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MAGNESIUM CONCENTRATIONS IN THE PLASMA AND ERYTHROCYTES OF YOUNG COMPETITIVE SWIMMERS AND CONTROLS AND THEIR RELATIONSHIP TO MAXIMUM OXYGEN CONSUMPTION

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By

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ABSTRACT

MAGNESIUM CONCENTRATIONS IN THE PLASMA AND ERYTHROCYTES OF YOUNG COMPETITIVE SWIMMERS AND CONTROLS AND THEIR RELATIONSHIP TO MAXIMUM OXYGEN CONSUMPTION

By

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Magnesium concentrations in the plasma and erythrocytes of twenty-two 9-13 year old competitive swimmers and of eighteen children of similar age, gender, height and weight, but not in training for any sport, were determined by atomic absorption spectrophotometry, compared, and then correlated with maximum oxygen consumption obtained by treadmill testing. Plasma magnesium was significantly ($P<0.05$) greater in male swimmers than in female swimmers (2.11 ± 0.11 vs 1.95 ± 0.15 mg/dl), but not different between male and female controls. Plasma magnesium was significantly ($P<0.05$) correlated with maximum oxygen consumption in all controls, $r=0.44$, in all males, $r=0.42$, and in control males, $r=0.58$, but there was an inverse correlation between plasma magnesium and maximum oxygen consumption in female swimmers and in all females. Adaptation of magnesium metabolism in response to endurance training may differ in pre-teen males and females.

DEDICATION

To my parents,

Earl and Estle Winegardner

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To my husband, Maurice, and my children, Ellen, Sara, Nathan, and Susannah, I express sincere gratitude for the support and freedom they gave me when I most needed it.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER I

THE PROBLEM

Magnesium has been known to be important to man for a long time. As early as 1810, magnesium oxide was used to treat patients with uric acid stones (102,p.1). Although magnesium deficiency was described in animals in 1932, description of the deficiency in man and research concerning the biological functions of magnesium was hampered until the development of atomic absorption spectrophotometry during the 1950's (102,p.2). Magnesium has been found to be obligatory wherever there is adenosine triphosphate (ATP), the source of useable energy within the body (1,p.11). Magnesium is necessary for all enzymes for which phosphate or a phosphate-compound is the substrate or thiamine pyrophosphate is a cofactor; therefore, it is necessary for oxidative phosphorylation, nerve conduction, muscle contraction, membrane transport, and protein synthesis (102,p.3). In 1975, Rubin (87), proposed that the concentration of free Mg ions within the cells seems to regulate the metabolic activity of the cells.

Dietary magnesium on the order of 6 mg/kg body weight/day has been reported to be sufficient to produce magnesium balance in humans; obligatory daily losses seem to be more for adult males than for adult females (88).

Recommended daily allowances have been set at 350 mg/day for adult males and 300 mg/day for adult females (70). Although magnesium is known to be necessary for growth in young animals (102,p.2;42;43) and necessary in the refeeding of young victims of kwashiorkor (89,p.123), there is little information on the requirements of magnesium and the factors that influence those requirements during childhood and adolescence. "Allowances for children and adolescents are only estimates but are intended to allow for increased needs during rapid bone growth"(70).

It is difficult to produce a symptomatic magnesium deficiency in man by simply restricting dietary intake (102,p.72;1,p.57). However, both Seelig (88,89) and Holtmeir and Kuhn (56) have suggested that a chronic sub-optimal intake of magnesium is widespread throughout occidental societies and that the "relative magnesium deficiency" which results may play a role in the development of cardiovascular diseases. Clinical observations, epidemiological evidence, and some experimental evidence have been reported which support continued study of magnesium's possible role in prevention of chronic diseases (106;96;5;1,p.69,70;76;101).

Several factors increase the excretion of magnesium from the body or result in a need for more magnesium. Dietary factors such as high intakes of fats, phytates, calcium, zinc, and phosphates decrease absorption of magnesium; high intakes of protein, carbohydrates, vitamin

D, and calcium increase renal excretion of magnesium; and high intakes of protein, carbohydrates, thiamine, vitamin D, and pyridoxine increase the need for magnesium (61). Other factors which cause a greater excretion of or need for magnesium are increased levels of growth hormone, thyroid hormone, and aldosterone (32). Sometimes conditions which result in adrenergic stress have been observed to be accompanied by signs of magnesium deficiency and are, therefore, thought either to increase the need for magnesium, or to influence the metabolism or distribution of magnesium in the acute situation in such a way as to mimic symptoms of deficiency (33). Among these conditions are exposure to cold, psychological stress, and prolonged physical exercise.

Several investigators have reported a significant fall of approximately 15% in the plasma magnesium concentration of male subjects as an acute response to exercise bouts lasting one and one-half to six and one-half hours (74,83,84,112). In addition, both Wolfswinkel, Van der Walt and Van der Linde (112) and Refsum, Meen and Stromme (83) have measured a quantitatively similar rise in erythrocyte magnesium during prolonged exercise. In the only study that tested a control group, there was less decrease in the plasma magnesium of the control males than in the plasma magnesium of the athletes during the same period of exercise (74). An acute response to prolonged exercise, particularly when the response is unequal in athletes and in untrained men, suggests that a chronic adaptation to training may

occur. Lukaski et al (62) found evidence of a possible adaption of magnesium metabolism to physical training in the positive correlation of plasma magnesium with maximal oxygen consumption in trained athletes.

The participation of children in vigorous training in preparation for competitions in various sports raises questions of how that training influences their growth and their nutritional needs. Because growth seems to increase the need for magnesium in young animals (102,p.2), because exercise may alter magnesium metabolism in adult males (62), and because a relative deficiency of magnesium may predispose humans to chronic diseases later in life (89), it is important to ask if there is evidence that the magnesium metabolism of growing children is altered by the exercise involved in training.

PURPOSE OF THE STUDY

The purpose of this study was to ascertain whether evidence of cellular adaptation of magnesium metabolism in the form of a positive correlation between plasma magnesium and maximal oxygen consumption could be found in pre-teen, endurance trained, competitive swimmers. It was also designed to determine if differences in plasma, erythrocyte and whole blood magnesium concentrations exist between 9-13 year old swimmers and controls of similar age, gender, height and weight who were normally active in recreational games but not in training for any sport. The differences

between males and females as well as the differences between older and younger members of the experimental and control groups were considered.

RESEARCH HYPOTHESES

The specific hypotheses which were tested were:

1. There is a positive correlation between plasma magnesium and maximal oxgen uptake of the 9-13 year old swimmers, the male swimmers and the female swimmers, but no correlation between these variables in the respective control groups.

2. The concentration of magnesium in the plasma of pre-teen swimmers, male swimmers, and female swimmers is greater than the concentration of magnesium in the plasma of their controls, but the males do not differ from the females in plasma magnesium.

3. The pre-teen swimmers, male swimmers and female swimmers do not differ from their respective controls, and the males do not differ from the females, in concentrations of magnesium in their erythrocytes or whole blood, or in hematocrits, or in concentrations of hemoglobin.

4. There is no correlation between red blood cell magnesium or whole blood magnesium and maximum oxygen uptake in either the swimmers or the controls.

5. Plasma magnesium, erythrocyte magnesium and whole blood magnesium are not correlated with hemoglobin or hematocrit in the pre-teen swimmers, male swimmers, female swimmers or

in their respective control groups.

6. The 9-13 year old swimmers do not differ from the controls in consumption of nutrients considered in this study: kilocalories of energy, kilocalories per kilogram of body weight, percentage of energy consumed as fats, carbohydrates, and protein, dietary cholesterol, ratio of polyunsaturated to saturated fat, protein, calcium, magnesium, phosphorus, thiamin and vitamin D.

7. Pre-teen males eat more total kilocalories and more kilocalories per kilogram of body weight than similarly aged females, but do not differ from each other in consumption of the other selected nutrients when intakes have been expressed as a percentage of their recommended daily allowance (%RDA).

8. In the limited age group considered in this study, younger swimmers do not differ from older swimmers, younger controls do not differ from older controls, younger males do not differ from older males, and younger females do not differ from older females in whole blood, erythrocyte, or plasma magnesium, or in nutrient intake expressed as %RDA.

RESEARCH PLAN

Fasting blood samples were drawn from each of twenty-two 9.5-12.9 year old swimmers, 9 males and 13 females, who met the criteria of training intensity and duration set for inclusion in the study, and from eighteen control children, 11 males and 7 females, who were not training. Whole blood,

erythrocyte, and plasma magnesium concentrations were determined by atomic absorption spectrophotometry (86,51). Hematocrits (67) and hemoglobin (51) determinations were also made.

Dietary intake was estimated from a three-day food record kept by each subject, clarified by interview, and analyzed for nutrients by means of the Michigan State University Nutrient Data Bank (69).

The subjects for this study were recruited for another study (94). Written, informed consent was obtained prior to their participation. Measurements of physical characteristics and the other data used for correlation with magnesium concentrations were obtained from that study.

Maximum oxygen uptake was calculated from percentages of oxygen and carbon dioxide in the expired air collected while subjects ran to exhaustion on a treadmill (23). Protocol for the run was continuous with constant speed and increasing grade.

Body fat percentages were determined by underwater weighing. Residual lung volume was determined at the time of underwater weighing by the rebreathing method (63) as modified by Rahn, Fenn, and Otis (79). Buskirk's formula (14) for body density and the Siri equation (92) for body fat were used in the calculations.

Three way analysis of variance, Student's t-test and the Pearson product-moment correlation coefficient were used to analyze results (72).

LIMITATIONS OF THE STUDY

A true control group, i.e. swim team members with the same characteristics, abilities and aspirations but restricted from training and competition, was not possible. The comparison group used in this study may have been different from the experimental group in prior factors that caused the swimmers to choose competitive swimming.

It was necessary to use an insufficient sample size of 40 subjects in this study. Necessary and sufficient sample size was calculated to be 63 to detect a difference between means of one-half standard deviation.

Restriction of physical activity and eating prior to the fasting blood draw and testing was not monitored.

Accuracy of the daily food records was subject to the motivation of the children and assistance of their parents in keeping the records and to their ability to estimate quantities, even though great care was exercised to obtain the most complete records possible by interview. The magnesium content of the water supply can provide 0-96 mg/day (1,p.72). No attempt was made to determine the magnesium content of the various water supplies used by these young swimmers and controls, nor to determine the quantity of water consumed.

Hemoglobin determinations were made with two different Drabkin solutions. The time that the blood samples were refrigerated before separation varied from one to ten hours. The contribution to differences made by these two

limitations are thought to be very minor.

Although the relative proportion of younger and older subjects was similar in both the experimental and control groups, there were more older subjects than younger subjects.

The results of this study are applicable only to children between the ages of 9 and 13 years.

SIGNIFICANCE OF THE STUDY

The role of magnesium in growth and development is only beginning to be explored (25). The central involvement of magnesium in energy production and protein synthesis (42,43) makes it a very important consideration when the stress of training for several months is superimposed upon growth. It is important to know if physical training for competition over a prolonged period of time alters magnesium metabolism in children.

CHAPTER II

REVIEW OF LITERATURE

Magnesium is an important metal in the life of man. Aikawa (1,p.6) suggests that this importance may be due to the abundance of magnesium in the environment at the time life on earth began so that biological systems evolved with a heavy dependence on its availability. Not only is it the central cation in the structure of chlorophyll (1,p.15), but the whole process of photosynthesis cannot proceed without magnesium (1,p.18;102,p.46,50,51).

Within the physical body of man, magnesium has many important biochemical roles. In intracellular metabolism it is essential in the process of oxidative phosphorylation. Magnesium serves as an activator for enzyme systems, particularly those reactions involving adenosine triphosphate (ATP) (1,p.23). It has been suggested that free magnesium ions regulate overall cellular metabolic activity by affecting the rate-limiting transphosphorylation reactions (102,p.50). Magnesium bound to the inner mitochondrial membrane plays a role in the permeability of the membrane to monovalent cations (102,p.19). Magnesium is necessary for protein synthesis (102,p.29). Since ATP is required for the utilization of glucose, for the synthesis of fat, protein, nucleic acid and coenzymes, and for the

contraction of muscle, magnesium metabolism also affects all of these functions secondarily (1,p.27).

Physiological roles of magnesium are many and varied. These roles include stabilization of the electrochemical equilibria of the axonal membrane; nerve impulses are normal only when sufficient magnesium is present (102,p.70). Magnesium affects the release of acetylcholine at the neuromuscular junction (103), influences skeletal muscle contraction (102,p.50), and may act as a second messenger for insulin (102,p.50;1,p.27). Magnesium also is necessary for mobilization of calcium from bone (91), and it may play a central role in prevention of heart disease (1,p.72-76;89,p.141-156,186-252), atherosclerosis (89,p.161-177), and hypertension (1,p.77;89,p.178-183;65;66).

In a discussion of these many roles of magnesium, it is also pertinent to consider the body content of magnesium and the way it is measured, the intake of magnesium required daily for maintainence, and the factors which affect influences on absorption and excretion of magnesium.

CELL METABOLISM

Heaton (54) has reviewed the studies of the activation of enzymes _in vitro_ by magnesium and the metabolic disturbance during magnesium deficiency _in vivo_. The more than 100 enzymes activated by magnesium can be divided into five groups according to the type of reaction they catalyze. The first group catalyzes transfer of substituted phosphoric

acid, is the largest group, and includes kinases, synthetases, and phosphatases but not phosphorylases (which do not seem to require Mg ions for activity). The other four groups of enzymes catalyze transfer of acyl groups, hydration-dehydration reactions, carbonyl addition reactions and dehydrogenations.

Magnesium activates seven of the enzymes involved in the glycolytic pathway and three enzymes in the oxidation of pyruvate by the tricarboxylic acid cycle (54;102,p.47). Magnesium appears to have two mechanisms in activating enzyme systems (102,p.33;34). If it reacts with the enzyme substrate, the reaction just needs a divalent cation and calcium can substitute for magnesium. If it reacts with the enzyme directly, Mg ions are necessary and calcium acts as a competitive inhibitor for those enzyme systems. Vitale, Nakamura, and Hegsted (101) were able to demonstrate the uncoupling of oxidative phosphorylation in very young rats after only four days of a magnesium deficient diet. The effect was reversible by magnesium administration. Heart mitochondria were more susceptible than were kidney or liver mitochondria.

A model for the control of metabolism and growth through the activity of the Mg ion has been proposed by Rubin (87). In this model, alterations in the cell membrane where Mg ions bind, by effectors such as insulin, lead to an increase in the free Mg ions within the cell. These free Mg ions, in conjunction with the ATP concentration of the cell,

regulate the rate-limiting transphosphorylation reactions. It is the alteration of the membrane by insulin and subsequent release or influx of Mg ions that led to the designation of Mg ions as the "second messenger" (1,p.27;102,p.50).

Magnesium also acts at the mitochondrial membrane to monitor permeability to monovalent cations. This mechanism was studied by Wehrle et al (108) subsequent to much earlier observations that intracellular potassium (K) could not be maintained in the presence of severe magnesium deficiency (24;109). However, intracellular K was found to return to normal when the magnesium deficiency was relieved. Gunther, Averdunk and Ising (52) also found a low intracellular K level secondary to magnesium deficiency. They postulated that this low intracellular K concentration further depresses decreased protein synthesis which is due to magnesium deficiency.

PROTEIN SYNTHESIS

Protein synthesis occurs in the ribosome and magnesium plays a vital role. The structure of the ribosome itself is dependent upon Mg ions (102,p.29). The size of ribonucleic acid (RNA) aggregates is controlled by the concentration of magnesium (1,p.27); when the concentration of magnesium is too low, the aggregates are too small for synthesis to continue. Magnesium links the template and transfer ribo-

nucleic acid (tRNA) to the ribosome (103). It may serve as a coenzyme in the synthesis of polypeptides (1,p.25). In order for polypeptide formation to proceed, magnesium must activate aminoacyltRNA synthetase, but the amount of magnesium required depends upon the amino acid being activated (102,p.27; 91). Within the cell nucleus, magnesium may be responsible for the unwinding of the parent deoxyribonucleic acid (DNA) which is required before cell duplication can occur (1,p.25;103).

Alteration of protein synthesis in magnesium deficiency is shown by lack of growth and occurs quickly and dramatically in young animals. This phenomenon has been known since 1926 (103,p.64). As a result of their studies with rats, George and Heaton (42,43) proposed that the primary lesion in magnesium deficiency occurs in the mitochondria rather than the ribosome and that decreased protein synthesis is secondary to the impairment of energy metabolism. They suggest that the metabolic disturbances, therefore, are likely to vary between different tissues according to the energy requirements of the particular cells.

REGULATION OF CONDUCTIVITY

As early as 1869, intravenously administered magnesium was found to mimic curare in animals (102,p.2). Calcium and magnesium act antagonistically in stimulating and inhibiting the release of acetylcholine and norepinephrine at nerve endings. The flaccid, anesthesia-like state observed in

animals after intravenous administration of magnesium sulfate is thought to be due to this peripheral neuromuscular blockade (45,p.880). However, Somjen, Hilmy and Stephen (95) demonstrated that the muscular paralysis is not accompanied by loss of sensation or consciousness in humans.

Clinical manifestations of magnesium deficiency, while not presented by all patients with magnesium deficiency, serve to illustrate the role of magnesium in maintaining the integrity of the nervous system. These symptoms are discussed by Flink (38) and include muscular twitching, purposeless movements, muscle cramps, weakness, irritability and restlessness, and convulsions. The basis of magnesium's effect on the neuromuscular system is not understood at present but may include alteration of calcium metabolism (102,p.68).

Both Wacker (102) and Chadda, Lichstein and Gupta (16) discuss the independent, clinical observations of cardiac arrythmias (especially tachyarrythmias) associated with low serum magnesium levels which are recorded in the literature. Raising the level of magnesium to 10-15 mEq/l causes increased conduction time in the heart and slows the rate of S-A nodal impulse formation. Magnesium may be used to abolish premature ventricular contractions but, at 15 mEq/l, magnesium causes cardiac arrest in diastole (45,p.880).

Although the role of magnesium in stabilization of neural and muscular cellular membranes, in chemical transmission at synapses and the neuromuscular junction, and

in muscular contraction and relaxation has been established, the mechanisms are as yet largely speculative. Chutkow (19) has reviewed in detail the physiologic role and biochemical functions of magnesium in the central and peripheral nervous systems and the proposed mechanisms for alterations due to an excess or deficiency of magnesium.

MUSCLE CONTRACTION

Wacker (102,p.50) reviews several studies which show that magnesium is involved in muscle contraction through increased reuptake of calcium by the sarcoplasmic reticulum. However, magnesium affects heart muscle ATPase and skeletal muscle ATPase differently. A change in the concentration of free Mg ions can alter the net release of calcium, can change the sensitivity of heart muscle to alterations in the calcium concentration, and can change the amount of calcium required to activate heart muscle. Other studies suggest that the concentration of free Mg ions regulates the trigger mechanism mediated by the binding of calcium to troponin.

A high level of magnesium also seems to be necessary for adequate removal of calcium from troponin in resting muscle. Contraction persists when the magnesium concentration is too low. Both Mg ions and MgATP are necessary for muscle relaxation (107,p.645).

BONE METABOLISM

Magnesium deficiency causes major metabolic disturbances of bone, producing brittle and hypermineralized bones, particularly in those which are more metabolically active. That is, there is more effect in the mandible than in the femur (89,p.278). This disturbance in skeletal growth may be due to the importance of magnesium in enzyme systems which synthesize protein polysaccharides and collagen, thereby producing hypermineralized bones with little matrix (1,p.110). The effect of magnesium deficiency also may be due to its effect on synthesis or activation of alkaline and pyrophosphatases which are involved in bone mineralization (89,p.281,289).

Animal studies of the effect of magnesium deficiency on bone mineralization have led to conflicting results. Seelig (89,p.268-275) reviews these studies and suggests that the inconsistant results may be due to the widely differing composition of the diets used: from 3200 to 8000 ppm calcium, 1900-5100 ppm phosphorus, 1150-1,000,000 IU vitamin D per kg of diet mix, and 3-100 ppm magnesium. These studies showed that a high intake of vitamin D and calcium and a low magnesium intake resulted in decreased mobilization of bone calcium and replacement of surface bone magnesium by calcium (89,p.270). High intake of vitamin D and low intake of both calcium and magnesium resulted in resorption of bone and calcification of soft tissue either due to a direct effect of vitamin D or to a secondary hyper-

parathyroidism caused by hypocalcemia. The effect of hypomagnesemia on parathyroidism is not clear-cut because in severe magnesium deficiency, increased magnesium deposition in bones occurs (89,p.272). Increasing dietary phosphate increased the need for magnesium for survival and produced bone and tooth mineral changes in guinea pigs. Increasing magnesium along with the phosphate prevented the mineralization changes. High phosphorus/calcium and high phosphorus/magnesium ratios have been found to cause bone wasting and renal calcification in several species which may be due to the phosphate-induced depletion of magnesium. Other studies have produced increased bone formation and mineralization with phosphate loading and have explained the findings by noting that phosphate is bound to collagen and initiates crystal nucleation and growth (89,p.275).

