MAGNESIUM STATUS AND PEROXIDATIVE STRESS IN GOLDEN SYRIAN HAMSTERS FED CASEIN AND SOY BASED DIETS

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Abstract

The overall objective of this thesis was to utilize casein and soy based diets containing different levels of magnesium and/or phytate to show the effect of magnesium status and bioavailability on the susceptibility to lipid peroxidation in hamsters. Magnesium deficiency has been shown to enhance oxidative stress in experimental animals. Since cholesterol metabolism in hamsters closely resembles that of humans, in contrast to other rodent species, the Golden-Syrian hamster lipid model, under casein and soy fed magnesium sufficient and magnesium deficient diets, was used to test the hypothesis that magnesium deficiency may enhance oxidative stress. In experiment 1, 32 male Golden-Syrian Hamsters (n = 8) were randomly assigned to magnesium-depleted (185 $\pm$ 3 ppm) and magnesium-repleted (653 $\pm$ 4 ppm) diets alongside supplemented phytate (0.5%) and non-supplemented phytate (0%) diets. Diets in experiment 1 were casein based. After 4 weeks on the experimental diets, hamster magnesium status, as shown by magnesium concentrations in the kidney and heart, was mainly affected by dietary magnesium ($p<0.05$) in hamsters not by supplemented dietary phytate. Oxidative stress was evaluated in terms of levels of thiobarbituric acid-reactive substances (TBARS) in the liver as well as modified ApoB levels in Hamster LDL during time dependent forced peroxidation with hydrogen peroxide (H_2O_2). Both dietary magnesium and dietary supplemented phytate were determining factors in the hamster's ability to cope with oxidative stress ($p<0.05$). In experiment 2, 32 male Golden-Syrian Hamsters (n = 8) were randomly assigned to magnesium-depleted (191 $\pm$ 2 ppm) and magnesium-repleted (761 $\pm$ 4 ppm) diets alongside supplemented phytate (0.5%) and non-

supplemented phytate (0%) diets. Diets in experiment 2 were soy based. After 4 weeks on the experimental diets, hamster magnesium status was mainly affected by dietary magnesium ($p<0.05$) in hamsters not by supplemented dietary phytate. Both dietary magnesium and dietary supplemented phytate were determining factors in the hamster's ability to cope with oxidative stress ($p<0.05$).

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List of Abbreviations and Acronyms

apoB	apoprotein B
%	percent
μM	micromolar
ATP	adenosine triphosphate
EDTA	ethylenediaminetetraacetic acid
g	grams
H_2O_2	hydrogen peroxide
HDL	HDL - high density lipoprotein
IP_6	inositol hexaphosphate
KBr	potassium bromide
LDL	LDL - low density lipoprotein
mg	milligram
min	minutes
ml	milliliter
NaCl	sodium chloride
NaN_3	sodium azide
nm	nanometer
°C	degree Celsius
PBS	phosphate-buffered saline
PTH	parathyroid hormone
RF	RF - relative fluorescence
TBARS	thiobarbituric acid reactant substances
TC	TC - total cholesterol
TG	triglyceride
VLDL	very low density lipoprotein

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1. INTRODUCTION

According to 1997 statistics, cardiovascular disease (heart disease and stroke) is Canada's number one killer, claiming 79,457 lives. Approximately 36% of all deaths (35.6% male: 38.2% female) in Canada are caused by heart and blood vessel disease and stroke (Heart and Stroke Foundation of Alberta, NWT and Nunavut, 1997). Despite this high incidence of fatalities, millions of Canadians are unaware that 75% of Canada's population exhibit at least one risk factor for cardiovascular disease. The primary risk factors for cardiovascular disease are hypertension, high cholesterol, diabetes, overweight, cigarette smoking, and physical inactivity. The first four of these risk factors may cluster in some persons and have been identified as components of a syndrome known as metabolic cardiovascular syndrome (Arnesen, 1992) or the "deadly quartet" (Kaplan, 1989). This syndrome is characterized by a persistent state of insulin resistance and compensatory hyperinsulinemia that may be etiologically related to the four risk factors. Persons with one of the four risk factors are at increased risk for having any of the other three (Pi-Sunyer, 1993). Excessive caloric intake resulting in weight gain has been postulated as the event leading to the emergence of the other risk factors in metabolic cardiovascular syndrome (DeFronzo and Ferrannini, 1991).

