosphate
Ca^{2+}	- Calcium Ion
DBP	- Diastolic Blood Pressure
DNA	- Deoxyribose Nucleic Acid
DOMS	- Delayed Onset Muscle Soreness
EMg	- Erythrocyte Magnesium
GI	- Gastrointestinal
Hb	- Hemoglobin
Hct	- Hematocrit
iCa	- Ionic Calcium
iMg	- Ionic Magnesium
K^+	- Potassium Ion
MBC	- Mononuclear Blood Cell
M	- Magnesium-treated
Mg	- Magnesium
Mg^{2+}	- Magnesium Ion
Mg^{2+}-ATP	- Magnesium-Adenosine Triphosphate
M/P	- Magnesium-treated First / Placebo-treated Second
Na^+	- Sodium Ion
P	- Placebo-treated
P/M	- Placebo-treated First / Magnesium-treated Second
RBC	- Red Blood Cell
RDA	- Recommended Daily Allowance
RNA	- Ribose Nucleic Acid
RNI	- Recommended Nutrient Intake
SBP	- Systolic Blood Pressure
T1	- First Testing Session
T2	- Second Testing Session
T3	- Third Testing Session
T4	- Fourth Testing Session
TMg	- Total Serum Magnesium
2,3-DPG	- 2,3-Diphosphoglycerate
V_E	- Ventilation
$V_E$peak	- Peak Ventilation
VO_2	- Volume of Oxygen Uptake
VO_2max	- Maximum Volume of Oxygen Uptake
WBC	- White Blood Cell

1.5 LIMITATIONS

(1) Ionic Mg^{2+} levels were indicated to have some degree of variability within individuals. The reasons for this variability are unclear but this factor limited the ability to accurately diagnose Mg^{2+} deficiencies and classify subjects based on iMg status.

(2) The reference values for [iMg] are not well established, especially for the physically active.

(3) The analyses of the hematological parameters were dependent upon the accuracy of the Stat Profile™ Ultra Analyzer® model 11-3C (Nova Biomedical Canada Ltd., Mississauga, ON).

(4) The analyses of the dietary intakes were dependent upon the accuracy of the information recorded by each athlete, on whether the three days analyzed represented true usual intakes, on the accuracy and consistency of the investigators' interpretations, and on the accuracy of the software Diet Analysis Plus™ ©1996 by West Publishing Co., St. Paul, MN.

(5) The recording of objective and subjective measures in the training diary were dependent upon the accuracy and consistency of the information recorded by each athlete.

(6) The analyses of expired gases were restricted to the accuracy of the SensorMedics Vmax System® metabolic cart ©1996 (Yorba Linda, CA).

(7) The analyses of the maximal parameters for the treadmill tests were restricted to the subjects' motivation to reach their potential.

1.6 DELIMITATIONS

(1) Participants included 32 apparently healthy and physically active females who ranged in age from 17 to 29 years of age and who all resided in Thunder Bay, Ontario, Canada during the study period.

(2) Screening included [iMg] levels.

(3) Hematological assays included [iMg], [iCa], [Na^+], [K^+], [Hb], Hct, [plasma lactate] and [plasma glucose].

(4) Dietary analyses were conducted for three days each at both T1 and T3.

(5) Self-reported variables in the training log included length of workout(s), length of sleep, training willingness, appetite, irritability, muscle soreness, minor illnesses and injuries, gastrointestinal (GI) difficulties, menstrual irregularities, and stressful events.

(6) Anthropometric, health and exercise performance tests included weight, height, five skinfold measures, blood pressure, and incremental (aerobic) and anaerobic treadmill tests. Aerobic versus anaerobic athletes were not distinguished, thus, these groups were not compared. It has been suggested that the more anaerobic the exercise, the more Mg^{2+} is lost in the urine (Deuster et al, 1987).

(7) Only 212 mg elemental Mg^{2+} per day dosage amount in the form Mg oxide was administered as supplementation.

(8) Only 4-week treatment periods were utilized with a 6-week washout period between.

CHAPTER TWO: REVIEW OF LITERATURE

2.1 INTRODUCTION

The purpose of this chapter is to first review the roles of Mg^{2+} in the body in order to show how Mg^{2+} status can affect exercise performance and recovery measures. Secondly, intakes of Mg^{2+} are reviewed for their effectiveness in achieving adequate Mg^{2+} stores. Thirdly, the techniques for assessing Mg^{2+} stores are compared with one another. Next, the causes and effects of Mg^{2+} deficiency are discussed in order to emphasize the need for measuring Mg^{2+} status and for maintaining sufficient stores of the mineral. All of these concepts then form the basis for discussing the various studies dealing with measures of Mg^{2+} status and/or Mg^{2+} supplementation and their relation to indices of exercise performance and recovery.

2.2 PHYSIOLOGICAL ROLES OF MAGNESIUM

Magnesium is an essential mineral and is a cofactor for over 325 enzymatic reactions involved in cellular energy production and storage, protein synthesis, deoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA) synthesis, cell growth and reproduction, adenylate cyclase synthesis, maintenance of cellular electrolyte composition, and stabilization of mitochondrial membranes. The physiological consequences of these biochemical activities include Mg^{2+}'s central roles in the control of neuronal activity, cardiac excitability, neuromuscular transmission, muscular contraction, vasomotor tone, and blood pressure. (Altura, Shirey et al., 1994). The intracellular functions of Mg^{2+} are achieved through the formation of magnesium adenosine triphosphate (Mg^{2+}-ATP) - a substrate for a wide variety of enzymes (e.g., phosphatases and

phosphokinases) located in cell membranes or intracellular compartments (Al-Ghamdi, Cameron and Sutton, 1994).

As a cofactor to enzymes that require ATP, Mg^{2+} exercises control over electrolyte balance across cell membranes. Conduction is a function of an electrical gradient across a nerve or a muscle cell membrane. The sodium-potassium (Na^{2+}-K^{+}) pump which is ATP-dependent establishes this electrical gradient by pumping ionized K^{+} into the cell. The contraction mechanism of any muscle tissue (cardiac, smooth and skeletal) depends on extracellular calcium (Ca^{2+}). Similar to the Na^{2+}-K^{+} pump, there is a Mg^{2+}-ATP pump that forces ionized Ca^{2+} (iCa) out of the cell. As a muscle cell depolarizes, iCa rushes in and binds to troponin which results in rotation of the troponin-tropomyosin complex, thereby exposing the myosin binding sites on actin and the subsequent binding of myosin to actin. The actin-myosin crossbridge formations are the basis of a muscle's contraction. Magnesium also serves as a Ca^{2+}-channel blocker to modulate cardiac, vascular and skeletal muscle contraction. If an individual has too little Mg^{2+}, then too much Ca^{2+} is admitted into the cell. Too much contraction leads to hypertension, tachycardia, neuromuscular irritability and muscle cramps. Conversely if one has too much Mg^{2+} in the cell, then too little Ca^{2+} is being admitted and too little contraction results leading to hypotension and bradycardia (Shirey, 1995).

The role which Mg^{2+} plays in controlling cell membrane permeability also relates to its speculated role in preventing delayed onset muscle soreness (DOMS) after a bout of unaccustomed exercise. Alterations in cell membrane permeability results in a significant increase in Ca^{2+} influx from the extracellular fluid to the muscle fibres. The Ca^{2+} ions activate a Ca^{2+}-dependent proteolytic enzyme responsible for selective degradation of Z-lines, troponin and

tropomyosin. Progressive deterioration would then permit diffusion of the intracellular components into the plasma. This would then induce chemotactic activity by white blood cells (WBCs) which would be converted into macrophages and activate mast cell degranulation in the areas of damage (Dragani, Giamberardino and Vecchiet, 1994).

Magnesium is necessary for the transfer, storage and utilization of energy. Intracellular Mg^{2+} activates enzymes in the breakdown of fatty acids, amino acids and glucose during energy metabolism. The intracellular [iMg] serves to regulate intermediary metabolism through the activation of rate limiting enzymes such as creatine kinase, hexokinase, pyruvate dehydrogenase and enolase (Altura, 1991-92).

Magnesium controls hemoglobin (Hb) function since most of the ATP in red blood cells (RBCs) is bound to the mineral in the Mg^{2+}-ATP complex (Bunn, Ransil and Chao, 1971). There is also evidence that the synthesis of erythrocyte 2,3-diphosphoglycerate (2,3-DPG) is Mg^{2+}-dependent (McDonald and Keen, 1988; Resina et al., 1994) through the activation of hexokinase (Casoni et al., 1990). Thus Mg^{2+} may control Hb's oxygen delivery to the working muscles (Resina et al., 1994; 1995). It seems that the maintenance of an adequate Mg^{2+} status is of utmost importance for efficient delivery of oxygen for rapid energy release so that performance is optimal. The mineral also plays a vital role in regulating cell growth, reproduction and membrane structure, by regulating DNA and RNA synthesis and structure (Altura, 1991-92).

2.3 INTAKE OF MAGNESIUM

2.3.1 Absorption and Excretion of Magnesium

Normal absorption is dependent upon total Mg^{2+} intake, intestinal transit time, and the absorption rate of water (Health and Welfare Canada [HWC], 1990). Absorption of Mg^{2+} through the jejunum and ileum is reported to be in the 40 to 50% range in normal dietary conditions (Elin, 1991-92). However, with increasing intakes, absorption has been shown to increase whereas fractional absorption has been shown to decrease, ranging from a fractional absorption of 65% at the lowest intake (a standard meal with no supplemental Mg^{2+}) to 11% at the highest intake (a standard meal with 40 mmol Mg acetate added) (Fine, Santa Ana, Porter and Fordtran, 1991). In conditions of extremely low intakes, the kidney can conserve all but 12 mg of Mg^{2+} per day (HWC, 1990).

Urinary Mg^{2+} excretion has been correlated with Mg^{2+} intake and has shown to have increased significantly with supplementation (Borella, Bargellini and Ambrosini, 1994). Spencer, Fuller, Norris and Williams (1994) studied five male subjects who exhibited an increase in absorption with 800 mg/day intake as compared to that noted with their normal intakes which were below the RDA of 350 mg/day. These subjects showed increases in both urinary and fecal Mg^{2+} excretion but were also able to develop a positive Mg^{2+} balance through supplementation.

Although bone and the GI tract play a part in Mg^{2+} homeostasis, the kidney is the prime regulator of Mg^{2+} balance (HWC, 1990). In the kidney, normally 3 to 6% of the filtered load is excreted (Altura, 1991-92). Approximately 25% of the filtered Mg^{2+} is reabsorbed in the proximal tubule while 50 to 60% is reabsorbed in the ascending loop of Henle.

2.3.2 Dietary and Supplemental Intakes

Lukaski (1995b) mentions that the best way to achieve an adequate Mg^{2+} intake is through a balanced diet if at all possible. Some good dietary sources of Mg^{2+} are found in whole grains, raw nuts, milk, seafood, bananas, potatoes, seeds and leafy green vegetables. Some examples of foods with high Mg^{2+} content are spinach (1 cup cooked = 150 mg), beans and black-eyed peas (1 cup cooked = 70-100 mg), and tofu (0.5 cup = 125 mg).

Lukaski (1995b) goes on to review that excessive fibre intake may decrease Mg^{2+} absorption since Mg^{2+} binds to fibre resulting in its rapid transit through the GI tract. Typical western diets which are high in protein and/or fat do not seem to harm Mg^{2+} absorption or excretion, although these diets may not contain sufficient amounts of the mineral. Increases of both dietary Ca^{2+} and phosphate do not adversely affect Mg^{2+} balance. If an individual is not able to acquire sufficient Mg^{2+} from their diet, Mg^{2+} supplementation may be beneficial. Supplemental Mg^{2+} may be taken as gelatin capsules or tablets with Mg^{2+} salts. In general, Mg^{2+} salts have a fractional absorption of about 20%. Most Mg^{2+} supplements which are available to the public are combined with other minerals such as Ca^{2+}, K^+, phosphorus, and zinc, and/or with vitamins such as D and B-6. This suggests that Mg^{2+} supplementation may be widespread.

2.4 ASSESSMENT OF MAGNESIUM STATUS

2.4.1 Distribution of Magnesium

Magnesium is second only to K^+ in intracellular composition and it resembles K^+ in distribution about the body (Altura, 1991-92). Magnesium amounts to about 21 to 28 g (or about 1% of the mineral content) in the average adult man (Altura; Elin, 1991-92), and is the fourth

most abundant macromineral in the human body. Sixty percent of it is stored, and is relatively unexchangeable, in bone along with Ca^{2+} and phosphorus, and about 20 to 27% is found in skeletal muscle (Altura; Elin). Less than 1% of the total body Mg^{2+} exists in blood, and about 0.3% is present in the blood serum (Elin). The cells of the body contain about 38% of Mg^{2+} whereas the extracellular fluid contains only 1 to 2%. There is still much uncertainty as to the exact proportions of Mg^{2+} distribution in the body, and only recently have measures been made for [iMg] in the serum and in whole blood. The preliminary data show that [iMg] comprises about 71% of [TMg] in serum (Altura, 1991-92). The remainder of the TMg is bound to nonspecific proteins or is complexed.