CARDIOVASCULAR DISEASE

Ventricular muscle has a high magnesium content (106), and left ventricular muscle has a higher magnesium content than right ventricular muscle (96). Victims of heart attacks have low levels of myocardial magnesium and even lower levels in infarcted areas of their hearts (96). Anderson, Leriche, Hewitt and Neri (5) noted that fatal cardiac arrythmias are less common in hard-water than in soft-water areas. Their autopsies, performed over a four-month period in two areas of Canada, showed that the magnesium concentration of heart muscle was greater in hard-

water areas than in soft-water areas. Aikawa (1,p.69,70) reviews other evidence suggesting an inverse relationship of magnesium in drinking water and cardiovascular diseases. In addition to the Canadian studies, he cites a Finnish investigation, English studies, and a World Health Organization study of five European cities that provide epidemiological evidence suggesting that lack of magnesium may play a role in heart disease. These discoveries led to the hypothesis that magnesium deficiency might be a predisposing condition for various forms of heart disease. However, it also is possible that a redistribution of tissue magnesium takes place as a result of the heart attack (17).

The possible mechanisms for the involvement of lack of magnesium in heart disease may include: (a) prolongation of the QT interval resulting from delayed repolarization of the ventricular myocardium thus enhancing the likelihood of cardiac arrythmia, myocardial infarction and sudden death (1,p.73); (b) alteration of myofibrillar contraction and relaxation and of uptake and release of calcium from the sarcotubules (76); and (c) alteration of the energy provided to myocardial cells from the aerobic oxidation of fatty acids and glucose and of the synthesis of ATP by the uncoupling of oxidative phosphorylation (101). Seelig (89) devotes one chapter to a review of the literature concerning magnesium deficiency and cardiac dysrhythmia.

Hypertension is considered to be part of the complex of cardiovascular diseases. Indirect evidence that lack of

magnesium contributes to hypertension during pregnancy is the long-standing successful use of magnesium sulfate in the treatment of eclampsia (65). Acute hypomagnesia in man and animals is associated with rises in blood pressure and elevations of peripheral vascular resistance (1,p.77). A study by Altura and Altura (3) suggests that Mg ions may be important in regulating permeability, in translocating and binding of Ca ions in vascular muscle, and in regulating vascular tone and blood pressure. Seelig (89,p.178-180) also presents evidence from the literature of experimental magnesium deficiency which is associated with increased blood pressure; however, she discusses other studies that offer experimental and clinical evidence that magnesium deficiency has decreased blood pressure, perhaps due to decreased activity of neurohypophyseal peptides in response to the magnesium deficiency (89,p.182). Altura, Altura, and Waldemar (4) suggest another hormonal-magnesium mechanism for control of blood pressure: without optimal (neither too high nor too low) magnesium concentrations, prostaglandin cannot evoke arterial muscle relaxation. The relationship of magnesium and hypertension remains to be clarified.

Seelig (89,p.162-169) describes the similarities found in arterial lesions of infantile arteriosclerosis and in the coronary and myocardial lesions produced in animals on magnesium deficient diets and in herds grazing on magnesium-poor lands, especially during early lactation. She further describes a cardiovasopathic diet that causes spontaneous

myocardial infarction. atherosclerosis, hyperlipidemia and elevated blood pressure when fed to rats, dogs and cocks (89,p.172-173). Except for being low in chloride, this diet possesses the characteristics of diets consumed by many people in an affluent society: high in saturated fat, cholesterol, protein, vitamin D, sodium, and phosphate; low in magnesium, potassium, and chloride; normal in calcium. "Increasing the dietary intake of magnesium chloride five-fold over the normal requirement mitigated, significantly, the cardiopathic changes as well as the coronary and aortic pathology, which had included thickening of the small coronary arteries, with marked increase of the arterial wall/lumen ratio" (89,p.173).

A different issue, but related to vessel damage and wall lesions observed in atherosclerosis, is the elevated cholesterol and blood lipids associated with atherosclerosis. Much of the impetus for correlating serum magnesium with serum cholesterol came from clinical reports that parenteral magnesium administration was helpful in treating patients with myocardial infarction. Seelig (89,p.145-147) presents evidence from several clinical studies conducted from 1956-1971 that magnesium administration induces decreased total serum cholesterol, decreased low density lipoproteins (LDL), and/or increased high density lipoproteins (HDL) in patients with myocardial infarction, angina, ischemic heart disease, or peripheral arterial disease. More cholesterol in the LDL fraction

appears to be atherogenic, while elevated HDL may be protective (48). The relationship of serum magnesium and serum cholesterol and lipoprotein fractions in apparantly healthy individuals is unclear. Bersohn and Oelofse (9) found lower serum cholesterol, higher serum magnesium, lower incidence of arteriosclerosis, higher dietary intake of magnesium in Bantus than in white South Africans. In an Australian study, Charnock, Casley-Smith, and Schwartz (18) found lower incidence of cardiovascular disease, lower serum cholesterol and higher serum magnesium among the aborigines than among other Australians living in the same locality. However, they found very high serum cholesterol associated with high serum magnesium levels in Australians from a northern area 1000 miles away, which is in contrast to the low serum cholesterol associated with high serum magnesium among the aborigines. Jankelson, Vitale and Hegsted (58) compared serum magnesium and lipid fractions of atherosclerotic patients and controls and found higher magnesium levels and lower lipoprotein levels (both HDL and LDL) in the control subjects than in the patients. The cholesterol level (normal, 204 mg/dl) was reported to be the same in both. However, patients who were alcoholics or nonalcoholics with liver disease did not yield consistant correlations and these investigators concluded that they were unable to demonstrate a correlation between serum magnesium and serum cholesterol levels. Mondschein (68), in investigations carried out on 32 random human subjects aged

40-60 years, found 50% of the serum magnesium levels to be below the defined normal range and none to be above. He discovered highly significant negative correlations between serum magnesium and cholesterol and between serum magnesium and LDL. He suggested that low serum magnesium may affect the conformational integrity of LDL apoprotein and may attenuate its binding efficiency for dietary cholesterol thus decreasing lipid transport and increasing both serum cholesterol and LDL through a feedback mechanism to the hepatic cells.

The influence of diet on serum lipids has been the focus of various investigations attempting to find a means of preventing cardiovascular disease through changes in diet. Seelig (89,p.137-141) describes a variety of studies on the effects of dietary fat, vitamins, and minerals on serum lipids that have produced conflicting results. Of interest in the present discussion is the hypercholesteremic effect of vitamin D, perhaps partly because of the influence of vitamin D on magnesium metabolism. Seelig (89,p.140) cites several studies confirming the increase in urinary loss of magnesium when vitamin D intake is in excess and confirming the production of a high calcium/magnesium ratio due to enhanced calcium absorption. (Rather than influencing serum cholesterol, this ratio appears to increase arterial resistance.) Lindeman (61) also describes the same vitamin D effect on magnesium metabolism as well as an effect of high dietary fat in decreasing the absorption of magnesium.

Hence a high fat diet may influence serum lipid levels partly by causing a decrease in magnesium absorption.

Rats were used by Rademeyer and Booyens (78) to confirm the inverse relationship between serum magnesium and serum cholesterol found by Bersohn and Oelofse (9) in man. They also altered amounts of saturated and unsaturated fat in the rats' diets and substituted maize meal for glucose to determine if maize meal might have contributed to the low incidence of circulatory diseases in the Bantu. They found that addition of saturated fats to the diet caused hypercholesterolemia accompanied by low serum magnesium levels in the rats. Unsaturated fat caused hypocholesterolemia and increased serum magnesium levels. Substituting maize meal for glucose countered the hypercholesterolemia and decreased serum magnesium levels which had followed the addition of saturated fats. They attributed the hypocholesterolemic effect of maize meal to its high magnesium content, its stimulating effect on intestinal flora which enhances excretion of cholesterol, and its high fiber content which also increases cholesterol excretion.

In a more recent study of rats to determine the effects on blood lipids of a high carbohydrate but magnesium-deficient diet, the investigators found that such a diet, particularly if the carbohydrate was sucrose, greatly increased the cholesterol and triglycerides in LDL and very low density lipoproteins (VLDL), and decreased the amounts in HDL (81). In addition, these increased blood lipid

levels were accompanied by decreased serum magnesium levels. Because this study used diets which were very deficient in magnesium, it still leaves in question the relationship of serum magnesium and serum lipids when diets are sufficient, or nearly sufficient, in magnesium.

Further study is necessary to determine whether, in humans, serum magnesium levels are related to serum cholesterol or lipoprotein fractions and to identify what mechanisms might be involved in such a relationship.

BODY CONTENT AND DISTRIBUTION OF MAGNESIUM

Magnesium is the fourth most abundant cation in the body (102,p.52). A 70-kg adult contains about 2000 mEq or 24 gm of magnesium (103). About 60% of this total is found in bone and most of the rest is distributed equally between muscle and non-muscular soft tissue. Aikawa (1,p.44) provides a table of magnesium content in human tissues developed from assays of adult accident victims in India. Tissue levels range from 11.7 mEq/kg wet weight in uterine tissue to 28.3 mEq/kg in breast tissue. Approximately 1% is found in the extracellular fluid (1,p.43). Plasma magnesium has a narrow normal range within 0.75-1.05 mM (1.5-2.1 mEq/l or 1.8-2.52 mg/dl) but varies with the investigator (89,p.359). Each laboratory is urged to establish its own "normal" range with a coefficient of variation of 10%-20%. About 33% of plasma magnesium is bound to protein, 55% of the ions are free and the rest is complexed with citrates,

phosphates and other anions (34;102,p.53). Erythrocyte magnesium varies greatly with the investigator, but the normal range appears to be about 4.4-8.0 mg/dl (89,p.362).

Henrotte, Benech and Pineau (55) demonstrated significant differences in male and female plasma magnesium concentrations and in male and female red blood cell magnesium concentrations between the ages of 21 and 36 years. Males had significantly higher values in both compartments. Before age 22, there were no significant differences in plasma or erythrocyte magnesium. They suggest that there may be a modification of magnesium metabolism at about the age of 20 in both males and females, possibly related to gonadal or adrenal activity. After menopause, the red blood cell magnesium of females may exceed that of males.

Magnesium is the second most abundant cation within the body cells, exceeded only by potassium (102,p.52). Intracellular magnesium is 80% bound and this buffers the free ion at a relatively constant concentration which is optimum for enzyme activity. Subcellular distribution of magnesium is not homogeneous but appears to be mostly bound to microsomes. Even within the mitochondria, 4% is in the outer membrane, 50% is in the intermembranous compartment, 5% is in the inner membrane and 41% is in the matrix (34).

Bone magnesium exchanges with serum magnesium but the rapidity of exchange declines with age and the stable magnesium of bone increases with age (1,p.48). After

intakes of diets moderately deficient in magnesium for 18 weeks, weanling rats maintained magnesium values in soft tissue at the expense of bone stores (37). The investigators suggested that this protects against cardiac abnormalities found in extreme deficiency. Seelig (89,p.277) describes several studies in cattle that showed lactating cows with grass tetany and hypomagnesemia had normal amounts of magnesium in their bones whereas magnesium-deficient calves had soft tissue levels that were not significantly depleted but had up to a 56% loss of bone magnesium.

MEASUREMENT OF MAGNESIUM

Both in clinical chemistry and in research, the current method of choice for determining magnesium in body fluids is atomic absorption spectrophotometry. It is highly specific; sample preparation is minimal; instrumentation is simple to operate; precision can be obtained in small samples; and determinations can be performed rapidly (1,p.120). Aikawa (1,p.119) discusses three chemical procedures previously in use, and Wacker (102,p.8) describes a flame emission spectrophotometric procedure. Ebel and Gunther (34) discuss procedures for specialized uses and their limitations: electron probe analysis, helium glow photometry, and a flurometric technique.

The radioisotope ^{28}Mg has been used since 1957 in clinical studies to determine the distribution of a

parenteral load of magnesium. The 24-hour urinary excretion following an infusion approximates the infused amount but only 20% of the radioactive magnesium is recovered (1,p.47). It appears that the ions excreted are not the ions that are administered. It is necessary to use isotope ^{26}Mg for the study of absorption because of its longer half-life (1,p.49).

Magnesium deficiency is difficult to diagnose because: (a) serum levels of magnesium have been found to be an unreliable indicator of magnesium status of the body (89,p.357; 103,p.661;25); (b) the magnesium concentration of erythrocytes is not a valid indicator of muscle and bone tissue stores, nor of total body magnesium, but is thought to be a reflection of the concentration of plasma at the time of erythropoiesis (35); and (c) clinical symptoms may or may not be present and also may take various forms (33). At present, the most practical means of evaluating magnesium status is the determination of 24-hour urinary magnesium output before and after a parenteral magnesium load. Those who retain more than 20-25% are thought to be repleting a deficit. Patients with magnesium deficiency due to renal loss will not be detected by this test (89,p.367).

DIETARY MAGNESIUM

Magnesium is widespread throughout the four food groups (grain products, milk products, meat and other protein foods, fruits and vegetables). Seelig (89,p.13,14) provides a table of foods rich in magnesium (over 100 mg/100 ml),

moderate in magnesium, and relatively poor in magnesium (under 25 mg/100 ml). This table can be summarized as follows: cocoa and chocolate, nuts including peanuts, other legumes (soybeans, butter beans, dried peas and beans), and whole unrefined grains are the richest sources of magnesium; those foods relatively poor in magnesium are meats and fish, fruits and vegetables, dairy products (eggs, milk, cream, butter) and refined cereals. Aikawa (1,p.44) ranks foods by meq of magnesium per kg of food in the following order: nuts,162 meq/kg; cereals,66; seafoods,29; meats,22; legumes,20; vegetables,14; dairy products,15; fruits,6; refined sugars,5; fats,0.6. However, when ranked on the basis of energy value of the food, the order becomes: vegetables, legumes, seafoods, nuts, cereals, dairy products, fruit, meat, refined sugars and fats.

Pao and Mickle (75) using data from the 1977-78 Nationwide Food Consumption Survey, identified the food sources contributing most to the diets of individuals with the highest and lowest intakes of magnesium. Those individuals with the highest intakes of magnesium were infants, many children and one-third of adult men under 65. Those with the lowest intakes were teenagers and adults (one-third of the adult males and 41-62% of the adult females). The fruits and vegetables group proved to be the largest source of magnesium for both the lowest and highest intake groups, contributing 23-25% of the total intake except among children under 14 where the milk group contributed most.

Grain products supplied about the same percentage for both the lowest and highest intake groups, ranging from 18-30% of the total (excluding infants). Surprisingly, beverages (mainly coffee) contributed 16-25% of the total for males and females age 23-64 years. Meat/eggs were more important sources for the lowest intake group, contributing 17-27% of total intake versus 10-17% for the highest intake group.

The Food and Nutrition Board (FNB) of the National Academy of Sciences reports that the average American diet has been estimated to contain about 120 mg of magnesium per 1000 kilocalories (70).

RECOMMENDED INTAKE AND USUAL INTAKE

The FNB sets the Recommended Dietary Allowances (RDA) high enough to meet the nutritional needs of nearly all healthy persons (70). RDA's are meant to indicate amounts of nutrients recommended for populations grouped by sex and age and are not requirements for specific individuals. Individual variations in requirements are not known and the RDA's for all nutrients except calories are planned to exceed the probable requirement. Researchers have used 80%, 70%, and 66.7% of the RDA as the level below which diets are thought to need improvement (75). For magnesium, the RDA for adult males is 350 mg/day and for adult females is 300 mg/day. "Allowances for children and adolescents are only estimates but are intended to allow for increased needs during bone growth" (70). The recommended amount for

children age 7-10 years is 250 mg/day; for males age 11-14 years, 350 mg/day; and for females age 11-14 years, 300 mg/day.

Estimates of requirements for adult males based on balance studies range from 200 mg/day (3.0 mg/kg of body-weight/day) (88) up to as high as 700 mg/day (7-10 mg/kg/day) (89,p.10). In her analysis of published balance studies, Seelig (89,p.9) found that more young women than men remained in balance at usual intakes. Using the intake at which at least three-fourths of the subjects remained in balance, she found the minimum daily requirement to be 6 mg/kg/day . Aikawa (1,p.43) gives 3.6-4.92 mg/kg/day as adequate and 3.0 mg/kg/day as possibly adequate.

The typical American intake is given by Seelig (89,p.9) as 4.0-4.9 mg/kg/day. NAS/NRC (70) reports that intake of 3 mg/kg/day is average for subjects consuming common institutional diets. Aikawa (1,p.43) states that the average American eats 240-480 mg/day. Wacker (102,p.55) reports 12.5 mM or 300 mg/day as average daily intake. Pao and Mickle (75) reported that magnesium intakes from U.S. data during 1977-78 were 65-69% of RDA for females age 15-22 years, 71-80% of RDA for all other females 12 years and older, 77-88% of RDA for males 12 years and older, and 87-209% of RDA for children under 12 years.

Ebel and Gunther (34) caution that large quantities of magnesium may be extracted in cooking water and lost so that estimates of average daily intakes may be too high. Hard

drinking water may contribute 39-96 mg/day of magnesium (1,p.72). Among those using only hard water, drinking water may supply up to 18% of the daily intake (89,p.16).

ABSORPTION AND EXCRETION

Once consumed, approximately 44% of the magnesium in an ordinary diet of about 240 mg/day is absorbed mainly in the small intestine (1,p.49). Normal absorption is influenced by total magnesium intake: with higher than normal intakes, a smaller percentage is absorbed; with lower intakes, a larger percentage is absorbed (49). Absorption also is influenced by intestinal transit time, rate of water absorption and resultant luminal magnesium concentration (91,p.311).

The mechanism of absorption is not known, but results of experiments in several species including man are non-linear and saturable, and may indicate that magnesium is taken up by means of a channel or carrier with limited capacity. The data may indicate a mechanism of simple diffusion limited at high magnesium concentrations by a permeability factor (34).

Intestinal absorption of magnesium has been shown to be decreased by high intakes of calcium, phosphate, zinc, phytates and fat in the diet (61). Calcium and magnesium may be transported across the intestinal wall by the same mechanism but evidence is inconclusive (102,p.57). A study by Coburn et al (20), which showed no effect on magnesium

absorption of two forms of vitamin D that increased calcium absorption, led them to suggest that the intestinal transport of calcium and magnesium are independent of one another. Magnesium forms insoluble magnesium phosphate complexes with phosphate (13). No mechanism is postulated for the zinc interference (61). Phytates produce insoluble and unabsorbable complexes with calcium and magnesium resulting in decreased absorption and increased fecal loss (64;28). However, after several weeks, adaptation occurs and magnesium absorption in the presence of phytates is improved (104). Dietary fat may decrease magnesium absorption by formation of fecal fatty acid magnesium soap (34;89,p.42).

Dietary fluoride has been shown to increase absorption of magnesium in rats (61). Fluoride also is thought to spare magnesium by an effect of fluoride on magnesium dependent metabolic processes.

The source of dietary protein has been found important in retention of magnesium. In persons receiving adequate protein and magnesium, animal protein causes more magnesium to be retained than does plant protein (61,2).

Protein, carbohydrate, vitamin D, pyridoxine and thiamine are thought to increase need for magnesium by increasing metabolic processes requiring magnesium, i.e. metabolic breakdown of foods, protein synthesis and growth, and bone deposition of magnesium. Refeeding of children with kwashiorkor, malnourished alcoholics, and starvation

victims with large loads of carbohydrate, protein, and thiamine precipitates symptoms of magnesium deficiency which have been found to be preventable with magnesium supplementation (61).

Excretion of the magnesium which is absorbed or given intravenously is almost entirely renal (103). There are various influences which increase renal excretion: extracellular volume expansion, diuretics, aldosterone, thyroid hormone, growth hormone (32). Vitamin D, calcium, sodium, protein, carbohydrate and ethanol increase the excretion, whereas zinc salts decrease the excretion of magnesium (61).