Magnesium is abundant in nature and the major routes of intake are through food and water. Through changes in the treatment of foodstuffs and altered diets, as well as increased use of surface water with low magnesium content, magnesium deficiency is present in modern society. More recently, there has been increasing evidence linking deficient dietary magnesium to cardiovascular disease in areas where the water is low in

magnesium (Marx and Neutra, 1997; Yang et al., 1997; Yang, 1998; Rubenowitz et al., 1999; Rubenowitz et al., 2000). These results show that there may be significant protective effects of dietary magnesium intake on the risk of cardiovascular diseases. Despite the apparent association between magnesium and cardiovascular diseases, the relationship between dietary magnesium intake and cardiovascular disease is still obscure.

1.1. The Hypothesis

It is hypothesized that hamsters fed magnesium deplete diets experience a negative magnesium balance and are thus more susceptible to lipid peroxidation than counterparts fed magnesium replete diets. Since dietary magnesium affects multiple homeostatic metabolic reactions, it is possible that dietary magnesium deficiency or reduced bioavailability could ultimately influence the Syrian hamster's susceptibility to lipid peroxidation. In addition, dietary magnesium may affect calcium bioavailabilty in hamsters. It is also hypothesized that the presence of phytate in the diet reduces magnesium bioavailability and contributes to a greater susceptibility to lipid peroxidation. Lipid peroxidation has been implicated in adverse tissue changes, one of which includes LDL-oxidation.

1.2. The Objectives

The overall objective of this thesis was to utilize casein and soy based diets containing different levels of magnesium and/or phytate to show the effect of magnesium status and bioavailability on the susceptibility to lipid peroxidation in hamsters.

The specific objectives were:

To investigate the effect of dietary magnesium on magnesium and calcium status and peroxidation parameters in hamsters fed casein/soy based diets.

To investigate the possible interactions between dietary magnesium and calcium in hamsters fed casein/soy based diets.

To investigate the effect of dietary phytate on magnesium and calcium status and peroxidation parameters in hamsters fed casein/soy based diets.

2. LITERATURE REVIEW

2.1. Biological functions of magnesium

Magnesium is a ubiquitous element. As a cation, it ranks fourth in overall abundance within the human body, but intracellularly it is second only to potassium as the most abundant metal in the body. Total body magnesium pool is approximately 25 g, of which 50 to 60 percent resides in the bone of normal adults (Brady et al., 1987; Wester, 1987). The word magnesium comes from the name of the Greek city, Magnesia, where large deposits of magnesium carbonate were found. Magnesium carbonate salt has been traditionally used as a laxative; this salt is still used in this way. Magnesium has been deemed the "iron" of the plant world. As iron is the central structure of hemoglobin, the central atom of the chlorophyll structure is magnesium (Wester, 1987).

Extensive studies of the physiological and biochemical functions of magnesium were impaired until about 45 years ago. In mid 1950's, Walsh introduced an analytical magnesium measurement method using atomic absorption spectroscopy. Rapid advances in computer technology and electronics of the past few decades further developed Walsh's atomic absorption spectroscopy method. Magnesium may now be measured easily and accurately in all biological material in small amounts and at low concentrations. As a result, the body of knowledge about its biological role has increased exponentially. We now know that magnesium is a required cofactor for more than 300 enzymes (Wester, 1987) and, in particular, those involved in cellular energy provision. A primary function of magnesium in this role is to provide stability to the structure of adenosine triphosphate (ATP) in the numerous ATP-dependent enzyme reactions. In

addition, magnesium has a role in cell gene replication, muscle contraction, and the biosynthesis of lipids and essential skeletal components.

