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VARIABILITY OF IONIC MAGNESIUM

by

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A Thesis

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ABSTRACT

With the invention of the ion-selective electrode, ionic magnesium (iMg), the biologically active form of Mg, is a common blood assay. There is some evidence that iMg has considerable within subject variability. To further assess this, blood samples were collected from 13 healthy volunteers 6X/day (7:00 – 22:00, every 3 hours) for 3 consecutive days and analysed for iMg (NOVA Stat 8 Analyser). Individual ranges averaged .08 mmol/L (range .05 to .14). Coefficients of variation (CV) ranged from 3% to 7% (mean 4%) while analytical variation was determined to be 2.3%. Biological variability thus accounts for almost half of the variability which is clinically significant as 9 of the 13 subjects recorded at least one value below a reference range of .46 – .60 mmol/L. A significant within day variation ($p<.001$) was noted, with differences between 7:00 and 10:00 as well as 10:00 and 22:00. Between day variations were not significant ($p=.56$). A plausible explanation of this data is that iMg has a circadian rhythm, but a sinusoidal curve of best fit computed for each subject did not correlate highly to iMg ($r=.20$). Thus, cautious interpretation of iMg values is warranted until future research determines the nature of iMg variability.

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Chapter One: Introduction

1.1 STATEMENT OF THE PROBLEM

With the invention of the ionic sensitive electrode (ISE), ionic magnesium (iMg) has recently become a common measure for magnesium assessment and has the potential of becoming the most accepted method. However, some evidence has shown that iMg may be quite variable and thus not a reliable measure. Therefore, the primary purpose of this study is to analyze the variability of iMg to determine its utility. If iMg is variable within a day, a secondary purpose will be to determine if a diurnal pattern exists over the course of three days.

1.2 IMPORTANCE OF THE PROBLEM

The rationale for studying the variability of iMg lies in the physiological importance of magnesium, the prevalence of Mg deficiencies and the emergence of iMg as a potentially sensitive measure of Mg status. The usefulness of iMg in both clinical or research settings hinges on the establishment of the assay's variability.

Magnesium is the second most abundant cation found in the intracellular fluid and the fourth most abundant cation in the extracellular fluid (Lewenstam, Blomqvist & Ost, 1994). It is a cofactor for more than 325 enzymatic reactions including adenosine triphosphate (ATP) metabolism, glucose utilization, muscle contraction and synthesis of fat, protein, and nucleic acids (Altura & Altura, 1994). It is also involved in intermediary metabolism, neuromuscular activity, secretion, excitation-secretion coupling, cardiovascular health and bone metabolism (Muneyyirci-Delale, Nacharaju, Altura and Altura, 1998).

A number of disease states have been associated with magnesium imbalances and

include: cardiovascular diseases, neuromuscular disorders, higher mortality rates (Greenway, Hindmarsh, Wang, Khodadeen & Hebert, 1996), renal diseases, drug toxicities, asthma (Altura & Altura, 1991-92), migraines, premenstrual syndrome, pre-eclampsia, eclampsia, menopausal bone problems (Muneyyirci-Delale et al., 1998), atherosclerosis, diabetes mellitus, obesity (Djurhuus et al., 1995), and hypertension (Paolisso & Barbagello, 1997). As well, Lukaski (1995b) has noted a decrease in athletic performance.

Altura (1994) found that dietary intakes of magnesium in the North America have been declining since the turn of the century from about 500 mg/day to 175-225 mg/day. According to the National Research Council of Canada, 1979, this is due to the increasing use of fertilizers (lacking Mg) and food processing (removing Mg). Some investigators believe that the current RDA of 350 mg/day for men and 300 mg/day for women as recommended by the US National Academy of Sciences is too low and should be 450-500 mg/day (Altura and Altura, 1996).

According to Djurhuus et al., (1995) there is no consensus regarding measurement of magnesium. Although muscle Mg, obtained through a needle biopsy, is thought to be reliable, it is time consuming to perform, very invasive and causes discomfort to the patient. Magnesium status can also be measured in the serum, erythrocytes, lymphocytes or through a magnesium load test with urinary excretion (Djurhuus et al., 1995). However, Djurhuus et al., (1995) have found that urinary Mg is very variable, so generally it cannot be used to evaluate Mg status. The total amount of Mg in serum (TMg) is the most common means for measuring magnesium status. Although TMg has been the most common measure for magnesium status, since the introduction of the ISE for Mg, iMg has been widely used and may become the new standard.

There is approximately 1000 mmol of magnesium in the human body (Touitou, Touitou,

Bogdan, Beck & Reinberg, 1978), with muscle and bone compromising approximately 80% of total body Mg (Djurhuus et al., 1995). The serum portion of blood contains less than 1 percent, yet is the most accessible source for Mg measurement. Serum Mg can further be subdivided into its component parts: ionic, complex-bound, and protein-bound. It is the free (ionic) portion, however, that is most important because it is physiologically active (Hoshino et al., 1998). Ionic magnesium levels have been found by Altura & Altura, (1991-92) to be altered in some disease states and should prove to be of importance in disease management. They also found that iMg may be measured in plasma, serum or whole blood with virtually no differences. If abnormalities in magnesium are suspected, iMg is the suggested method for assessment as it can be measured rapidly on whole blood, near the patient, and may theoretically be more clinically relevant (Greenway, Hindmarsh, Wang, Khodadeen & Hebert, 1996).

Despite the clinical advantages that iMg has to offer, its usefulness may be compromised by physiological variability. One potential source of physiological variability is a circadian rhythm. In 1978, Touitou et al. found a significant circadian rhythm in TMg. As well, Willimzig, Latz, Vierling & Mutschler (1996) found a noticeable circadian fluctuation of TMg with a peak in evening hours and strong fluctuations in the morning. Ising, Bertschat, Gunther, Jeremias & Jeremias (1995) were the first to discover a significant circadian rhythm in iMg and observed the highest concentrations around 9:00 and the lowest concentrations around 15:00. A further study relating to the discoveries by Ising et al., (1995) was conducted by Jacomella et al., (1997) to see if glucose loading affected the circadian rhythm of iMg and found no significant results. The present study, built on the research to date measured for three consecutive days.

Many analytes show cyclical rhythms which can be circadian, monthly, or seasonal in

nature (Reilly, 1996). Knowledge of these changes over time is vital to the collection of specimens at appropriate times, selection of relevant reference values, and in diagnosis, because the absence of the expected rhythm may indicate the presence of disease (Fraser & Harris, 1989). A problem, however, is that the rhythms are variable from one individual to another, so that no particular time represents a universal maximum or minimum (Fraser & Harris, 1989).

Although assessment of iMg would appear to have obvious clinical and research implications in the screening, monitoring, diagnosis and treatment of individuals, determination of an intra-individual variability would confound appropriate interpretation of iMg values. This research thus represents a critical step in the validation of iMg measurements.

1.3 DEFINITIONS

Analytical variation - The variation due to the testing procedure and the instrument (Fraser and Harris, 1989).

Biological variation - the variation that is explained by biological processes, not from analytical error (Fraser and Harris, 1989).

Circadian Rhythm - cyclical changes that recur regularly over a given day (Reilly, 1996).

Coefficient of variation - a score that represents the percent variance, calculated from the standard deviation (s) and the mean (X). Equal to s / X.

Index of individuality - calculated from the ratio of total within-subject standard deviation and between subject standard deviation (Fraser and Harris, 1989). Compares the usefulness of a given reference range for each subject.

Ionic Magnesium (iMg) - a form of magnesium that is physiologically active intracellularly, yet

can be measured extracellularly in the blood. Normal range: 0.46 - 0.60 mmol/L (Ising et al., 1995).

Ion Sensitive Electrode (ISE) - a measuring device that is used to measure ionic magnesium (or other hematological variables such as ionic calcium, potassium, or sodium). (Altura & Altura, 1991-92).

Total Magnesium (TMg) - magnesium values represented by the total amount in the blood which include ionic magnesium, complexed magnesium and protein bound magnesium. Normal Range: 0.73 - 0.94 mmol/L (Ising et al., 1995). Also referred to as serum magnesium (SMg) or plasma magnesium (PMg).

Total variation - the variation that comprises both physiological and analytical variation (Fraser and Harris, 1989).

Variability - the quality or state of being inconsistent, unsteady, changeable (adapted from Webster's Dictionary, 1998). Variability can range over a spectrum of time. Short-term variability occurs within a day or less, whereas long-term variability, occurs over a longer period of time.

Within-subject variation - variation in a series of samples from one individual. Also called intra-individual, intra-subject or within-person (Fraser and Harris, 1989).

1.4 LIMITATIONS

- The analysis of ionic magnesium and other hematological variables was dependent on the accuracy of the Nova 8 stat analyzer (Nova Biomedical Canada Ltd., Mississauga, ON).