There does not appear to be any hormonal mechanism for regulating serum magnesium (91). In his review of the interrelation of hormones and magnesium, Wallach (105) concludes that very little is known about how magnesium interacts with the endocrine system, although magnesium metabolism is clearly altered by some hormones and variations in magnesium homeostasis can influence changes in hormone secretion. No single hormone appears to exert primary control of magnesium homeostasis, and alterations in hormonal secretion caused by magnesium may be so small as to be indiscernable under physiologic conditions.

The renal reabsorption of filtered magnesium seems to be the mechanism responsible for maintaining magnesium homeostasis (34). It can restrict losses to as low as one mEq (12 mg) per day during magnesium deprivation (103) or cause excretion of up to 164 mEq (1968 mg) per day (1,p.46).

This tremendous flexibility makes the occurrence of an excess or deficiency of magnesium rare in human adults.

EXCESS AND DEFICIENCY IN MAN

Magnesium excess usually is caused by impaired renal function or is found in association with magnesium-containing medications. Symptoms arise when serum magnesium levels reach 4 mEq/l and progress to heart block at 15 mEq/l. Dialysis is used to relieve magnesium toxicity.

It is difficult to produce a symptomatic magnesium deficiency in man by simply restricting dietary intake (102,p.72;1,p.57). Urinary excretion falls to 12 mg/day within a few days of dietary restriction. Deficiency usually is seen in individuals with a condition that either interferes with absorption, enhances excretion, or increases need. Such conditions include: severe prolonged diarrhea or laxative abuse, cancer of the colon, fasting or parenteral feeding with inadequate magnesium, chronic alcoholism, diabetic ketoacidosis, diuretics (especially furosimide and ethacrynic acid), chronic glomerulonephritis, familial renal magnesium wastage, hyperthyroidism, aldosteronism, and excessive lactation (38). Aikawa (1,p.62-63) discusses the conditions that produce deficiency in infants and children: primary hypomagnesemia due to malabsorption, neonatal deficiency due to certain surgical procedures on the gastrointestinal tract, sudden infant death syndrome, kwashiorkor, and familial disorder of magnesium metabolism.

Clinical manifestations of magnesium deficiency have previously been discussed (see Regulation of Conductivity).

Shils (91) was able to produce an experimental magnesium deficiency in human volunteers using a severely restricted diet containing 9.6 mg/day of magnesium. Urine and fecal levels fell to extremely low levels, plasma magnesium fell progressively to 10-30% of pre-experimental levels, hypocalcemia occurred in six of the seven subjects, serum sodium and phosphate did not change except in one subject, and most subjects developed hypokalemia. Neurologic signs appeared in five of the seven subjects after 25-110 days of deficiency and reverted to normal with reinstitution of magnesium.

RELATIVE MAGNESIUM DEFICIENCY

Seelig (88,89) proposes the idea that a relative or subacute magnesium deficiency may contribute to early establishment of cardiovascular, skeletal and renal lesions that lay the groundwork for disease processes that become overt later in life. Holtmeir and Kuhn (56) agree and provide a survey of causes of decreased magnesium supply in foods and diets over the past few decades:

1. Magnesium depletion of the soil has been produced by intensified agriculture, by fertilization with low magnesium fertilizers, and by use of quick-lime which increases the pH of the soil and allows removal of magnesium

in greater quantities.

2. Cultivation of fodder plants (hay, sugar beets, potatoes) removes great quantities of magnesium from the soil. These fodder plants, grown on magnesium-depleted soils in the last 100 years, contain 20-30% less magnesium than previously. The magnesium-poor feeds contribute to the "grass staggers" magnesium deficiency in animals fed them.
3. Magnesium deficiency in cattle, in turn, leads to less magnesium in the milk and meat consumed by humans.
4. Industrial food processing also reduces magnesium in the food supply by refinement of grain.
5. With a rising standard of living, increased intake of calcium, protein and fat increases human requirements for magnesium.
6. Both alcohol and oral contraceptives increase the need for magnesium.
7. Reducing diets of 1050-1450 kcal typically supply only 200-264 mg of magnesium.

In his discussion of chronic magnesium deficit, Durlach (33) lists common decompensation factors that produce a secondary or "conditioned" magnesium deficiency which masks and aggravates a chronic magnesium deficiency. Two of these factors, psychological stress and hypersecretion of

antidiuretic diencephalohypophyseal hormone (ADH), produce urinary magnesium wastage. Decompensation also can occur during physiological adaptations that increase the need for magnesium. Durlach (33) summarizes these examples from nine studies conducted between 1970 and 1975: in adaptation to cold, tissue magnesium is mobilized; in adaptation to heat, magnesium is lost in sweat and urine; in adaptation to different altitudes, magnesium exerts anti-anoxic and metabolic inhibitory activities; and in adaptation to physical strain, magnesium requirements are increased. Therefore, seasonal variations (15) and athletic activities (26) may both be pre-conditioners for magnesium deficiency.

Gunther, Averdunk and Ising (52) studied the effect of stress on magnesium deficient rats and found increased urinary excretion of adrenaline and increased cyclic AMP in some tissues in stressed animals. The increased cyclic AMP found in magnesium deficient rats may be mediated by catecholamines. That is, because an influx of Ca ions is necessary for the release of catecholamines and because Mg ions may compete with Ca at the influx site or channel, reduced serum magnesium would allow for greater catecholamine release. In this experiment, stress-induced cardiac fibrosis was enhanced in magnesium deficient rats and was thought to be due to increased catecholamine stimulation of fibroblasts.

In a study of fasted lambs stressed by cold, Rayssiguier and Larvor (82) found significant hypomagnesemia and increased non-esterified fatty acids. They concluded

that situations which stimulate lipolysis cause reductions in the level of plasma magnesium in ruminants. They postulate that grass tetany and transport tetany may be a result of adrenergic stress and lipomobilization; however, they also indicate that the actual role of magnesium in lipolysis remains to be determined.

Elliot and Rizach (36) found that magnesium accumulates in the adipocytes during adrenalin lipomobilization. Cells from the epidiymal fat pad of 6-week old rats were incubated in a buffer solution with and without epinephrine. Magnesium was measured in the fat cells and the extracellular buffer after 30 minutes and was found to accumulate in the cells. Intact adipocytes also showed an accumulation of ^{28}Mg following stimulation by epinephrine, norepinephrine and ACTH. The investigators suggested that one mechanism for hormonal control of the enzymes involved in fatty acid synthesis may involve magnesium.

Flink et al (38) have described the precipitation of magnesium oleate from aqueous solution at physiological concentrations. They also demonstrated an in vivo decrease in divalent cation activity with serum fatty acid elevation, which supports the theory of a formation of a magnesium and free fatty acid complex. These investigators concluded that the neurological symptoms associated with alcohol withdrawal may well be because the increased levels of circulating free fatty acids during alcohol withdrawal chelate sufficient magnesium ions to produce those symptoms.

Seelig (89,p.154) discusses the long-chain free fatty acid chelation of serum magnesium. Circulating long-chain free fatty acids are increased by catecholamines produced during stress. She suggests that arrythmia following myocardial infarction could be due to inactivation of serum magnesium by the catecholamine-induced increase in circulating free fatty acids. She speculates that ". . . the availability of more fat for lipolysis under stressful situations might explain the greater susceptibility of the obese individual to fatal ischemic heart disease."

Prolonged exercise in man also elevates epinephrine levels and mobilizes fatty acid from adipose tissue (7,p.500). Several investigators have reported decreased plasma magnesium levels in men during prolonged exercise (74,83,84,112). However, none of these investigators linked the decreased plasma magnesium to increased levels of circulating free fatty acids.

Rose et al (84) appear to be the first to document a decrease in serum magnesium after prolonged exercise. They hypothesized that it might be due to sweat loss and noted that the muscle cramps and nausea experienced by some subjects (eight conditioned adult males) might have been due in part to the magnesium changes. They also noted that it was unusual that serum magnesium decreased and serum potassium increased because changes in serum levels of magnesium and potassium usually parallel each other.

Refsum et al (83) also documented a decrease in serum

magnesium in 16 trained adult males age 21-58 years as well as a parallel increase in erythrocyte magnesium and no change in whole blood magnesium during each of two long distance skiing competitions that took place one week apart. Because urine losses of magnesium were low and because serum levels normalized rather quickly after exercise without any magnesium intake, they suggested that loss of magnesium in sweat was unlikely and that there probably was a transient shift from the serum into the red blood cells. They hypothesized several reasons: higher metabolic need for magnesium in the red blood cell during exercise; increase in the serum concentration of various hormones known to reduce serum magnesium (aldosterone, cortisol, growth hormone, thyroxin); increase of glucagon secretion. They noted that the effect of the mechanism tending to reduce serum magnesium was stronger than the magnesium-mobilizing effect associated with depletion of glycogen stores. They also confirmed the finding that changes in serum magnesium and potassium were in the opposite direction.

It should be mentioned here that local hypoxia, even that produced by a blood pressure cuff, has been found to cause an increase of 9.5% in plasma magnesium at the local site (110). Although egress of cellular magnesium might have produced this elevation, the increase in serum magnesium was of the same magnitude as the changes in the hematocrit and osmolality suggesting that perhaps it was due to intracellular movement of water which ocurred as a

result of a pH change. Serum potassium increased 19.0% and was thought to be due to cellular efflux.

Olha et al (74) tested five male middle and long distance runners and five sedentary males, 20-34 years of age, in two exercise protocols, a graded anaerobic threshold test and a 90 minute test at 65-70% $\dot{V}O_2$max. Both tests made use of bicycles with ergometers to alter workload. Significant differences in the mean $\dot{V}O_2$max demonstrated that the runners had adapted to aerobic training. Serum magnesium increased in both the trained and untrained subjects during the graded test but less increase was seen in the trained. Values were 2.4% $\pm$ 2.4% and 6.4% $\pm$ 3.2% for trained and sedentary, respectively. During the prolonged test, the serum magnesium of the trained group decreased 6.7% $\pm$ 2.1% and that of the untrained 2.4% $\pm$ 2.0%. This difference was statistically significant. The investigators suggested that continuous stress may be necessary to produce a decrease in serum magnesium and that the intermittant nature of the graded protocol may have prevented a decrease in magnesium levels. They suggested that the increases in serum magnesium noted during the graded protocol might be due to hemoconcentration brought about by a decrease in plasma volume, to magnesium release from exercising muscle, or to magnesium release secondary to increase in extracellular pH. Mg ions and K ions are released to aid neutralization of H ions. The attenuated increase in serum magnesium in the trained group may indicate a tendency to

maintain Mg ions intracellularly for proper enzyme function assuming that greater contractile forces produced in the trained group would result in a larger requirement for magnesium. These investigators attributed the greater decrease in serum magnesium in the trained group to the greater absolute work intensity that they performed and further postulated that serum magnesium entered the active tissue and perhaps accumulated in the mitochondria. They also found evidence in the lower respiratory exchange ratios in the trained group of greater reliance on fat as a source of energy. K ions also were increased significantly during both exercise tests in both groups of subjects, but the difference in increase between groups was not significant.

The most recent report of decreased serum magnesium during exercise is that of Wolfswinkel, Van Der Walt and Van Der Linde (112). They cite a clinical observation of an apparantly normal man who developed epileptic-type convulsions in a hot environment and who exhibited hypo-magnesemia following prolonged exercise. In their study, eight physically fit males ($\dot{V}O_2$max 56-68 ml/kg/min) ran for three hours at 66% of $\dot{V}O_2$max and rested for the three hours following. Serum magnesium levels decreased gradually until exercise ceased and gradually returned to normal at the end of the three hour rest period. Erythrocyte magnesium increased by the same amount as the amount leaving the serum, while the whole blood magnesium concentration was approximately the same. They concluded that the magnesium

which leaves the serum during exercise is stored temporarily in the red blood cells and not in skeletal muscle. They also make two other interesting observations: (a) the increase percentage change in total circulating blood magnesium during and after exercise indicates that the magnesium shifts were not restricted to the intravascular space, but that there was influx from extravascular sources and (b) the origin of additional magnesium in muscle during exercise is not the serum. Aikawa(1,p.48) discusses the results of ^{28}Mg studies which have shown a labile pool of magnesium and which suggest that it is contained primarily in connective tissue, skin, and soft tissue of the abdominal cavity (i.e. liver and intestine).

Although it seems likely that alterations in blood compartment magnesium levels actually do take place during prolonged exercise and possibly during short-term intense exercise, the reasons for the changes, the mechanisms by which they occur, and the source of the magnesium are not known at present.

ADAPTATION OF MAGNESIUM METABOLISM TO TRAINING

The investigation of Olha et al (74) suggests that there are differences in the acute response of trained and untrained males to exercise, both prolonged and intense, with regard to magnesium distribution in the blood compartments. Such occurrence raises the question of the possibility of chronic adaptations of magnesium metabolism.

Very little information is available with regard to adaptations which might alter magnesium metabolism, status, or needs as a result of exercise during prolonged periods of training.

There appear to be only two studies which have addressed the issue of alteration of magnesium status during a prolonged training period. Reported in an abstract were findings of decreased magnesium concentrations in the hair, plasma and erythrocytes of collegiate wrestlers during pre-season determinations, and decreased plasma and erythrocyte levels late in the competitive season and following it (73). In a study by Lukaski et al (62), 44 male athletes from a variety of sports (mean $\dot{V}O_2max$, 55.5±7.1 ml/kg/min) had normal mean levels of magnesium in plasma and erythrocytes which were not different from mean levels in the plasma and erythrocytes of twenty similarly aged untrained men (mean $\dot{V}O_2max$, 47.0±6.0 ml/kg/min). These investigators did find a significant correlation between the plasma magnesium and $\dot{V}O_2max$ of r=0.46 among the athletes and no relationship among controls. They proposed that this relationship may represent a cellular adaptation of magnesium metabolism to training which would facilitate oxygen delivery by enhancing the production of 2,3-diphosphoglycerate (DPG) in the erythrocyte.

The theory proposed by Lukaski et al (62) is supported by the previously discussed studies which found decreases in serum magnesium, increases in erythrocyte magnesium and no

change in whole blood magnesium during prolonged submaximal exercise (74,83,83,112). It is further supported by two studies which assessed erythrocyte DPG during exercise or after training. Boswart et al (10) found that the mean level of DPG increased in the red blood cells of eight trained speed canoists, 15-25 years of age, after a short bout of maximum intensity exercise. Resting values also increased after six months of training. From results of a study of college and high school female cross country runners, Smalley, Runyan, and Puhl (93) suggested that erythrocyte DPG increases in response to sports anemia and that trained individuals have elevated levels of erythrocyte DPG. They proposed two roles for erythrocyte DPG in prolonged physical training: compensation for loss of oxygen transport capacity during sports anemia and augmentation of oxygen utilization efficiency in trained individuals.

The mechanism by which DPG facilitates oxygen release to the tissues is established, but the role of magnesium in this process is less clear (30). DPG binds reversibly to deoxyhemoglobin but not to oxyhemoglobin (8). This binding lowers the affinity of hemoglobin for oxygen with the result that the greater the concentration of DPG within the cell, the higher the partial pressure at which hemoglobin will release oxygen to the tissues. Conditions of hypoxia cause a progressive increase in the levels of erythrocyte DPG, but the exact control mechanism is not fully understood (60). Darley (30), in studies on the storage of blood, found that

addition of free magnesium ions produced an increase in DPG within one hour at 37°C. The effect was even more pronounced when adequate glucose was ensured. When deoxygenation of hemoglobin occurs, free magnesium ions are released within the red cell, and this relieves the inhibition of hexokinase which increases glycolysis (21,44). Increased glycolysis in the presence of deoxygenated hemoglobin (which binds DPG preventing accumulation of DPG which would inhibit diphosphoglyceromutase), would favor the Rapoport-Luebering shunt and the production of DPG (85). Brewer (11) has suggested that the 5% of erythrocyte magnesium which is unbound is the agent of this regulation of glycolysis.

SUMMARY

Magnesium is integrally related to a myriad of metabolic functions in man, particularly all those involved with ATP, the energy currency of the body. Perhaps its most important role is control of the rate-limiting transphosphorylation reactions within the cells. A modification or adaptation of magnesium metabolism could therefore be expected to have widespread consequences.

Although dietary magnesium is available in a great variety of foodstuffs, there is some doubt whether human beings in affluent societies consume adequate amounts. Such individuals may be in a relative magnesium deficiency which presents no problems or symptoms until an added need for

magnesium arises such as exposure to cold, long term physical exercise, or other forms of adrenergic stress. Insufficient magnesium also may predispose people to chronic diseases of the cardiovascular system although the mechanism of this involvement is quite unclear at present.

The discovery of altered concentrations of magnesium in plasma and erythrocytes during endurance exercise and a significant positive correlation between plasma magnesium and maximal oxygen consumption in athletes have led to the suggestion that an adaptation of magnesium metabolism may occur in response to prolonged physical training.

CHAPTER III

RESEARCH METHODS

This study was designed to compare the magnesium concentrations in the plasma, erythrocytes, and whole blood of 9-13 year-old competitive swimmers and controls, and to determine whether the plasma or red cell magnesium concentrations were positively correlated with maximum oxygen consumption.

SUBJECTS

Forty Caucasian children ranging in age from 9.5 to 12.9 years participated in this study. The experimental group consisted of volunteers from swim clubs in the Lansing, Michigan, area who met the criteria of swimming at least 3500 yards per day, at least four days per week for a minimum of 35 weeks. Included were 9 males and 13 females. Control subjects were selected from the Motor Performance Study conducted by Michigan State University Department of Health and Physical Education. These 11 males and 7 females were required to be similar in age, height and weight to the young swimmers. They were normally active but not training vigorously for competition. Daily activity was determined by a questionnaire (94). Subjects for this investigation

were recruited for a different study (94) and selected data from that study were used for correlation with plasma and erythrocyte magnesium levels. Written, informed consent was obtained from all subjects and their parents prior to testing.

BLOOD ANALYSES

A 10 milliliter blood sample was drawn with stasis into a heparinized vacutainer from the antecubital vein of each experimental and control subject after a 12-hour fast. Blood samples were inverted gently to prevent clotting and were immediately stored at 4° C.

One to ten hours after the blood samples were refrigerated, triplicates of 0.5 ml whole blood were pipetted into polystyrene tubes (99) and were stored at -20° for later analysis. Hemoglobin was measured by the cyanomethemoglobin method (27) and hematocrit was determined by the method of McGovern, Jones, and Steinberg (67). Plasma was harvested from the remaining whole blood after centrifugation at 2400 RPM (1300g) for 15 minutes at 5°C in an IEC Model PR-6000 centrifuge[1]. Plasma samples were stored at -20° in polystyrene tubes for later analysis (57).

Plasma magnesium was determined by atomic absorption spectrophotometry using an Instrumentation Laboratory

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[1]Damon/IEC Division Needham Hts.,MA

spectrophotometer (Model 951)[2]. Aliquots of plasma samples were diluted 1:100 with 10,000ppm strontium chloride using a Labindustries repipet diluter[3]. They were read against aqueous standards prepared from magnesium stock solution (J.T.Baker Chemical Co.[4]) and diluted in the same manner with 10,000ppm strontium chloride (J.T.Baker Chemical Co.[4]). Plasma samples from all subjects were read on the same day directly after preparation of the standard curve. Experimental and control samples were read alternately in duplicate and the values averaged. Any duplicates that differed by more than 7% were discarded and new samples were prepared.

Whole blood magnesium was determined by the method described by Rosner and Gorfien (86) with the following modification: trichloracetic acid (TCA) (Mallinkrodt[5]) was added to the whole blood aliquots. This modification was necessary because the blood had been pre-measured before storage. The final dilution of 1:4 in 10% TCA before centrifugation was achieved by first adding 1 ml of distilled, deionized water, vortexing, and then adding 1 ml of 20% TCA to each aliquot of whole blood. Supernatants were diluted 1:1 with distilled, deionized water. These

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[2]Instrumentation Laboratory,Inc. Wilmington,MA

[3]Labindustries Berkely,CA

[4]J.T.Baker Chemical Co. Phillipsburg,NY

[5]Mallinkrodt,Inc. Paris,KY

dilute supernatants were further diluted 1:19 with 10,000ppm strontium chloride to fall into the sensitive range of measurability of the spectrophotometer. Aqueous standards were diluted in the same manner as the samples. Samples from all subjects were read according to the protocol described for plasma magnesium determinations. Final duplicates agreed within 94%. Erythrocyte magnesium was calculated by the following formula (51).

$$\text{erythrocyte magnesium} = \frac{\text{whole blood Mg} - \text{plasma Mg}(1 - \text{hematocrit})}{\text{hematocrit}}$$

OTHER MEASUREMENTS

Nutrient intake was estimated from three diet diaries kept by each subject on a Wednesday, Thursday and Friday. Each food record was clarified at the time of collection by discussion with the subject concerning portion sizes, condiments, vitamin supplements, any possible exclusions, brand names, etc. Foods listed in each diary were coded and analysed by means of the Michigan State University Nutrient Data Bank (69). Nutrients in supplements were added to food nutrients to arrive at total daily consumption before intake was expressed as percent of recommended daily allowance (%RDA).