2.4.2 Tissue Magnesium

There are two tissues which have been primarily used for clinical medicine - blood and muscle. Within the blood, assays can be derived from serum, RBCs and mononuclear blood cells (MBCs), while the muscle measure can be obtained from a biopsy. The combination of all of these measurements usually represents the total Mg^{2+} content of the body, and that one measure does not necessarily relate to another (Elin, 1991-92). Al-Ghamdi et al. (1994) report that the [TMg] is maintained within a narrow range by the kidney and small intestine. In Mg^{2+} depletion, both organs increase the absorption of Mg^{2+}. If further depletion occurs, bone stores give up Mg^{2+} to the plasma. Thus [TMg] can be normal in the presence of Mg^{2+} deficiency. If the depletion is severe enough the result is Mg^{2+} deficiency. The reference interval for [TMg] in human adults is 0.75-0.96 mmol/L (Elin, 1991-92). Values below this range are considered to be Mg^{2+}-deficient. Borella et al. (1994) found that only subjects with the lowest Mg^{2+} intake had

lower values of [TMg]. Similarly for [EMg], Borella and colleagues discovered a 10% lower value in marginally deficient subjects as compared to controls. Elin (1991-92) reports that a fairly new test for assessing Mg^{2+} status is the determination of Mg^{2+} in MBCs which is taken from the leucocytes in blood. Magnesium levels in MBCs have not correlated highly with [TMg] or [EMg] and additional studies will need to ascertain the correlation between MBCs and muscle tissue. The biopsy method of measuring muscle indices is tedious and costly and uncomfortable for subjects therefore this measure is left out of most studies in this area.

The majority of studies to date have measured [TMg], however, [iMg] is the most important fraction of the measure since it is the physiologically active portion (Elin, 1991-92). This portion of Mg^{2+} serves to regulate metabolism through activation of rate-limiting enzymes such as hexokinase, pyruvate dehydrogenase, enolase and creatine kinase (Altura, 1991-92). Moreover, the method of measuring [iMg] has recently become more available to perform. This measure can be determined by the use of an electrode in which iMg is separated out of the serum in a blood sample. Newer ion-sensitive electrodes have been designed to function in the presence of iCa and other potential cationic interferences found in the blood of normal and diseased human subjects and animals. The speed of determining [iMg] with these ion-sensitive electrodes has aided the clinical application of the [iMg] measure to numerous conditions. Some of these syndromes include diabetes, cardiopulmonary bypass, organ transplantation, neonatal distress, migraine headaches, atherogenesis and hypertension (Altura, 1994). Plasma iMg can be determined using a plasma, serum or whole blood sample. Ionized Mg^{2+} appears to cross the cell membrane relatively quickly which would suggest that the two reservoirs are in dynamic equilibrium (Shirey, 1995). This has important implications when trying to assess whole body

Mg^{2+} status, since, as stated previously, [TMg] measurements are not reflective of intracellular concentrations. For a more thorough review of iMg sensitive electrodes and their clinical applications, the reader is referred to the Scandinavian Journal of Clinical and Laboratory Investigation, Vol. 54, Suppl. 217, 1994.

2.4.3 Physiological Assessment of Magnesium Status

Elin (1991-92) reports four other tests for the physiological assessment of Mg^{2+} status, i.e., those requiring Mg^{2+} to be metabolized during testing - balance studies, renal excretion studies, isotope studies, and retention of Mg^{2+} following acute administration of isotopes. These measures all require normal absorption, tissue uptake and excretion rates for proper accuracy. Balance studies are very costly and are not available for routine assessment of Mg^{2+} status but would provide important questions about Mg^{2+} metabolism. Renal excretion measures often involve a 24-hour period of urine collection. Excretion studies depend on intake, absorption and renal function and, thus, can detect any failures in renal function. Under normal circumstances Elin mentions that daily excretion rates of Mg^{2+} are in the range of 3.6 ± 1.4 mmol for females and 4.8 ± 1.5 mmol Mg^{2+} for males. Isotope studies are used for clinical research. They involve injecting a Mg^{2+} isotope into the antecubital vein and measuring excretion (and thus retention) rates in the urine for 24 to 48 hours (Holm et al., 1987). Following the parenteral administration of a Mg^{2+} load, the retention rate can be measured and this has recently become a very valuable tool in Mg^{2+} status assessment. Magnesium deficiencies are said to be indicated if retention is higher than 40% in a 24-hour period (Hinds, Bell, McMaster and McCluskey, 1994). Holm and coworkers (1987) suggest that since there exists a relation between the concentration of Mg^{2+} in

muscle and Mg^{2+} retention, the Mg^{2+} load test could be used to diagnose even marginal Mg^{2+} deficiencies. The test is also much easier and less costly that the muscle biopsy procedure. Lastly, the retention of Mg^{2+} following acute administration of a Mg^{2+} load is becoming more popular in diagnosing deficiencies. If the amount of the load, the length of time to infuse the load, and the length of time for urine collection becomes standardized, this test will have high clinical value in detecting deficiencies (Elin, 1991-92).

2.4.4 Summary

Research in Mg^{2+} status has been unclear and has been hampered in part by the lack of an accurate and sensitive indicator of Mg^{2+} status and deficiency (Elin, 1991-92; Holm et al., 1987; Lukaski, 1995a). It seems that the hematological indices of [TMg] and [EMg] are not negatively impacted until a very severe state of Mg^{2+} deprivation. Thus any deficiencies would not be discovered early enough by using only these indices. It is evident that the utilization of [iMg] and/or physiological tests for assessing the body's Mg^{2+} is in order for studies in sports medicine.

2.5 MAGNESIUM DEFICIENCY

2.5.1 Causes of Magnesium Deficiency

Hypomagnesemia can arise from inadequate intakes of the mineral but severe deficiencies of the mineral are rare because of the remarkable ability of the kidney to conserve Mg^{2+}. The condition may also result from malabsorption due to various disorders of the GI tract (Al-Ghamdi et al., 1994), renal dysfunction, burns, diabetic keto-acidosis, alcoholism, long-term diuretic use, hyperparathyroidism and cirrhosis (HWC, 1990; Kreg and Murray, 1986). By far

the most important cause of clinical hypomagnesemia is from excessive loss through severe and prolonged diarrhea (Zilva and Pannall, 1979). Genetic differences in Mg^{2+} utilization may also be a factor in accounting for differences in stress reactions and in vulnerability to Mg^{2+} deficiency. It is also evident that intense prolonged physical exercise can cause Mg^{2+} deficiency (Lijnen et al., 1988; Rose et al., 1970; Stendig-Lindberg, 1991).

The daily intake of Mg^{2+} has been declining steadily in North America since the turn of the century. Due to the increasing use of fertilizers (lacking Mg^{2+}) and food processing (removing Mg^{2+}) in practice today, intakes have declined from 500 mg/day to about 175 to 225 mg/day (Altura, 1994; NRCC, 1979). Daily intakes of Mg^{2+} can be evaluated from self-report dietary records. As values from 70 to 100% of the RDA are considered adequate for an individual, cause for concern results when intakes are less than 70% of the RDA (Lukaski, 1995a). Well over 50% of the normal population have Mg^{2+} intakes below that of the RDA which is in the range of 280-350 mg/day depending on sex, age and condition (McDonald and Keen, 1988). Severe Mg^{2+} deficiency, however, is rare in developed countries because of the increased availability of most foods (Seelig, 1994).

Clarkson and Haymes (1995) indicate that athletes appear to have adequate Mg^{2+} status, however, those who are on calorie-restricted diets may not be obtaining sufficient Mg^{2+} supply. Lukaski (1995b) claims that based on population surveys and data from dietary histories, some athletes show intakes which are less than 70% that of the RDA. Seelig (1994) claims that as many as half of the athletes she has studied consume diets containing less than 50% of the RDA for Mg^{2+}. Lukaski (1995b) reports that in general, Mg^{2+} intakes for male athletes equal or exceed the RDA of 350 mg/day as compared to females who reveal intakes of about 60 to 65% of the

RDA of 280 mg/day. It has been shown that female field athletes (Faber and Spinnler-Benade, 1991), bodybuilders (Kleiner, Bazzarre and Ainsworth, 1994), and cross-country runners (Powell and Tucker, 1991) exhibit dietary Mg^{2-} below RDA levels which did not hold the same for their male counterparts. Newhouse, Clement and Lai (1993) could not corroborate these findings as the mean Mg^{2-} intake of the recreational level female athletes whom they studied was 342 $\pm$ 83 mg/day. No subjects failed to meet the Canadian Recommended Nutrient Intake (RNI) of 200 mg/day. Singh, Deuster, Day and Moser-Veillon (1990) actually discovered a higher Mg^{2-} intake in highly-trained athletes than untrained ones. Deuster et al. (1986) also found intakes above the RDA (i.e., 410 mg/day) in 51 highly-trained female runners. Thus it seems that intakes are highly variable indicating that individualized assessment is required. Also, since recommended intakes are based on non-athletic populations, and the literature regarding the actual Mg^{2-} status of athletes is limited, one can still question whether the observed dietary intakes are sufficient for the amount of energy that the athletes are expending and the potential increased Mg^{2-} losses. It is evident that further investigations should be conducted in this area.

In an athletic population, increased Mg^{2-} loss usually occurs through urine, sometimes through sweat (Rose et al., 1970), or through feces due to diarrhea (Al-Ghamdi et al., 1994; Spencer et al., 1994; Zilva and Pannall, 1979). The amount lost through urine has been found to increase on exercising vs. control days and with the degree of anaerobic activity (Deuster et al., 1987). These authors also found that urinary loss returns to normal the day after exercise. However, repeated bouts of exercise which do not allow a day to recover may result in accumulated Mg^{2-} loss and perhaps an eventual deficiency. Indeed, Resina et al. (1995) found that one month of daily endurance training led to significant decreases in Mg^{2-} stores through

urinary losses causing a marginal Mg^{2+} deficiency. On the other hand, urinary Mg^{2+} excretion did not differ for highly-trained vs. untrained female runners according to a study done by Singh and colleagues (1990). Hypoxia may be the mechanism behind a urinary Mg^{2+} loss. Hypoxia results in the breakdown of ATP causing the release of iMg into the cytosol and then into the plasma. Ionized Mg^{2+} is subsequently lost at the kidneys. The resynthesis of ATP during reperfusion would reverse the flow of iMg back into the cell leaving a plasma deficit (Shirey, 1995). This model also explains the Mg^{2+} loss that has been observed following cardiopulmonary bypass surgery and the redistribution of Mg^{2+} immediately after exercise (to be discussed later).

Controversial data thus exists regarding the possibility of intense endurance exercise decreasing the Mg^{2+} balance. An inadequate dietary intake coupled with potential increased losses could certainly put the athlete at risk of deficiency.

2.5.2 Effects of Magnesium Deficiency

As stated previously, Mg^{2+} is involved in the control of neuronal activity, neuromuscular transmission, muscular contraction, cardiac excitability, vasomotor tone, blood pressure and peripheral blood flow (Altura, 1991-92). Since Mg^{2+} plays such a vital role in so many physiological activities, a deficiency of the mineral can cause many negative consequences for an individual. Hypomagnesemia could present itself through a wide range of clinical features as depicted in Table 1.

Table 1. Clinical features of hypomagnesemia (from Polancic, 1991; as cited by Shirey, 1995)

Neuromuscular	**Cardiovascular**	**Musculoskeletal**
- tremors	- cardiac arrhythmias	- weakness
- seizures	- hypertension	- cramps
- paralysis	- digitalis toxicity	- ataxia
Metabolic	**Psychiatric**	- spasms
- hypocalcemia	- depression	- tetany
- hypokalemia	- agitation	
- hyponatremia	- psychosis	
- hypophosphatemia		

Most hypomagnesemia in clinical practice is asymptomatic with the signs and symptoms becoming evident once [TMg] drops below 0.5 mmol/L (Al-Ghamdi et al., 1994). This is not to say that milder cases of hypomagnesemia are not without consequence, but rather [TMg] determinations have not routinely been determined to establish the mineral's association with various disorders. To correct this situation, Al-Ghamdi and co-workers suggest that [TMg] be routinely ordered in all acute care units and in all patients with conditions or medications that may predispose them to Mg^{2+} deficiency. Taking the suggestion one step further, [iMg] determinations could add more sensitivity to Mg^{2+} status changes.

Altura and coworkers have been pioneering the research linking more subtle degrees of hypomagnesemia to a variety of pathological syndromes. With the utilization of [iMg] measures, they have shown that a chronic deficiency of Mg^{2+} is associated with atherogenesis, hypertension, type II diabetes, eclampsia, migraine, headaches, decreased resistance to endotoxins, cardiac arrhythmias, asthma and complications from cardiac surgery. The clinical course for some of these disorders is correlated with [iMg] and not [TMg] (Altura, Shirey et al., 1994).