- All assays were assayed within one hour after collection and not frozen for later simultaneous analysis, which could lead to increased (between-run) analytical variation.
- The diet analysis was dependent on the consistency and accuracy of the subjects filling out the self-report journals, the accuracy of the investigators to transfer the data to the Diet Analysis Plus program, and the accuracy of the software Diet Analysis Plus™ 1996 by West Publishing Co., St. Paul, MN.
- Accuracy of exercise and sleep analysis was dependent on self-report records by each subject.
- Analysis of between-day variation was limited to the three days of testing.

1.5 DELIMITATIONS

Thirteen healthy volunteers from Lakehead University (nine males and four females) were selected for participation in this study. All subjects were non-smokers between the ages of 19-41 and resided in Thunder Bay, Ontario, Canada during the course of the study. Measurements of iMg variability were delimited to six measures per day (from 7:00 to 10:00) for three consecutive days using the NOVA 8 stat analyzer (NOVA Biomedical).

Chapter Two: Review of Literature

2.1 INTRODUCTION

The purpose of this chapter is to review the literature relevant to an understanding of iMg measures. In particular, factors that could potentially lead to variable iMg results within an individual will be examined. As an overview, the distribution of Mg in the body and the various assay options available to the clinician and researcher will be noted.

2.2 DISTRIBUTION OF MAGNESIUM

Magnesium imbalances have been associated with many disorders and disease states. Therefore, an effective and accurate means of diagnosing Mg status must be found. There are a number of methods presently available but there is no consensus on the Mg index that best reflects Mg stores, due mainly to the distribution of Mg in the organism and the variable relations between the different compartments (Meladu, Manuel, Keenoy & DeLeeuw, 1996).

Magnesium is found in the body either intracellularly or extracellularly. Intracellular Mg consists of approximately 99% of the total body Mg stores - 53% is found in bone, 27% in muscle, 19% in soft tissue and .5% in red blood cells (Elin, 1991-92). Intracellular Mg stores can be measured through red blood cells, mononuclear blood cells, muscle, bone, renal excretion and Mg retention tests. The final source of Mg in the body (less than 1%) is the extracellular component and is found in serum (Elin, 1994). Serum Mg (also called total Mg (TMg)) can be further divided into its component parts - 15% is complexed to small anion ligands such as bicarbonate, 30% is protein-bound (mostly albumin) and 55% is found in the ionic form.

2.3 INTRACELLULAR SOURCES OF MAGNESIUM

2.3.1 Erythrocyte Magnesium

Erythrocyte Mg (EMg) has been used in a variety of clinical situations on the basis that it provides an indication of intracellular Mg status, yet there is virtually no exchange between erythrocyte and plasma (Martin, Lyon, Fell & McKay, 1997). The study by Martin et al. (1997) found that changes in EMg occur relatively slowly, tending to be more moderate than changes in serum, due to the fact that the amount of Mg in red blood cells is largely a function of the age of the cells and the amount of Mg available in bone marrow at the time of erythropoiesis. They concluded that numerous technical errors were documented in previous studies and doubt was cast on the reliability of EMg as a guide to Mg status.

2.3.2 Mononuclear Blood Cells and Lymphocyte Magnesium

Although Mg assays from blood would be easier to obtain than assays from bone or muscle, opinions differ as to the clinical significance of the various blood options. Lymphocyte magnesium (LMg), has been suggested as a good indication of intracellular Mg levels (Dyckner and Wester, 1985; Gunther, Vormann and Forster, 1985; Tyan, Ryan, Thornton and Counihan, 1981) but Meladu, Manuel, Keenoy & DeLeeuw (1996) reported that LMg is not a good indicator of intracellular Mg concentrations but rather a sensitive index of TMg concentration, even though they found an excellent correlation between LMg and heart Mg in magnesium deficient rats.

2.3.3 Ionized Mononuclear Blood Cell Magnesium

Another possibility of a total body magnesium status indicator is the use of ionized mononuclear blood cell magnesium (iMBCMg). Huijgen et al., (1998) researched hemodialysis patients to determine if the ionized fraction of Mg was an excess and found that neither ionic source of Mg (serum nor MBC) was able to discriminate fully between normal Mg homeostasis and Mg excess, and suggest that TMg remains the measure of choice. Also, because the measure of iMBCMg is rather complicated, this intracellular marker is not really suitable as a routine lab test (Huijgen et al., 1998).

2.3.4 Muscle and Bone Biopsies

Muscle or bone biopsies seem to be good samples for Mg analysis as they contain 80% of the total Mg body stores (27% and 53% respectively) but are not suited for routine measurements (Huijgen et al., 1997), as they are invasive, time consuming and have a poor correlation with each other (Gullestad, Midtvedt, Dolva, Norseth & Kjekshus, 1994). Meladu, Manuel, Keenoy & DeLeeuw (1996) found that biopsy of muscle cells causes discomfort and some risk to the patient and results are complicated by contamination of the sample with blood and connective tissue. Meladu et al. (1996) further states that bone Mg analysis, apart from the need for a biopsy, also has the disadvantage that different types of bone may contain different concentrations of Mg and these bones respond with different time intervals to differences in Mg supply.

2.3.5 Urinary Excretion and Load Retention

The last two methods of intracellular Mg analysis, urinary excretion and the load retention test have been used by many researchers. Urinary excretion follows a circadian rhythm (Graham, Caesar and Burgen, 1960) thus a 24-hour specimen is required to accurately assess renal magnesium excretion (Elin, 1991-92). The result of the excretion depends on intake, absorption and renal function, but is also a valuable tool for documenting magnesium wasting by the kidneys due to medication or aberrant kidney function (Elin, 1991-92). The daily normal excretion of Mg in humans is 3.6 +/- 1.4 mmol for females and 4.8 +/- 1.5 mmol for males (Elin, 1994).

An extension of the urinary excretion test, measuring the percentage of Mg retained after parenteral administration of a Mg load, is becoming a more popular method to diagnose total body Mg deficiency (Elin, 1994). Previous studies (of the load test) have different test procedures with various doses of Mg given, differing routes of administration, differing durations of urine sampling and no general agreement for normal and pathological retention (Gullestad, Midtvedt, Dolva, Norseth & Kjekshus, 1994). They conclude that the Mg load retention test is a valuable tool in the estimation of deficiency and has an advantage (over other measures) because it serves as a concomitant treatment in subjects suspected with Mg deficiencies. Both Gullestad et al., (1994) and Romamo (1997) state that the load retention test is a much better predictor of tissue Mg stores than TMg concentrations. Also, this test assesses the loss of Mg from the major exchangeable Mg pool found in bone, as reported by Cohen and Laor (1990), who found a significant inverse correlation ($r = -0.992$) between the results of the Mg load retention test and the concentration of Mg in bone. However, further investigation into this Mg marker is needed,

for this method has not been standardized and has not generally correlated with other indices (Djurhuus, Gram, Petersen, Klitgaard, Bollerslev & Beck-Nielsen, 1995).

In regards to urinary excretion, nearly all of the Mg absorbed from the gut is excreted in the urine and therefore urinary excretion of Mg in a healthy individual is a reflection of the adequacy of the diet (Djurhuus et al., 1995). Djurhuus et al., (1995) notes that urinary Mg excretion is very variable, so generally cannot be used in the evaluation of an individual's Mg status.

2.4 EXTRACELLULAR SOURCES OF MAGNESIUM

2.4.1 Serum Magnesium

Measuring extracellular sources of Mg (TMg) has been reported in many studies, yet there have been mixed reports on its effectiveness as a tool to assess Mg status. According to Meladu, Manuel, Keenoy & DeLeeuw (1996), TMg remains the best and most accurately determinable index of Mg. Yet, TMg analysis, although readily available, widely used in a clinical setting and often acted on by physicians, does not correlate with any other tissue pool of Mg except for interstitial fluid (Gullestad et al., 1994; Romano, 1997). Also, in a study by Kulpmann & Gerlach (1996) it was found that total TMg determinations cannot be used to predict iMg values, as total and ionized magnesium were only closely related in marked hypermagnesaemia, and correlation was poor in samples with slightly elevated Mg concentrations or in hypomagnesaemic conditions. Total magnesium concentration varies very little within an individual (due possibly to a release of Mg from bone stores), hence a change in TMg within the limits of a reference interval might indicate a significant change in that

individual's Mg status (Djurhuus et al., 1995). In the above study by Djurhuus et al., (1995), they concluded that a single determination of TMg cannot be used in the evaluation of Mg status for an individual.

2.4.2 Ionic Magnesium

Ionic magnesium, measured in the extracellular portion of the blood, may be the best marker for Mg status since it is the physiologically active portion of Mg in the body (Elin, 1994). Ionic Mg can be measured using an ion sensitive electrode (ISE), which was first introduced around 1990. A study by Altura & Altura (1991-92) showed that within-run precision of the ISE is excellent and that precision of the new ISE on different levels of aqueous controls is quite remarkable. Altura & Altura (1991-92) also showed that a wide range of potential physiologic concentrations of ionic calcium, sodium or potassium does not interfere with the ability of the NOVA ISE to selectively sense free iMg. When comparing measurements of aqueous solutions of iMg obtained with the electrode versus that obtained from atomic absorption spectroscopy (previous method of measuring iMg), a perfect regression correlation of 1.0 was reported (Altura & Altura, 1990). Another benefit of measuring iMg with the ISE, as mentioned by Altura & Altura, (1996) is that there is no difference in iMg irrespective of whether one uses samples of serum, plasma or whole blood.