Body fat percentages were determined by underwater weighing. Residual lung volume was estimated at the time of

the underwater weight measurement using the rebreathing method of Lundsgaard and Van Slyke (63) as modified by Rahn, Fenn, and Otis (79). Samples of gases from the closed bag used for oxygen rebreathing were analyzed for nitrogen content with a MedScience 505 Nitralyzer[6]. Underwater weight was taken with the subject seated in an erect position on a bench suspended from a strain gauge. A chart recorder[7] received signals from the gauge through a Wheatstone bridge. Body density was calculated using the formula of Buskirk (14) and body fat was calculated by the Siri formula (92).

Respiratory variables for determining maximum oxygen consumption were obtained during each subject's continuous treadmill run to perceived exhaustion. Treadmill speed was 5 MPH at onset and remained constant. Beginning grade was 0% and increased 1% each minute. The open-circuit Douglas bag method was used to collect expired gases (23). The subjects breathed through a two-way Daniels respiratory valve[8]. It was connected through two feet of 1 and 1/4 inch (interior diameter) corrugated tubing to a four-way automated switching valve[9]. Neoprene weather balloons were used to collect gases (41) and were changed every 30 seconds.

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[6]MedScience Electronics St.Louis,MO

[7]Model 2115M, Allen Datagraph Corp.

[8]Model VS4S, Cambridge Instrument Co.

[9]Van Huss-Wells Automated Switching Valve.

An Applied Electrochemistry CD-3A infrared CO_2 analyzer[10] and an Applied Electrochemistry S-3A electrochemical O_2 analyzer[10] were used to analyze expired gases for percentages of carbon dioxide and oxygen. Helium was used to zero the analyzers prior to calibration using a known standard gas sample verified for CO_2 and O_2 with a Haldane Chemical Analyzer[11]. Gases were pumped through a DTM-115[12] dry gas meter at a constant rate of 50 liters per minute for measurement of gas volumes. Pulmonary ventilation ($\dot{V}_E$) and oxygen uptake ($\dot{V}O_2$) were calculated by the equations of Consolazio, Johnson and Pecora (23).

STATISTICAL ANALYSES

A three-way analysis of variance was used to assess the effects of training, gender, and the younger(<11.2 years)-vs-older($\geq$11.2 years) portion of the sample on the dependent variables. Where appropriate, the t-test for independent samples with unequal sample sizes was used. A significance level of 0.05 was set for all analyses. The maximum acceptable probability of making a type II error was set at 0.20. Necessary and sufficient sample size to detect as significant a difference between means of one-half standard deviation under these conditions was calculated to be 63.

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[10]Applied Electrochemistry Sunnyvale,CA

[11]Arthur H. Thomas Co. Philadelphia,PA

[12]American Meter Co. (Singer)

Variable pairs were correlated by the Pearson product-moment correlation coefficient (72). Scattergrams of correlations found to be significant were plotted to check linearity and homoscedasticity.

CHAPTER IV

RESULTS AND DISCUSSION

In this chapter the physical characteristics of the subjects are described first. Then the results of measurements and correlations made to test the research hypotheses are presented under major subheadings. A discussion of these results can be found in the last half of the chapter.

RESULTS

Differences between means which were not statistically significant ($P<0.05$) but were greater than one-half standard deviation could not be judged to be statistically insignificant because of the small group sizes. In the following presentation, these differences are designated "judgement reserved".

PHYSICAL CHARACTERISTICS

Among the youngsters in this study, the physical characteristics which were significantly affected by treatment, i.e. by being a swimmer or control, were lean body weight ($F=6.608, P<0.05$), $\dot{V}O_2max$, l/min ($F=15.080, P<0.01$), and $\dot{V}O_2max$, ml/kg/min ($F=12.779, P<0.01$). Being male or

female affected $\dot{V}O_2max$, l/min ($F=6.025, P<0.05$), and $\dot{V}O_2max$, ml/kg/min ($F=9.075, P<0.01$), but did not affect height, weight, or lean weight. There was an interaction effect of treatment and gender on weight ($F=3.733, P<0.05$) and body fat ($F=5.220, P<0.05$).

Selection of control subjects was based on the fact that the subject was the same gender, age, height, and weight as a swimmer (Table 1). Sampling losses made the group of male swimmers smaller than the group of male controls. It was difficult to locate female control subjects who matched the female swimmers by age, height and weight, and therefore the female control group was small in number, and the mean weight of the female controls was 7 kg less ($P<0.05$) than female swimmers (Table 1). However, the slightly lower percentage of body fat (2.6%) in female controls compared to female swimmers was not significant. The percentage of body fat was significantly less ($P<0.05$) in male swimmers than in male controls (17.2 vs 23.7%). The percentage of body fat was also significantly less ($P<0.05$) in male swimmers than in female swimmers (17.2 vs 24.8%). There were no differences in percentage of body fat between male and female controls. The fact that the lean body mass was significantly greater in all (male and female together) swimmers than in all controls reflected the difference in lean body mass in females. Lean body weight was the same in male swimmers and in male controls. However, the female swimmers had 4.1 kg greater mean lean body weight than the

female controls ($P<0.05$).

Although there was a significant difference ($P<0.05$) in maximum oxygen consumption of 0.35 l/min between all swimmers and all controls, it was a reflection of the large difference between the female swimmers and controls in absolute oxygen consumption (Table 1). Female swimmers had a $\dot{V}O_2max$ greater by 0.59 l/min than female controls ($P<0.05$). However, the greater uptake of 0.24 l/min of oxygen by male swimmers than by male controls was not significant. There was no significant difference in $\dot{V}O_2max$, l/min, for male vs female swimmers, whereas there was a difference in maximum oxygen uptake of 0.38 l/min ($P<0.05$) between male and female controls. Relative uptake of oxygen, i.e. $\dot{V}O_2max$, ml/kg of body weight/min, was significantly greater ($P<0.05$) in male swimmers than in male controls, in female swimmers than in female controls, and in male controls than in female controls (Table 1). However, while the difference between male and female swimmers was not significant, it was large enough that it cannot be judged insignificant due to the small sample sizes of male and female swimmers.

Whether a subject was older or younger than 11.2 years made a difference in height ($F=25.325, P<0.01$), in weight ($F=11.103, P<0.01$), in lean body weight ($F=14.934, P<0.01$), and in $\dot{V}O_2max$, l/min, ($F=10.830, P<0.01$), but not in body fat nor in $\dot{V}O_2max$, ml/kg/min. Male and female swimmers and controls were also divided into groups based on age in order

to identify trends within each group associated with aging (Table 2). Mean height and weight were greater by one-half standard deviation or more in older subjects compared with younger subjects in all groups. There was no difference in percent body fat between older and younger members of the male controls or the female controls, but the older male swimmers had 3% less fat, judgement reserved, than the younger male swimmers, and the older female swimmers had 4.7% more fat, judgement reserved, than the younger female swimmers. Lean weight was significantly ($P<0.05$) greater by 9.7 kg in older male swimmers than in younger male swimmers, but the 2 kg more lean weight of older female swimmers than younger female swimmers was not significant. Older members of the male controls and female controls had 4.1 kg and 5.3 kg more lean weight, respectively, than younger members of their groups, judgement reserved. $\dot{V}O_2max$ expressed in l/min was greater for the older subjects than younger subjects in all groups, but the difference was not significant in the female swimmers. When expressed as ml/kg/min, $\dot{V}O_2max$ was not significantly different between older and younger members of the male swimmers, male controls, and female swimmers; judgement was reserved for the female controls.

HEMOGLOBIN LEVELS AND HEMATOCRITS

All subjects except one had normal hemoglobin concentrations ranging from 14.5-16.6 g/dl. One male control had

a slightly low hemoglobin value of 12.3 g/dl. The mean concentrations of hemoglobin were not different between male swimmers and controls or between female swimmers and controls (Table 3). Similarly, venous hematocrits ranged from 39 to 48 except for the one subject with low hemoglobin. His hematocrit was 33. The mean hematocrits for all groups were nearly identical (Table 3). There were no differences between older and younger members of each group in either hemoglobin concentration or hematocrit.

MAGNESIUM CONCENTRATIONS

There were no treatment effects on plasma, red cell, or whole blood magnesium concentrations. There was no gender effect on erythrocyte or whole blood magnesium, but the gender effect on plasma magnesium was large enough that judgement must be reserved ($F=3.513, P<0.10$).

Male swimmers and controls had similar magnesium concentrations in plasma and erythrocytes (Table 4). In the whole blood, however, the difference was large enough that judgement must be reserved as to whether the concentration of magnesium in the whole blood of the male swimmers was the same or may have been greater than the magnesium concentration in the whole blood of male controls. Female swimmers and controls were not different in any magnesium concentration (Table 4). Male and female controls were not different in any magnesium concentration (Table 4, absence of superscripts). However, the male swimmers had significantly

greater ($P<0.05$) plasma magnesium than female swimmers, 2.11 $\pm$ 0.11 mg/dl and 1.95 $\pm$ 0.15 mg/dl respectively (Table 4). The female swimmers had one-half standard deviation more erythrocyte magnesium than the male swimmers, and judgement must be reserved as to whether or not these mean concentrations are the same.

There was a pronounced trend toward lower levels of magnesium in the erythrocytes and whole blood of older subjects when compared with younger subjects. In fact, when all subjects were taken together, the difference was significant ($F=4.69, P<0.05$) in whole blood (Figure 1). This trend seemed somewhat attenuated in the erythrocytes and the whole blood of male controls and female swimmers. Plasma magnesium concentrations also were decreased in the older females, older female swimmers, and older female controls when compared with younger girls in those groups (Figure 1). However, the older members of all males, male swimmers and male controls had increased levels of plasma magnesium when compared with younger boys in those groups (Figure 1).

Trends in the plasma magnesium differences can be examined more closely in Figure 2. Younger male swimmers had higher plasma magnesium than younger male controls, older male swimmers had higher plasma magnesium than older male controls, while younger female swimmers had lower plasma magnesium than younger female controls, and older female swimmers had lower levels than older female controls.

CORRELATIONS OF PLASMA MAGNESIUM

Significant ($P<0.05$) positive correlations between plasma magnesium and $\dot{V}O_2max$, ml/kg/min, were found in all controls, r=+0.44, in all males, r=+0.42, and in male controls, r=+0.59, (Table 5). Unexpectedly, no correlation between plasma magnesium and $\dot{V}O_2max$, ml/kg/min, was observed in all swimmers, r=+0.02, and in male swimmers, r=+0.05. Had a one-tailed test for inverse correlation been used, plasma magnesium and $\dot{V}O_2max$, ml/kg/min, would have been significantly but inversely correlated ($P<0.05$) in all females, r=-0.40, and in female swimmers, r=-0.54. This correlation in female controls was weak, r=+0.12. Similar relationships between plasma magnesium and $\dot{V}O_2max$, l/min, were observed (Table 5). The greatest differences between correlations of plasma magnesium and $\dot{V}O_2max$, ml/kg/min, and correlations of plasma magnesium with $\dot{V}O_2max$, l/min, were found in two groups: in the male swimmers where the coefficient for plasma magnesium and $\dot{V}O_2max$, l/min, was r=+0.34, and in the female controls where that coefficient was r=-0.17.

The scattergrams for correlation of plasma magnesium and $\dot{V}O_2max$, ml/kg/min, in male swimmers and controls (shown in Figure 3 as different symbols) and in female swimmers and controls (shown in Figure 4 as different symbols), as well as in all males (Figure 3) and in all females (Figure 4) were examined. Among the male swimmers, one value seemed to deviate from the pattern of the rest. The one male swimmer

with the lowest plasma magnesium had the highest $\dot{V}O_2max$. By deleting that one male from the calculations, the correlation coefficient of plasma magnesium and $\dot{V}O_2max$, ml/kg/min, among the remaining eight male swimmers was r=+0.42, which was equivalent to the coefficient for all males. However, when tested by the procedure described by Grubbs (50) for both plasma magnesium and for $\dot{V}O_2max$, this subject was not a statistical outlyer. One male control had a plasma magnesium concentration that was a statistical outlyer for the group of male controls and also for all males. When he was deleted from the calculations, the relationship between plasma magnesium and $\dot{V}O_2max$, ml/kg/min, for male controls was decreased to r=+0.41. For the group of all males, the correlation coefficient without his values was also r=+0.41.

Examination of the scattergram of plasma magnesium and $\dot{V}O_2max$, ml/kg/min, for females (Figure 4) revealed no apparant relationship between these two variables in the female controls. The strong negative relationship between plasma magnesium and $\dot{V}O_2max$, ml/kg/min, in female swimmers appeared to be influenced by one female swimmer with the highest plasma magnesium and the lowest $\dot{V}O_2max$. Her oxygen consumption was found to be a statistical outlyer for the group of female swimmers. When this subject was deleted from the calculations, the correlation coefficient between plasma magnesium and $\dot{V}O_2max$, ml/kg/min, for the remaining 12 female swimmers was r=-0.30. Scattergrams of the

relationship of plasma magnesium and $\dot{V}O_2$max, l/min, are found in appendix A.

Age was more strongly associated with plasma magnesium in females, and in female swimmers than any of the other groups (Table 5). A scattergram is provided in appendix A. The association between plasma magnesium and hemoglobin (and also between plasma magnesium and hematocrit) was very similar to the association between plasma magnesium and $\dot{V}O_2$max (Table 5). However, the inverse correlation between plasma magnesium and hemoglobin among females appeared to be homoscedastic (Figure 5), while this correlation among males appeared to be set up by two male control subjects (scattergram included in appendix A).

The similar relationship of plasma magnesium with hemoglobin and hematocrit and of plasma magnesium with $\dot{V}O_2$max among females suggested that hemoglobin and $\dot{V}O_2$ max might be highly correlated among these pre-teen females. However, that did not prove to be the case. The correlation between $\dot{V}O_2$max, ml/kg/min, and hemoglobin among female swimmers was r=+0.41, and among female controls was r=-0.02. The correlation of $\dot{V}O_2$max, l/min, with hemoglobin among female swimmers was r=+0.30, and among female controls was r=-0.10. Holding hemoglobin constant and computing the partial correlation of plasma magnesium and maximum oxygen consumption did not substantially change the relationship in these two female groups. The results of the partial correlations were: $\dot{V}O_2$max, ml/kg/min, with plasma magnesium

among female swimmers r=-0.45, among female controls r=+0.14; $\dot{V}O_2max$, l/min, with plasma magnesium among female swimmers r=-0.51, among female controls r=-0.34.

CORRELATIONS OF ERYTHROCYTE MAGNESIUM

The strong significant correlation between erythrocyte and whole blood magnesium resulted in very similar correlation coefficients between red cell magnesium with the other variables and whole blood magnesium with the other variables. Correlations of red cell magnesium are presented in Table 6.

Red cell magnesium was not importantly related to any physical characteristic. The generally negative correlations shown across Table 6 simply reflect the fact that red cell magnesium was decreased in the older subjects compared to the younger subjects of each group while age, weight, lean weight and maximum oxygen consumption, l/min, all increased. The three significant ($P<0.05$) correlations in the female control group could be explained by that observation and the fact that age, lean weight and weight were strongly correlated,i.e. age vs lean weight r=+0.90,P=0.006, in that small female control group. (Scattergrams for the relationship of erythrocyte magnesium and weight among females and among males are provided in appendix B.) However, the correlation of r=-0.74, $P<0.05$, for percent body fat and red cell magnesium in the male swimmers was surprising. The mean percent body fat and the

mean red cell magnesium were both less in the older than younger male swimmers and a positive correlation between these two variables might be expected. Examination of the scattergram (appendix B) provided insight. The youngest male swimmer with the highest percent fat had to be deleted because his red cell magnesium concentration was lost during analysis. Placing the point representing his hypothetical red cell magnesium along the line representing 26.5% body fat anywhere above the mean for all male swimmers, under the assumption that his red cell magnesium would have been similar to the other younger members of the male swimmers, greatly decreases the correlation between these two variables. This correlation, although significant, is probably an artifact of the small group size and selective sampling losses and not important.

The strong negative correlations in the control males between red cell magnesium and hemoglobin and hematocrit were similarly explained by examining scattergrams (appendix B). One subject had both the highest hemoglobin, 17.8 gm/dl, and hematocrit values and the second lowest concentration of erythrocyte magnesium while another subject had the lowest hemoglobin, 12.3 gm/dl, and hematocrit and the highest erythrocyte magnesium. All other subjects clustered between 14.8 and 16.2 mg/dl for hemoglobin with intermediate red cell magnesium values and no apparant correlation. These same two subjects set up the correlation for all males. Similarly, two female control subjects were

responsible for the strong positive correlations of red cell magnesium with hemoglobin and hematocrit among females. Appendix B has the scattergram showing the relationship of erythrocyte magnesium and hematocrit among female subjects.

The correlation between red cell magnesium and maximum oxygen consumption of r=+0.45 in the male swimmers appeared to be possibly important. A negative correlation would be expected since red cell magnesium was less while maximum oxygen consumption, ml/kg/min, was greater in older than in younger subjects. However, if it is assumed that the sample of red cell magnesium from the second youngest subject which was lost might have been an average of the first and third youngest subjects' red cell magnesium values and that value of 5.70 mg/dl is placed along the line on the scattergram (appendix B) representing his 43.4 ml/kg/min of oxygen consumption, the resulting correlation coefficient for the 9 male swimmers is r=+0.15.

NUTRIENT CONSUMPTION

Meal patterns among the forty subjects were very similar. Virtually all subjects ate breakfast. Only one swimmer skipped breakfast once in the three days of reporting. No control skipped any breakfast, but three controls regularly consumed just one item for breakfast, i.e. a breakfast bar or a glass of juice or an apple.

There were no significant treatment effects on consumption of the selected nutrients listed in Table 7.

However, when compared with females, males consumed more total kilocalories (kcal) of energy ($F=7.246, P<0.05$), but not more total kcal when expressed as %RDA ($F=0.719, P>0.05$). Males, compared with females, also ate more kcal per kg of bodyweight (kcal/kg) ($F=5.249, P<0.05$), greater %RDA of protein ($F=5.362, P<0.05$), and greater %RDA of phosphorus ($F=6.584, P<0.05$). ANOVA did not show any significant effects on nutrient consumption of being older or younger than 11.2 years. The greatest age-related difference was in kcal/kg ($F=3.763, P<0.10$).

The male swimmers and controls ate similar diets, and the female swimmers and controls ate remarkably similar diets (Table 7). The percentage of energy from macronutrients in the diet was the same for male swimmers, male controls, female swimmers, and female controls (Table 7). Mean consumption of kcal was close to 100 %RDA and the standard deviations were similar among all groups (Table 7). Significant differences ($P<0.05$) in consumption of kcal/ kg and %RDA of protein were found between male and female swimmers, but not between male and female controls (Table 7, superscripts). As shown in Table 9, four individuals consumed less than 67 %RDA for kilocalories, but all four were near the 50th percentile for height and weight except one who was near the 95th percentile for weight (70,p.21). Older male swimmers ate more kilocalories than younger male swimmers, and the difference was large enough that judgement of non-significance must be reserved

due to insufficient sample size (Table 8). However, older and younger female swimmers consumed similar kcal. Older controls of both genders ate slightly less kcal than younger controls. In all groups, older subjects ate fewer kcal/kg than younger subjects, and the differences were large enough that the means cannot be said to be the same in all groups except the female swimmers (Table 8).

Male swimmers ate significantly ($P<0.05$) more protein than female swimmers, but male and female controls ate similar quantities of protein (Table 7, superscripts). All male and female swimmers and male controls, and 6 of the 7 female controls, consumed more than 100 %RDA for protein (Table 9). Only one female control consumed less than 67 %RDA for protein (Table 9). All subjects ate 23 - 93 g of animal protein each day. Older subjects consumed somewhat less protein than younger subjects, but mean protein consumption of all groups was greater than 165 %RDA (Table 8).