2.2. Factors influencing magnesium and calcium bioavailability

2.2.1. Magnesium absorption

Little agreement exists about the absorption of magnesium. Metabolic studies show a very wide range of average magnesium absorption rates (15% to 80%) with an average of 35% (Wester, 1987). The mechanism of absorption is not much more definitive than the efficiency of absorption. Some researchers believe that absorption occurs both by active transport and facilitated diffusion (Brady et al., 1987), whereas others maintain that absorption occurs by simple and facilitated diffusion (Shils, 1988). Shils reported that the particular transport system (simple diffusion and/or facilitated diffusion) is operative, depending upon the concentration of magnesium presented to the small intestine. The facilitated system becomes saturated at a lower intraluminal concentration, whereas simple diffusion occurs at higher or pharmacological concentrations. The absorption of magnesium is also influenced by the amount of water in the intestine (Brady, 1987; Wester, 1987).

The site of magnesium absorption is also not definitive. Magnesium is absorbed throughout the small intestine, but more absorption appears to occur in the jejunum than in the ileum (Shils, 1988). The colon may also play a role in the absorption of magnesium when disease has interfered with magnesium absorption in the small intestine (Wester, 1987).

Many factors, such as magnesium status in the body, the amount of magnesium ingested and the overall composition of the diet as a whole, affect the absorption efficiency of magnesium. Magnesium absorption is thought to be more efficient when magnesium status is poor or marginal and/or when magnesium intake is low. Since vitamin D influences the absorption of calcium, it is suggested that vitamin D may also play a role in magnesium absorption. This postulation is based on the two cations being antagonists of one another in the human but this presumption is still uncertain (Shils, 1988). Certain nutrients of the diet, when ingested in excessive amounts, can have adverse effects on magnesium absorption; excessive amounts of calcium, phosphate and phytate appear to increase magnesium requirements (Wester, 1987).

The first information regarding hormonal control of magnesium transport in the kidney was obtained from several in vivo renal clearance and micropuncture studies. Parathyroid hormone (PTH) was the first hormone tested and shown to reduce magnesium excretion by enhancing reabsorption within the kidney (Harris et al., 1984). Subsequently, calcitonin was observed to reduce magnesium excretion in thyro-parathyro-hyponized rats (Poujel et al., 1980). Moreover, micropuncture studies in hormone deprived rats revealed that both PTH and calcitonin increased magnesium reabsorption in the loop of Henle. This was seen in parallel with the decreased excretion of magnesium in the urine (Elalouf et al., 1984a, 1986b; Bailly et al., 1984).

2.2.2. Calcium absorption

Calcium is the most abundant cation in the human body, averaging approximately 1 kg in a 70 kg adult (Avioli, 1988; Lemann et al., 1979). Bones and teeth which contain

about 99% of the calcium, are primarily dependent upon this mineral for their strength and structure. The other 1% of the body's calcium is distributed between the extracellular fluids and various soft tissues, where it performs a variety of regulatory functions.

As chyme enters the small intestine, it is subject to mechanical peristalsis and enzymatic chemical action. As a result, calcium is released and absorbed across the intestinal epithelium into the lymph and blood plasma. Calcium is ingested in the form of relatively insoluble salts, whether the source is food or dietary supplements. Because the mineral is absorbed only in the ionized form, it must first be released from these salts; therefore, the bioavailability of calcium assumes much importance in calcium nutriture (Sheikh et al., 1987).

Greater calcium absorption does not necessarily mean greater retention. Sometimes, increased absorption of calcium is offset by its increased excretion in the urine and/or digestive juices (Avioli, 1988). Two processes are responsible for the absorption of calcium. The first is a saturable one, operative primarily in the proximal intestine (duodenum and proximal jejunum). It is transcellular, energy requiring and is regulated by vitamin D. The second process is a non-saturable one, where calcium moves down a concentration gradient from the lumen to the body fluids. It varies in intensity throughout the small intestine and is characterized as being paracellular (Bronner , 1986). Three sequential steps are involved in the saturable absorptive process: brush border entry, intracellular movement, and finally, extrusion at the basolateral membrane. Whenever available calcium is insufficient for body needs, absorption by the saturable mechanism accelerates. This acceleration is due to the action of calcitriol. Calcitriol, the form of vitamin D that is biologically active in intestinal transport and calcium resorption

by bone, is produced in response to an increase in parathyroid hormone (PTH) secretion caused by a reduction in plasma levels of ionized calcium. Calcitriol accelerates the rate of absorption of calcium from the gastrointestinal tract and plays a positive role in the action of PTH on bone. PTH acts to conserve body calcium and to increase extracellular fluid calcium concentration by promoting resorption of calcium from the bone, increasing reabsorption of calcium by kidney tubules, and increasing the rate of calcitriol formation in the kidney (Bronner , 1986).