Blood iMg determinations could be very useful as diagnostic and prognostic guides to clinical management as iMg concentrations have been found to be altered in some disease states and should prove to be of importance in disease management (Altura & Altura, 1991-92). A recent clinical study by Greenway, Hindman, Wang, Khodadeen & Hebert (1996) demonstrated

that iMg, but not TMg, was depressed in a number of conditions (migraine, head trauma, non-insulin-dependent diabetes mellitus, asthma and women with high risk pregnancies) and suggest that further studies along these lines be performed if abnormalities in Mg metabolism are suspected. 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.

2.5 MAGNESIUM VARIABILITY

2.5.1 Introduction

The purpose of the present study was to establish the short term variability within subjects for iMg. Only two other studies have addressed this question and these will be reviewed, though two other topics relevant to iMg variability will be discussed first. The significance of iMg variability needs to be viewed in context with the variability of other Mg status indicators. In doing so, the variability of Mg can be surmised. The second topic that may shed more light on iMg variability is an analysis of iMg reference ranges reported in the literature. Discrepant and large ranges could be a reflection of variable iMg measures and an examination of what exclusion and/or partitioning factors were used (or not used) in calculating the various reference ranges will highlight which factors may contribute to iMg variability.

2.5.2 Variability of Mononuclear Blood Cell, Erythrocyte, Urinary & Serum Magnesium

Huijgen et al., (1997), determined the precision of Mg determination in mononuclear blood cells and in erythrocytes and found that the coefficients of variation for the reproducibility

of the complete Mg assay were rather high (MBC: 8% within-day, 10% between-day; RBC: 4.3% within-day, 11.1% between-day). Since the coefficients of variation were obtained using blood from healthy patients, these results cannot be extrapolated for patients with abnormal intracellular Mg concentrations. Huijgen et al., (1997) further conclude that intracellular Mg concentrations obtained in daily practice should be interpreted with care.

Urinary Mg measurements have been considered as another method of diagnosing Mg status but as mentioned earlier UMg is quite variable and is not a good indicator of Mg status. A study by Djurhuus et al, (1995) comparing UMg (24 hour excretion without a load) and TMg found that a change in TMg concentrations of 9.5% is considered significant whereas 24 hour UMg excretion must exceed a change of 101% before it reaches statistical significance. This further solidifies the above statement that UMg is not a good indicator of Mg status.

Total Mg variability has been studied more than any other Mg marker, with the majority of the studies focussing on circadian rhythm, yet very little information is available. In 1978, Touitou, Touitou, Bogdan, Beck & Reinberg studied TMg circadian rhythm in human adults. They discovered a statistically significant circadian rhythm in healthy young males, elderly females and elderly males. Similar studies have been performed that have shown a circadian rhythm (Szyszka, Brocki, Mrozikiewicz & Paradowski, 1992; Ebel, Classen, Marquardt & Spath, 1975). Willimzig, Latz, Vierling, Mutschler, Trnovec & Nyulassy (1996) constructed a baseline plasma profile before studying Mg supplementation on TMg levels. They report that usually no measurements of the diurnal variation of the Mg values during a control phase are carried out.

2.5.3 Reference Ranges

To use iMg as a valid biochemical marker, Greenway et al., (1996) suggest that it is essential to establish a reliable reference interval in a healthy population. The production of health-associated reference values and the subsequent estimation of the reference interval for a given analyte must be carried out in accordance with a well-defined protocol as outlined below in NCCLS document C28-A, 1995.

(1) Establish an appropriate list of biological variations and analytical interferences from medical and scientific literature (in the case of a totally new analyte, the literature may not be helpful, which necessitates a new laboratory investigation of these matters).

(2) Establish selection (or exclusion) and partition criteria and an appropriate questionnaire designed to reveal these criteria in the potential reference individuals.

(3) Execute an appropriate written consent form for participation in the reference interval study and have the reference individual complete the questionnaire.

(4) Categorize the potential reference individuals based on the questionnaire findings and results of other appropriate health assessments.

(5) Exclude individuals from the reference sample group based on the exclusion criteria or other assessments indicating a lack of good health.

(6) Decide on an appropriate number of reference individuals in consideration of desired confidence intervals.

(7) Prepare, properly and consistently, the selected persons for specimen collection for the measurement of a given analyte consistent with the routine practice for patients.

(8) Collect and handle the biological specimens properly and in a manner consistent with

the routine practice for patient specimens.

(9) Collect the reference values by analysing the specimens according to the respective analytical methodology under well-defined conditions and consistent with the routine practice for patient specimens.

(10) Inspect the reference value data and prepare a histogram to evaluate the distribution of data.

(11) identify possible data errors and/or outliers.

(12) Analyse the reference values, i.e., select a method of estimation and estimate reference limits and the reference interval (including partitioning into subclasses for separate reference intervals, if appropriate).

(13) Document all of the previously mentioned steps and procedures

With a reference interval in place, it appears that iMg provides physicians and laboratory personnel with a useful test near the patient with measurements which may theoretically be more relevant (Greenway et al., 1996). A number of studies have calculated a reference range for iMg and are listed in table 2. Looking closely at the many references ranges shows discrepancies between the ranges. A subject with an iMg value of 0.53 mmol/l will have a midrange value in most of the reference ranges (even considered high in some) yet it is considered deficient in other ranges. This may be due to the subject pool used to calculate the reference ranges, possible exclusion criteria (see table 3) or partitioning factors (see table 4). A possible explanation to the variation in reference ranges may be explained by the lack of partitioning and exclusion criteria reported in the studies. Only one study, (Greenway et al., 1996), gave any elaboration on the

subject pool used to calculate the reference range. The 125 subjects consisted of hospital employees - laboratory personnel, nurses, physicians, medical students, clerks and porters. Significant disease, drug and/or alcohol use in volunteers were ruled out from questionnaire response and common biochemical test results.

Table 1. Reference Ranges for iMg

Authors	Instrument	Population	Reference Range	Calculation
Altura & Altura (1991-92)	NOVA	60 healthy	0.53 - 0.67 mmol/l	(95% CI)
Altura et al. (1994)	NOVA	60 healthy	0.53 - 0.67 mmol/l	(95% CI)
Ising et al. (1995)	Microlyte	179 healthy	0.46 - 0.60 mmol/l	*
Kulpman & Gerlach (1996)	Microlyte	60 healthy	0.49 - 0.72 mmol/l	(2.5-97.5%)
Saha et al. (1996)	Microlyte	47 dialysis	0.44 - 0.68 mmol/l	(X+/-2SD)
Greenway et al. (1996)	Microlyte	125 healthy	0.43 - 0.59 mmol/l	(X+/-2SD)
Altura & Altura (1996)	NOVA	60 healthy	0.54 - 0.65 mmol/l	(95% CI)
Fogh-Andersen et al. (1996)	NOVA	26 bl donors	0.55 - 0.66 mmol/l	(95% CI)
Muneyyirci-Delale et al.(1998)	NOVA	*	0.54 - 0.64 mmol/l	lab reference
Kone	Microlyte	*	0.51 - 0.61 mmol/l	from company
AVL	AVL	*	0.46 - 0.60 mmol/l	from company
NOVA Biomedical	NOVA	*	0.45 - 0.60 mmol/l	from company

CI = confidence interval, X = mean, SD = standard deviation, * = unknown

Table 2. Examples of Possible Exclusion Criteria

Alcohol consumption	Illness, recent
Blood donor	Lactation
Blood pressure, abnormal	Obesity
Drug abuse	Occupation
Drugs, prescription	Oral contraceptives
Drugs, over the counter	Pregnancy
Environment	Surgery, recent
Fasting, non-fasting	Tobacco use
Genetic factors	Transfusion, recent
Hospitalization, current/recent	Vitamin abuse

** from NCCLS document C28-A, 1995*

Table 3. Possible Partitioning Factors

Age	Posture when sampled
Blood group	Race
Circadian variation	Sex
Diet	Stage of menstrual cycle
Ethnic background	Stage of pregnancy
Exercise	Time of day when sampled
Fasting or non-fasting	Tobacco use
Geographic location	

** from NCCLS document C28-A, 1995*

2.5.4 Ionic Magnesium Variability

Studies examining the variability of Mg assays are scarce (Huijgen et al., 1997) and this is particularly true of iMg. Ionic magnesium may possibly be the best indicator of total body magnesium stores because it is the physiologically active portion of magnesium. However, there

has been some evidence that it may be variable, thus jeopardizing its effectiveness as an indicator of magnesium status. Finstad (1997), noticed that subjects screened for borderline iMg deficiency showed differing iMg values three weeks later when retested.