Diet cholesterol had a very large range of variability and means were not different between either male or female swimmers and controls (Table 7). As shown in Table 8, all group means were less than 300 mg per day of cholesterol except the younger male swimmers and older male controls. There was a larger difference in the polyunsaturated vs saturated fat (poly/sat) ratios of the male swimmers and controls than of the female swimmers and controls (Table 7). Table 8 shows that the 3 younger male controls consumed the most favorable ratio of fats, while the younger male

swimmers, the younger female swimmers, and the older female controls ate similarly unfavorable ratios. None of these differences in poly/sat ratios were significant, but some were as great as one-half standard deviation (Tables 7 and 8).

Swimmers of both genders were slightly more likely to use supplements than controls (Figure 6). Male and females were very similar in supplement consumption.

The mean consumption of calcium, magnesium, phosphorus, thiamin, and vitamin D was greater than 67 %RDA in male swimmers, male controls, female swimmers, and female controls (Table 7). Mean consumption of magnesium, when expressed as %RDA, was the same across the groups of male swimmers, male controls, female swimmers, and female controls (Table 7). The male swimmers had the fewest members who consumed less than 67 %RDA and the most members who consumed more than 100 %RDA for magnesium in contrast to the male controls whose consumption pattern was reversed (Table 9). Both female groups were split approximately by thirds in magnesium consumption of <67 %RDA, 67-100 %RDA, or >100 %RDA (Table 9).

A larger percentage of subjects had an individual three-day average consumption for magnesium and vitamin D than for calcium, thiamin and phosphorus which fell below 67 %RDA (Table 9). Average consumption of vitamin D over three days for individuals ranged from 5 IU to 753 IU. Three subjects or 7.5% consumed less than 100 IU; twenty subjects

or 50% consumed 100-400 IU; eight subjects or 20% consumed 400-600 IU; and nine subjects or 22.5% consumed 600-753 IU daily over three days. Many children reported fruit juice or soft drink as a beverage with meals rather than fluid milk, the major source of Vitamin D. Calcium consumption was higher than vitamin D consumption because many children reported frequent servings of pizza, cheese, yoghurt, tacos, macaroni and cheese, and ice cream. For example, 100% of the female controls consumed less than 67 %RDA for vitamin D, but only 14% consumed less than 67 %RDA for calcium (Table 9). Examination of the 21 daily diets of these seven subjects revealed an average fluid milk consumption of 1.25 cups per child per day. Of those 21 diet-days only two days were devoid of other calcium-containing foodstuffs. Two control females used a daily multi-vitamin containing vitamin D and none took supplements of calcium. Phosphorus and thiamin were consumed adequately by all but one control female (Table 9).

Mean consumption of all vitamins and minerals was greater than 67 %RDA in all groups of younger and older subjects except the mean for total dietary vitamin D in younger female controls (n=2) which was 48 %RDA (Table 8).

DISCUSSION

PHYSICAL CHARACTERISTICS

The fact that it was difficult to locate female controls of the same weight as swimmers when age and height were the same raises the question as to whether or not exercise increases lean body mass in females. Dissimilarity in size between girl swimmers and age-matched controls has been found by other investigators. Astrand et al (6) reported that 30 girl swimmers were taller and heavier than their age-matched female controls from age 7, before beginning their training (data at younger ages was obtained from elementary school records), and throughout adolescence. The height difference between swimmers and controls accelerated from 1 cm at 7 years to 2.8 cm at 14 years, but the weight/height ratio was the same for both groups of girls. These investigators could not determine if the accelerated growth of the swimmers was a constitutional trait, having begun as early as age 7, or if it was correlated with their vigorous training (6000-65,000 meters in 6-28 hours/week, 3-7 days/week). Therefore, one must consider whether or not 9-13 year old females who do match female competitive swimmers in age, height and weight might be an extreme group of controls. In the same study, 84 older women, aged 20-40 years who had trained as swimmers from 12.3-18.7 years of age were found to have attained a

final height of 167.8 ± 0.42 cm, SD 3.8 cm. They were only 1 cm taller than school girls, age 19-20, who had not trained (166.9 ± 0.82 cm, SD 5.1 cm), and they had the same weight/height ratio as the school girls. This suggests that the swimmers were early maturers, but not larger after maturity than females who did not train. However, the 1 cm difference between these two groups might have been larger if the 19-20 year old school girls had been compared with 19-20 year old female swimmers of their own generation, which would suggest that females who are physically stronger may excel in sports and may therefore choose to compete.

The significantly smaller percentage of fat in the male swimmers when compared with the male controls observed in this investigation was not found in a previous study of fifty-five 8-11 year old males divided into a higher active group and a lower active group (98). However, because the maximum oxygen uptake was not measured in those boys, it is difficult to assess whether their "higher activity" was similar in physical stress to the training by swimmers in the present study. Other studies which measured maximal oxygen consumption did not determine percentage of body fat (29,59). Notice should be taken that the same training regimen which appears to have produced a reduction in percentage of body fat in male swimmers did not have the same effect on the female swimmers, who were not different from either the male or female controls in percentage of body fat.

When compared with their respective controls in maximum oxygen uptake, ml/kg/min, both the male and female swimmers clearly showed the well-known adaptation to aerobic training (40,p.220). The male swimmers' $\dot{V}O_2max$ of 54.7 ml/kg/min compared quite closely with the 52.5 ml/kg/min found in ten 11.8 year old male swimmers by Cunningham and Eynon (29), as well as with the 55.5 ml/kg/min found by Lukaski et al (62) in male athletes, age 20. The female swimmers' $\dot{V}O_2max$ of 48.5 ml/kg/min was similar to the 49.5 ml/kg/min found in six female swim-team members, age 14.7 years, by Kramer and Lurie (59) and to the 46.2 ml/kg/min found by Cunningham and Eynon (29) in eight female swimmers, age 12.2 years.

While caution must be exercised in studying groups of such small size, two interesting observations can be made from Table 2. Although height, weight, and lean weight were increased in every group from the younger to the older subjects, percent body fat decreased from younger to older male swimmers and increased from younger to older male controls. Percent body fat increased from younger to older female swimmers and stayed the same among female controls. Apparantly the training regimen may affect the body composition of both males and females differently directly by different adaptations of fat metabolism , or affect it indirectly by accelerating maturation. However, use of underwater weighing to characterize fatness levels of children may be biased by differences in bone mineralization which causes overestimation of adiposity in the less mature

subjects (111). Therefore, the significance of this observation cannot be fully evaluated by the results of this study. Also, $\dot{V}O_2max$ expressed in ml/kg/min was decreased in the older female swimmers when compared with the younger female swimmers (Table 2). This has been found previously by Astrand et al (6): in 10-15 year old swimmers, maximum oxygen uptake, ml/kg/min, decreased 12% when older girls were compared with younger ones. Presumably, this reflects the fact that body weight is increasing faster than absolute oxygen uptake during female adolescence. The fact that the $\dot{V}O_2max$, ml/kg/min, was greater in the older than in the younger female controls in this study may be further evidence that these girls were not as mature as the female swimmers.

MAGNESIUM CONCENTRATIONS

Mean values for erythrocyte magnesium in subjects under 20 have been found to be 5.42 mg/dl for males and 5.62 mg/dl for females (55). The mean values for red cell magnesium found in this study (see Table 4) were slightly lower. However, they were within the normal adult range for means, 4.56-7.44 mg/dl (89,p.361). In comparison with the mean value of 4.7 $\pm$ 0.64 mg/dl found in college-age male athletes (62), the mean values of red cell magnesium of children in this study were slightly higher and the variability slightly greater. The children were healthy, as determined by physical examination (94). There was no reason to conclude

that these are not normal values for pre-teenage children.

The wider variablity of erythrocyte magnesium compared with plasma magnesium found in this study (see Figure 1) is consistant with other studies (31;89,p.359,362). Serum magnesium is normally maintained within a narrow range having a coefficient of variability of only 10%-20% (89,p.358). Therefore, the coefficient of variability for plasma magnesium in this study, which was 8.6%, was a reasonable value. All individual plasma magnesium values were normal when compared with published values for adults, and the mean group values for male and female controls (Table 4) were consistant with means for males and females under 20 years, 2.01 mg/dl for males and 2.02 mg/dl for females (55). However, the pre-teen male and female swimmers had significant differences in mean plasma magnesium of the same magnitude as the significant differences between males and females in their early twenties reported by Henrotte, Benech and Pineau (55). The mean plasma magnesium of the male swimmers in this study was 2.11 mg/dl and the mean plasma magnesium of the 23 year old French males was 2.09 mg/dl (55). The female swimmers' mean plasma magnesium was 1.95 mg/dl and that of the 23 year old French females was 1.93 mg/dl (55). These French subjects were a combination of apparently healthy volunteers (university students and professors) and of outpatients with various psychosomatic disorders. Their fitness levels were not reported. Further comparisons can be made for the male

swimmers. Eight physically fit South African males, mean $\dot{V}O_2$max 59.1 ml/kg/min, mean age 25 years, had a mean plasma magnesium concentration of 2.14 $\pm$ 0.13 (112). Forty-four American male athletes, mean $\dot{V}O_2$max 55.5 ml/kg/min, mean age 20 years, had mean plasma magnesium of 2.0 $\pm$ 0.23 mg/dl (62). In contrast to the current study, another investigation found that five male athletes, mean $\dot{V}O_2$max 68.1 ml/kg/min, mean age 23 years, had mean plasma magnesium of 2.08 mg/dl, which was slightly, but not significantly, lower than the plasma magnesium of 2.11 mg/dl in their five male controls, mean $\dot{V}O_2$max 45 ml/kg/min, mean age 24 years (74). Unfortunately, plasma magnesium concentrations are not strictly comparable across studies even when atomic absorption spectrophotometry is used (89,p.358).

Henrotte, Benech and Pineau (55) pointed out the coincidence that the 21-24 year age span in which they found the greatest differences between males and females in red cell and plasma magnesium, with females significantly lower in both variables than males, was also the age span during which the excretion of 17-ketosteroids are highest in both males and females (53). Since exercise involves stimulation of the adrenal cortex (71,p.285), it is probable that the swimmers in this study also had greater 17-ketosteroid excretion per day than their controls. This observation lends support to the speculation of Henrotte, Benech and Pineau (55) that adrenal or gonadal activity may play a role in variations of plasma and erythrocyte magnesium between

males and females. This observation also suggests that exercise may, indeed, alter magnesium metabolism in children.

Several questions arise from observations of differences in magnesium levels between older and younger subjects. The significant difference in whole blood magnesium concentration between older and younger subjects could not be explained by any hemodilution due to growth, because mean hematocrits of younger and older subjects were nearly identical. Certainly this difference in the current study seemed to be the algebraic sum of the lower concentrations of in erythrocyte magnesium of older members of all groups, except possibly the female swimmers and male controls, and of the lower levels of plasma magnesium of older females, especially female swimmers, and the higher levels of plasma magnesium of older males, especially male swimmers. Since the 23 year old French females (55) had both lower plasma magnesium and lower red cell magnesium concentrations than their male counterparts, it must be pointed out that the older female controls in the present study had lower mean levels of magnesium in both red cell and plasma when compared with the younger female controls. It seems reasonable to suppose that this might be normal female adolescent growth toward the mature female blood magnesium levels. However, although the older female swimmers had lower mean levels of plasma magnesium, they had slightly greater mean concentrations of red cell magnesium,

when compared with the younger female swimmers (see Figure 1). The opposite situation occurred in males. It would seem reasonable that male values of both plasma and red cell magnesium would increase during adolescence until magnesium concentrations found in adult males (55) were attained. In this study, the older control males, with their higher mean plasma magnesium and equivalent red cell magnesium when compared with the younger male controls, more closely approximated this prediction than did the older male swimmers. The older male swimmers did approach adult male plasma magnesium levels, but had greatly decreased red cell magnesium levels when compared with either the younger male swimmers, the male controls or the female swimmers (see Figure 1). Further research is necessary to determine whether these differences are due to exercise or are artifacts of the small groups resulting from age/gender/treatment division of subjects in this study.

CORRELATIONS OF PLASMA MAGNESIUM

Correlations between plasma magnesium and maximum oxygen utilization in this study (see Table 5) contrast with the published correlations found for 20 year-old male athletes, $r=+0.46, P<0.002$, and 23 year-old male controls, $r=-0.32, P=0.17$ (62). The results of the current study suggest that there may be differences between males and females in the relationship of plasma magnesium with maximum

oxygen uptake, and that combining males and females in the swimmer and control groups obscures results. Unfortunately, dividing each training group between males and females leaves very small sample sizes to be used in the comparisons, and so the results must be interpreted with extreme caution. Reserve is particularly necessary in those groups left with less than 10 subjects, where inclusion or deletion of values for one subject may change the correlation substantially. When the two groups of pre-teen males in this study were combined into one group of 20 males, the effect of any one (perhaps deviant) subject was less influential and the resultant $r=+0.47$, $P=0.018$, for plasma magnesium and oxygen uptake, l/min, and the $r=+0.42$, $P=0.032$, for plasma magnesium and oxygen uptake, ml/kg/min, were quite similar to the $r=+0.46$, $P<0.002$, for plasma magnesium and oxygen uptake, ml/kg/min, found in the 44 twenty-year-old male athletes (62).

Substantial quantities of growth hormone should be present in the serum of both the male swimmers and controls, aged 9.5-12.9 years,in this study. Since the stress of exercise increases the secretion of growth hormone (71,p.688), serum growth hormone concentrations would be expected to be higher in the 20 year old male athletes studied by Lukaski et al (62) than in their controls, who were found to have no correlation between oxygen consumption and plasma magnesium. In order to verify this, growth hormone levels in the serum should be analyzed. Elevated

levels of growth hormone in the plasma may be the reason that oxygen consumption and plasma magnesium show a correlation in all the pre-teen males and the older male athletes but not in the older male controls.

The weaker correlation between the plasma magnesium and oxygen uptake of the male swimmers in this study and the male athletes in the study by Lukaski et al (62) than that of the pre-teen control males in this investigation might be due to the large influence of one male control and one male swimmer on the correlations of the small groups of male controls and male swimmers. However, if growth hormone or the somatomedins produced in response to growth hormone are involved in the correlation between plasma magnesium and maximum oxygen consumption, that weaker correlation might be due to the daily increase in glucocorticoids and androgens from the adrenal cortex in the exercised subjects. Both glucocorticoid and androgen production are stimulated by exercise-induced increases in adrenocorticotropin hormone (ACTH) (12,p.183; 100,p.742). Glucocorticoids decrease the rate of secretion of growth hormone and also the release of the somatomedins produced by the liver in response to growth hormone (71,p.694).

Female estrogen also decreases the rate of release of somatomedins from the liver, and this is one proposed mechanism for the suppression of growth by estrogen (71,p.694). Estrogen-inhibited release of somatomedins might account for the weaker correlation of plasma magnesium

with oxygen consumption in the female controls than in the male controls. Like the female controls, the female swimmers would have some estrogen present simply because of their sex and age, but also would have increased ACTH stimulation of the production of cortisol and of androgens during their daily exercise. Androgens can be converted to estrogen in adipose tissue by the enzyme aromatase (71,p.707; 12,p.647). In addition to suppression of release of somatomedins by the liver, estrogen also has been found to increase the uptake of magnesium to the cytoplasm of estrogen-sensitive cells and thereby decrease serum magnesium (47). These two factors may account for the stronger negative correlation of plasma magnesium and oxygen uptake in the female swimmers than in the female controls, and the latter may explain in part the lower plasma magnesium levels in female swimmers than in male swimmers.

These observations do not conflict with the idea that plasma magnesium may be utilized in the production of DPG in the erythrocyte during prolonged exercise which was proposed by Lukaski et al (62), only add that other factors, influenced by exercise, may alter the availability of magnesium for such use. The lesser ability of the female than the similarly trained male to increase extraction of oxygen from the blood through training (40,p.220) might be due in part to the lower quantities of plasma magnesium for use in DPG production.

In the study by Smalley, Runyan and Puhl (93), serum

concentrations of DPG at rest in female, 20-year old, cross-country runners was elevated from sedentary levels and did not increase with eight weeks of further training. In the same study, 15-year old untrained females had lower resting levels of DPG than the 20-year old athletes, but, after one week of endurance training, had levels of DPG equal to the trained subjects, and these higher levels of DPG remained, but did not increase, throughout the rest of the eight week training period. These female DPG concentrations reported by Smalley, Runyan, and Puhl (93) were lower than the DPG concentrations found in middle distance and marathon athletes, presumably male, at rest (97) and in formerly sedentary males after eight weeks of training (90), but are comparable to eight trained male speed canoists (10) and 36 world class white athletes, 25 endurance and 11 non-endurance, all presumably male (80). In studies of adults whose training were not reported which were reviewed by Purcell and Brozovic (77) , some investigators found significant differences between men and women and others did not. No other studies of DPG concentrations in trained females were found in the literature, and therefore the question remains: can the same elevations of DPG found in some trained males be produced by females through training?

The inverse correlation between plasma magnesium and hemoglobin found in these pre-teen female swimmers and controls also may be related to DPG production. Smalley, Runyon, and Puhl (93) suggested that DPG elevations are a

response to sports anemia that enhances oxygen transport in the presence of decreased hemoglobin. If plasma magnesium is involved in DPG production as suggested by Lukaski et al (62), it follows that a lower hemoglobin concentration might be correlated with higher plasma magnesium. Further research is necessary to confirm this observation among females and to explore why the relationship did not appear in the pre-teen males.

Both Wolfswinkel, Van Der Walt and Van Der Linde (112) and Refsum, Meen and Stromme (83) have documented a decrease of plasma magnesium and a quantitatively similar increase in erythrocyte magnesium during prolonged exercise, as well as a gradual return to resting levels during recovery. Refsum, Meen and Stromme (83) suggested that growth hormone or cortisol, aldosterone, or thyroxin, which were found to be elevated in skiers after 70 and 90-km races, might be involved in induction of the shift of magnesium into the red cells. Surely something during prolonged exercise must alter the permeability of the red cell membrane to magnesium because studies with radioactive magnesium have established that uptake of magnesium from plasma by the red cell occurs very slowly (1,p.48). In another investigation, intravenous infusion of magnesium resulting in a two to four-fold increase in plasma magnesium was not accompanied by changes in erythrocyte levels (89,p.364). In the investigations by Darley (30) of the influence of magnesium on the preservation of DPG in stored blood, adding 4 mMol/l or 96 mg/dl of

magnesium to the plasma did not result in increased red cell magnesium over a 77 day period.

It has been observed that the inorganic content of the cell, i.e. magnesium, potassium, and phosphorus, is a reflection of its ability to use energy in growth and has been suggested that the limitation of ion uptake controls growth (1,p.85). Growth hormone is a family of polypeptides with a number of metabolic functions, some unrelated to growth, but related to production of energy during exercise, such as stimulation of lipolysis in adipocytes for release of fatty acids (71,p.689). The multiple effects caused by growth hormone may be due to different forms of growth hormone produced by the pituitary, or to the formation of different somatomedins, at least three of which have been characterized (100,p.785,786). Since one of the mechanisms by which somatomedins stimulate cell growth is believed to be by increasing membrane transport of amino acids, sugar, nucleotides and ions (46,p.97), and since regulation of red cell glycolysis may be controlled by the concentration of magnesium ions (11), it seems reasonable to propose that the mechanism of one type of somatomedin, perhaps yet to be characterized, perhaps released during prolonged exercise, and the production of which perhaps is suppressed by estrogen, might be to alter the permeability of the red cell membrane to magnesium in support of enhanced energy utilization during exercise. A study by Fornaini et al (39) has demonstrated that red cells from 25 male subjects accustomed

to physical exercise (middle and long distance runners) consumed 44% more glucose in vitro than did red cells from 25 untrained males, and results utilizing blood drawn immediately after a race suggested that the phenomenon was not related to acute physical effort but was an adaptation to chronic exercise. Although the buffer solution in which the red cells were suspended contained 1.1 mMol/l of $MgCl_2$, no tests were made to see whether the uptake of Mg ions by red cells from the exercised subjects was different from that of red cells from the untrained subjects during the 120 minute incubation period. This might prove to be a fruitful direction for further research.