On the contrary, calcitonin suppresses resorption of bone by inhibiting the activity of osteoclasts, a cell type that "digests" the bone matrix and hence, releases calcium into the bloodstream. In addition, calcitonin inhibits tubular reabsorption of magnesium and calcium, leading to increased rates of loss in urine.

In essence, PTH, calcitriol and calcitonin are involved in calcium homeostasis. The amount of calcium absorbed via the nonsaturable, paracellular mechanism is dependent upon an adequate supply of calcium in the intestinal lumen. Increased absorption via this mechanism becomes possible only when there is an increased intake of the mineral. Although absorption of calcium is variable among individuals, the average absorption rate is approximately 30%, with absorption being more efficient in males than females (Avioli, 1988).

2.2.3. Magnesium and calcium interrelationship regulating absorption

An interrelationship exists between magnesium and a number of other nutrients, but the relationship is very complex. One of the most obvious and complex relationships exists between magnesium and calcium (Wester, 1987) and is still incompletely

understood. Magnesium and calcium, both divalent cations, appear to compete with each other for absorption when an excess of either is present in the gut. In addition, magnesium is necessary for the secretion of PTH, hence this ion has an important role in the regulation of serum calcium.

It is thought that alterations of intracellular and/or extracellular magnesium concentrations influence the handling of calcium, thereby affecting cell function (White and Hartzell, 1988). There are several possibilities that may exist affecting the interaction of magnesium on calcium in the cell. Firstly, some suggest that magnesium may bind competitively to the same sites as calcium, either producing the appropriate physiologic response or inhibiting the response (Weaver, 1987). Secondly, magnesium may cause an alteration in calcium distribution by changing flux of calcium across the cell membrane or by replacing calcium on its intracellular binding sites. This will result in a rise in intracellular calcium concentration (White and Hartzell, 1988).

2.3. Effect of phytate on magnesium and calcium absorption

Minerals in food and supplements are subject to a variety of conditions in the gastrointestinal tract that influence and complicate their absorption. The form of the mineral compound, the degree of dietary deficiency, the presence of dietary ingredients that can impede or enhance absorption, and the health of the gastrointestinal tissues all play an important role in determining how much of each ingested mineral will actually be absorbed. The impact of low mineral intake on development and health may be magnified by the presence of food components that interfere with intestinal bioavailabilty of minerals. Some scientists expressed concern about the adequacy of magnesium and

calcium intake from the vegetarian diet due to its relatively high level of whole grains and legumes consumption (Pallauf and Rimbach, 1997). Such foods are the sources of phytate (inositol hexaphosphate or IP6), which may impair the absorption of magnesium and calcium in humans as well as monogastric animals (Erdman, 1979; Pallauf and Rimbach, 1997). Plant fiber contain large amounts of phytate, which is the major storage form of phosphorus in plants. IP6 possesses a high potential for chelating minerals such as Mg^{2+} and Ca^{2+}, therefore, inhibiting their bioavailability. Indeed, a high intake of dietary phytate (15% dry weight) from a diet marginal in magnesium (300 ppm dry weight) resulted in a significant reduction of body weight gain in rats (Rimbach and Pallauf, 1999). Current consumer preferences for plant and plant/meat products could be a nutritional concern since phytate, if ingested in high enough amounts, can potentially bind minerals and prevent their absorption into the body. This is a cause for concern especially when dietary magnesium is at a marginal level.

2.4. Role of magnesium and heart disease

Magnesium depletion may result from decreased intake or absorption, internal redistribution or increased losses of this element. Although dietary factors which play an important role in the occurrence of heart disease have been well documented (Leaf, 1999; Menotti, 1999; Satter, 2000), magnesium deficiency may be a contributing factor that has been overlooked.