There are only two studies that examined the variability of ionic magnesium. Ising, Bertschat, Gunther, Jeremias & Jeremias (1995), drew blood from seven healthy volunteers every three hours over a 24h period and discovered a significant circadian variation of iMg with a max difference of approximately 10% (compared to a non-significant difference of only 4% in TMg). The highest iMg concentrations were observed at 9:00 and the lowest concentrations at 16:00 (no iMg values given). They found a significant correlation between iMg and free fatty acids indicating that Mg is bound to free fatty acids, thus reducing iMg.

The other study (Jacomella et al., 1997) that examined iMg variability hypothesized that Mg metabolism is linked at least in part to the mechanisms that regulate the concentration of blood glucose loading on free plasma Mg. However, they found that glucose loading failed to modify TMg or iMg, indicating that in healthy individuals, the rise in circulating insulin induced by a carbohydrate meal does not induce a significant shift of Mg from blood to tissue, thus the circadian pattern in extracellular Mg is not modulated by the metabolic and hormonal mechanisms that adjust the concentration of glucose.

2.5.5 Potential Factors Affecting Magnesium Variability

There are a number of factors (acute and chronic) that may influence iMg variability. Acute factors (blood donation, menstruation, tobacco use, blood pressure and exercise) will be discussed first, followed by factors exhibiting both acute and chronic characteristics (diet), with

chronic factors (circadian rhythm and age) discussed last.

2.5.5.1 Blood Donors

Blood donors are often used as a reference population for clinical chemistry yet the effect of blood donation on participation in a clinical study has not been well examined. Fogh-Andersen et al., (1996) researched the changes in plasma ionized calcium and magnesium in blood donors after donation of 450 ml of blood. They found ionized magnesium in blood donors decreased 0.01 mmol/l after blood donation (a significance of $p < 0.001$) and concluded those blood donors immediately after blood donations are unsuited as a reference population for proteins and ions.

2.5.5.2 Menstrual Cycle

A study by Muneyyirci-Delale et al., (1998) found a significant decrease in iMg around the time of ovulation ($p = 0.046$) as well as when progesterone (luteal phase) concentration peaked ($p = 0.024$) and when testosterone levels peaked (p level not given), but not during the other stages of the female sex cycle. These ionic changes probably result in the various pre-menstrual syndrome (PMS) symptoms, provided that there is an underlying deficiency of the biologically active Mg or an excess of ionized calcium, which may lead to a better diagnosing and managing women with PMS-related complaints and symptoms (Muneyyirci-Delale et al., 1998).

2.5.5.3 Tobacco use

The effect of smoking on serum iMg concentration was studied by Niemala, Cecco, Rehak & Elin (1997). They discovered that smoking significantly inversely affects the determination of iMg concentration by the NOVA Mg ISE, yet does not affect the relationship between smoking status and the determination of iMg by the AVL Mg ISE. Analysis of the data disclosed two noteworthy findings: first, the number of cigarettes per day had a significant inverse effect on the NOVA Mg ISE, and second, that the variable 'minutes since last cigarette' had no effect on the NOVA Mg ISE indicating that the difference in iMg concentration is not due to the immediate effects of smoking. They also observed a relationship between white blood cell (WBC) counts and serum NOVA iMg concentration (linking electrolyte concentration and WBC counts) suggesting that smokers may have a stable serum factor related to an elevated WBC count that negatively interferes with the determination of iMg by the NOVA Mg ISE. Thiocyanate, a metabolite of nicotine, was reported by Rehak, Cecco, Niemala & Elin (1997) to interfere with the NOVA ion-selective electrode. McHale (1997), a technical product manager and field support analyst for NOVA Biomedical responded (in a letter to the editor) by saying that thiocyanate indeed affects the NOVA 8 Mg sensor (a decrease of 0.02 mmol/l iMg when 0.5 mmol/l of thiocyanate is added to the sample) and that appropriate action to resolve the problem is underway.

2.5.5.2 Blood Pressure

Evidence has shown that Mg plays an important role in regulating ion transport and vascular tone and reactivity. As such, Mg deficiency may be an important factor in the development and treatment of hypertension. Altura & Altura (1996) report that in 28

independent clinical studies, patients with hypertension exhibit decreased serum TMg. On average, the reduction in TMg was 15% from normal. They further report that even borderline hypertensive individuals (systolic blood pressure/ diastolic blood pressure =140/90 - 150/95) usually exhibit significantly depressed iMg, but not TMg. A variety of such clinical studies as well as observations from clinical experiences, support the idea that Mg deficiency is linked to hypertension.

2.5.5.5 Exercise

Buchman et al., (1998) investigated the effect of a marathon run on plasma and urine mineral and metal concentrations and observed that TMg concentrations decreased significantly in marathon runners following their race to levels consistent with mild deficiency. Other investigators have described similar percent decreases in TMg, not only in endurance running, but other endurance activities as well including bicycle ergometry, swimming and treadmill exercise (as cited in Buchman et al., 1998). According to Golf, Happel, and Graef (1984) when the duration of physical exercise is longer than one hour and involves more muscle tissues, a significant decrease in Mg in the plasma may occur, probably caused by an exhaustion of the erythrocyte reservoir. Yet evidence has shown that the intracellular concentration of Mg is not altered by exercise (Lijnen, 1995). The data from various studies designed to monitor shifts in Mg after exercise are inconsistent, which may be related to differences in experimental design, work intensity and duration, type of exercise, timing of blood sampling, environmental conditions and dietary intake (Lijnen, 1995).

2.5.5.6 Diet

Diet may be the largest factor to contribute to magnesium variability. A study by Mullis & Bianchetti (1998) reported that high-fibre diets may be responsible for hypomagnesaemia in diabetic patients. Dietary fibre is a subject of considerable interest, for it may be beneficial in treating or preventing several gastro-intestinal disorders; as well, large amounts of water-soluble fibres have a positive effect on serum lipids and may delay glucose absorption without inducing malabsorption (Mullis & Bianchetti, 1998). Increasing dietary fibre may have the above-mentioned positive effects, yet not all fibre has the same effect, for they conclude that an increased intake of water-insoluble fibres may lead to hypomagnesaemia (a well-accepted risk factor for cardiovascular abnormalities) and recommend that the desirable fibre intake be achieved not by adding water-insoluble fibre to the diet but by consuming fruit, vegetables, legumes, and wheat grain cereals, which in addition provides minerals and vitamins.

Ising et al., (1995) reported a significant negative correlation between iMg and free fatty acids, indicating that Mg is bound to free fatty acids, thus reducing iMg. Jacomella et al (1997) hypothesized that Mg metabolism is linked at least in part to the mechanism that regulates the concentration of blood glucose loading on free plasma Mg. However, they found that glucose loading failed to modify TMg or iMg, indicating that in healthy individuals, the rise in circulating insulin induced by a carbohydrate meal does not induce a shift of Mg from blood to tissue, thus the circadian pattern in extracellular Mg is not modulated by the metabolic and hormonal mechanisms that adjust the concentration of glucose.

2.5.5.7 Circadian Rhythm

Circadian rhythm of serum Mg has been shown in prior research (Touitou et al., 1978; Szyszka et al., 1992; Willimzig et al., 1996; Selmaoui et al., 1999) and may explain some of the variability shown in magnesium studies. The scientific study of biorhythms (known as chronobiology) is now a respected field in its own right (Reilly, 1996). Cyclical changes that recur regularly over a given length of time are related to underlying physiological processes with a cycle length (period) that can range from a fraction of a second (neural firing rates) to slow changes in harmony with the seasons (Reilly, 1996). A rhythm is characterized by its length, its amplitude (half the variation from peak to trough), its mesor (rhythm adjusted mean) and its acrophase (time the peak occurs) (Touitou, Touitou, Bogdan, Beck and Reinberg, 1978). Circadian rhythms, which influence biological function (impacting performance and physical skills), are determined by the spin of the earth about its vertical axis (Reilly, 1996). Humans have adapted by timing sleep and wakefulness with darkness and light.

2.5.5.8 Age

In the human, magnesium absorption decreases with age. Between the ages of 30 and 70 Mg absorption drops to one-third of normal (Durlach et al., 1993). Magnesium exchangeable pools become reduced in elderly patients (Alfray, Miller & Butkus, 1974) and in some cases urinary magnesium leakage may be increased (Durlach et al., 1993). The problem may be compounded in some elderly individuals who use diuretics for other medical ailments. Hoshino et al. (1998) studied the iMg level in whole blood of healthy Japanese children (aged zero to 19 years), and reported no significant correlation between iMg and height, weight, age and sex and

that iMg was constant irrespective of growth (age and body weight).

2.7 SUMMARY

In summary, it is important that an accurate and efficient means of measuring magnesium status is found. Ionic magnesium has the potential of being the dominant measure for magnesium status as it has been linked to both the diagnosis and treatment changes of various diseases. Some literature though, has shown that iMg measurements may be jeopardized by physiological variability. The literature also shows that, although a number of reference ranges have been developed, they do not show consistency which may confound the utility of iMg measurements.

Chapter Three: Methodology

3.1 SAMPLE

A sample of thirteen healthy subjects (nine male and four female) volunteered for this study.