NUTRIENT CONSUMPTION

The percentage of energy from macronutrients in the diets of the male swimmers, male controls, female swimmers, and female controls at 14% protein, 35-37% fat and 50-51% carbohydrate were all similar to each other. This pattern was similar to, but slightly more favorable in the ratio of fats to carbohyhrates than, the common American intake of 15% protein, 40% fat, and 45% carbohydrate (22). Few children skipped breakfast, the most commonly neglected meal, and so a favorable meal pattern was common to all groups of subjects.

The magnesium intake of subjects in this study was similar to the usual intake of Americans in their age range. The mean magnesium intakes of male swimmers, 95 $\pm$ 19 %RDA,

of male controls, 90 ± 61 %RDA, of female swimmers, 90 ± 32 %RDA, and female controls , 79 ± 33 %RDA, were all greater than 67 %RDA, the level at which daily intake might be considered to be inadequate (75). These figures were comparable to the daily dietary intakes of 9-11 year old and 12-14 year old males and females in the 1977-78 Nationwide Food Consumption Survey (NFCS) in which male means were 88 %RDA and 79 %RDA respectively for the two age groups, and female means were 87 %RDA and 75 %RDA respectively (75). In the present study, 11% of the male swimmers and 36% of the male controls consumed less than 67 %RDA for magnesium while 89% of the male swimmers and 45% of the male controls consumed more than 100 %RDA for magnesium. In both the female swimmer group and the female control group, approximately 30% of the individuals consumed less than 67 %RDA for magnesium, and 30% consumed more than 100 %RDA. In the NFCS, 27% of the 9-11 year old males and 43% of the 12-14 year old males consumed less than 70 %RDA for magnesium, while 30% of the 9-11 year old and 19% of the 12-14 year old males consumed more than 100 %RDA for magnesium. Among the females, 27% of the 9-11 year olds and 45% Of the 12-14 year olds consumed less than 70 %RDA for magnesium while 28% of the 9-11 year olds and 16% of the 12-14 year olds consumed more than 100 %RDA for magnesium (75).

The nutrients selected for consideration in this study are those that have been shown to have an influence on magnesium absorption or excretion or on intracellular need

for magnesium.

The use of animal protein in the presence of adequate protein and magnesium enhances magnesium retention (61,2). All subjects in this investigation consumed animal protein. All but one exceeded 67 %RDA for protein and the mean consumption of protein for the male swimmers, male controls, female swimmers, and female controls exceeded 165 %RDA for protein. Protein also has been found to increase the need for magnesium and the excretion of magnesium (61). It was assumed that subjects in this study were not eating inadequate amounts of magnesium in relation to their large intakes of protein because the substantial amount of animal protein, in the presence of the 79-95 %RDA for magnesium consumed by these subjects, would enhance magnesium retention. The male swimmers, who ate the most protein, also ate the greatest amount of magnesium.

As the quantity of fat in the diet increases, magnesium absorption decreases (34;89,p.142). The diets of all groups were equivalent in fat with 35-37% of kilocalories from fat, which was less than the American average of 40% (22).

Excessive calcium consumption decreases the absorption and increases the excretion of magnesium in the feces (61). The subjects in this study had a mean consumption of calcium of close to 100 %RDA across all groups. The male swimmers had the highest mean consumption of 115 %RDA which was judged not excessive.

Dietary phosphorus decreases the intestinal absorption

of magnesium by formation of insoluble magnesium phosphate complexes (13). In this study, mean consumption of phosphorus ranged from 98 %RDA for the female controls to 148 %RDA for the male swimmers. The significant difference ($F=6.584, P<0.05$) between males and females in total dietary phosphorus probably reflects the greater protein consumption of the males since phosphorus is plentiful in milk, cheese, meat, fish, and poultry. The calcium/phosphorus ratio was maintained at 1:1 for setting the recommended allowances for calcium and phosphorus, and the range which can be tolerated by man is 2:1 to 1:2 (70). The calcium/phosphorus ratio in this study ranged from 1:1.15 to 1:1.27 and was closer to unity than the American average of 1:1.5 to 1:1.6 (70). Therefore the higher percentage of RDA for phosphorus than for calcium consumed by subjects in this study was judged to be not excessive.

Vitamin D in excess, especially if calcium is also taken in excess, causes magnesium loss (89,p.270). Food composition values are less reliable for magnesium and vitamin D than for the other nutrients examined in this study (75) so the results must be interpreted with restraint. The intakes of vitamin D in food and vitamin supplements by subjects in this study must be regarded as minimum amounts because not all foods in the data bank had values for vitamin D and because there was no way of estimating vitamin D from exposure to the sun. Concern has been expressed by the American Pediatric Association, which

recommends consumption up to 400 IU per day, that total vitamin D consumption in the U.S. might range from 600-4000 IU daily (89,p.114). In this study, nine subjects or 22.5 % had between 600 and 753 IU daily which might have been excessive, while three subjects or 7.5 % consumed less than 100 IU daily and might have been deficient.

When intracellular availability and utilization of thiamin is increased, there is very likely an increased need for magnesium (61). Although mean consumption of thiamin across groups was more than 175 %RDA while mean consumption of magnesium was less than 105 %RDA, need for additional magnesium cannot be ascertained for three reasons: (1) the ratio of thiamin/magnesium needs has not been established; (2) some foods in the data bank had not been analyzed for magnesium content, while thiamin in foodstuffs was more likely to have been assessed; and (3) drinking water may contribute up to 96 mg/day of magnesium (1,p.72), but was not evaluated in the diets of these subjects.

Table 1. Physical characteristics of competitive swimmers, aged 9.5-12.9 years, and controls.

	Male Subjects (n=20)			Female Subjects (n=20)		
	Swimmers (n=9)	Controls (n=11)	sig	Swimmers (n=13)	Controls (n=7)	sig
Age (years)	11.5 ± 1.1	11.5 ± 1.0	ns	11.7 ± 0.9	11.6 ± 0.7	ns
Height (cm)	150 ± 9	147 ± 6	ns	150 ± 8	147 ± 6	ns
Weight (kg)	38.4 ± 6.7 *s	39.9 ± 7.4 c	ns	42.1 ± 7.9 *s	35.0 ± 5.0 c	*
Body fat (%)	17.2 ± 4.2 *s	23.7 ± 6.9 c	*	24.8 ± 7.1 *s	22.2 ± 4.0 c	ns
Lean weight (kg)	31.9 ± 6.0	30.0 ± 3.8 c	ns	31.3 ± 4.0	27.2 ± 3.8 c	*
$\dot{V}O_2$max						
(l/min)	2.12 ± 0.54 s	1.88 ± 0.36 *c	ns	2.01 ± 0.27	1.42 ± 0.32 *c	*
(ml/kg/min)	54.7 ± 7.9 s	47.3 ± 6.7 *c	*	48.5 ± 6.0 s	40.3 ± 4.9 *c	*

	All Subjects (n=40)		
	Swimmers (n=22)	Controls (n=18)	
Age (years)	11.9 ± 0.9	11.5 ± 0.9	ns
Height (cm)	150 ± 8	147 ± 6	ns
Weight (kg)	40.6 ± 7.5	38.0 ± 6.9	ns
Body fat (%)	21.7 ± 7.1	23.2 ± 5.9	ns
Lean weight (kg)	31.5 ± 4.9	28.9 ± 4.0	*
$\dot{V}O_2$max			
(l/min)	2.05 ± 0.40	1.70 ± 0.41	*
(ml/kg/min)	51.1 ± 7.4	44.6 ± 6.9	*

Values are Mean ± SD. $\dot{V}O_2$max is maximum oxygen consumption.
ns not significant
* P<0.05
*s P<0.05 between male and female swimmers
*c P<0.05 between male and female controls
s Sample size was too small to declare non-significance between male and female swimmers.
c Sample size was too small to declare non-significance between male and female controls.

Table 2. Physical characteristics of younger (<11.2 years) and older (≥11.2 years) members of the male and female swimmers and controls.

	MALE SUBJECTS (n=20)											
	Swimmers (n=9)					Controls (n=11)						
	Younger (n=3)		Older (n=6)		sig	Younger (n=3)		Older (n=8)		sig
Age (years)	10.2	± 0.8	12.1	± 0.6	*	10.2	± 0.7	12.0	± 0.6	*
Height (cm)	139.6	± 2.2	154.8	± 5.4	*	138.6	± 6.4	150.4	± 2.9	*
Weight (kg)	31.5	± 1.1	41.9	± 5.2	*	34.9	± 9.0	41.7	± 6.4	R
Body fat (%)	19.2	± 6.4	16.2	± 2.9	R	22.0	± 4.3	24.4	± 7.8	ns
Lean weight (kg)	25.4	± 1.2	35.1	± 4.3	*	27.1	± 6.3	31.2	± 2.1	R
$\dot{V}O_2max$ (l/min)	1.66	± 0.20	2.35	± 0.52	R	1.61	± 0.52	1.98	± 0.26	R
(ml/kg/min)	52.9	± 8.2	55.6	± 8.4	ns	45.6	± 3.6	48.0	± 7.7	ns

	FEMALE SUBJECTS (n=20)											
	Swimmers (n=13)					Controls (n=7)						
	Younger (n=4)		Older (n=9)		sig	Younger (n=2)		Older (n=5)		sig
Age (years)	10.8	± 0.1	12.0	± 0.8	*	10.8	± 0.4	11.8	± 0.6	R
Height (cm)	147.1	± 6.2	152.1	± 8.4	R	139.7	± 4.1	149.4	± 4.2	*
Weight (kg)	38.2	± 4.7	43.9	± 8.6	R	30.1	± 3.2	37.0	± 4.3	R
Body fat (%)	21.6	± 2.1	26.3	± 8.2	R	22.3	± 2.3	22.2	± 5.0	ns
Lean weight (kg)	29.9	± 3.0	31.9	± 4.7	ns	23.4	± 1.9	28.7	± 3.2	R
$\dot{V}O_2max$ (l/min)	1.92	± 0.33	2.05	± 0.25	ns	1.12	± 0.28	1.54	± 0.26	R
(ml/kg/min)	50.1	± 3.6	47.8	± 6.9	ns	37.0	± 4.6	41.6	± 4.8	R

Values are Mean ± SD.
* = P<0.05
ns = not significant
R = Sample size was too small to declare non-significance.

Table 3. Concentrations of hemoglobin and hematocrits of male swimmers, female swimmers and their respective control groups.

	Male Subjects (n=20)			Female Subjects (n=20)		
	Swimmers (n=9)	Controls (n=11)	sig	Swimmers (n=13)	Controls (n=7)	sig
Hemoglobin (g/dl)	15.2 ± 0.5	15.4 ± 1.3	ns	15.4 ± 0.6	15.5 ± 0.7	ns
Venous hematocrit (% packed cell volume)	43 ± 1	42 ± 4	ns	43 ± 1	43 ± 2	ns

Values are Mean ± SD. ns = not significant

Table 4. Magnesium concentrations in the erythrocytes, whole blood, and plasma of male swimmers, female swimmers and their respective control groups.

	Male Subjects (n=20)			Female Subjects (n=20)		
	Swimmers (n=9)	Controls (n=11)	sig	Swimmers (n=13)	Controls (n=7)	sig
Erythrocyte Magnesium (mg/dl)	5.06 ± 0.71[S]	5.03 ± 0.61	ns	5.36 ± 0.49[S]	5.08 ± 0.98	ns
Whole Blood Magnesium (mg/dl)	3.39 ± 0.28	3.27 ± 0.20	R	3.42 ± 0.23	3.34 ± 0.48	ns
Plasma Magnesium (mg/dl)	2.11 ± 0.11[*]	2.04 ± 0.21	ns	1.95 ± 0.15[*]	2.01 ± 0.16	ns

Values are Mean ± SD.
ns = not significant
R = Sample size was too small to declare non-significance between male swimmers and controls.
s = Sample size was too small to declare that male swimmers and female swimmers did not differ.
* = Male swimmers differed from female swimmers ($P<0.05$).

Table 5. Correlation coefficients between plasma magnesium concentrations and physical characteristics, hemoglobin levels and hematocrits in preteen swimmers and controls.

	Treatment		Gender		Treatment and Gender			
Variables	S[1] (n=22)	C (18)	M (20)	F (20)	MS (9)	MC (11)	FS (13)	FC (7)
Plasma Magnesium vs								
Age	-0.37	+0.25	+0.26	-0.51*	+0.05	+0.39	-0.66*	-0.15
Weight	-0.01	+0.09	+0.22	-0.15	+0.41	+0.19	+0.02	-0.34
Lean weight	-0.01	+0.29	+0.40	-0.32	+0.31	+0.50	-0.30	-0.21
Body fat	-0.04	-0.24	-0.21	+0.13	+0.30	-0.24	+0.33	-0.31
Hemoglobin	-0.30	+0.21	+0.36	-0.48*	+0.18	+0.43	-0.41	-0.69
Hematocrit	-0.36	+0.21	+0.33	-0.45*	+0.47	+0.42	-0.41	-0.53
$\dot{V}O_2max$ (l/min)	-0.02	+0.36	+0.47*	-0.43*	+0.34	+0.60*	-0.57*	-0.17
(ml/kg/min)	-0.02	+0.44*	+0.42*	-0.40*	+0.05	+0.59*	-0.54*	+0.12

1 S = Swimmers, C = Controls, M = Males, F = Females * P<0.05

Table 6. Correlation coefficients between erythrocyte magnesium concentrations and whole blood magnesium, plasma magnesium, physical characteristics, hemoglobin levels and hematocrits in pre-teen swimmers and controls.

	Treatment		Gender		Treatment and Gender			
Variables	S[1] (n=22)	C (18)	M (20)	F (20)	MS (9)	MC (11)	FS (13)	FC (7)
Erythrocyte Magnesium vs								
Wh.Blood Mg	+0.94*	+0.87*	+0.81*	+0.97*	+0.98*	+0.66*	+0.93*	+0.99*
Plasma Mg	-0.28	-0.08	-0.34	+0.08	-0.66	-0.24	+0.13	+0.14
Age	-0.15	-0.44	-0.29	-0.30	-0.39	-0.21	+0.08	-0.86*
Weight	-0.36	-0.23	-0.21	-0.29	-0.69	+0.14	-0.27	-0.86*
Lean weight	-0.37	-0.36	-0.27	-0.26	-0.57	+0.04	-0.08	-0.85*
Body fat	-0.13	+0.03	-0.05	-0.19	-0.74*	+0.15	-0.36	-0.15
Hemoglobin	-0.00	-0.27	-0.52*	+0.23	-0.22	-0.69*	+0.08	-0.45
Hematocrit	+0.05	-0.16	-0.38	+0.37	+0.45	-0.69*	-0.12	+0.72
VO_2max (l/min)	-0.31	-0.25	-0.10	-0.18	-0.28	+0.07	-0.25	-0.71
(ml/kg/min)	+0.10	-0.19	+0.12	+0.05	+0.45	-0.13	+0.12	-0.35

1 S = Swimmers, C = Controls, M = Males, F = Females * P<0.05

Table 7. Consumption of selected nutrients: comparisons between swimmers and controls within the male and female subjects, and comparisons between male and female swimmers and between male and female controls.

	Male Subjects (n=20)			Female Subjects (n=20)		
	Swimmers (n=9)	Controls (n=11)	sig	Swimmers (n=13)	Controls (n=7)	sig
Total Kcal (kcal)	2535 ± 459[s]	2325 ± 530[c]	ns	2085 ± 528[s]	1814 ± 604[c]	ns
Total Kcal (%RDA[1])	104 ± 23	94 ± 26[c]	ns	106 ± 31	110 ± 38[c]	ns
Kcal/Kg[2](kcal)	67 ± 13*	60 ± 18	ns	51 ± 16*	53 ± 18	ns
Protein (%RDA)	227 ± 44*	199 ± 43	R	178 ± 37*	183 ± 63	ns
Protein (%Kcal[3])	14 ± 1.9	14 ± 2.5	ns	14 ± 2.6	14 ± 1.6	ns
Fat (%Kcal)	37 ± 5.5	36 ± 3.8	ns	37 ± 4.5	35 ± 6.9	ns
Carbohydrate (%Kcal)	50 ± 6.3	52 ± 4.8	ns	50 ± 6.6	51 ± 5.6	ns
Diet Chol[4] (mg)	309 ± 207	272 ± 160[c]	ns	222 ± 110	192 ± 82[c]	ns
Poly/Sat[5] (ratio)	0.42 ± .13	0.55 ± .37[c]	R	0.41 ± .16	0.36 ± .15[c]	ns
Calcium (%RDA)	115 ± 25[s]	97 ± 26	R	99 ± 46[s]	88 ± 39	ns
Magnesium (%RDA)	95 ± 19	90 ± 61	ns	90 ± 32	79 ± 33	ns
Phosphorus (%RDA)	148 ± 32[s]	122 ± 29[c]	R	116 ± 37[s]	98 ± 38[c]	ns
Thiamin (%RDA)	190 ± 67	191 ± 93	ns	182 ± 72	166 ± 85	ns
Vitamin D (%RDA)	121 ± 55[s]	86 ± 53	R	97 ± 44[s]	70 ± 61	R

Values are Mean ± SD.

1 = Percent of recommended daily allowance
2 = Kilocalories per kilogram of body weight
3 = Percent of total kilocalories
4 = Diet cholesterol
5 = Ratio of polyunsaturated to saturated fats

ns = not significant
R = Sample size was too small to declare non-significance.
s = Sample size was too small to declare non-significance between male and female swimmers.
c = Sample size was too small to declare non-significance between male and female controls.
* = $P < 0.05$ between male and female swimmers.

Table 8. Nutrient consumption of younger (<11.2) and older (≥11.2) male and female swimmers and controls.

	Male Swimmers (n=9) Younger (n=3)	Male Swimmers (n=9) Older (n=6)	sig	Male Controls (n=11) Younger (n=3)	Male Controls (n=11) Older (n=8)	sig
Total Kcal (kcal)	2308 ± 498	2648 ± 441	R	2466 ± 272	2272 ± 607	R
Total Kcal (%RDA[1])	100 ± 37	106 ± 18	ns	95 ± 8	94 ± 30	R
Kcal/Kg[2] (kcal)	74 ± 18	64 ± 11	R	72 ± 10	56 ± 18	R
Protein (%RDA)	246 ± 62	218 ± 35	R	191 ± 36	202 ± 48	ns
Protein (%Kcal[3])	15 ± 2.3	14 ± 1.8	ns	12 ± 1.2	15 ± 2.4	R
Fat (%Kcal)	40 ± 6.8	36 ± 4.7	R	35 ± 0.9	36 ± 4.4	ns
Carbohydrate (%Kcal)	46 ± 9.4	52 ± 4.2	R	54 ± 1.3	51 ± 5.4	R
Diet Chol[4] (mg)	421 ± 357	253 ± 78	R	188 ± 40	304 ± 179	R
Poly/Sat[5] (ratio)	0.32 ± .05	0.47 ± .12	R	0.68 ± .15	0.50 ± .42	R
Calcium (%RDA)	127 ± 43	109 ± 11	ns	92 ± 20	99 ± 29	ns
Magnesium (%RDA)	95 ± 28	95 ± 16	ns	133 ± 110	75 ± 28	R
Phosphorus (%RDA)	167 ± 52	138 ± 15	R	137 ± 41	116 ± 25	R
Thiamin (%RDA)	185 ± 79	192 ± 69	ns	220 ± 77	180 ± 101	ns
Vitamin D (%RDA)	126 ± 81	118 ± 46	ns	119 ± 60	73 ± 14	R

	Female Swimmers (n=13) Younger (n=4)	Female Swimmers (n=13) Older (n=9)	sig	Female Controls (n=7) Younger (n=2)	Female Controls (n=7) Older (n=5)	sig
Total Kcal (kcal)	2042 ± 468	2104 ± 578	ns	1932 ± 422	1767 ± 702	ns
Total Kcal (%RDA[1])	110 ± 26	105 ± 34	ns	130 ± 18	102 ± 42	R
Kcal/Kg[2] (kcal)	54 ± 14	50 ± 17	ns	64 ± 7	49 ± 20	R
Protein (%RDA)	189 ± 16	173 ± 43	R	220 ± 41	168 ± 67	R
Protein (%Kcal[3])	15 ± 2.9	14 ± 2.6	ns	14 ± 0.5	14 ± 1.9	ns
Fat (%Kcal)	35 ± 2.9	38 ± 5.0	R	38 ± 9.1	33 ± 6.4	R
Carbohydrate (%Kcal)	52 ± 1.6	49 ± 7.8	R	49 ± 9.4	52 ± 4.7	R
Diet Chol[4] (mg)	232 ± 80	218 ± 115	ns	218 ± 70	181 ± 92	ns
Poly/Sat[5] (ratio)	0.33 ± .02	0.44 ± .19	R	0.42 ± .23	0.34 ± .13	ns
Calcium (%RDA)	81 ± 16	107 ± 53	R	105 ± 31	82 ± 43	R
Magnesium (%RDA)	72 ± 20	98 ± 35	R	93 ± 35	74 ± 34	R
Phosphorus (%RDA)	103 ± 7	122 ± 44	R	119 ± 34	89 ± 38	R
Thiamin (%RDA)	238 ± 80	157 ± 56	R	160 ± 77	168 ± 97	ns
Vitamin D (%RDA	92 ± 52	99 ± 43	ns	48 ± 12	79 ± 73	R

Values are Mean ± SD.
ns = not significant
R = Sample size was too small to declare non-significance.
1 = Percent of Recommended Daily Allowance
2 = Kilocalories per kilogram of body weight
3 = Percent of total kilocalories
4 = Diet cholesterol
5 = Ratio of polyunsaturated to saturated fats

Table 9. Number and percent of subjects consuming less than 67% recommended daily allowance or more than 100% recommended daily allowance of selected nutrients over a three-day period.