The effects of hypomagnesemia on athletic performance are quite speculative due to problems involving the precise determination of Mg^{2+} status in athletes, the confounding effects that exercise might have on the redistribution of Mg^{2+}, and the equivocal studies to date on the beneficial effects of Mg^{2+} supplementation. These topics will now be reviewed.

2.6 MAGNESIUM AND EXERCISE

2.6.1 Magnesium Status in Athletes

Many studies involving exercise performance and recovery do not include any measurements of Mg^{2+} status. In addition, for those studies that do, the precise determination of Mg^{2+} status in athletes has been lacking insofar as normal blood indices are usually used to assess whole body nutriture. McDonald and Keen (1988) mention that there is growing evidence that exercise causes a decrease in the blood indices of Mg^{2+}, and that marginal deficiencies may be common among endurance athletes (whose dietary intakes of Mg^{2+} are inadequate). The reference interval for normal [TMg] in adults is from 0.75-0.96 mmol/L (Elin, 1991-92). Resina et al. (1994) noted that in their sample of 20 well-trained middle-distance and long-distance runners, the mean [TMg] at baseline was 0.786 ± 0.08 mmol/L with 25% of the athletes having values representing marginal deficiencies. A one-month period of training did not change the mean values although hypomagnesemia was noted in 40% of the post-test sample. It has also been found that 111 mostly moderately-trained females had a mean [TMg] value of 0.79 mmol/L with 14 subjects (12.6%) having values below 0.73 mmol/L (unpublished raw data from Newhouse et al., 1993).

2.6.2 Acute Effects of Exercise on Magnesium Distribution

There is typically a fall in [TMg] after intense exercise (Rayssiguier, Guezennec and Durlach, 1990). Stendig-Lindberg (1991) claimed that low [TMg] is a significant factor in lowering maximum voluntary contraction thus the lowering of [TMg] signifies that strenuous exercise induces Mg^{2+} deficiency. Rose and coworkers (1970) postulated that an observed TMg loss was due to sweat after a marathon race. However, more recent studies indicate that decreases in [TMg] are more likely due to the redistribution of Mg^{2+} from the plasma to the RBCs for working muscles to utilize the Mg^{2+} for energy processes (Al-Ghamdi et al., 1994; Casoni et al., 1990; Costill, Cote and Fink, 1976). This redistribution is apparently short-lived as studies have found that [TMg] usually returns to pre-exercise values within two hours after exercise (Deuster et al., 1987; Lijnen, 1995; Lukaski, 1995a). Yet other studies reveal that the Mg^{2+} shift is from the plasma to other tissue compartments other than the RBCs (Resina et al., 1995). Lijnen and coworkers (1988) suggest that adipose cells uptake the Mg^{2+} in these situations. Dressendorfer, Wade, Keen and Scaff (1982) studied subjects during a 20-day road race: Pre-race [TMg] levels were marginally low, yet commencing on day 2, [TMg] levels were significantly higher and remained that way. Magnesium intake was not controlled in this study and thus it is postulated that a greater Mg^{2+} intake likely caused the higher [TMg] levels during the race. Golf, Happel, Graef and Seim (1984) found that after ergometer exercise, [TMg] was unchanged and rather [EMg] had decreased significantly. It is evident from these conflicting results that much remains to be done to clarify the compartmental shifts of Mg^{2+} during exercise and this can be done by utilizing more of the various aforementioned assays for Mg^{2+} status.

It is evident that studies of exercise's effect on Mg^{2+} status and distribution are lacking due to methodological problems. By the same token it is also difficult to draw conclusions from the literature examining the reverse relationship - that being the effect of Mg^{2+} status or Mg^{2+} supplementation on exercise performance and recovery indices. This relationship will now be discussed.

2.6.3 Magnesium Supplementation's Effects on Exercise

It is well documented that an optimal Mg^{2+} status will be beneficial for exercise (Brilla and Gunter, 1995; Dragani et al., 1995; Ripari, Pieralisi, Giamberardino, Resina and Vecchiet, 1989; Vecchiet et al., 1995). What can be misleading to some is the myth of which the popular press seems to promote, that Mg^{2+} or any other mineral supplementation can improve one's health no matter what one's dietary intake is already (McDonald and Keen, 1988). There are few studies which reveal that excessive Mg^{2+} supplementation actually improves performance. It is important to remember that if an athlete is to suppiement, he or she should be focusing on correcting a Mg^{2+} deficiency. Too many athletes are convinced that by merely taking nutritional supplements their performance will be enhanced. McDonald and Keen claim that excessive Mg^{2+} supplementation is not thought to be a serious problem, yet Al-Ghamdi et al. (1994) show that over 500 mg/day supplemental Mg^{2+} may result in GI difficulties. In one study it was found that after 3 months of supplementation of a multivitamin and mineral supplement including 116 mg of Mg^{2+}, no change in Mg^{2+} status was indicated (Weight, Noakes et al., 1988), and no ergogenic effect was indicated (Weight, Myburgh and Noakes, 1988). Yet these subjects' Mg^{2+} statuses were reported to be in the normal range throughout the study. On the other hand, Classen et al.

(1986) claim that many experiments indicate that Mg^{2+} supplementation may benefit normomagnesemic subjects. The issue with this statement, again, is that measures of Mg^{2+} status may have been inadequate to detect the presence of Mg^{2+} deficiencies in some subjects participating in the investigations.

A prolonged Mg^{2+} deficit may progressively impair athletic performance (Golf, Bohmer and Nowacki, 1993). Ripari et al. (1989) found that supplementation with 4.5 g/day Mg pidolate (a Mg^{2+} salt) resulted in significantly reduced physiological stress in submaximal exercise. Brilla and Gunter (1995) discovered similar results with daily supplementation of 8 mg per kg body weight Mg oxide in a crossover study. Golf and coworkers summarize that Mg^{2+} supplementation can improve performance in many sports either in competition or during maximal and submaximal exercise tests. Indeed, without Mg^{2+}'s effective control over energy transport pathways to provide sufficient energy supply, peak performance is not possible.

The studies just cited did not include any measures of Mg^{2+} status which makes it uncertain whether the subjects were originally deficient or not. Supplementation has been shown to increase performance in athletes with [TMg] at the low end of the normal range. A group of male competitive rowers supplemented with 360 mg/day Mg^{2+} for four weeks showed a decrease in oxygen consumption at the same submaximal workload (Golf et al., 1993). Some subjects with normal [TMg] revealed enhanced maximal volume of oxygen uptake (VO_2max) and physical work capacity with supplementation (Steinacker, Grunert-Fuchs, Steininger and Wodick, 1987; Vecchiet et al., 1995). This phenomenon is, again, most likely due to an intracellular deficiency of Mg^{2+}. These results suggest the potential benefit of Mg^{2+} supplementation on energy metabolism and work efficiency for intense endurance exercise.

The measure of plasma glucose has been found to be decreased during and after intense endurance exercise (MacKinnon and Hooper, 1991). Golf and co-workers (1993) review that physical stress induces glucose uptake by muscle cells and its oxidation to pyruvate and lactate, and therefore is involved in the acceleration of glycolysis. The functions of glucose are insulin-dependent, and insulin, in turn, is dependent on Mg^{2+} for it to bind to the cell membrane receptor for its activation. Thus, an increase in Mg^{2+} supply will increase insulin responsiveness for glucose. Moreover, for glucose to be taken up by muscle cells, hexokinase is needed. This enzyme is also dependent on the presence of Mg^{2+} as previously stated. Seelig (1994) states that low Mg^{2+} levels may also aggravate emotional stress which, in turn, further lowers Mg^{2+} levels. This subsequently leads to a Mg^{2+}-stress cycle whereby the more stressed one gets the more Mg^{2+} is excreted from the body. Thus, stress (whether physical or mental) increases the need for Mg^{2+}.

Golf and colleagues (1993) review that Mg^{2+} also affects lactate metabolism after exercise. They reported that after competitive swimming and rowing, a concurrent accelerated lactate elimination from blood was observed after Mg^{2+} treatment. Seeing as lactate concentration in plasma represents the extent of acidosis and the stress of the lactic acid system during physical stress, lowered post-exercise lactate levels are said to be indicative of improved recovery.

An adverse effect of some exercise is Delayed Onset Muscle Soreness (DOMS) which is a complex of muscle soreness symptoms (Dragani et al., 1995). The symptoms begin about 8-10 hours after exercise, peak at about 24-48 hours, and start to decrease gradually after 3-4 days so that 5-7 days post-exercise, they are gone (Armstrong, 1984). Armstrong hypothesized that the DOMS symptoms start from the rupture of structural proteins and associated connective tissue in

muscle fibres. These symptoms are induced by the high mechanical forces developed during the physical activity. Dragani et al. (1995) go on to discuss the mechanisms of DOMS and how Mg^{2+} is involved in them: Alterations in the cell membrane permeability results in a significant increase in Ca^{2+} influx from the extracellular fluid to the muscle fibres. The Ca^{2+} ions accumulate in the mitochondria inhibiting cell respiratory functions and resulting in painful cramps. An ATP deficit subsequently arises and this makes it more difficult for the ridding of Ca^{2+}. However, Mg^{2+} is known to favour cellular energy production since it is essential in the regulation of metabolic pathways involving ATP and erythrocyte 2,3-DPG (Resina et al., 1995). Therefore, an adequate Mg^{2+} supply serves to effectively counteract the Ca^{2+} influx in the cell at the membrane level to decrease symptoms of DOMS. Dragani et al. undertook a study which tested the hypothesis that a 3-week intake of 387 mg/day Mg pidolate supplement by non Mg^{2+}-deficient subjects would minimize DOMS after eccentric stepping exercises. They found that the treatment group experienced lower spontaneously perceived pain on the first day and that it decreased rapidly remaining constantly less severe than the values found for the placebo group.

It seems that there is an increased energy available to muscle cells as well as a protective effect that goes along with the Mg^{2+} supplementation vs. muscle damage due to exercise. Therefore it is proposed that there is a requirement to have additional Mg^{2+} intake to prevent muscle damage, and a need for supplementation in periods of intense exercise. The controversy with this hypothesis is that the singular measure of [TMg] has been inadequate in detecting deficiencies. Bohmer (1995) reminds us that Mg^{2+} fluxes from the plasma take time to return to pre-exercise values (normally occurring within 24 hours), thus, there must be enough TMg

supply to combat the repeated exercise bouts inherent in training so that an athlete experiences less muscle damage and soreness for improved recovery.

2.7 RECOVERY AND OVERTRAINING VARIABLES

From the literature cited up to this point, it has been indicated that Mg^{2+} supplementation may result in increased effectiveness of recovery from exercise stress. (Recovery is the process of reestablishing homeostasis within the muscle.) The recovery parameters which have already been discussed include oxygen consumption, work capacity, and the concentrations of lactate and glucose in the blood. Although there is considerable speculation, negative changes in these variables are also said to be indicators of overtraining. Overtraining is the condition in which there is an imbalance between exercise and recovery resulting in severe and prolonged fatigue (Kuipers and Keizer, 1988). This condition directly involves performance decrements and ineffective recovery from exercise. Other indicators of overtraining are now discussed.

As alluded to earlier when discussing DOMS, persistent muscle soreness is one valid indicator of chronic physical exertion in athletes. MacKinnon and Hooper (1991) review that an elevated resting heart rate also appears to be a strong indicator. Stray-Gundersen, Videman and Snell (1986) found that when they purposely overtrained runners for two weeks, resting heart rates remained significantly elevated even following another two weeks of recovery. Systolic blood pressure is also a good indicator of overtraining although it is usually not readily available for daily monitoring. However, elevated pulses and blood pressures can stem from other stressors in life, from illnesses, or from certain medications, thus caution must be in interpreting such data.

Loss of appetite and unexplained weight loss are often used as signs of overtraining and should be measured together. The loss of body weight may be due to a decreased appetite and increased resting metabolic rate, both reported to occur with overtraining. Psychological effects have also been related to the syndrome and these include increased irritability, lack of motivation to exercise, and sleep disturbances. These effects may be due to changes in hormones, e.g., cortisol, which is a hormone that is linked to depression.

In addition to all of the markers discussed so far, it has been found that an increased incidence of minor illnesses, GI difficulties, and menstrual irregularities have been linked to overtraining (MacKinnon and Hooper, 1991). These effects may be due to altered metabolism and hormonal status from exercise, or from other forms of stress. Higher incidences of minor injuries may also result from decreased coordination in a fatigued state. Finally, MacKinnon and Hooper advise of the importance of monitoring a wide variety of overtraining and recovery markers since subjects hardly ever exhibit every one of the signs and symptoms which have been mentioned. Studies have been lacking with respect to Mg^{2+} supplementation's effects on certain markers of recovery and overtraining as recorded in a daily training diary thus further study needs to be done in this area.