3.2 PROCEDURES

3.2.1 Test Item Protocols

The study was approved by the Lakehead University ethics committee. After informed consent was obtained from each subject, three consecutive days of testing occurred with six blood samples taken each day for a total of eighteen blood samples. The first blood sample was taken at 7:00 and every three hours thereafter (7:00, 10:00, 13:00, 16:00, 19:00, 22:00). Blood samples were collected throughout the study in 7 ml green topped Vacutainer tubes® (lithium-heparin added) by antecubital venipuncture. Prior to blood withdrawal, the subjects were seated for ten minutes. When blood was collected, a tourniquet was applied gently to the upper arm and released prior to actual blood flow.

The Nova 8 stat analyzer® (Nova Biomedical Canada Ltd., Mississauga, Ontario) housed in the same laboratory that testing occurred, was used for immediate analysis of [iMg] and Hct, from whole blood samples. All testing was done by the same technician throughout the study. Within-run variation (analytical error) was calculated by replicate analysis of the specimens and control standards (i.e., ten samples in a row of the same blood).

During the three days of testing, subjects recorded diet, sleep, exercise and stress in a self-monitored log book (see Appendix A). Subjects received information that explained the

procedure and importance of accuracy for the self-reported logs and were instructed to continue to follow their normal routine as close as possible (see cover letter in Appendix B).

Dietary intakes were analyzed using computerized diet software (Diet Analysis Plus®, 1996, West Publishing Co, St. Paul, MN). Subjects recorded three consecutive days of testing (the same days as the blood samples - two weekdays and one weekend day) and all data was entered into the computer by the same technician.

3.2.2 Daily Log

The variables included in the daily log include: (1) quality of sleep, (2) number of hours of sleep, (3) length of exercise session, (4) intensity of exercise session, (5) minor illnesses, (6) minor injuries, (7) menstruation, and (8) major stressful events.

3.3 VARIABLES

3.3.1 Dependent Variables

(1) Ionic magnesium (mmol/L) - corrected for hematocrit

(2) Hematocrit (%)

3.3.2 Independent Variable

(1) Time

3.3.3 Monitored Variables

(1) Sleep quality

(2) Sleep quantity

(3) Exercise duration

(4) Exercise intensity

(5) Illnesses

(6) Injury

(7) Menstruation

(8) Stress

(9) Diet

3.4 STATISTICAL ANALYSIS

The primary purpose of this study was to determine the variability of iMg in order to determine its utility. Descriptive statistics (mean, standard deviation, range and coefficient of variation (CV)) and graphic presentations of the data were used to gain an initial appreciation of the variability of iMg. CVs were computed to assess analytical error (from repeated measures of control samples as well as repeated measures of subject samples (subjects as their own control)), as well as to assess the variability for each of the six time periods. To calculate the CV for the six time periods, all blood values calculated at each time period were pooled together (13 subjects * 3 days = 39 values). An index of individuality was calculated using a ratio of the total within-subject standard deviation and the between-subject standard deviation, in order to assess the utility of iMg in either monitoring a patient or classifying them based on reference ranges.

A 13 (subjects) X 6 (time periods) repeated measures analysis of variance (ANOVA) was used to calculate the within-day variability. Calculation of the between-day analysis requires a

data transformation to provide a score for each subject that represents the events of each day across each time measurement. It is important to recognize that the score must consider the events within the measurement interval as a function of events in the preceding interval but while impacting events in each subsequent interval. Plotting the data across time points in a given day for a given subject indicated that there was an inherent rhythm for the measured variable. Therefore, a linear (summative) transformation was inappropriate for the data. A summative scalar such as the coefficient of variation (standard deviation / mean) only considers the range of the variance distributed over the group mean, thus, a transformation that considers range as well as rhythmicity of responses over the entire collection period was needed. Considering the above, the raw data was transformed using a cosine function (transformed score = ½ sin (raw score)). The slope of the transformed data curve was then calculated for each subject, for each day, and the slope scores used in a two-way 3 (days) X 13 (subjects) ANOVA.

The secondary purpose of this study was to examine the possibility of a diurnal pattern to iMg measurements. To do this the transformed data was pooled into each of the six time periods and correlated to a sinusoidal curve of best fit. As well, correlations were calculated for each subject for each day.

CHAPTER FOUR: RESULTS

4.1 DESCRIPTION OF SUBJECTS

The characteristics of the 13 subjects (nine males and four females) that volunteered for the study are listed in Table 4. Subject selection for this study was based largely on volunteers who were willing to donate 18 blood samples over three days, but subjects were also screened for smoking (nonsmokers), blood pressure (resting blood pressure not higher than 144/94), drug (prescription and non-prescription), vitamin and/or mineral use. As well, alcohol consumption was not permitted throughout the duration of the study. Subjects' self-report journals revealed that no unforseen circumstances occurred during the three days of testing. All subjects maintained normal routines of exercise and lifestyle (work, diet, sleep), with no unforseen or unexpected changes in their daily routines (other than the six blood samples per day).

The mean dietary magnesium intakes were 378.2 +/- 157.7 mg/day. The dietary analyses showed that four subjects (two males and two females) had intakes less than the RDA of 350 mg/day for men and 300 mg/day for women, with both above mentioned male subjects having less than 70% of the RDA. Values over 70% of the RDA are in the acceptable range for adequate nutrition (Lukaski, 1995a).

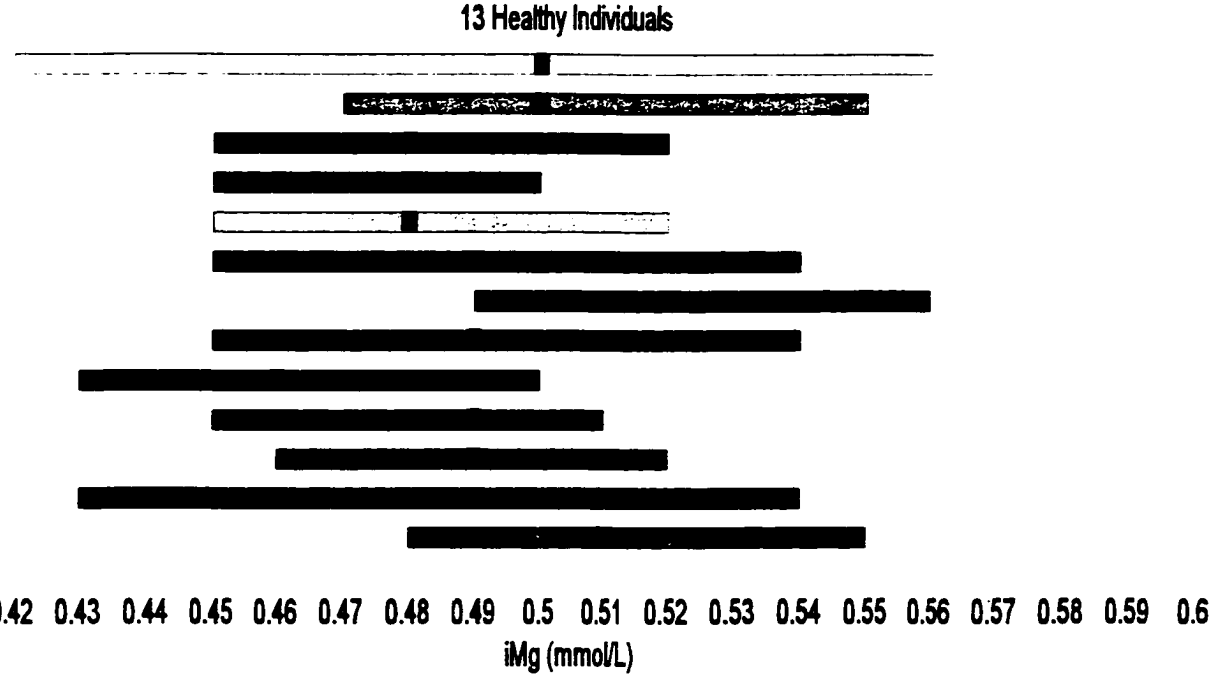
Table 4. Characteristics of Volunteers

PARAMETER	MEAN +/- SD	RANGE
Age (yrs)	24.8 +/- 5.7	21 - 41
Height (cm)	173.5 +/- 8.7	165.5 - 184.0
Weight (kg)	67.1 +/- 12.5	47.7 - 89.3
Systolic Blood Pressure (mmHg)	115.3 +/- 8.2	103 - 125
Diastolic Blood pressure (mmHg)	72.9 +/- 8.2	58 - 80
Dietary Mg Status (mg/day)	378.2 +/- 157.7	132 - 533

Mean iMg values and ranges are presented in Figure 1. Nine of the thirteen subjects

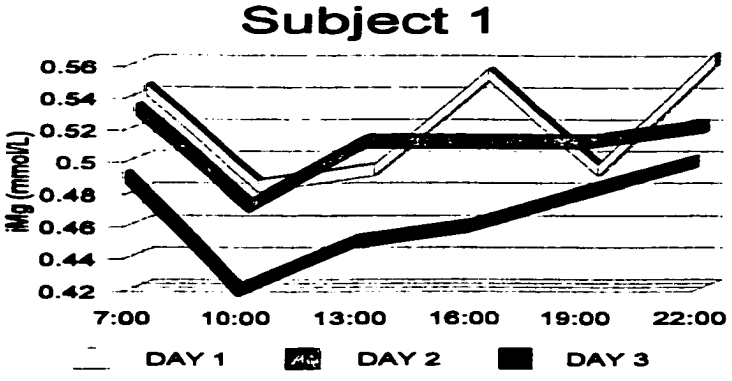
(69.2%) recorded at least one value below the reference range (0.46 - 0.60 mmol/L) suggested by Ising et al., (1995). The highest value recorded (0.56 mmol/L) was reached by two subjects.