	Male Subjects				Female Subjects			
	Swimmers (n=9)		Controls (n=11)		Swimmers (n=13)		Controls (n=7)	
	#	%	#	%	#	%	#	%
Kilocalories								
< 67%	1	11%	1	9%	1	8%	1	6%
>100%	6	67%	4	36%	8	62%	6	86%
Protein								
< 67%	0	0%	0	0%	0	0%	1	14%
>100%	8	89%	11	100%	12	92%	5	71%
Calcium								
< 67%	0	0%	1	9%	4	31%	1	14%
>100%	8	89%	5	45%	4	31%	2	29%
Magnesium								
< 67%	1	11%	4	36%	4	31%	2	29%
>100%	5	56%	2	18%	4	31%	2	29%
Phosphorus								
< 67%	0	0%	0	0%	0	0%	1	14%
>100%	9	100%	8	73%	8	62%	3	43%
Thiamin								
< 67%	0	0%	0	0%	0	0%	1	14%
>100%	8	89%	11	100%	12	92%	5	71%
Vitamin D								
< 67%	5	56%	7	64%	9	69%	7	100%
>100%	0	0%	2	18%	3	23%	0	0%

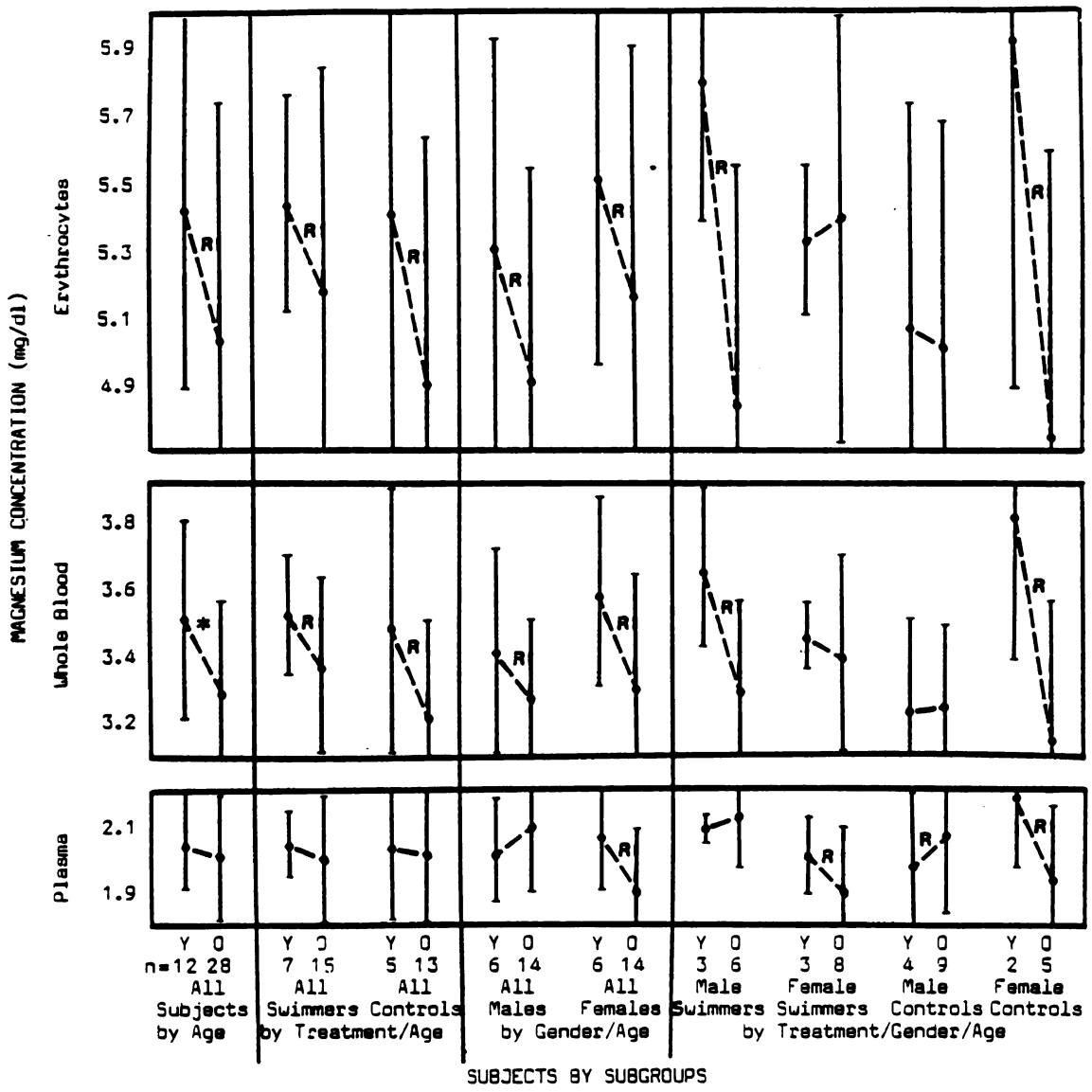

Figure 1. Magnesium concentrations of erythrocytes, whole blood, and plasma (mean ± SD) in younger and older subjects by gender and treatment.

n=number in group Y=younger (<11.2 years) O=older (≥11.2 years)
R=difference between means was one-half standard deviation
*=whole blood magnesium was significantly less in the older members of all subjects ($P<0.05$)
Dashed lines between means illustrate the trend to lower magnesium concentrations in older subjects when compared with younger subjects. However, older members of all male subgroups had greater plasma magnesium concentrations than younger members of those groups.

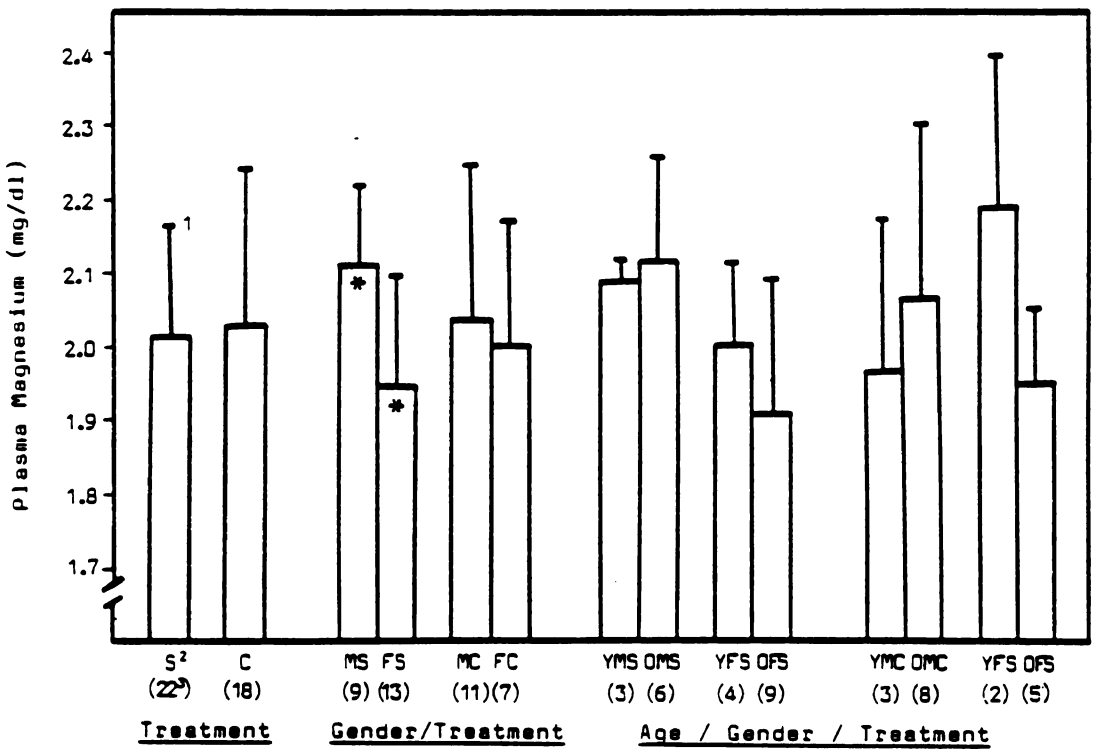

Figure 2. Mean plasma magnesium concentrations of pre-teen swimmers and controls, expressed by treatment, gender/treatment, and age/gender/treatment subgroups.

1 Mean values ± SD

2 S=swimmers C=controls M=male F=female Y=younger O=older

3 Number in parentheses is number of subjects in subgroup.

* Mean values of male and female swimmers were significantly different ($P<0.05$).

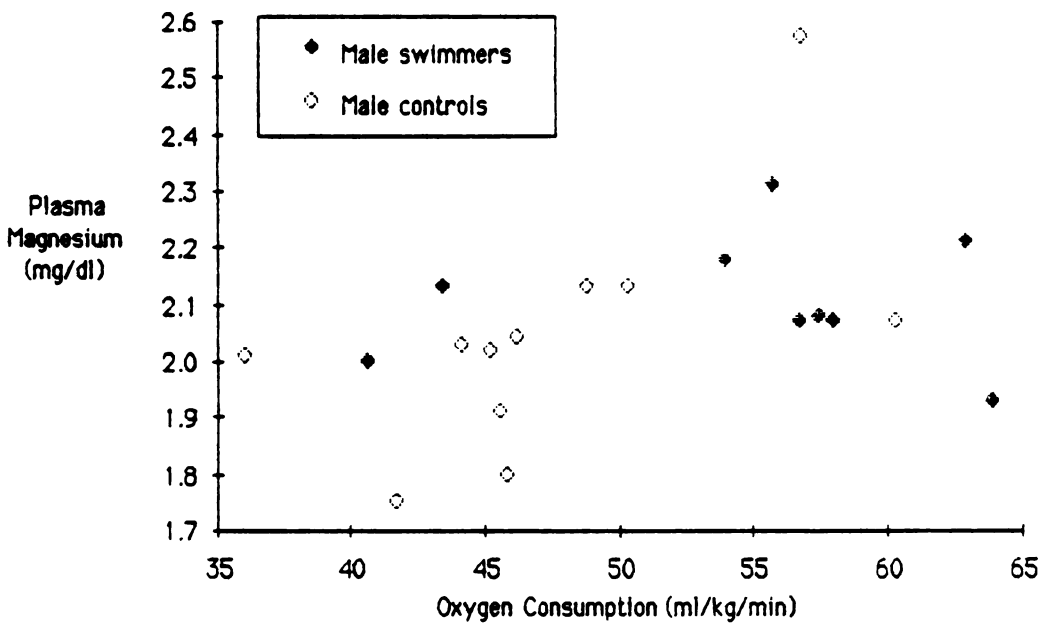

Figure 3 . Relationship of plasma magnesium and oxygen consumption among pre-teen males.

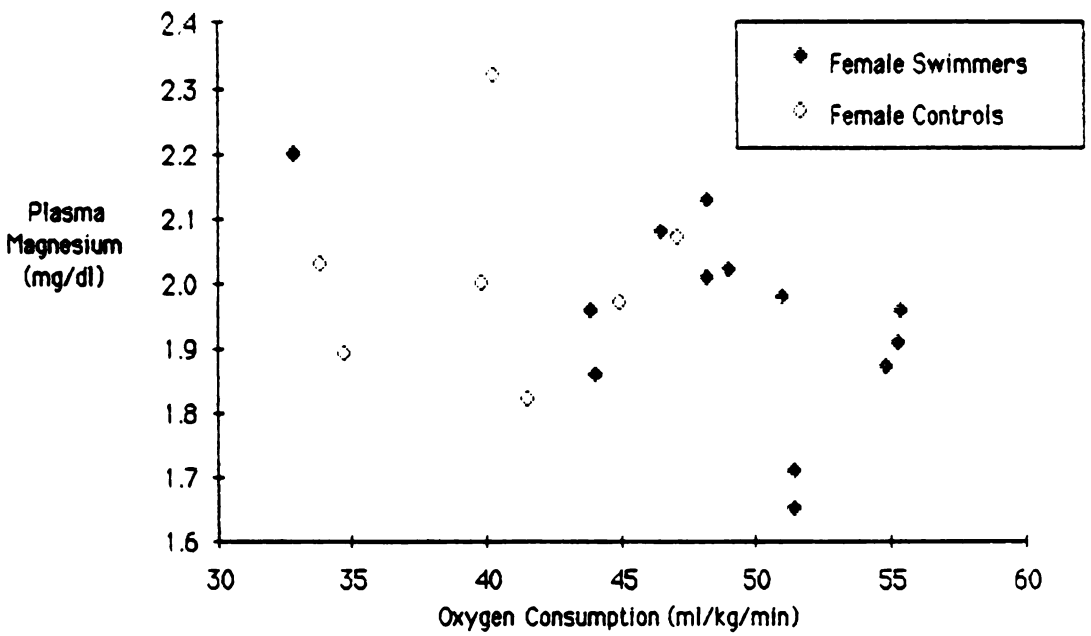

Figure 4. Relationship of plasma magnesium and oxygen consumption among pre-teen females

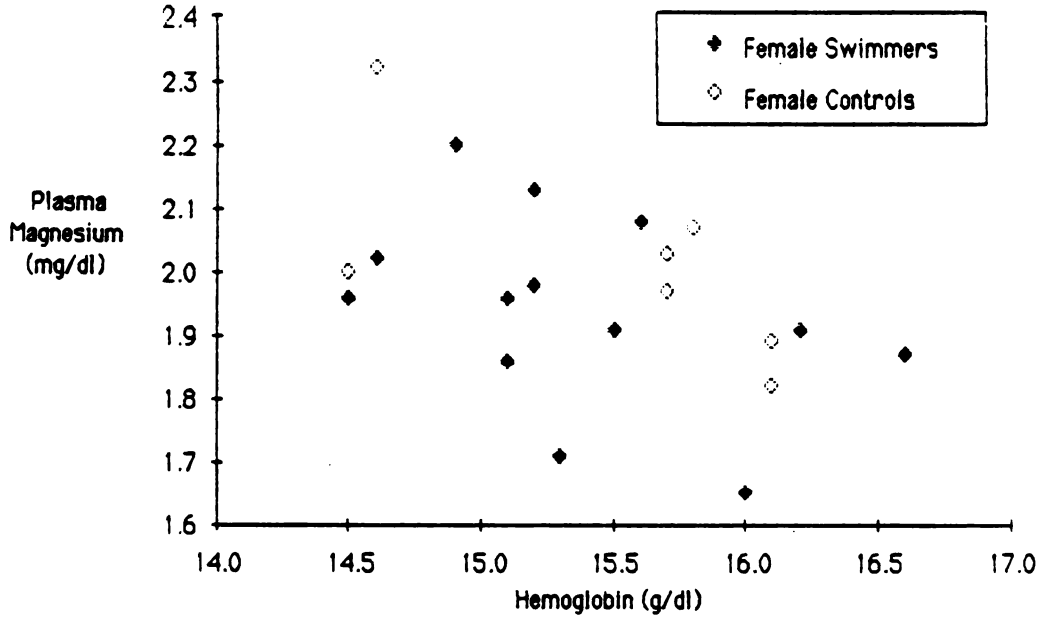

Figure 5. Relationship of plasma magnesium and hemoglobin among pre-teen females

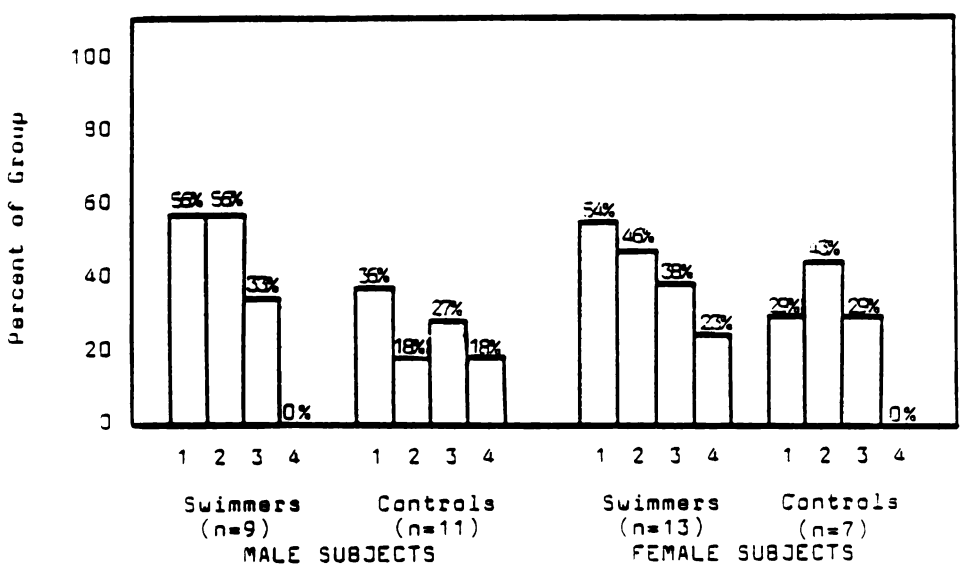

Figure 6. Supplementation of diet with vitamins and minerals in pre-teen male and female swimmers and controls.

1 = multiple vitamins 2 = vitamin C

3 = iron 4 = other vitamins or minerals

CHAPTER V

SUMMARY, CONCLUSIONS, RECOMMENDATIONS

The purpose of this study was to determine if a possible cellular adaptation of magnesium metabolism to physical training, a positive correlation between plasma magnesium and maximum oxygen consumption, could be found in pre-teen children who were training for competitive swimming. Eight hypotheses, listed in Chapter I, were tested.

METHODS

Forty caucasian children between 9.5 and 12.9 years of age participated in the study. Twenty-two swim club members, 9 males and 13 females, who swam at least 3500 yards per day, five days per week, for at least 35 weeks comprised the experimental group. Eighteen control subjects, 11 males and 7 females, who were not in training for any sport, were chosen based on the fact that they were similar in age, height and weight to a swimmer.

Blood, drawn from all subjects after a 12-hour fast, was analyzed for magnesium by atomic absorption spectrophotometry. Hemoglobin concentration and venous hematocrits were also determined by standard methods.

Body fat was calculated from body density which was determined by underwater weighing. Maximum oxygen consumption was obtained by a treadmill run using a continuous protocol, a constant speed, and an increasing grade. Nutrient intake was estimated from a three-day food record analyzed by means of the Michigan State Nutrient Data Bank.

RESULTS

The following results were obtained.

1. A positive correlation ($P<0.05$) between plasma magnesium and maximum oxygen consumption was found in all control subjects, in all males and in control males.

   A positive correlation between plasma magnesium and maximum oxygen consumption was not found in all swimmers, in male swimmers, in all females, in female swimmers, and in female controls.

   Had a one-tailed test for a inverse correlation been used, a significant inverse correlation would have been found in all females and in female swimmers.

2. Male swimmers had significantly ($P<0.05$) greater plasma magnesium than female swimmers. All males had a concentration of plasma magnesium greater by one-half standard deviation than all females. Judgement must be reserved as to the significance of this difference due

to insufficient sample size.

There were no differences in the plasma magnesium concentrations between all swimmers and all controls, between male swimmers and male controls, between female swimmers and female controls, and between male and female controls.