2.8 SUMMARY

In summary of the literature, it is apparent that some studies show that Mg^{2+} supplementation results in increased performance and recovery measures whereas others do not. It is also noted that most studies involving exercise do not use measures of Mg^{2+} status, or else use insensitive measures in testing for Mg^{2+} deficiencies in subjects. A number of studies have

shown improvements in exercise performance and recovery, yet Mg^{2-} statuses of the subjects involved have been relatively unknown. It is therefore not proven that Mg^{2-} supplementation, beyond the maintenance of an adequate dietary intake of the mineral, is effective in enhancing performance and recovery from exercise. In addition, little research has concentrated on physically active females who may be at the highest risk for Mg^{2-} deficiency.

CHAPTER THREE: METHODS

3.1 SUBJECTS

After informed consent was obtained (Ethics Advisory Committee, Lakehead University), 121 apparently healthy, physically active females between the ages of 17 and 43 residing in the Thunder Bay, Ontario, Canada region, were screened for participation based on iMg status. Those who qualified to continue with the study were twenty marginally Mg^{2+}-deficient subjects and twenty who possessed [iMg] levels in the upper range of normal. The normal range for [iMg] is 0.53 - 0.67 mmol/L (Altura, Shirey et al., 1994). Selection criteria for subjects included:

(1) Having no clinical history of proven or suspected hypersensitivity to Mg^{2+} supplements.
(2) The expectation and willingness to maintain regular physical activity (i.e., performing at least three workouts at or above 75% maximum intensity, for at least 2 hr total, per week) throughout the study.
(3) The expectation and willingness to maintain regular dietary intakes throughout the study.
(4) Having filled out a questionnaire (refer to Appendix A) and consent to participate (refer to Appendix B), and having read the instructions for participants (refer to Appendix C).

Exclusion criteria for subjects included:

(1) Not performing the incremental treadmill test at all four test sessions.
(2) Not ingesting at least 75% of pills given per treatment period (as verified by pill count).
(3) Being ill or injured (enough to prevent exercising) for more than one week during the treatment periods.
(4) Not maintaining a training load equal to $\pm$ 25% of their regular load.

A power test utilizing previous test data on VO_2max values from Brilla and Gunter (1995) indicated the sample size of 40 should be sufficient to reach a power of 0.80 in detecting

an effect size of 3 ml/kg/min. Enrolment continued until the requisite number of subjects had been identified. (The categories based on iMg status were later altered; refer to data analyses section for explanation.)

3.2 PROCEDURES

3.2.1 Test Items

Selected subjects underwent the following tests:

a. Dietary analysis:
   I. Three-day self-reported dietary records (refer to Appendix D)
b. Anthropometrics:
   II. Height
   III. Weight
   IV. Skinfolds (sum of five skinfolds; predicted % body fat - Durnin-Womersley method)
c. Physiological tests:
   V. Resting blood pressure
   VI. Incremental treadmill test with measurement of workload, heart rate, and expired gases - for assessment of VO_2max, anaerobic threshold, maximal workload, and submaximal running efficiency. Blood was sampled pre-test, and 4, 10, 30 min and 24 hr post-test, for assessment of:
      i. [plasma lactate]
      ii. [plasma glucose]
      iii. [iMg]
      iv. [iCa]
      v. [plasma K^+]
      vi. [plasma Na^+]
      vii. [hemoglobin]
      viii. hematocrit
   VII. Anaerobic treadmill test (a test of lactacid capacity performed on a treadmill)
   VIII. Subjective and objective measures of overtraining to be recorded daily in a training diary (refer to Appendix E)

3.2.2 Test Item Protocols

Four testing periods (T1, T2, T3 and T4) were scheduled from October 1996 to February 1997 with testing taking place at the Human Performance Laboratory at Lakehead University. During the weeks of T1 and T3, subjects filled out three consecutive days (including one weekend day) of self-report dietary records. Dietary intakes were assessed using computerized diet analysis software (Diet Analysis Plus™ ©1996, West Publishing Co., St. Paul, MN). The Mg^{2+} supplements were not included in the analyses. The primary interest of this portion of the study was to monitor Mg^{2+} intakes between groups, although macronutrient intake and dietary factors that affect Mg^{2+} absorption were also examined.

Subjects were asked to refrain from unaccustomed strenuous exercise for 48 hours (and any strenuous exercise 24 hours) prior to exercise testing (and the blood withdrawal associated with it). They were also asked to refrain from alcohol consumption 24 hours (and caffeine consumption six hours) prior to testing. They were advised to consume a light meal in carbohydrate 2-3 hours prior to reporting to the exercise tests which took place between 1:00 and 9:00 p.m. Anthropometric measurements were performed as detailed in the Certified Fitness Appraiser Resource Manual [CFARM] (1995) by a Certified Fitness Appraiser. Harpenden calipers were used to measure skinfolds which were taken from the triceps, biceps, subscapular, iliac crest and medial calf regions. Percent body fat was predicted using the Durnin-Womersley method (CFARM).

Resting blood pressure (performed by a Certified Fitness Appraiser using an AMG Med. Professional Series sphygmomanometer), and resting heart rate (using a Polar Vantage XL® heart rate monitor from Polar CIC Inc.), were obtained after the subject rested in the sitting

position for 10 minutes. Blood was collected in 7 ml green-topped Vacutainer® tubes (lithium-heparin added) by antecubital venipuncture (Pendergraph, 1984). The tourniquet was applied gently and released prior to the actual blood draw.

The multi-test Stat Profile™ Ultra Analyzer® model 11-3C (Nova Biomedical Canada Ltd., Mississauga, Ontario) was utilized for the immediate analyses of [iMg], [iCa], [Na^{2-}], [K^{-}], [Hb], Hct, [glucose] and [lactate] from 25 μg of whole blood. The instrument was housed in the same laboratory as the exercise testing, and the analyses were performed by the same technician throughout the study. The precision of the instrument for [iMg] was estimated at $\pm$ 0.03 mmol/L, as determined by repeat testing, both within and between days, on an individual not involved in the study. All analytes were within their respective reference ranges as determined by NOVA quality control substances.

Prior to the incremental treadmill test, the subjects warmed up on the treadmill (Quinton Instruments, Seattle, WA) for five minutes. The initial treadmill speed was 2.22 m/s (5 mph) and was increased by 0.22 m/s (0.5 mph) every minute. The treadmill grade remained horizontal until the subject had completed two workloads past the workload at which the subject's respiratory exchange ratio (expired CO_2 / inspired O_2) passed a value of 1.0. At that time, the speed no longer increased but the grade increased by 2 percent each minute. Subjects continued until exhaustion. Expired gases were sampled and analyzed by a SensorMedics Vmax System® metabolic cart ©1996 (Yorba Linda, CA) with VO_2max and $V_E$peak determined as the highest 30 second mean. Heart rates were monitored by a telemetric heart rate monitor and recorded 30 seconds into each workload. Anaerobic thresholds were assessed via examination of expired gas values according to the method described by Beaver, Wasserman and Whipp (1986). Two

investigators blinded to the study, and familiar with anaerobic threshold estimations, independently analyzed the V_E/V_{CO2} curves to pick out the lowest point. These researchers then met to compare estimates. Discrepancies (more than one minute difference) were resolved by examining other expired gas indices of anaerobic threshold. Efficiency of running at a submaximal workload was assessed by noting the workload during the T1 test that corresponds to 65% of VO_2max. At T2, T3, and T4, the oxygen consumption was noted at this same workload.

The anaerobic treadmill test was performed 48 hours after the incremental treadmill test, and according to the protocol described by Bouchard, Taylor and Dulac (1982). In brief, the test required the subjects to exert a maximal effort on the treadmill with the speed set at 3.31 m/s (7 mph) and the grade set at 20%. Time to exhaustion (approximately 30 to 90 s) was the only variable measured.

Encouragement was given equally to all subjects for all treadmill tests. The protocols described above remained identical for each of the four testing periods. The timing in terms of day of week and time of day were consistent for each subject with a few exceptions to accommodate subjects' schedules.

3.2.3 Schedule of Events for Testing Sessions

The screening of the 121 subjects for [iMg] (and administration of consent forms, questionnaires and instructions) was conducted over three days in early October. Three weeks later, the first testing session (T1) began. Each of the four testing sessions (T1, T2, T3 and T4) involved the following: Day One consisted of the distribution of dietary records (T1 and T3

only), and submission to anthropometric tests, resting heart rate and blood pressure measurement, and an incremental treadmill test with pre-test, and 4, 10, and 30 min post-test blood withdrawal. Day Two involved only the 24 hr post-test blood withdrawal. Day Three consisted of the anaerobic treadmill test, and the dispensing of pills (Mg^{2+} supplement or placebo) (T1 and T3 only), as well as training logs (T1, T2, and T3 only).

3.2.4 Treatment

In a double-blind manner, subjects were randomly assigned to begin treatment with either 212 mg/day (two pills of 106 mg elemental Mg^{2+}) Mg oxide (C.E. Jamieson and Company Ltd., Windsor, Ontario) (Group M/P) or matching placebo (Group P/M). Both the Mg^{2+} supplement and the placebo were in tablet form, and were identical in appearance, consistency, and taste. The placebo contained 222 mg dicalcium phosphate, 12 mg purified stearic acid, 6 mg coscarmellose sodium, 4 mg silicon dioxide, and 276 mg microcrystalline cellulose. Subjects were instructed to consume the contents of one tablet twice per day (one just before breakfast and one just before dinner). The treatment lasted four weeks (28 ± 3 days) which was followed by a 6-week (42 ± 3 days) washout period. Treatments were then reversed for the final four weeks.

3.2.5 Training Diary

A total of 11 subjective and 4 objective variables were recorded daily by the subjects in a standardized diary (refer to Appendix D). The variables were as follows: (1) length of training workout(s), (2) length of intensity section of workout(s), (3) morning rest pulse, (4) morning

body weight, (5) energy, (6) quality of sleep, (7) training or competing willingness, (8) appetite, (9) irritability, (10) muscle soreness, (11) minor illnesses, (12) minor injuries, (13) GI difficulties, (14) menstrual difficulties, and (15) stressful events. Of these, the first two and last five variables were used as control measures whereas the others were dependent (objective or subjective) measures of recovery.

3.3 EXPERIMENTAL DESIGN

A randomized, double-blind, crossover (Mg oxide vs. placebo) design was employed. Testing (identical to that noted above) occurred every four weeks (at T1, T2, T3 and T4). Figure 1 shows the 14-week experimental design including the treatment and washout periods for each group. Subjects were grouped according to both treatment and Mg^{2+} status. There were two treatment groups, one for which the order of pill administration was Mg^{2+} supplement first and placebo second (group M/P), and the other, vice versa (group P/M). As for Mg^{2+} status, subjects were re-classified (from the screening results) into three pools exhibiting either low, medium, or high [iMg] levels. (The reasoning for this is explained in the results section.)

Figure 1. Experimental Design

WEEK #		0	4	10	14
SESSION		T1	T2	T3	T4
GROUP M/P (n=13)	High iMg (n=4)	212 mg Mg oxide	[Washout]	Placebo	
	Medium iMg (n=4)				
	Low iMg (n=5)				
GROUP P/M (n=19)	High iMg (n=8)	Placebo	[Washout]	212 mg Mg oxide	
	Medium iMg (n=6)				
	Low iMg (n=5)				

Note. Group M/P = Mg^{2+}-treated first, placebo-treated second; group P/M = placebo-treated first, Mg^{2+}-treated second.

3.4 DEPENDENT VARIABLES

(1) VO_2max (ml/kg/min)
(2) Maximal workload (% grade)
(3) $V_E$peak (L/min)
(4) Workload at anaerobic threshold (mph)
(5) VO_2 at anaerobic threshold (ml/kg/min)
(6) Anaerobic treadmill test - time to exhaustion (s)
(7) Systolic blood pressure (mmHg)
(8) Diastolic blood pressure (mmHg)
[Remaining measures were performed five times, i.e., pre, 4, 10, 30 min, and 24 hr post-incremental treadmill test]
(9) [iMg] (mmol/L) X 5
(10) [plasma lactate] (mmol/L) X 5
(11) [plasma glucose] (mg/dl) X 5
(12) [Hb] (g/dl) X 5
(13) Hct (%) X 5
(14) [iCa] (mmol/L) X 5
(15) [Na+] (mmol/L) X 5
(16) [K+] (mmol/L) X 5

3.5 DATA ANALYSES

Data analyses were performed utilizing the statistical software of STATISTICA™ ©1995 (StatSoft, Inc., Tulsa, OK). Correlational coefficients were used to establish relationships between the baseline measures of dietary intakes, hematological assays and performance variables. The dietary intakes and training diaries were examined so that any discrepancies in routine would be taken into account. In order to test if a carryover effect was present (i.e., if the washout period was ineffective) an independent t-test was performed on the [iMg] change scores from pre to post-treatment (Armitage and Hills, 1982). Once the carry-over effect was disclaimed (see results section), the data were pooled to test for differences between groups based on treatment (Mg^{2-} or placebo) and/or iMg status (high, medium and low). The differences between treatments were measured using Student's t-test for paired samples (see also Dragani et al., 1995) on change scores from baseline values to treated values.[2] The differences between status groups were measured using repeated measures analyses of variance (ANOVA).[3] The accepted level of significance was $p < .05$ for all statistical tests. Since the analyses did not reveal any differences between groups based on Mg^{2-} status (see results section), results concentrate on the treatment effects.