Figure 1. Means and Ranges of iMg



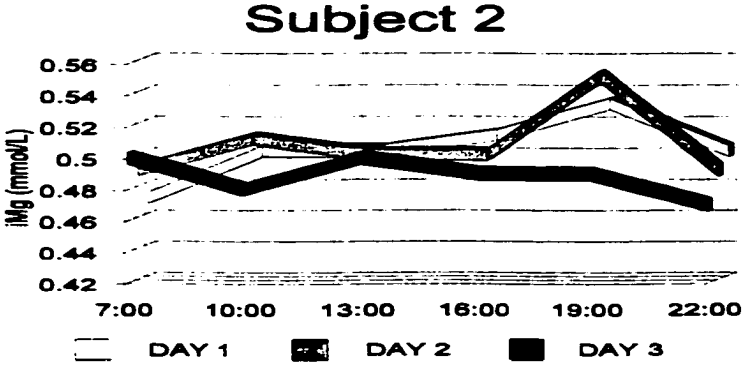
Graphic presentations for all subject's iMg values (all three days) can be seen on pages 32-35.

Figure 2. Individual Subject Data Plots

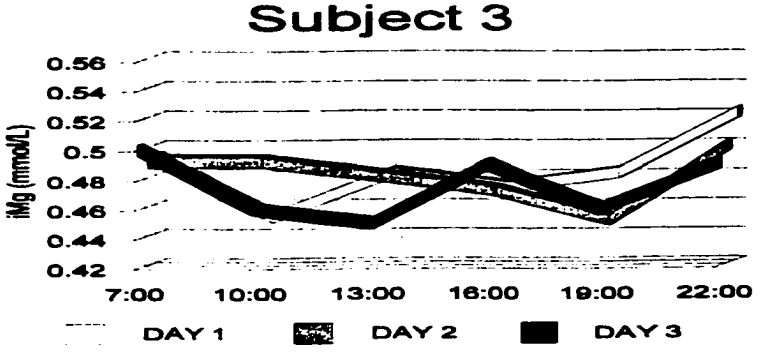


Day 1: CV = 6.8%
Day 2: CV = 4.0%
Day 3: CV = 6.3%

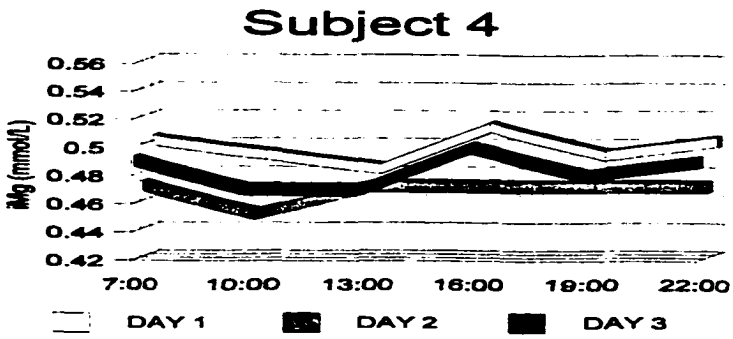
This subject was menstruating during the time of the study and recorded the highest mean within-day CV of 5.7% Recorded the highest value of the study (along with subject 7) as well as the lowest.



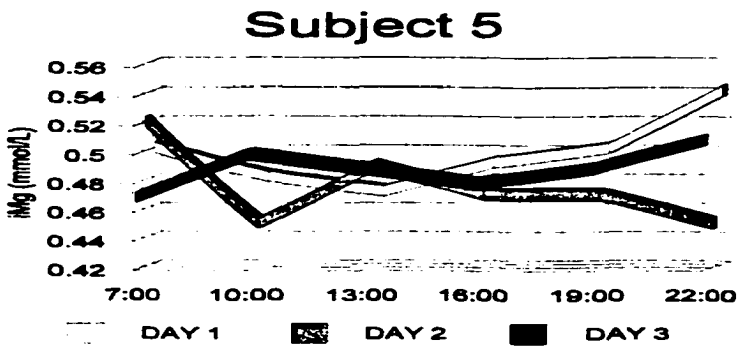
Day 1: CV = 3.9%
Day 2: CV = 4.4%
Day 3: CV = 2.4%



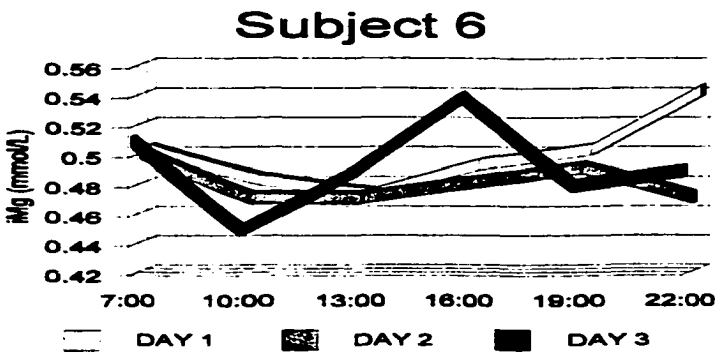
Day 1: CV = 4.8%
Day 2: CV = 3.7%
Day 3: CV = 4.4%



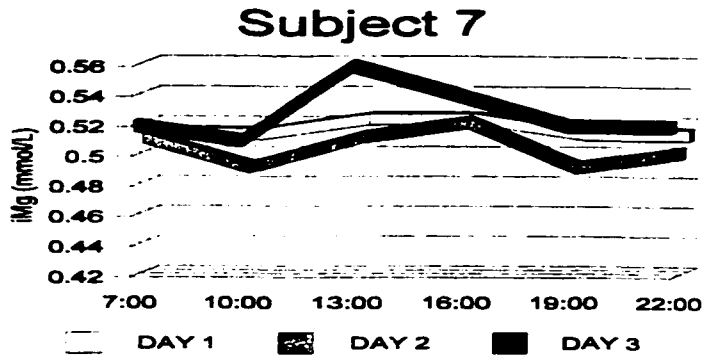
Day 1: CV = 2.1%
Day 2: CV = 1.7%
Day 3: CV = 2.5%



Day 1: CV = 3.1%
Day 2: CV = 5.6%
Day 3: CV = 2.9%

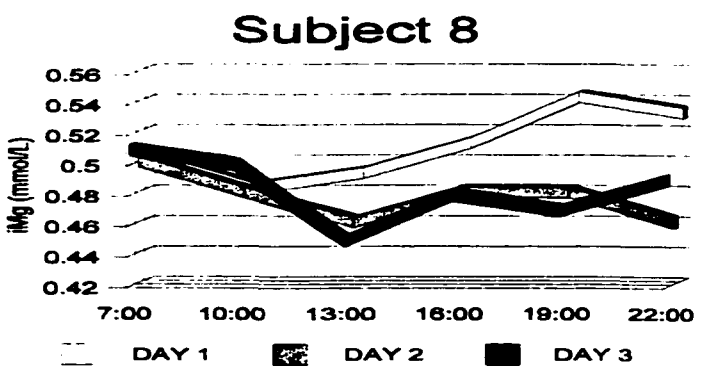


Day 1: CV = 4.9%
Day 2: CV = 2.6%
Day 3: CV = 6.1%

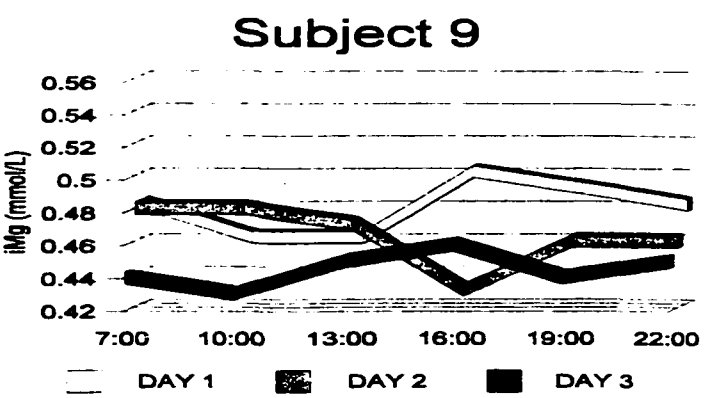


Day 1: CV = 1.0%
Day 2: CV = 2.4%
Day 3: CV = 3.5%

Recorded the highest value in the study (along with subject 1).