There is increasing evidence that magnesium deficiency is a possible contributor to the development of atherosclerosis and cardiovascular damage (Sellig, 1980; Berthelot and Eoprito, 1983; Rayssiguier and Gueux, 1988). Magnesium deficiency induces

alterations in the main metabolic processes of the cells resulting in morphological and functional changes in the arteries (Seelig, 1980). These alterations may cause a predisposition of these tissues to the accumulation of lipids. Fatty deposits build up on the inner artery walls causing blood vessels to narrow, and blood flow to decrease. In turn, thrombosis, coronary disease, and strokes may occur.

2.5. Lipid peroxidation and atherosclerosis

It is evident that lipoprotein peroxidation may be involved in the onset of atherosclerosis (Steinberg et al. 1989). Through in vitro modification by peroxidation, low density lipoprotein (LDL) are taken up by scavenger receptors on the surface of monocytes and macrophages (Heinecke, 1987; Steinbrecher et al., 1990). This uptake may lead to the formation of fatty streaks, the initial step of atherosclerosis (Faggiotto and Ross, 1984). In 1989, both Steinberg and co-workers and Palinsky and co-workers confirmed that oxidized LDL is generated in vivo, and furthermore demonstrated that proteins with malondialdehyde-modified lysine residues were present in atherosclerotic lesions of rabbit aortas. This finding suggested that in vivo peroxidation of LDL may lead to atherosclerotic lesions in the aorta.

Oxygen free radicals (superoxide [O_2^-] and hydroxyl radical [OH•]) and hydrogen peroxide (H_2O_2), called reactive oxygen species (ROS), play a significant role in the antibacterial and antitumorigenic capacity of macrophages and neutrophils, but they are also capable of presenting a toxic action on self tissues causing lipid peroxidation. Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues (Halliwell and

Chirico, 1993). Lipid peroxides are unstable, and decompose to form a complex series of compounds including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malonaldehyde (MDA) and 4-hydroxyalkenals upon decomposition. Measurement of malonaldehyde has been used as an indicator of lipid peroxidation. More recently, there is an increasing recognition that impaired antioxidant status and prevailing levels of lipid peroxidation are associated with the development of atherosclerosis (Prasad and Kalra, 1993; Bonithon-Kopp et al., 1997), hypertension (Papies et al., 1989), and diabetes (Godin et al., 1988; Morel and Chisolm, 1989). Pathological processes have also been associated with oxidative stress and in particular to lipid peroxidation damage (Saccini et al. 1992).

Lipid peroxidation has been implicated in adverse tissue changes in aging as well as in certain diseases (Halliwell and Gutteridge, 1986). Plasma lipid peroxides have also been reported to be elevated in humans with diabetes (Sato et al., 1979; Nishiki et al., 1981). There is also indication that secondary complications of diabetes mellitus are strong indications of increased or uncontrolled oxidative activity (Godin et al., 1988). In addition to this effect, Type II diabetic patients with retinopathy have significantly elevated levels of plasma peroxides relative to patients without evidence of retinopathy (Sato et al., 1979; Uzel et al., 1987). Furthermore, atherosclerotic patients show a positive correlation between elevations in lipid peroxide levels in plasma and in arterial walls (Ledwozwy et al., 1986), both of these paralleling the severity of coronary heart disease.

Lipid peroxides are the products of the chemical damage done by oxygen free radicals to the lipid components of cell membranes. A serum lipid peroxide level,

therefore, measures the overall potential for oxygen free radical pathology, the risk for degenerative processes, and the need for compensatory antioxidant supplementation. High serum lipid peroxide levels indicate excessive oxygen free radical lipid peroxidation.

This fundamental process underlying pathological conditions demonstrates the widespread application of this concept to many different diseases. Chemically, a substance is oxidized when electrons are removed and reduced when electrons are added. All chemical reactions involve the transfer of electrons. The body generates energy by gradually oxidizing its food in a controlled fashion and storing it in the form of chemical potential energy, adenosine tri-phosphate (ATP). This oxidation process removes electrons sequentially in a kind of bucket brigade, passing the electrons to their final recipient, molecular oxygen, forming water and generating ATP. Ironically, this energy generation mechanism that is so essential to life can also set the stage for cell damage. The oxidation of foodstuffs is a controlled reaction that liberates energy but can also release reactive substances, giving rise to potential damage. These substances are free electrons escaping the transport system or electrons freed by lack of chain terminating oxygen (hypoxic conditions). These unpaired electrons readily form free radical molecules that are highly unstable and chemically reactive.