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[2]Alternative analyses in the form of mixed factorial ANOVAs on nonpooled data were also performed. Appendix H displays these chronological changes over the study.

[3]Alternative analyses in the form of mixed factorial ANOVAs on nonpooled data were also performed.

CHAPTER FOUR: RESULTS

4.1 DESCRIPTION OF SUBJECTS

The characteristics of the 121 subjects screened for the study are listed in Table 2. Of the 121 originally screened for this study, 44 (or 36.4%) were marginally Mg^{2+}-deficient according the criteria set by Altura, Shirey et al. (1994), i.e., they exhibited an [iMg] of less than 0.53 mmol/L. Most subjects maintained their training regimes throughout the study, although there was some seasonal fluctuation, especially as several did not exercise consistently during the washout period which took place over the Christmas season. However, no differences in training, lifestyle, or dietary intake were apparent between treatment groups. Subjects participated in sporting activities which were aerobic (most often running, cross-country skiing and swimming) and/or anaerobic (most often weight training, soccer, basketball and volleyball). All participants possessed regular menstrual patterns and no gastrointestinal distress throughout.

The study was originally planned so that a group of marginally Mg^{2+}-deficient individuals would be compared to a group with adequate stores of the mineral, as determined from screening results. Yet, since for some subjects, [iMg] levels had changed over the 3-week interval between the initial screening and the start of the main study (T1), and the T1 results showed a normal distribution, three groups were formed according to [iMg] level: low (below 0.53 mmol/L) (n = 10), medium (0.53 to 0.58 mmol/L) (n = 10), and high (above 0.58 mmol/L) (n = 12). As mentioned in the methods section, since there were no differences found between groups based on Mg^{2+} status, results focus on the treatment effects.

Before completing the study, seven subjects dropped out from group M/P (n = 13 as a result), and one dropped out from group P/M (n = 19 as a result). Of these eight individuals, two

became injured, three became ill, and three experienced scheduling difficulties; none were included in the analyses. Other data for various parameters were discarded in the case of a temporary equipment failure or subject noncompliance for a particular test.

Table 3 lists the characteristics of the 32 subjects completing the entire study as well as the characteristics of both treatment groups, M/P and P/M, with independent t-tests performed between groups. Since there were no significant differences between treatment groups on baseline measures, further analyses were justified. The mean dietary Mg^{2+} intakes were 320 $\pm$ 123 mg/day at T1 and 333 $\pm$ 127 mg/day at T2. Each of the two sets of dietary analyses revealed that six (or 20%) had intakes less than the RNI amount of 200 mg of Mg^{2+}, and the same number had less than the 70% RDA amount[4]. Complete dietary intake data may be found in Appendix F. There was no correlation between Mg^{2+} intake and [iMg] at T1 ($r = -.27$).

4.2 TEST FOR CARRY-OVER EFFECT

In studies with crossover designs such as in this study, it is necessary to test for a carry-over effect, i.e., to see if the supplemental Mg^{2+} (or the placebo) still carried any effect from the first treatment period to the second treatment period (Armitage and Berry, 1987). Armitage and Hills (1982) also refer to this effect as the 'treatment by period' interaction, where 'treatment' is the factor representing the Mg^{2+} supplementation or placebo, and 'period' is the factor representing the two periods of treatment. An independent t-test was performed between treatment groups on [iMg] change scores from pre to post-treatment ($t_{(30)} = -0.05$, $p > .05$). The

[4] Values over 70% of the RDA amount are in the acceptable range for adequate nutrition (Lukaski, 1995a).

Table 2. Characteristics of screened subjects

PARAMETER	Means ± SD	Range
Age (yrs)	21.5 ± 4.2	17 - 43
Height (cm)	165.8 ± 6.2	151.5 - 178.0
Weight (kg)	63.2 ± 8.2	45.0 - 87.0
Resting [iMg] Level (mmol/L)	0.538 ± 0.040	0.46 - 0.69
Systolic Blood Pressure (mmHg)	109.9 ± 8.8	94 - 130
Diastolic Blood Pressure (mmHg)	66.8 ± 7.9	50 - 90

Note. (121 subjects)

Table 3. Baseline characteristics of subjects completing entire study

PARAMETER	All Subjects	Group M/P	Group P/M	T-test Result
Age (yrs)	21.2 ± 3.1	20.8 ± 1.8	21.4 ± 3.8	$t(30) = -0.46$
Height (cm)	165.5 ± 5.6	166.3 ± 7.2	165.0 ± 4.2	$t(30) = 0.63$
Weight (kg)	61.5 ± 5.3	61.9 ± 7.1	61.2 ± 3.8	$t(30) = 0.34$
Predicted Percent Body Fat (%)	22.5 ± 4.0	23.1 ± 4.4	22.1 ± 3.8	$t(30) = 0.70$
Resting [iMg] Level (mmol/L)	0.567 ± 0.032	0.560 ± 0.037	0.572 ± 0.029	$t(30) = -1.04$
Mg^{2+} Intake (mg/day)	320.4 ± 123.2	305.8 ± 133.5	325.6 ± 115.0	$t(30) = -0.45$
VO_2max (ml/kg/min)	49.8 ± 6.3	49.4 ± 6.8	50.2 ± 6.1	$t(30) = -0.34$
$V_E$peak (L/min)	109.2 ± 12.3	109.3 ± 11.1	109.2 ± 13.3	$t(30) = 0.04$
Maximum Heart Rate (beats per min)	195.1 ± 9.5	200.0 ± 7.2	193.9 ± 9.5	$t(30) = 1.96$
VO_2 at Anaerobic Threshold (ml/kg/min)	37.4 ± 4.6	38.3 ± 4.9	36.7 ± 4.4	$t(30) = 0.94$
Anaerobic Treadmill Test Result (s)	41.7 ± 14.3	41.0 ± 16.8	42.2 ± 12.8	$t(26) = -0.24$
Systolic Blood Pressure (mmHg)	114.3 ± 8.9	116.3 ± 9.9	113.0 ± 8.1	$t(30) = 1.04$
Diastolic Blood Pressure (mmHg)	69.4 ± 9.5	70.2 ± 12.4	69.0 ± 7.3	$t(30) = 0.35$

Note. Group M/P = Magnesium-1st, Placebo-2nd (n = 13); Group P/M = Placebo-1st, Magnesium-2nd (n = 19). Total of 32 subjects or 30 for Mg^{2+} intake; means ± SD. Independent t-tests performed on treatment groups; *$p<.05$.

test did not provide evidence of a treatment by period interaction, thus, data from the two treatment periods could then be pooled (or combined) to form two categories - Mg^{2+}-treated (M), and placebo-treated (P).

4.3 MAGNESIUM STATUS

In the 3-week interval between the initial screening of potential subjects and T1, resting [iMg] significantly increased from 0.532 ± 0.046 mmol/L to 0.567 ± 0.032 mmol/L, $t_{(31)} = 4.70$; $p < .05$ (n=32). Only 15.6% were marginally deficient at T1. The correlation for resting [iMg] levels between screening and T1 was $r = .45$. Correlations between resting [iMg] levels from pre-treadmill test to 24-hr post-test were $r = .55$ (T1), $r = .35$ (T2), $r = .80$ (T3), and $r = .59$ (T4). Pooled data revealed that throughout treatment the increase in resting [iMg] was significantly more for M (+0.044 mmol/L) vs. P (+0.028 mmol/L), $t_{(31)} = 2.28$; $p < .05$ (see also Table 4).[5]

4.4 PERFORMANCE AND RECOVERY

For this section, the reader is, again, referred to Table 4 (or Appendix G for blood indices) for pooled raw data and change scores, and t-tests on the change scores. Values for performance and recovery indices did not differ significantly between treatments. However, a trend was indicated for $V_E$peak values in that they increased slightly more throughout treatment for M (+2.25 L/min) vs. P (-2.01 L/min), $t_{(25)} = 1.75$; $p = .09$.[6]

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[5]The change in [iMg] values over the natural progression of the study is displayed in Appendix H utilizing nonpooled data.

[6]The changes in values for $V_E$peak, VO_2max, and systolic blood pressure over the natural progression of the study are displayed in Appendix H utilizing nonpooled data.

Table 4. Physiological testing parameters by treatment

PHYSIOLOGICAL VARIABLE	TREAT-MENT	RAW SCORES Baseline	Treated	CHANGE Actual	%	T-TEST RESULT
Resting [iMg] (mmol/L)	M	0.563 ± 0.045	0.607 ± 0.037	+0.044	+7.82	t(31) = 2.28*
	P	0.563 ± 0.038	0.591 ± 0.043	+0.028	+4.97	
VO_2max (ml/kg/min)	M	49.66 ± 6.54	50.70 ± 6.65	+1.04	+2.09	t(26) = 0.95
	P	49.77 ± 5.96	50.20 ± 6.69	+0.43	+0.86	
$V_E$peak (L/min)	M	107.21 ± 13.59	109.46 ± 14.47	+2.25	+2.10	t(25) = 1.75
	P	107.54 ± 12.64	105.53 ± 13.95	- 2.01	- 1.87	
Maximal Workload (% grade)	M	3.25 ± 2.63	3.13 ± 2.43	- 0.12	- 3.69	t(28) = -0.14
	P	3.66 ± 1.86	3.59 ± 2.35	- 0.07	- 1.91	
Maximal Heart Rate (beats/min)	M	197.00 ± 8.35	195.81 ± 7.84	- 1.19	- 0.60	t(28) = -0.66
	P	195.34 ± 9.54	195.52 ± 6.99	+0.18	+0.09	
VO_2 at AT (ml/kg/min)	M	38.31 ± 3.95	39.12 ± 4.30	+0.81	+2.11	t(30) = 0.22
	P	38.16 ± 5.40	38.75 ± 4.20	+0.59	+1.55	
Anaerobic Test (s)	M	36.54 ± 14.74	37.18 ± 13.50	+0.64	+1.75	t(27) = 1.06
	P	38.47 ± 11.87	37.80 ± 13.72	- 0.67	- 1.74	
Systolic BP (mmHg)	M	115.09 ± 9.45	110.94 ± 7.72	- 4.15	- 3.61	t(31) = -1.26
	P	114.31 ± 8.53	112.69 ± 8.91	- 1.62	- 1.42	
Diastolic BP (mmHg)	M	68.94 ± 9.03	68.00 ± 7.73	- 0.94	- 1.36	t(31) = -0.36
	P	68.84 ± 6.91	68.59 ± 7.80	- 0.25	- 0.36	

Note. Pooled data based on treatment: M = Magnesium; P = Placebo. Baseline and Treated, tests performed before and after treatment, respectively (means ± SD). Student's t-tests performed on change scores; *p<.05.

CHAPTER FIVE: DISCUSSION

5.1 MAGNESIUM STATUS

Results indicated that four weeks of treatment with 212 mg/day Mg oxide was successful in raising resting [iMg] levels. Previous research has discovered slight but insignificant increases in [TMg] and [EMg] (after three weeks of supplementation with 387 mg/day Mg pidolate) (Vecchiet et al., 1995). It may be that the [iMg] measure is more sensitive than [TMg] or [EMg] as previously claimed (Altura, 1994; Altura, Burack et al., 1994). Therefore, the use of this assay to assess the effects of Mg^{2+} treatment on performance and recovery may be advantageous over other hematological measures of Mg^{2+}.

The significant increase in [iMg] levels from screening to T1 may be due to the subjects' increased awareness and interest about their nutritional habits from their involvement in such a study. Since there were mostly weak correlations between resting [iMg] values, it seems that these levels were fairly labile within individuals. The instrument itself does not appear to account for this as the precision (coefficient of variation) of the ion-selective electrode for Mg^{2+} has been reported to be excellent, i.e., less than 6% for control samples (Altura, Shirey et al., 1994), and indeed quality control samples and repeated sampling on the same blood yielded consistent values in the present study. The variability of [iMg] prevented effective analyses regarding the effects of Mg^{2+} status since subjects could not be consistently classified in the same status group throughout the study. The concept of natural regression toward the mean (Howell, 1987) may have also influenced the analyses as noted by the changes in [iMg] from screening to T1. The variability of [iMg] within individuals over time must be researched in more detail as the assessment, classification and diagnosis of Mg^{2+} status based on these values

are put in jeopardy. Moreover, the ratio of [iMg]/[TMg] has been reported to be consistent within individuals but not so between individuals (Altura, Shirey et al.); adding the [TMg] assay would thus further confirm the precision of the [iMg] measure. It would also be helpful to see whether the [iMg]/[TMg] ratio would be elevated (indicating increased mobilization of Mg^{2+}) in certain individuals. This method may add critical information about the existence of deficiencies in the subject pool (H.C. Lukaski, personal communication, October 7, 1997).