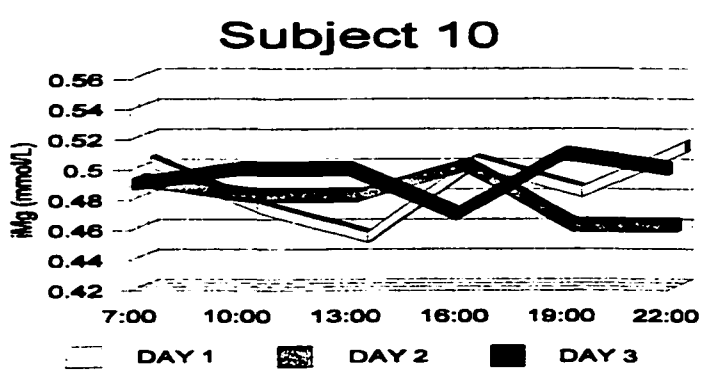


Day 1: CV = 4.6%
Day 2: CV = 3.2%
Day 3: CV = 4.5%

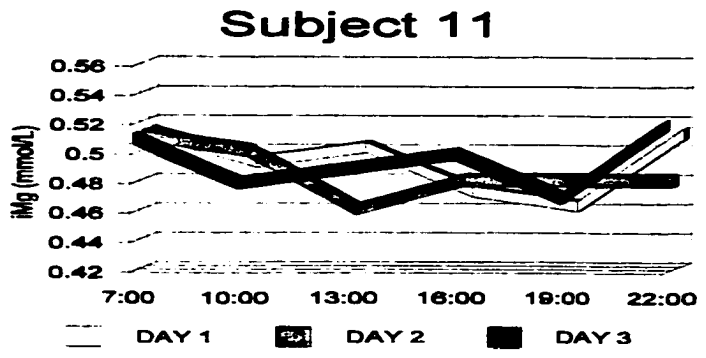


Day 1: CV = 3.3%
Day 2: CV = 4.0%
Day 3: CV = 2.4%

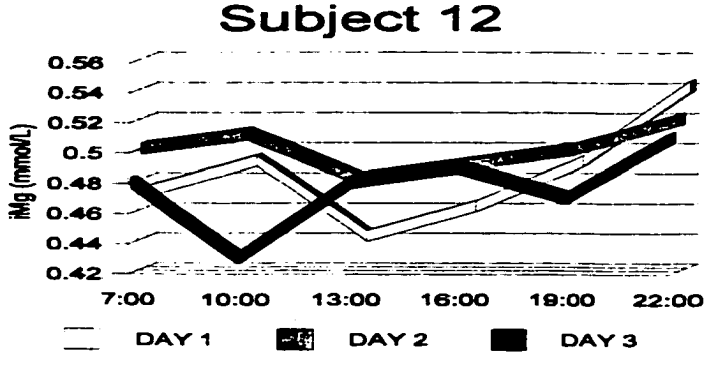
This subject was pregnant during this study (middle of the second trimester).



Day 1: CV = 4.7%
Day 2: CV = 3.3%
Day 3: CV = 2.8%

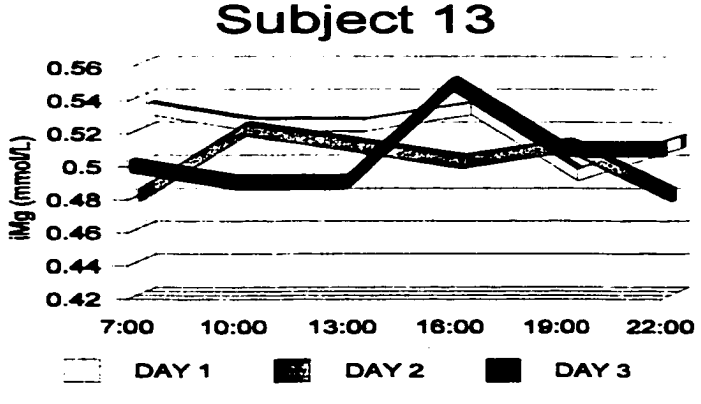


Day 1: CV = 4.3%
Day 2: CV = 3.6%
Day 3: CV = 3.8%



Day 1: CV = 7.9%
Day 2: CV = 2.8%
Day 3: CV = 5.6%

This subject missed a blood sample due to a prior commitment (day 1 - 10:00). The mean value is shown in the figure for the missed sample.



Day 1: CV = 2.9%
Day 2: CV = 3.3%
Day 3: CV = 5.7%

This subject missed the final two blood samples on the third day due to a severe migraine. An interesting find for this subject was noted in the final sample prior to the migraine, as the iMg value was the highest recorded for that subject (an increase of 0.05 mmol/L for that day; and 0.02 mmol/L from the next highest three-day recorded value)

4.2 WITHIN-DAY AND BETWEEN-DAY VARIABILITY

The two-way repeated measures analysis of variance (ANOVA) revealed a significant within-day variation of iMg ($F(5,2)=6.71$, $p<0.001$). A Tukey-HSD post hoc comparison with a significance level of .05 revealed a significant difference between time one (7:00) and time two (10:00) as well as time two (10:00) and time six (22:00). A between-day main effect ($F(2,5)=2.07$, $p=0.142$) was calculated but will be discussed later. As well, there was a significant interaction ($F=6.71$, $p<0.001$) but was not a purpose of the study and will not be discussed further. A homogeneity of variance test was calculated and revealed a non-significant value ($F(42,3391)= 0.868$, $p=0.712$). Mean values for the six time periods of data collection as well as the corresponding coefficients of variation (CV) are shown in Table 5.

As mentioned in the methods chapter, it was felt that a linear approach to the calculation of between-day variation was inappropriate, therefore between-day variation was re-calculated by transforming the raw data into sinusoidal curve data, with the resulting slope values for each subject for each day used in a 3 (day) X 13 (subject) factorial ANOVA. A non-significant result was obtained for between-day variation ($F(2, 12)=0.915$, $p=0.557$). The between-subject main effect ($F=0.915$) as well as interaction ($F=1.093$) statistics were calculated with non-significant results but are not a focus of this study and will not be discussed further.

Table 5. Mean iMg Values and Coefficients of Variation for Six Time Periods

	T 1(7:00)	T 2(10:00)	T 3(13:00)	T 4(16:00)	T 5(19:00)	T 6(22:00)
iMg	0.50	0.48	0.48	0.49	0.49	0.50
CV(%)	3.9	5.2	5.0	5.3	4.8	5.2

* all time periods are within a range of +/- 40 minutes.

Discovering a significant variation of iMg throughout the day, it was thought possible

that a circadian rhythm may be the possible reason for the variation. However, when correlating the mean values (from all subjects for each day) to a smoothed sinusoidal curve of best fit, a correlation coefficient of 0.1996 was produced. Therefore, circadian rhythm was not found to be the cause of the within-day variation. However, pooling the data may have had a limiting effect as correlations to curves of best fit were greater when looked at on an individual basis. Values can be seen in Appendix C.

4.3 INDEX OF INDIVIDUALITY

Calculation of the index of individuality (within-subject standard deviation / between-subject standard deviation) revealed that 10 subjects had a score of 0.33, 2 others had a score of 0.50, while one subject had a score of 0.67.

4.4 ANALYTICAL ERROR

Analytical error was calculated from a number of control samples, with a reported coefficient of variation of 2.3%.

CHAPTER FIVE: DISCUSSION

The key finding of this study is that iMg is variable in healthy subjects over the course of one day. The within-day variability is illuminated in both the descriptive statistics and the repeated-measures ANOVA statistic.

5.1 WITHIN-DAY AND BETWEEN-DAY VARIABILITY

In order to assess within-day variability, blood samples were taken from subjects six times a day, for three consecutive days. Results were viewed in both a descriptive manner and with an ANOVA. From Figure 2 one can note that there is a significant variation throughout the day, with a significant difference between time 1 (7:00) and time 2 (10:00), as well as between time 2 (10:00) and time 6 (22:00). Maximum mean values were recorded at 7:00 and 22:00 with the minimum mean value recorded at 10:00. Ising et al., (1995) similarly found a maximum value in the morning (9:00), yet their minimum value was reported later in the day than the present study (15:00 compared to 10:00).

Coefficients of variation (CV) were calculated for the combined set of blood samples taken during each time period which showed that the lowest variation (3.9%) occurred during the first blood sample (7:00), while the second, fourth and sixth blood samples (10:00, 16:00, and 22:00) had the highest CV at 5.2%, which suggests that the best time for blood collection is first thing in the morning. This is in agreement with Fraser & Harris (1989) who reported that the ideal method for specimen sampling is to collect the specimen from fasting, non-exercised subjects between 7:00 and 9:00.

The above results have a large impact on the utility of iMg as a means of measuring Mg status. The discrepancy between consecutive blood samples (7:00 and 10:00) indicates that iMg may not be a suitable means of diagnosing Mg status. A patient in a clinic may show a normal ionic magnesium level if tested at 7:00 but could be deficient if tested again at 10:00. More research in this area is needed for a better understanding of within-day iMg variability.