It is these free radical molecules which rapidly react with other molecules, setting off a chain reaction of radical formation similar to an atomic explosion. The unsaturated lipid molecules of cell membranes are particularly susceptible to this damaging reaction process and readily contribute to the uncontrolled chain reaction. However, other biological molecules are also susceptible to damage, including enzymes, DNA and RNA.

Hence, in one process, all levels of cell function may be disrupted. This is why free radical pathology is thought to be such a basic mechanism of tissue injury and end stage pathology. To prevent the free radical chain propagation effect, the body uses antioxidants (chemical electron sinks) that quench the biochemical fire. The antioxidants include enzymes such as glutathione peroxidase, superoxide dismutase and catalase. Vitamins A, C, and E, beta-carotene, and coenzyme Q10 are potent antioxidants which may be their principle role in the body. All these compounds help to control the propagation of free radical pathology in the tissues.

As mentioned previously, increased levels of lipid peroxidation products are associated with oxidative stress. Since clinical studies have shown that oxidative stress of the heart increases the risk of cardiovascular mortality and more importantly, decreases the cardiac adaptability to pathological conditions (Kannel, 1974; Thormann and Schlepper, 1979), lipid peroxidation products were measured in our study to give an indication as to how adapt the Syrian hamsters were at fighting oxidative stress when fed a magnesium deficient diet. A measure of total serum lipid peroxidation has proven to be a simple, inexpensive and accurate means of reflecting whole body free radical activity. This test is presently gaining general acceptance in the research laboratory as a simple, standard means of assessing the body's antioxidant capability or overall oxidative stress.

2.6. Dietary protein sources and plasma lipid response

In 1979 (Sautier et al.) and 1980 (Hugg and Carrol), data was published indicating that rabbits fed soy protein diets excreted more sterols and absorbed less cholesterol than counterparts fed casein diets. It was found that semi-hydrolyzed soy proteins may bind

cholesterol or bile acids within the intestine and thus, directly influence the process of absorption. It was also determined that lipid-binding proteins depend upon protein tertiary and quarternary structure and results could not be reproduced by feeding a diet of free amino acids. This was contradictory to results reported by Kazunari et al. in 1984 when in fact, an amino acid mixture simulating soy protein also had an hypocholesterolemic effect in rats. This 1984 study also concluded that rats fed low fat, cholesterol-free semi purified diets containing soy protein or casein did not exhibit a change in serum cholesterol levels. Jacques et al., 1986 also duplicated findings that rats fed soy and casein protein were observed to have significant changes in serum cholesterol only when diets were cholesterol-enriched.

More recently, hypocholesterolemic action of undigested fractions of soybean protein was shown to occur in rats (Michiro et al., 1990; Sugano et al., 1988). Michiro et al. reported a significant decrease in serum cholesterol of rats fed diets containing high-molecular weight soy protein fractions and cholesterol at 0.5%. It was noted that digested products obtained after pepsin and pancreatic treatments of soybean protein can bind bile acids more effectively than those obtained from other dietary proteins and thereby can decrease plasma cholesterol levels. Sugano et al., in 1988, reported that the high-molecular fractions of digested soybean protein, abundant in hydrophobic amino acids, was found to be substantially hypocholesterolemic when fed at the nitrogen level equivalent to that of 20% soybean protein diets. The researchers postulated this cholesterol-lowering action to be attributed to an increased fecal steroid excretion.

Previous data have indicated the cholesterol lowering effects of undigested fractions of soybean protein in rats and rabbits. In 1995, Wang et al. performed a human

subject study feeding female university students who had relatively high serum cholesterol level for their age. Subjects took 8% of the total energy from casein, soybean protein isolate and high-molecular fractions of soybean protein. Wang and workers reported a decrease in low density lipoprotein cholesterol and an increase in high-density lipoprotein cholesterol in women fed the high-molecular fraction protein group as compared to the casein or soybean protein isolate group. These results confirmed that high-molecular fractions of soybean protein increases fecal steroid excretion and reduces cholesterol levels in human.