The mean dietary Mg^{2+} intakes of 320 $\pm$ 123 mg/day (for T1) and 333 $\pm$ 127 mg/day (for T2) are well above both the RNI of 200 mg/day and the RDA of 280 mg/day. Altura (1994) claimed that females (without regard to activity level) ingest approximately 175-225 mg/day Mg^{2+} (60-80% of the RDA), while Lukaski (1995a) noted that female athletes take in about 168 - 182 mg/day of the mineral (60-65% of the RDA). It seems that participants in this study possess Mg^{2+} intakes which are higher than normal. Apart from being regular exercisers between the ages of 17 and 29, the subjects appeared to be quite diverse in regards to dietary habits. Some subjects gave conscientious effort to obtaining a healthy diet whereas others tended toward highly refined convenience foods that often lack Mg^{2+} in adequate amounts. In addition, since there was no correlation between Mg^{2+} intakes and [iMg] levels ($r = -.27$), it may be that there is a wide variability in the way in which Mg^{2+} is metabolized. This may have meant that the supplementation would have had less effect on some participants.

5.2 PERFORMANCE AND RECOVERY

There were no significant effects of Mg^{2+} supplementation on performance during (or recovery from) aerobic or anaerobic exercise. Previous studies have discovered benefits of Mg^{2+}

treatment on VO_2max (Steinacker et al., 1987), total workload (Vecchiet et al., 1995), and time to exhaustion (Brilla and Gunter, 1995). Magnesium treatment has also been shown to improve submaximal performance by decreasing submaximal VO_2, V_E, and HR (Brilla and Gunter; Ripari et al., 1989). Yet all of these studies involved higher dosages of Mg^{2+} supplementation and either reported subjects with lower mean Mg^{2+} intakes or did not report intakes at all. Moreover, only Vecchiet and colleagues incorporated the highly controlled crossover experimental design as utilized in this study. These choices may have contributed extensively to reporting significant effects. In addition, it is difficult to speculate on the possibility of Mg^{2+} deficiencies in the research of Brilla and Gunter as well as that of Ripari and coworkers since Mg^{2+} status was not assessed. The results of the present study are more in line with those of Weight, Noakes et al. (1988). These investigators utilized a crossover design and a lower dose of Mg^{2+} (116 mg/day) and found no significant effects on performance in subjects with high Mg^{2+} intakes (372 $\pm$ 122 mg/day).

On the other hand, it is possible that the present study is lacking in certain areas which may have been critical for obtaining positive effects of Mg^{2+} supplementation; this is now discussed. The relatively high mean Mg^{2+} intake of the participants and the relatively low dosage of Mg^{2+} supplementation may have prevented the opportunity for significant increases in absorption of the mineral in the body. Although this study built on previous research in the area by utilizing the sensitive [iMg] measure to assess Mg^{2+} status, the discovery that [iMg] appears to be rather labile compromises its usefulness (at least until further research can elucidate all of the physiological factors contributing to the variability). As well, since reference ranges for [iMg] levels have not yet been well-established, it is difficult to confirm whether some subjects

were initially Mg^{2+}-deficient or not. It is still hypothesized that if those initially marginally Mg^{2+}-deficient had normal and controlled dietary intakes, they would benefit from supplementation with possibly a higher dosage of Mg^{2+}.

5.3 CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

This study concluded that four weeks of 212 mg/day Mg oxide supplementation significantly improved resting [iMg] levels but not performance or recovery in a group of physically active females. The conclusions appear to hold true regardless of initial iMg status (normal or marginally-deficient). Possible reasons for which the present study did not note a significant improvement in performance and recovery from exercise are as follows: Dietary intakes were higher than normal, the Mg^{2+} dosage (212 mg/day) may have been too low, and [iMg] levels were shown to be quite labile proving it difficult to diagnose deficiencies of the mineral. It may also be that the supplementation would have had less effect on some participants due to the wide variability in the way in which Mg^{2+} is metabolized. However, it appears that the [iMg] measure is more sensitive than [TMg] or [EMg] warranting the use of this assay to assess the effects of Mg^{2+} treatment in athletic situations.

This study was unique in utilizing the [iMg] assay in testing physically active females on exercise performance and recovery. A major recommendation for further research is to conduct repeated [iMg] assays within individuals to elucidate the degree of variability in [iMg] and to determine the causes of it. In addition, reference ranges for [iMg] must be described in more detail. Future studies similar to this should incorporate additional indices of Mg^{2+} status, indices of muscle damage, controlled diet and training regimes, more frequent dietary assessments,

different doses of Mg^{2-}, and blood sampling during treadmill testing. Also, an extra group may be added for control to which no treatments are given.

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APPENDICES

Appendix A

QUESTIONNAIRE

Name: _____ Age: _____

Please fill out the following questions as completely and accurately as possible.

1. Record the number of hours you spend exercising in an average week (7 days). Any exercising is applicable if it is performed at least a moderate (65% of maximum) intensity. Do not include stretching or lightly warming up or cooling down. If your weekly value varies during the year, record the suspected amount of hours that you would exercise during this study (autumn and winter of 1996).

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2. Record the number of sessions you spend exercising in an average week (as above).

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3. List each sport or type of exercise you are presently involved in plus the number of years you have been participating in each.

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4. Name all vitamin and/or mineral supplements you are presently taking. (Include brand names, names of vitamins and minerals, and quantities of each, if known.)

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5. List all medications and oral contraceptives taken presently.

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6. Have you had a history of menstrual difficulties (i.e., if menses are in excess or in absentia)?

-
-

7. List all known food allergies that you have.

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8. List all known medical conditions that you have had in your lifetime (including alcoholism, diabetes, etc.).

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Appendix B

PARTICIPANT CONSENT FORM

I, _____ (please print name), hereby consent to participate in this research study involving magnesium supplementation and its effect on exercise performance and recovery.

The purpose and procedures involved have been thoroughly explained to me. I will do my best in maintaining a consistent routine with respect to diet and training. I will not take any extra magnesium supplements for 4 weeks prior to the study as well as during the study. I will take 2 capsules of a supplement or placebo daily for 28 days at a time. I realize that at any, time, and for any reason, I can withdraw from the study.

Any risks involved in this study have been explained to me. The treatment dosage of magnesium (200 mg/day) is the Canadian Recommended intake and should pose no negative side effects. Blood sampling will be conducted by a phlebotomist. The amount of blood drawn will be small and there will be little discomfort with the procedure. There may be slight bruising and/or tenderness at the point of puncture. The treadmill tests involve maximal effort and thus the discomfort associated with temporary exhaustion will be felt. In healthy individuals this type of exercise carries no risk.

The data derived from individual subjects will remain confidential and publication of the results will not reveal subject identity as the subjects will be referenced by number. Upon the completion of the study, all subjects will be briefed on their individual results and, upon request, receive a summary of the project.

_____ _____

Signature of participant Date

Appendix C

INSTRUCTIONS FOR PARTICIPANTS

Please read carefully over all of the following instructions and ask the experimenters if any clarifications are needed. Before going any further, you will not be able to participate in this study if:

1) you are pregnant, and/ or
2) you have had any clinical history of proven or suspected hypersensitivity to Mg supplements (e.g., diarrhea), and/ or
3) you will be leaving the Thunder Bay area before the end of this study (which may extend into January or February, 1997).

1. Sign the consent form and fill out the questionnaire.

THE FOLLOWING ONLY APPLY TO THE RESULTANT 40 SUBJECTS:

2. Refrain from unaccustomed strenuous exercise for 48 hours and any strenuous exercise for 24 hours (and alcohol or caffeine products for 6 hours) prior to each treadmill test. Fill out a PAR-Q questionnaire for fitness testing upon arrival.

3. Consume a light carbohydrate meal (e.g., cereal and milk, and/or toast and/or fruit) about 2 to 3 hours prior to each treadmill test.

4. Fifty-six tablets will be given to you at the end of the first testing session as well as the third. The supplements are to be taken during each subsequent 4 week period. During these periods, ingest two tablets daily, one just before breakfast, and one just before dinner. No supplements are to be taken during the period in between the second and third testing sessions.

5. Leave the training log beside your bed so that you can fill out the first part of it in the morning upon awakening and the other part in the evening before retiring.

A) In order to get an accurate resting heart rate, lay down 5 minutes and then take pulse at wrist or neck and count the number of beats in 30 seconds. Multiply by 2 to get beats per minute.
B) Measure morning body weight (unclothed) before any food intake or excretion. Note if it is measured in kilograms or pounds and round off to the nearest half-kilogram or to the nearest pound. Use the same scale each day.
C) To record the number of minutes spent exercising, do not include the time spent stretching or lightly warming up or cooling down.
D) To record the number of minutes in the intensity section of exercise, the effort exerted during these sessions should prevent you from carrying on a normal conversation.

6. Keep exercise and dietary habits consistent throughout the study period. Report any major changes in these habits in the daily diary.

7. To make, confirm, or cancel your appointments for testing, or to ask any questions regarding the study, please call Eric at 343-8187 (graduate student office) or at 345-7025 (home). Please leave a message if I am not available.

Appendix D

DIETARY INTAKE FORM

The information we can produce for you is only as accurate as the information you provide. Please, therefore, **follow these instructions to the letter**. We will analyze 3 days. Two of these days will be weekdays and one day will be on a weekend (e.g., a Thursday, Friday and Saturday). Try to consume the amount and types of foods and drinks you would normally consume. Try to record the amounts as accurately as possible, i.e., weighing with a scale, using a measuring cup, using relative size (e.g., med. banana), and reporting each ingredient if item content is vague (e.g., 10 inch pizza with green peppers, olives and pepperoni). Also report, for example, if bread is white or whole wheat, milk is skim or whole, etc. Use back of page if needed.

Name: _____

Date/ day/ time	Amount	Brand Name	Food or Drink Item

Appendix E

TRAINING LOG

Group (A or B): _____ Session (1st, 2nd or 3rd): _____
Name: _____ Start and finish dates of session: _____

* Please fill out this training log as completely and accurately as possible.
* For questions with multiple choices (e.g., #3), **shade in** the most appropriate box.
* For questions with scales, normal = with respect to **only yourself.**
* For questions # 4, 12, 13, 14 & 15, **shade in** the box for any day that symptoms are occurring.
* Fill out first 4 questions (just this page) **every morning** upon awakening.

1. Morning resting heart rate (beats per minute) (Multiply 30 second count by 2)

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
X																												

2. Morning body weight (Indicate if in lb. or kg) (Record to nearest lb. or nearest half-kg)

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
X																												

3. Quality of sleep (1 = very, very low; 2 = very low; 3 = low; 4 = normal; 5 = high; 6 = very high; 7 = very, very high)

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
7																												
6																												
5																												
4																												
3																												
2																												
1																												

4. Minor illnesses (e.g.,cold, flu)

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
X																												

Comments regarding above (i.e., illness types and symptoms experienced)

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* Fill out the remaining questions **every evening** just before retiring to bed

5. Length of exercise session(s) (minutes)

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
X																												

6. Length of intensity section of exercise session(s) (minutes)

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
X																												

7. General energy level (1 = very, very low; 2 = very low; 3 = low; 4 = normal; 5 = high; 6 = very high; 7 = very, very high)

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
7																												
6																												
5																												
4																												
3																												
2																												
1																												

8. Training or competition willingness (1 = very, very low; 2 = very low; 3 = low; 4 = normal; 5 = high; 6 = very high; 7 = very, very high)

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
7																												
6																												
5																												
4																												
3																												
2																												
1																												

9. Appetite (1 = very, very low; 2 = very low; 3 = low; 4 = normal; 5 = high; 6 = very high; 7 = very, very high)

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
7																												
6																												
5																												
4																												
3																												
2																												
1																												

10. Irritability level (1 = very, very low; 2 = very low; 3 = low; 4 = normal; 5 = high; 6 = very high; 7 = very, very high)

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
7																												
6																												
5																												
4																												
3																												
2																												
1																												

11. Level of muscle soreness (1 = very, very much; 2 = very much; 3 = much ; 4 = normal; 5 = little ; 6 = very little; 7 = very, very little)

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
7																												
6																												
5																												
4																												
3																												
2																												
1																												

12. Minor injuries (e.g., shin splints)

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
X																												

Comments regarding above (i.e., injury types)

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13. Gastrointestinal difficulties

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
X																												

Comments regarding above (e.g., diarrhea)

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14. Menstruation

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
X																												

Comments regarding above (e.g., excessive)

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15. Major stressful events

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
X																												

Comments regarding above (e.g., exams, job and family troubles)

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16. Additional Comments (e.g., major changes in sleep or dietary routine)