Between-day variation, was calculated by transforming the raw data in order to come up with one value per day per subject. Results indicate there was no significant difference between days or subjects. The non-significant result of between-subject variation revealed that a homogeneous sample was used in the study, while the non-significant result of between-day variation revealed that iMg does not significantly change from day to day.

A non-significant between-day variability is important to the utility of iMg as a reliable means of measuring Mg status, as it shows that samples taken for three consecutive days will not significantly vary. This will add confidence to the clinician when measuring iMg in hospitalized patients, as stability can be monitored successfully from day to day, or more importantly when values are changing daily and are no longer stable.

5.2 CIRCADIAN RHYTHM

There are a number of reasons that could explain the discovered within-day variability, including analytical error or biological variability perhaps due to exercise, diet, stress or circadian rhythm. Speculation on causal factors that could explain iMg variability must be guarded as neither the purpose nor design of this study was geared toward causality. Analytical (experimental or mechanical) error can only account for a portion of the variability for control

samples and repeated tests from individual samples (subjects as their own control) were used to calculate within-run error, and revealed a coefficient of variation of 2.3%, which is less than the 3.0% considered acceptable by NOVA Biomedical. The possibility of a circadian rhythm has been found in previous research (Ising et al., 1995) and could explain the biological variability found in the subjects in this study. A preliminary look at plotted individual data showed an inherent rhythm to the data. However, when all data was pooled together and smoothed to a sinusoidal curve of best fit, the data no longer showed a rhythm to it. The curve of best fit showed a non-significant correlation coefficient of 0.1996. However, when looking specifically at an individual's three separate days, much stronger correlations were found for each separate day, for each subject (see Appendix D for each subject's values). Therefore, a circadian rhythm may be evident in each individual, which may explain the significant within-day variability. Also, as mentioned above, exercise and diet were not controlled throughout the study, which may influence the above-mentioned result. The fact that biological variability of iMg does exist but many biological causes are not controlled for in a clinical setting, points to the pressing need for more research along these lines.

5.3 INDEX OF INDIVIDUALITY

In order to assess the utility of iMg values, an index of individuality was calculated. When the index of individuality, expressed as S_w / S_b , is less than 0.6, conventional population-based reference values are of very limited diagnostic value (Fraser and Harris, 1989). On the other hand though, a low index of individuality means that the indice being measured could find value in the tracking of a disease progression or the effectiveness of the treatment. In contrast,

when S_w / S_b is more than 1.4, observed values can be compared usefully with reference values (Fraser and Harris, 1989). Using this index, all but one subject had an index score below 0.6, indicating that the reference values are of limited usefulness (one subject had a score of 0.67, which is just above the critical value, indicating that the reference range is also limited in its usefulness). Ionic Mg, especially with its noted association with various disease states could however be valuable in the monitoring of patients with Mg imbalances.

Looking at figure 2, the means and ranges of iMg, shows that the reference range suggested by Ising et al., (1995), is not useful with respect to each individual. Each subject's range falls on the low end of the spectrum, yet compared to other population-based reference ranges, this is one of the ranges with lower values. The individual ranges would fall completely out of some of the other population-based ranges. Therefore, the need for subject-based reference intervals may be necessary.

5.4 MAGNESIUM STATUS

Although measurement of the variability of iMg is the focus of the study, Mg status is also very important, as iMg is a means for determining total body magnesium status. Using the reference range by Ising et al. (1995) (0.46 -0.60 mmol/L) and the values obtained from the present study one is able to determine the status of each subject. The mean values for each subject (18 samples) revealed that one subject rests on the border of Mg deficiency (O.46 mmol/L) while all other subjects have normal mean iMg values but are on the low end of the range (highest individual mean is O.52 mmol/L). Throughout the three days of testing, however, nine of the subjects recorded one or more value below the reference range and would be

considered hypomagnesemic. The results of the diet analyses revealed that only two subjects had intakes of dietary Mg below the RDA, thus suggesting that diet may not be the cause of the low iMg values. One explanation could be that the NOVA instrument gives low values (compared to other instruments). This suggestion can be discounted though for testing of control samples of known iMg concentrations were always within acceptable ranges. A more likely explanation is that most published reference ranges for iMg are inappropriate, since all subjects in this study were healthy individuals.

Greenway et al., (1996) proposed that to use iMg as a valid biochemical marker, it is essential to establish a reliable reference interval in a healthy population. Close examination of previous studies that determined reference ranges for iMg has shown that a wide spectrum of ranges have been developed. This may be due to the lack of partitioning that has been reported and possibly due to the differences among the three different companies and the ISE that they use.

5.5 CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

This study concluded that there is a significant within-day variation in iMg (six blood samples per day, taken every three hours from 7:00 till 22:00), and a non-significant between-day variation of iMg (three consecutive days of sampling). A significant within-day variability of iMg could have a large impact on magnesium research. Ionic Mg has the potential of becoming the most widely accepted means for assessing Mg status, yet this variability may jeopardize the use of iMg in a clinical setting as well as question the validity of prior research that used iMg as a means of magnesium measurement.

Discovery a within-day variability triggers the necessity for more research into iMg. Circadian rhythm was considered a cause for the variability found in iMg, yet when data was pooled together a non-significant rhythm was found. However, a closer examination of an individual subject circadian rhythm may give greater insight to the possibility of a diurnal pattern. Knowledge of changes of any analyte is vital when collecting specimens, not only in research but also in clinical settings. A serial change in a patient's analyte level may be significant yet may not fall out of the population-based reference ranges. Therefore, individual reference ranges may be more suitable. Future study should examine the causal factors for physiological variability in iMg and the time course in which these changes take place.

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APPENDICES

Appendix A

Daily Log

Name:
Age:
Gender:

- Please fill out this log as completely and accurately as possible.
- For questions with scales normal = with respect to only yourself
- For questions # 5, 6, 7, answer yes or no, and add comments if yes

1. Length of exercise session (minutes):

DAY	1	2	3
X			

2. Length of intensity section of exercise session (minutes):

DAY	1	2	3
X			

3. Number of hours of sleep:

DAY	1	2	3
X			

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

DAY	1	2	3
X			

5. Minor illnesses:

DAY	1	2	34
X			

Comments regarding above - e.g., cold, flu, headache, sore throat

*
*
*

6. Major stressful events:

DAY	1	2	3
X			

Comments regarding above - e.g., exams, family, job

*
*
*

7. Menstruation:

DAY	1	2	3
X			

Comments regarding above

*
*
*

8. General energy level (1 = very low, 2 = low, 3 = normal, 4 = high, 5 = very high):

DAY	1	2	3
X			

9. **Willingness to train** (1 = very low, 2 = low, 3 = normal, 4 = high, 5 = very high):

DAY	1	2	3
X			

10. Additional comments

*
*
*

Appendix B

Participant Consent Form

I,_____ (please print name), hereby consent to participate in this research study which is trying to find if there is variability in ionic magnesium. The purpose of the study is to validate if ionic magnesium is a reliable measure for determining magnesium status.

The purpose and procedures have thoroughly been explained and I understand that I will be donating 18 blood samples (7 ml/sample) over a three day period. I will try to follow my normal daily routine as closely as possible, and keep accurate records in my daily log journal.

The amount of blood drawn for each sample will be small, with little discomfort during the procedure, however there may be slight bruising and/or tenderness at the point of puncture every time blood is drawn.

I understand that there will be no direct benefit to me from participating in this study, and realize that I can withdraw from the study at any time and for any reason. Any information collected from me will be keep strictly confidential and if the results are published, my identity will not be revealed.

Signature of Participant **Date**

Signature of Witness **Date**

I, Kris Johnson, have explained the nature of the study to the subject and believe he/she has understood it.

Signature of Researcher **Date**

Appendix C

Circadian Rhythm Correlation Coefficients

SUBJECT	DAY 1	DAY 2	DAY 3	MEAN
1	0.780	0.577	0.863	0.740
2	0.883	0.874	0.670	0.809
3	0.851	0.820	0.626	0.766
4	0.525	0.717	0.650	0.631
5	0.884	0.636	0.923	0.814
6	0.981	0.923	0.939	0.948
7	0.765	0.995	0.930	0.896
8	0.992	0.678	0.809	0.826
9	0.923	0.848	0.668	0.813
10	0.750	0.714	0.882	0.782
11	0.780	0.815	0.618	0.738
12	0.878	0.867	0.648	0.798
13	0.660	0.992	0.768	0.807

Appendix D

PARTITIONING THE VARIABILITY

Subject	Total Variation	Analytical Variation	Physiologic Variation
1	7.2	2.3	4.9
2	3.8	2.3	1.5
3	4.1	2.3	1.8
4	3.2	2.3	0.9
5	4.1	2.3	1.8
6	4.8	2.3	2.5
7	3.1	2.3	0.8
8	4.8	2.3	2.5
9	4.4	2.3	2.1
10	3.7	2.3	1.4
11	3.8	2.3	1.5
12	5.6	2.3	3.3
13	3.6	2.3	1.3
MEAN:	4.3	2.3	3.6

*Total, analytical and physiological variability expressed in percentages.