2.7. Hamsters as choice of experimental animal

The hamster has become widely studied as a model for cholesterol metabolism. In addition to the similarity in cholesterol synthesis, the Syrian hamster represents a lipoprotein dominant species similar to man (Nistor et al., 1987). Hence, the following experiments were conducted using this animal model. Syrian hamster LDL fractions were separated and peroxidized to analyze for peroxidation by-products. The level of lipid peroxidation products may be indicative of the hamster model's ability to adapt to oxidative stress. It is also noted that hamsters are herbivores (hindgut fermenters have a greater ability to digest phytate) as opposed to rats which are omnivores.

3. Experiment 1 - Golden Syrian Hamster Fed Casein Based Diets

3.1. Materials and Methods

3.1.1. Composition of casein diets

The composition of the four experimental casein diets are presented in Table 1. They consists of 43 % sucrose, 24 % vitamin free casein , 20 % corn starch (0.0 % exogenous phytate diet groups) or 19.5% corn starch (0.5% exogenous phytate diet groups), 5 % corn oil, 3.5 % mineral mixture AIN -76 (magnesium replete group) or 3.5 % modified mineral mixture containing no magnesium (magnesium deplete group), 3 % alphacel, 1 % vitamin mixture AIN-76, 0.5 % sodium phytate (0.5% exogenous phytate diet groups) or 0.0 % sodium phytate (0.0 % exogenous phytate diet groups), 0.3 % L-cystein, and 0.2 % choline bitartrate. All experimental diets were iso-nitrogenous and iso-caloric. The magnesium level varied in diets with the replete magnesium diets [653 $\pm$ 4 ppm (dry matter)], containing more magnesium than the deplete magnesium diets [185 $\pm$ 3 ppm (dry matter)]. Diets were prepared and stored at -18°C in a dark environment.

3.1.2. Experimental animals and housing

Thirty-two male Syrian hamsters (Charles River Breeding Laboratories, Montreal) at twenty-one-day of age weighing 89.1 $\pm$ 1.1g (mean + SEM) were individually housed in wire bottom stainless cages under controlled lighting (12 h light: 12 h dark cycle) and

Table 1. Composition of diets used in Experiment 1.

	Casein Protein Diets			
	Mg Deplete		Mg Replete	
Dietary Constituents[1]	Phy 0	Phy 0.5	Phy 0	Phy 0.5
Casein (vitamin free)	24	24	24	24
Sucrose	43	43	43	43
Corn Starch	20	19.5	20	19.5
Corn Oil	5	5	5	5
Mineral Mixture AIN-76	-	-	3.5	3.5
Modified Mineral Mix (Mg free)	3.5	3.5	-	-
Alphacel	3	3	3	3
Vitamin Mixture AIN-76	1	1	1	1
Sodium Phytate	-	0.5	-	0.5
Cystein	0.3	0.3	0.3	0.3
Choline	0.2	0.2	0.2	0.2
Total Percentage	100	100	100	100
Total Energy[2]	4672	4639	4692	4687
Mg[3]	183[a]	187[a]	657[b]	650[b]
Ca[3]	7758	7413	7413	7948

[a,b]Means within the same row sharing the same postscript are not significantly different ($p < 0.05$).

[1]All constituents are listed as % diet: Phy 0 = 0% phytate added; Phy 0.5 = 0.5% added

[2]cal/g dry matter (bomb calorimetry: calculation performed as dry matter basis)

[3]ppm (dry matter); Magnesium and Calcium content in experimental diets

temperature (23°C) conditions. The animals were randomly divided into eight groups of eight animals (n = 8 hamsters/group) and fed for 4 weeks on the specific experimental diets. All animals were given a 1 week adaptation period to the new environment prior to being introduced to experimental diets. All feed containers and water bottles were washed and rinsed with double-deionized water prior to use. All hamsters had free access to feed and water.

After 4 weeks of the experiment, each animal was transferred into an individual metabolic cage for 4 days prior to sacrificing, whereby body weight, fecal matter, urine, food and water intake were measured. Body weights of animals were measured immediately prior to sacrificing. Halothane was used as a general anesthetic and blood was drawn through cardiac puncture to exsanguinate the animal. Following removal of blood, organs and sections were removed in the following specific order; heart, liver, kidney, spleen, brain, and right femur. All samples were placed immediately on ice and later cryogenically frozen under liquid nitrogen. Samples were stored at -35°C until sample analysis was initiated.