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Appendix F

DAILY DIETARY CHARACTERISTICS OF PARTICIPANTS

DIETARY PARAMETER	T1	T3	FEMALE (19-24) NEEDS RNI (CAN)	RDA (US)
Energy (kcal)	2196 ± 765	2145 ± 791	—	—
Carbohydrates (g)	328 ± 124	319 ± 110	289	275
Proteins (g)	70.5 ± 29.7	73.7 ± 25.8	43	46
Fats (g)	65.8 ± 35.2	66.9 ± 44.3	70	73
% Carbohydrate	59.8 ± 9.8	59.4 ± 10.5	72	70
% Protein	12.8 ± 2.5	13.8 ± 2.6	11	12
% Fat	26.3 ± 8.1	26.2 ± 9.7	17	18
Fibre (g)	22.7 ± 9.9	22.4 ± 9.7	—	—
Cholesterol (mg)	189 ± 158	183 ± 152	—	300
Magnesium (mg)	320 ± 123	333 ± 127	200	280
Calcium (mg)	994 ± 352	1029 ± 434	700	1200
Sodium (mg)	2953 ± 1190	2796 ± 1170	—	500
Potassium (mg)	3211 ± 1756	3615 ± 1567	—	2000
Iron (mg)	16.0 ± 6.3	17.1 ± 8.8	13	15
Zinc (mg)	9.27 ± 3.50	9.64 ± 3.72	—	—
Phosphorus (mg)	1261 ± 365	1327 ± 486	850	1200
Vitamin A (RE)	1635 ± 1532	1726 ± 930	800	800
Thiamin-B1 (mg)	1.99 ± 1.05	2.40 ± 1.28	0.8	1.1
Riboflavin-B2 (mg)	2.19 ± 0.98	2.33 ± 0.96	1.1	1.3
Niacin-B3 (mg)	19.2 ± 6.9	24.0 ± 10.4	15.0	15.0
Vitamin B6 (mg)	1.75 ± 0.73	2.22 ± 0.96	—	1.6
Vitamin B12 (μg)	2.81 ± 1.53	3.50 ± 1.82	2.0	2.0
Vitamin C (mg)	217 ± 352	268 ± 236	30	60
Vitamin D (μg)	4.23 ± 2.18	5.85 ± 4.10	—	—
Vitamin E (mg)	6.92 ± 4.99	5.47 ± 3.07	—	—

Note. (30 subjects); means ± SD

Appendix G

HEMATOLOGICAL INDICES BY TREATMENT

PHYSIOLOGICAL VARIABLE	TREAT-MENT	RAW SCORES Baseline	Treated	CHANGE Actual	%	T-TEST RESULT
Resting [iMg] (mmol/L)	M	0.563 ± 0.045	0.607 ± 0.037	+0.044	+7.82	t(31) = 2.28*
	P	0.563 ± 0.038	0.591 ± 0.043	+0.028	+4.97	
4 min post-test [iMg] (mmol/L)	M	0.549 ± 0.043	0.597 ± 0.045	+0.048	+8.74	t(30) = 0.33
	P	0.543 ± 0.042	0.586 ± 0.042	+0.043	+7.92	
10 min post-test [iMg] (mmol/L)	M	0.526 ± 0.039	0.569 ± 0.038	+0.043	+8.17	t(30) = 0.16
	P	0.523 ± 0.036	0.562 ± 0.036	+0.039	+7.46	
30 min post-test [iMg] (mmol/L)	M	0.512 ± 0.034	0.553 ± 0.027	+0.041	+8.01	t(27) = 0.29
	P	0.506 ± 0.032	0.543 ± 0.031	+0.037	+7.31	
24 hr post-test [iMg] (mmol/L)	M	0.576 ± 0.040	0.623 ± 0.034	+0.047	+8.16	t(27) = -0.09
	P	0.569 ± 0.035	0.616 ± 0.054	+0.047	+8.27	
Resting lactate (mmol/L)	M	1.48 ± 0.49	1.24 ± 0.50	- 0.24	-16.22	t(28) = -1.17
	P	1.28 ± 0.45	1.24 ± 0.40	- 0.04	- 3.13	
4 min post-test lactate (mmol/L)	M	12.12 ± 3.18	12.71 ± 3.01	+0.59	+4.87	t(28) = 1.36
	P	12.45 ± 3.49	11.95 ± 3.72	- 0.50	- 4.03	
10 min post-test lactate (mmol/L)	M	10.41 ± 2.97	10.09 ± 3.41	- 0.32	- 3.07	t(28) = 0.98
	P	10.30 ± 3.59	9.24 ± 3.42	- 1.06	-10.30	
30 min post-test lactate (mmol/L)	M	4.04 ± 1.49	3.64 ± 1.34	- 0.40	- 9.90	t(26) = 0.75
	P	4.20 ± 1.77	3.54 ± 1.60	- 0.66	-15.71	
24 hr post-test lactate (mmol/L)	M	1.49 ± 0.92	1.44 ± 0.58	- 0.05	- 3.36	t(23) = 0.12
	P	1.60 ± 0.98	1.53 ± 0.91	- 0.07	- 4.38	
Resting glucose (mg/dl)	M	105.45 ± 12.93	112.21 ± 9.75	+6.76	+6.41	t(27) = 0.36
	P	103.33 ± 9.87	107.30 ± 19.09	+3.97	+3.84	
4 min post-test glucose (mg/dl)	M	136.76 ± 21.48	143.21 ± 18.06	+6.45	+4.72	t(27) = 1.18
	P	141.37 ± 17.00	140.83 ± 20.17	- 0.54	- 0.38	
10 min post-test glucose (mg/dl)	M	129.52 ± 19.47	134.83 ± 17.22	+5.31	+4.10	t(27) = 0.56
	P	131.53 ± 18.85	132.50 ± 18.52	+0.97	+0.74	
30 min post-test glucose (mg/dl)	M	104.64 ± 15.99	110.36 ± 10.62	+5.72	+5.47	t(25) = 0.26
	P	106.93 ± 15.21	111.71 ± 19.05	+4.78	+4.47	
24 hr post-test glucose (mg/dl)	M	94.13 ± 17.25	101.87 ± 15.45	+7.74	+8.22	t(26) = 1.47
	P	92.50 ± 13.00	96.43 ± 11.12	+3.93	+4.25	

Note. Pooled data based on treatment: M = Magnesium; P = Placebo. Baseline and Treated, tests performed before and after treatment, respectively (means ± SD). Student's *t*-tests performed on change scores; *p<.05.

Appendix G (Continued)

HEMATOLOGICAL INDICES BY TREATMENT

PHYSIOLOGICAL VARIABLE	TREAT-MENT	RAW SCORES Baseline	Treated	CHANGE Actual	%	T-TEST RESULT
Resting Ca^{2+} (mmol/L)	M	1.21 ± 0.03	1.22 ± 0.03	+0.01	+0.83	t(29) = -1.01
	P	1.20 ± 0.04	1.21 ± 0.03	+0.01	+0.83	
4 min post-test Ca^{2+} (mmol/L)	M	1.22 ± 0.03	1.22 ± 0.03	------	------	t(30) = -0.42
	P	1.21 ± 0.04	1.22 ± 0.04	+0.01	+0.83	
10 min post-test Ca^{2+} (mmol/L)	M	1.18 ± 0.03	1.19 ± 0.03	+0.01	+0.85	t(30) = 1.30
	P	1.19 ± 0.03	1.19 ± 0.03	------	------	
30 min post-test Ca^{2+} (mmol/L)	M	1.19 ± 0.03	1.19 ± 0.03	------	------	t(27) = -1.61
	P	1.18 ± 0.02	1.19 ± 0.03	+0.01	+0.85	
24 hr post-test Ca^{2+} (mmol/L)	M	1.21 ± 0.04	1.22 ± 0.03	+0.01	+0.83	t(27) = -1.29
	P	1.21 ± 0.03	1.22 ± 0.03	+0.01	+0.83	
Resting Na^{+} (mmol/L)	M	143 ± 2.3	143 ± 1.7	------	------	t(29) = 0.36
	P	143 ± 1.8	143 ± 1.6	------	------	
4 min post-test Na^{+} (mmol/L)	M	145 ± 1.8	145 ± 1.7	------	------	t(30) = 0.84
	P	145 ± 1.8	145 ± 2.2	------	------	
10 min post-test Na^{+} (mmol/L)	M	144 ± 1.8	144 ± 1.4	------	------	t(30) = 1.22
	P	144 ± 1.8	144 ± 1.9	------	------	
30 min post-test Na^{+} (mmol/L)	M	144 ± 1.6	144 ± 1.6	------	------	t(27) = 0.30
	P	144 ± 1.5	144 ± 1.7	------	------	
24 hr post-test Na^{+} (mmol/L)	M	144 ± 1.8	144 ± 1.7	------	------	t(27) = -0.46
	P	144 ± 1.2	144 ± 1.7	------	------	
Resting K^{+} (mmol/L)	M	4.26 ± 0.39	4.34 ± 0.32	+0.08	+1.88	t(28) = 0.69
	P	4.24 ± 0.29	4.27 ± 0.30	+0.03	+0.71	
4 min post-test K^{+} (mmol/L)	M	4.08 ± 0.30	4.15 ± 0.26	+0.07	+1.72	t(30) = -0.66
	P	3.99 ± 0.25	4.11 ± 0.22	+0.12	+3.01	
10 min post-test K^{+} (mmol/L)	M	4.12 ± 0.31	4.23 ± 0.30	+0.11	+2.67	t(30) = -0.04
	P	4.09 ± 0.27	4.20 ± 0.23	+0.11	+2.69	
30 min post-test K^{+} (mmol/L)	M	4.37 ± 0.28	4.49 ± 0.33	+0.12	+2.75	t(27) = -0.67
	P	4.27 ± 0.29	4.47 ± 0.33	+0.20	+4.68	
24 hr post-test K^{+} (mmol/L)	M	4.33 ± 0.33	4.45 ± 0.33	+0.12	+2.77	t(27) = -0.21
	P	4.28 ± 0.30	4.41 ± 0.28	+0.13	+3.04	

Note. Pooled data based on treatment: M = Magnesium; P = Placebo. Baseline and Treated, tests performed before and after treatment, respectively (means ± SD). Student's t-tests performed on change scores; *p<.05.

Appendix G (Continued)

HEMATOLOGICAL INDICES BY TREATMENT

PHYSIOLOGICAL VARIABLE	TREAT-MENT	RAW SCORES Baseline	Treated	CHANGE Actual	%	T-TEST RESULT
Resting Hb (g/dl)	M	13.78 ± 0.70	13.83 ± 0.68	+0.05	+0.36	t(29) = 0.81
	P	14.01 ± 0.65	13.94 ± 0.66	- 0.07	- 0.50	
4 min post-test Hb (g/dl)	M	15.16 ± 0.86	15.17 ± 0.92	+0.01	+0.07	t(30) = -0.44
	P	15.18 ± 0.73	15.28 ± 0.80	+0.10	+0.66	
10 min post-test Hb (g/dl)	M	14.85 ± 0.92	14.86 ± 0.92	+0.01	+0.07	t(30) = -0.37
	P	14.85 ± 0.68	14.93 ± 0.74	+0.08	+0.54	
30 min post-test Hb (g/dl)	M	14.08 ± 0.84	14.20 ± 0.86	+0.12	+0.85	t(26) = 0.30
	P	14.12 ± 0.69	14.22 ± 0.69	+0.10	+0.71	
24 hr post-test Hb (g/dl)	M	13.87 ± 0.74	13.79 ± 0.88	+0.08	+0.58	t(26) = -0.81
	P	13.74 ± 0.84	13.91 ± 0.75	+0.17	+1.24	
Resting Hct (% RBCs)	M	41.35 ± 2.14	41.47 ± 2.17	+0.12	+0.29	t(29) = 0.83
	P	42.10 ± 1.97	41.88 ± 2.06	- 0.22	- 0.52	
4 min post-test Hct (% RBCs)	M	45.50 ± 2.57	45.44 ± 2.73	- 0.06	- 0.13	t(30) = -0.67
	P	45.52 ± 2.08	45.84 ± 2.29	+0.32	+0.70	
10 min post-test Hct (% RBCs)	M	44.53 ± 2.84	44.63 ± 2.67	+0.10	+0.22	t(30) = -0.40
	P	44.52 ± 2.06	44.78 ± 2.18	+0.26	+0.58	
30 min post-test Hct (% RBCs)	M	42.25 ± 2.42	42.58 ± 2.58	+0.33	+0.78	t(27) = 0.35
	P	42.30 ± 2.10	42.58 ± 2.06	+0.28	+0.66	
24 hr post-test Hct (% RBCs)	M	41.63 ± 2.24	41.42 ± 2.67	- 0.21	- 0.50	t(25) = -0.14
	P	41.50 ± 2.06	41.71 ± 2.27	+0.21	+0.51	
Resting systolic BP (mmHg)	M	115.09 ± 9.45	110.94 ± 7.72	- 4.15	- 3.61	t(31) = -1.26
	P	114.31 ± 8.53	112.69 ± 8.91	- 1.62	- 1.42	
Resting diastolic BP (mmHg)	M	68.94 ± 9.03	68.00 ± 7.73	- 0.94	- 1.36	t(31) = -0.36
	P	68.84 ± 6.91	68.59 ± 7.80	- 0.25	- 0.36	

Note. Pooled data based on treatment: M = Magnesium; P = Placebo. Baseline and Treated, tests performed before and after treatment, respectively (means ± SD). Student's t-tests performed on change scores; *p<.05.