3. Female swimmers had a concentration of erythrocyte magnesium greater by one-half standard deviation than male swimmers. Judgement must be reserved as to the significance of this difference due to insufficient sample size.

No other differences between groups were found in erythrocyte magnesium concentrations or in whole blood concentrations.

There were no differences in hemoglobin concentrations or in venous hematocrits between any groups.

4. There was no correlation between red blood cell magnesium or whole blood magnesium and maximum oxygen uptake in the male or female swimmers or male or female controls.

5. Plasma magnesium was inversely correlated with hemoglobin and hematocrit among females ($P<0.05$), among female swimmers and female controls. Plasma magnesium was not related to hemoglobin or hematocrit in males. Red cell and whole blood magnesium was not related to

hemoglobin and hematocrit in any group.

6. Daily nutrient intake was not different between male swimmers and controls or female swimmers and controls in total kilocalories, kilocalories per kilogram of body weight, percentage of calories from proteins, fats and carbohydrates, cholesterol, magnesium, and thiamin.

   Female swimmers and controls differed by as much as one-half standard deviation only in consumption of vitamin D, whereas male swimmers consumed at least one-half standard deviation more than male controls of the following nutrients: protein, calcium, phosphorus, and vitamin D.

7. Male swimmers ate significantly more kilocalories per kilogram of body weight and a greater %RDA for protein than female swimmers ($P<0.05$). These differences did not occur between male and female controls.

   Male swimmers ate one-half standard deviation more total kilocalories and calcium than female swimmers.

   Male controls ate more total kilocalories, more phosphorus, more dietary cholesterol and a slightly greater ratio of polyunsaturated to saturated fats than female controls, but the significance of the difference could not be judged due to the small sample size.

8. Older subjects had significantly ($P<0.05$) less whole blood magnesium than younger subjects. The trend toward lower concentrations of whole blood magnesium in the blood of older members than in the blood of younger members was present in all subgroups except the male controls where older and younger members had similar concentrations. Differences in concentrations of whole blood magnesium of one-half standard deviation were present in all swimmers, all controls, all males, all females, male swimmers and female controls.

Erythrocyte magnesium was lower in the older members than in younger members of all groups except female swimmers, but no significant difference was found. Differences of one-half standard deviation in concentrations of erythrocyte magnesium were present in all groups except female swimmers and male controls.

Plasma magnesium concentrations were lower in the older members than in the younger members of all subjects, all swimmers, all controls, all females, female swimmers, and female controls. Plasma magnesium concentrations were higher in the older than in the younger members of all males, male swimmers and male controls. Differences of one-half standard deviation in plasma magnesium were observed between older and younger members of all females, female swimmers, male controls, and female controls. No significant difference between older and younger members of any

group in plasma magnesium was found.

No differences between younger and older members of any group were found in concentrations of hemoglobin or in venous hematocrit.

Comparisons of mean nutrient intakes of younger and older members of the various groups resulted in no significant differences.

CONCLUSIONS

This investigation provides evidence that the positive correlation between plasma magnesium and maximum oxygen consumption found previously in adult male athletes is also found in young, growing males. Whether it can be found in young, growing males who also are engaged in vigorous training cannot be determined from the present data because of the small sample of male swimmers. The data in this study suggest that a positive linear relationship between plasma magnesium and maximum oxygen consumption does not occur in young females, and that these variables may even be inversely related in young females who exercise vigorously. The significant difference in plasma magnesium concentrations between pre-teen male and female swimmers, but not between their male and female controls, which was found despite the small sample sizes, suggests that an adaptation of magnesium metabolism may occur in children as a result of endurance training and may affect young males and young females differently.

RECOMMENDATIONS

1. The positive correlation of plasma magnesium with maximum oxygen consumption among pre-teen males who are not in training and the lack of such a relationship in pre-teen males who do train, which were found in this study, should be carefully evaluated using a larger group of male swimmers and controls.

2. The absence of a positive correlation between plasma magnesium and maximum oxygen consumption among females, which was suggested by results of this study, should be investigated using a larger sample of female athletes and controls. The inverse correlation between plasma magnesium and hemoglobin and hematocrit found among these pre-teen females should also be confirmed.

3. The results of this study suggest the need to search for a mechanism that can explain the increase of plasma magnesium concentration in young males who are training and the decrease in plasma magnesium concentrations in young females who are training. Because the same direction of change in plasma magnesium levels was observed when the mean plasma levels of younger and older members of the male and female control groups were compared, it seems reasonable to search for a mechanism already present in growing individuals which may be enhanced by exercise.

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APPENDICES

APPENDIX A

Additional correlations of plasma magnesium

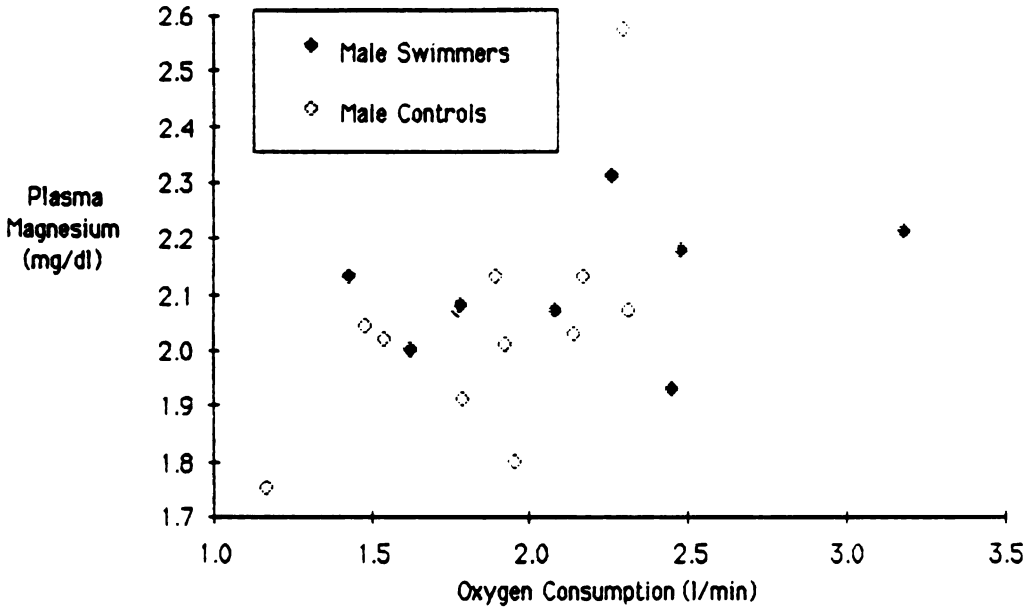

Figure A1. Relationship of plasma magnesium and oxygen consumption in l/min among pre-teen males.

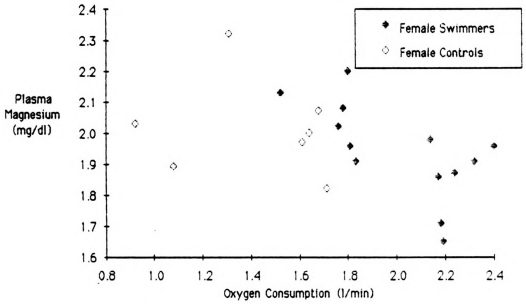

Figure A2. Relationship of plasma magnesium and oxygen consumption in l/min among pre-teen females

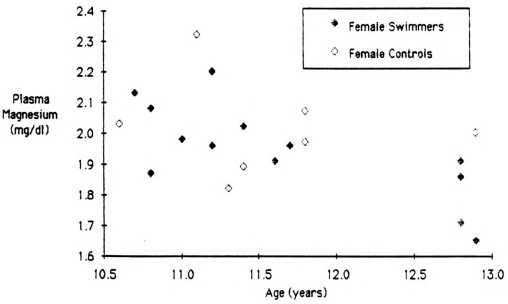

Figure A3. Relationship of plasma magnesium and age among pre-teen females

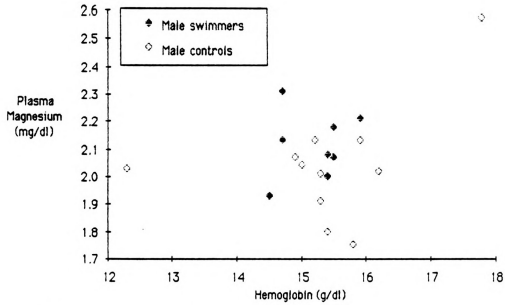

Figure A4. Relationship of plasma magnesium and hemoglobin among pre-teen males.

APPENDIX B

Additional correlations of erythrocyte magnesium

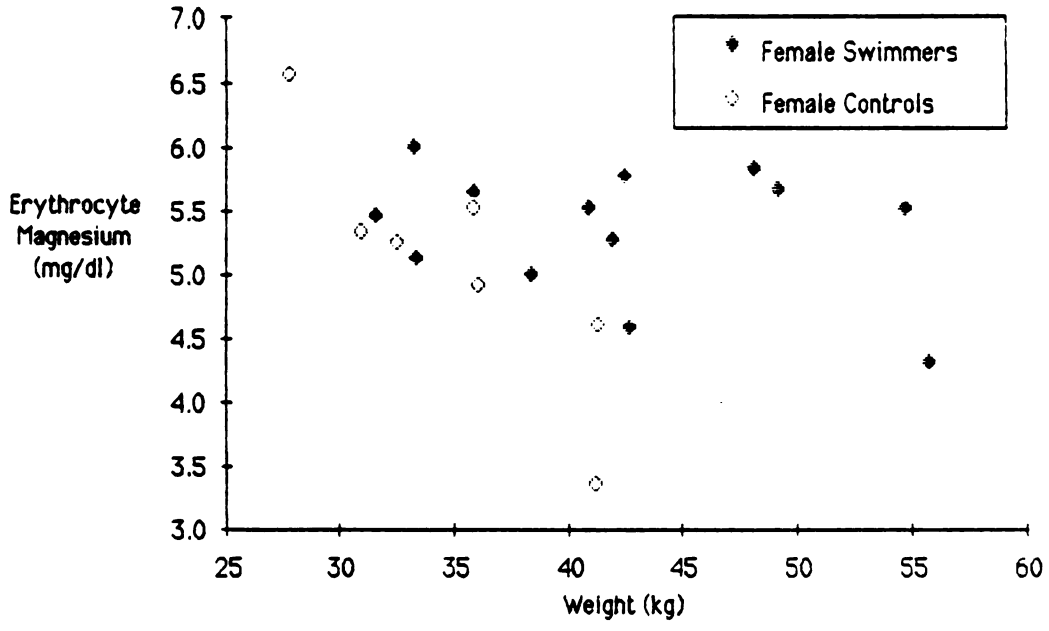

Figure B1. Relationship of erythrocyte magnesium and weight among pre-teen females.

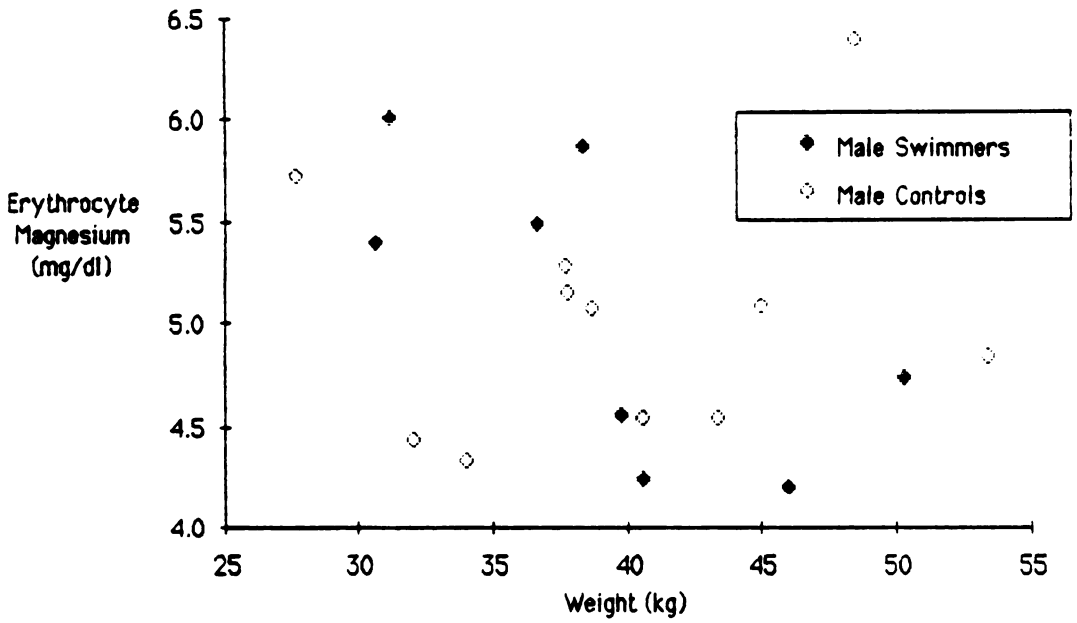

Figure B2. Relationship of erythrocyte magnesium and weight among pre-teen males.

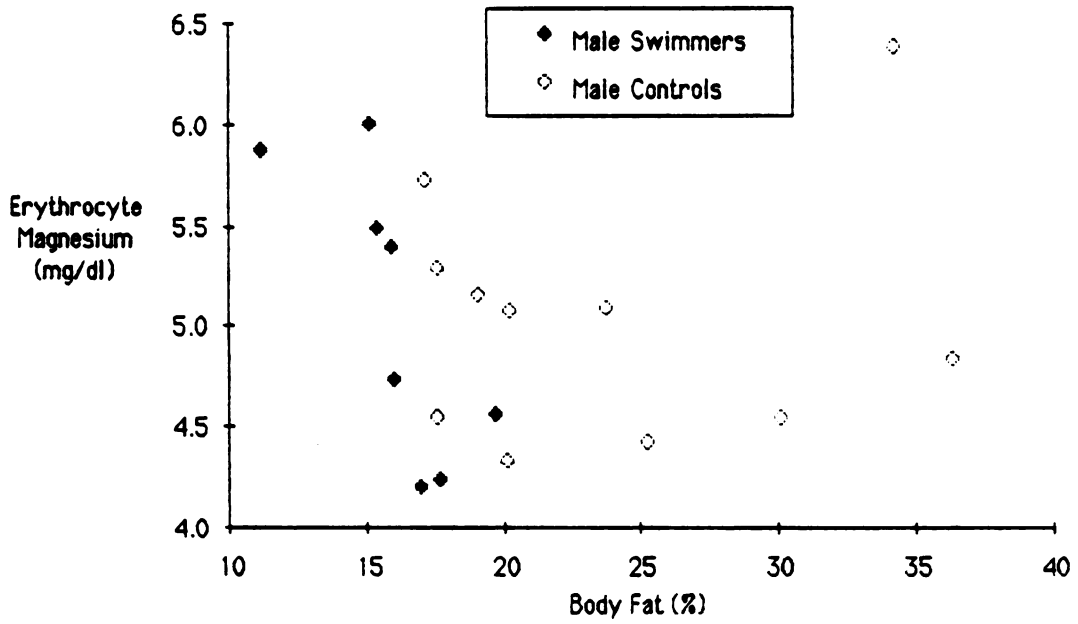

Figure B3. Relationship of erythrocyte magnesium and body fat among pre-teen males.

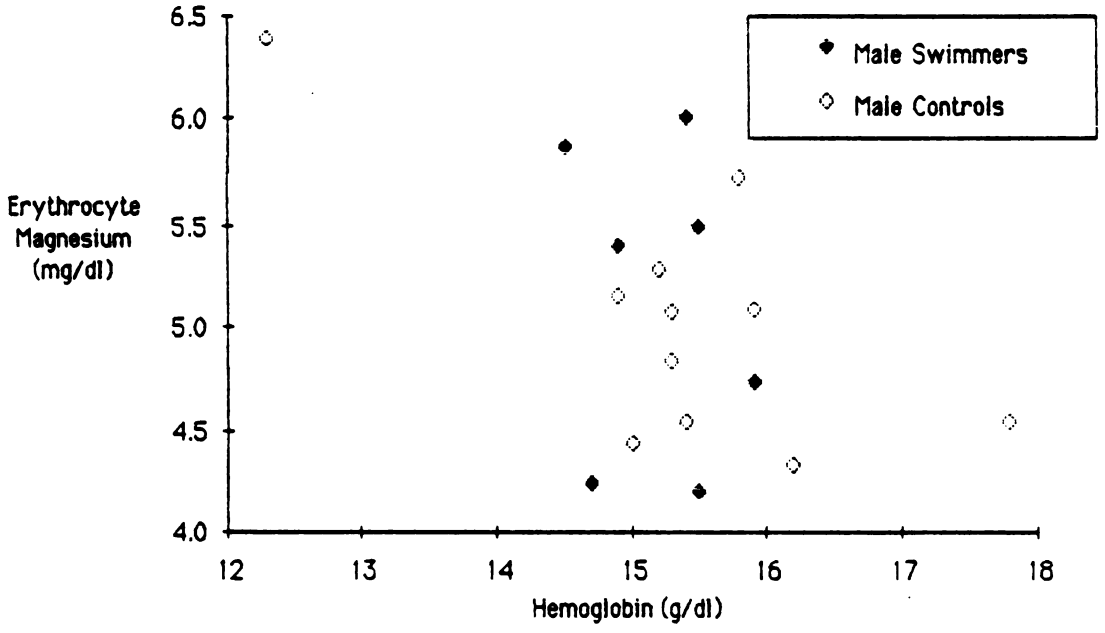

Figure B4. Relationship of erythrocyte magnesiumand hemoglobin among pre-teen males

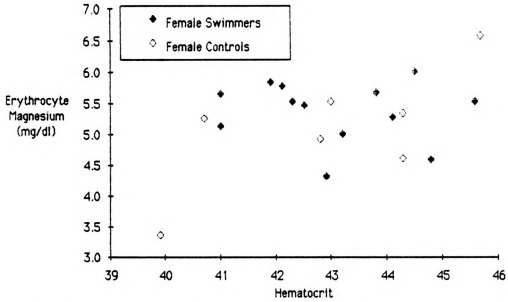

Figure B5. Relationship of erythrocyte magnesium and hematocrit among pre-teen females.

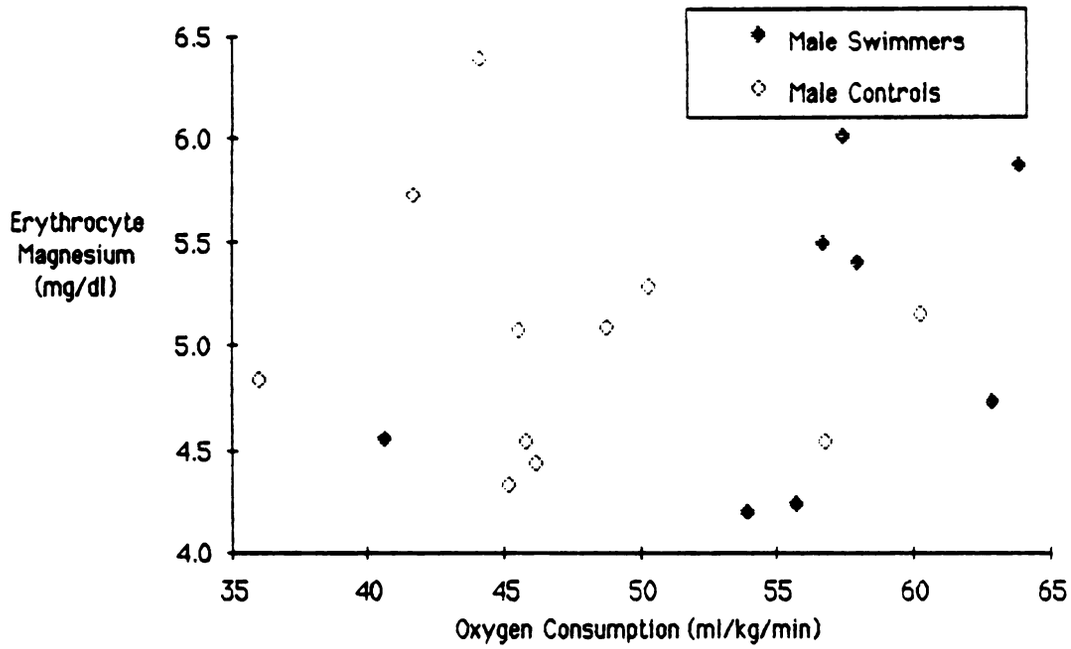

Figure B6. Relationship of erythrocyte magnesium and oxygen consumption among pre-teen males.