Appendix H

ANALYSES OF VARIANCE FOR NONPOOLED DATA

Figure 1. Resting [iMg]: Mixed factorial ANOVA: $F_{(3,90)} = 1.82$, $p > .05$

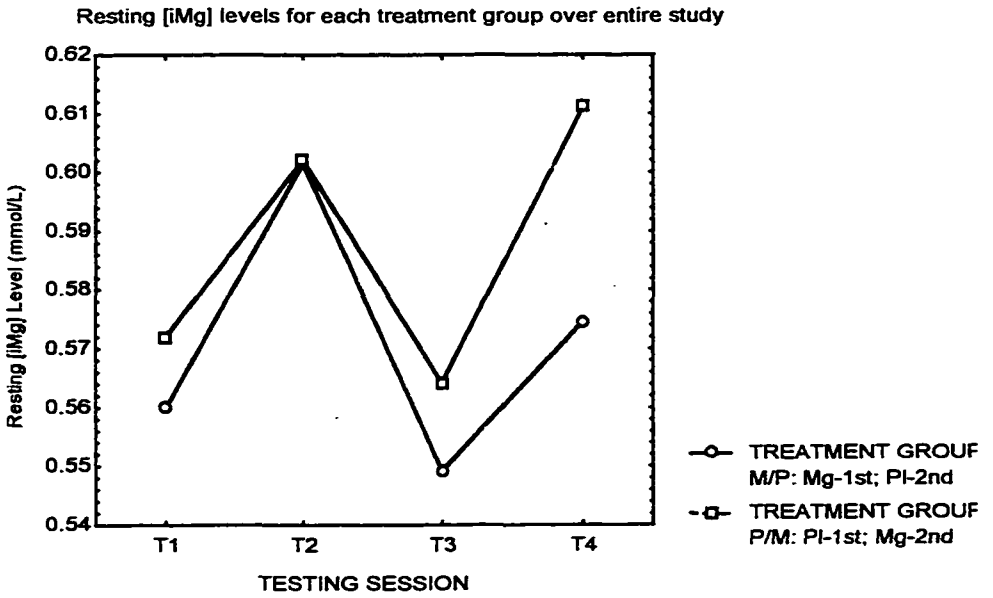
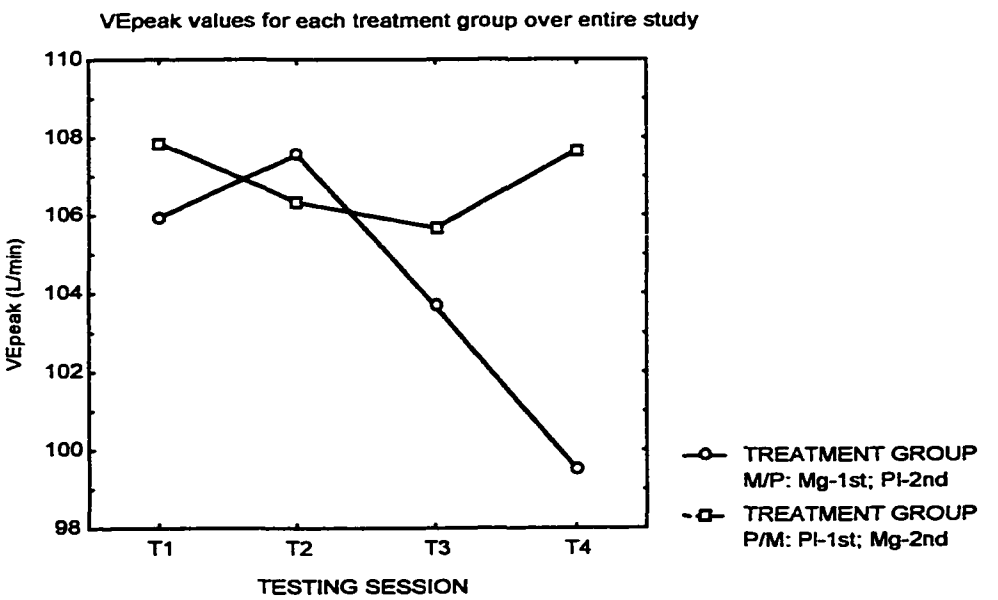


Figure 2. Peak ventilation: Mixed factorial ANOVA: $F_{(3,72)} = 2.51$, $p > .05$



Appendix H (Continued)

ANALYSES OF VARIANCE FOR NONPOOLED DATA

Figure 3. Maximum volume of oxygen uptake: Mixed factorial ANOVA: $F_{(3,75)} = 1.89$, $p > .05$

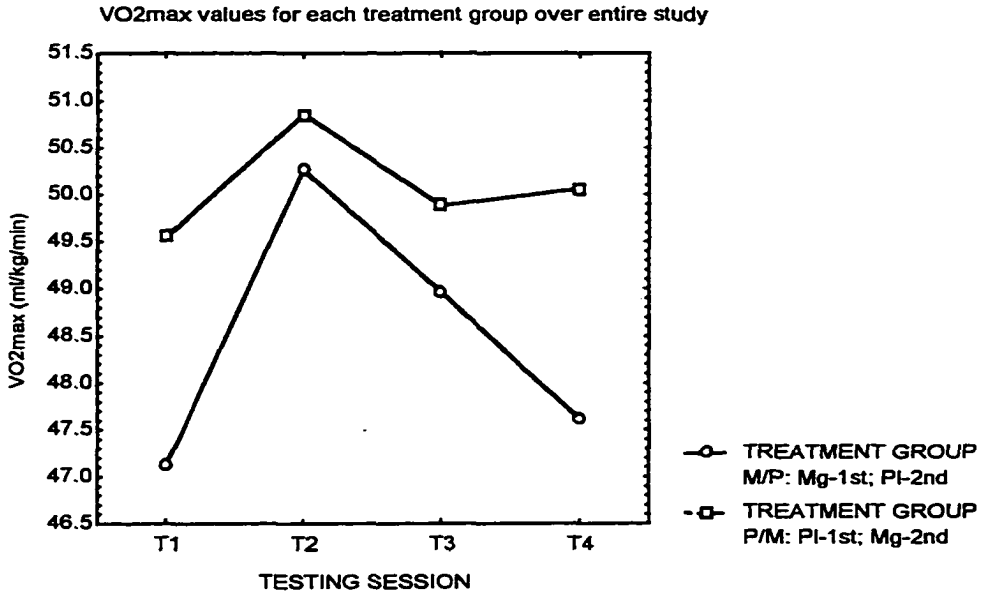


Figure 4. Resting systolic blood pressure: Mixed factorial ANOVA: $F_{(3,72)} = 2.51$, $p > .05$

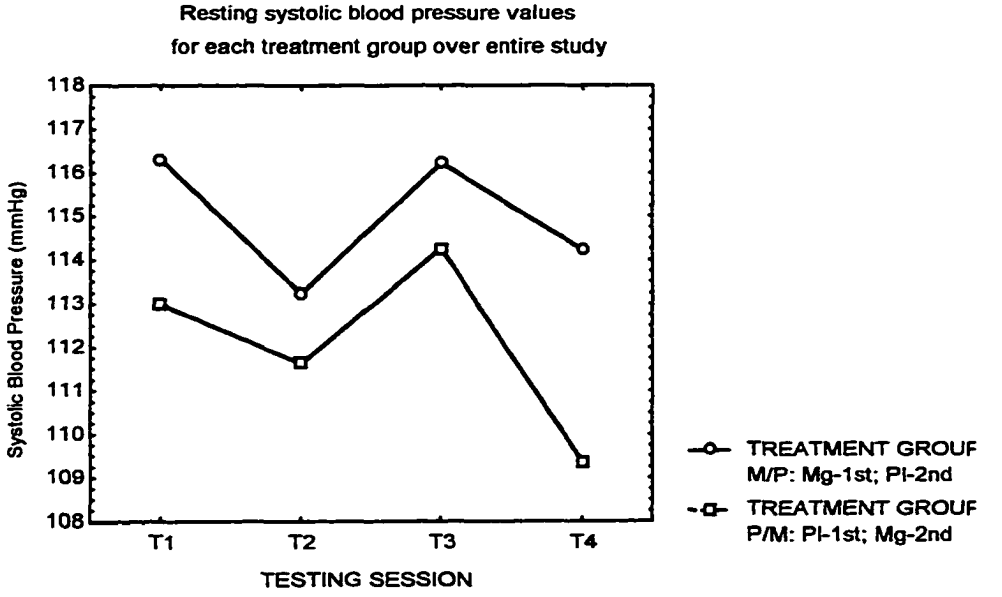
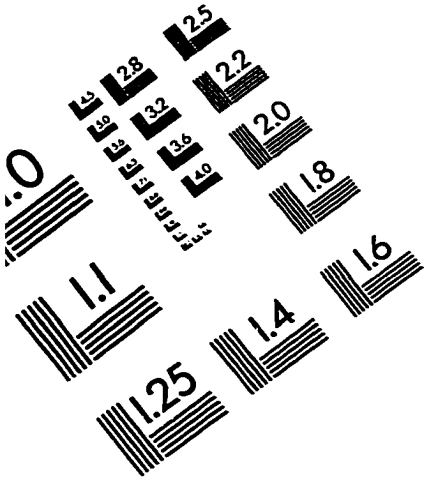
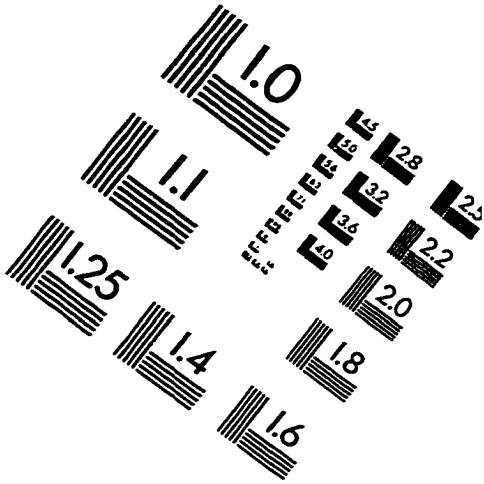
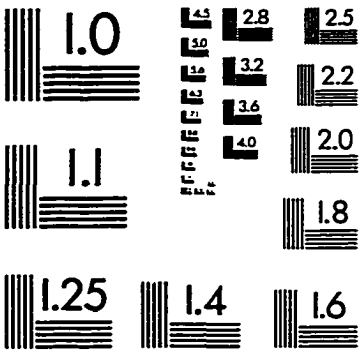
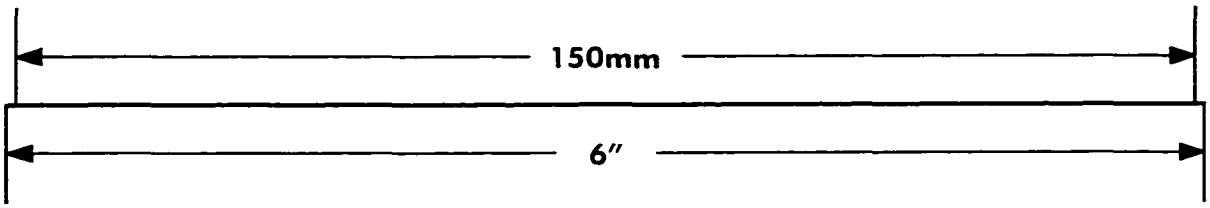


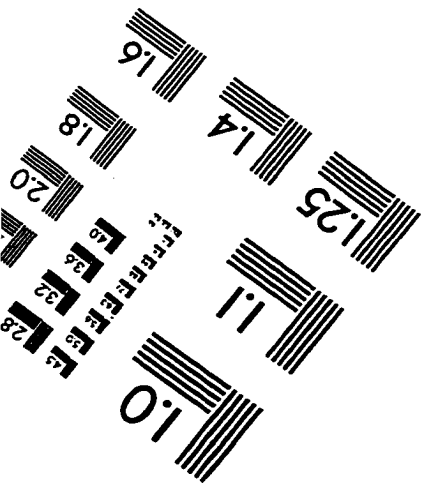
IMAGE EVALUATION TEST TARGET (QA-3)

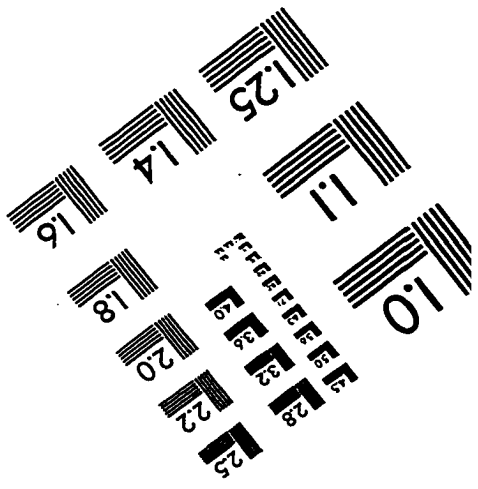












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