Whole blood, obtained through cardiac puncture from hamsters after 18 hours of fasting, was collected into heparinized ice-cold blood tubes and immediately centrifuged at 1,000 x g for 15 min at 4°C to collect plasma (Kitts et al., 1998). Samples were stored at 4°C under nitrogen in the dark and used within 4 days.

3.1.3. Mineral Analysis

Magnesium and calcium concentrations in feed, plasma, feces, urine, bone, liver, brain, and kidney were determined using a Perkin-Elmer series 400 atomic absorption

spectrophotometer (AAS) (Perkin-Elmer, Norwalk, CT). Plasma and urine magnesium were measured directly after dilution by lanthanum chloride {0.5 g La/100mL (Rock et al., 1995)}. All tissue and bone samples were dry ashed in 2 muffle furnaces; Thermolyne single point furnace and box-type muffle furnace (Bornstead Thermolyne Corporation, Dubuque, USA and Blue M, electric company, Blus Island Illinois, USA). The ash was dissolved in 6 N of hydrochloric acid before analysis of magnesium and calcium content by AAS (Vormann et al., 1995).

3.1.4. Plasma Lipid Analysis

Plasma was isolated by centrifugation at 1000 x g, 10 min, 4°C. Triglycerides (TG), total cholesterol (TC) and high density lipoprotein (HDL) cholesterol were determined enzymatically using standard enzymatic TG and CHOL Kits (Roche Diagnostics, Germany). The HDL cholesterol was determined after ultracentrifugation of the sodium density gradient containing plasma. The low density lipoprotein (LDL) fraction was isolated according to the method given below.

3.1.5. LDL isolation

Lipoprotein fractions were isolated by ultracentrifugation in a Beckman Model L2-65B ultracentrifugation (Beckman Instrument, Palo Alto, CA, USA) using a SW-41Ti swinging bucket rotor (Beckman Instrument, Palo Alto, CA, USA). Potassium bromide KBr (0.770 g) and sucrose (0.050 g) were placed in a SW 41 cellulose-nitrate centrifuge tube. Subsequently, 1 ml of serum and 1 ml of phosphate buffer of pH=7.4 were pipetted into the tube and finally 0.2 ml of Sudan black solution was added to prestain the serum.

The components were carefully mixed with a spatula. The prestained serum ($\rho 20\,^{\circ}C$ = 1.25 g/ml) was overlayered sequentially with 2 ml of a salt solution to yield a $\rho 20\,^{\circ}C$ = 1.225 g/ml (11.42×10^{-3} g NaCl and 315.54×10^{-3} g KBr/ml). This was overlayered with 4 ml of a salt solution of $\rho 20\,^{\circ}C$ = 1.10 g/ml (11.42×10^{-3} g NaCl and 133.48×10^{-3} g KBr/ml), and 4 ml of distilled deionized water (Terpstra et al., 1981). Only 1 sample was used in conjunction with the Sudan black solution for verification of protein bands. All solutions contained 10^{-4} g/ml ethylenediaminetetraacetic acid (EDTA) (disodium salt) (Terpstra et al., 1981; Kitts et al., 1998). The tubes were centrifuged for 23 hours (including acceleration time and 0.5 hours deceleration time) at 270,000 x g , 200 microns of vacuum, and at 4°C (Terpstra et al., 1981). A low deceleration rate without braking was used. During centrifugation, the salt solution layers diffused and a continuous gradient was formed. By visual comparison with the Sudan black standard sample and measuring the weight of each 1 ml aliquot from ultracentrifuge tubes, a gradient curve was developed to identify different lipoprotein classes (VLDL, $\rho 20 < 1.01$ g/ml; LDL, $1.02 < \rho 20 < 1.06$ g/ml; HDL, $1.09 < \rho 20 < 1.21$ g/ml) according to methods of Kitts et al. (1998). These density ranges were pooled (Figure 1) and analyzed for protein content (Appendix I) using the BioRad protein assay (BioRad Lab., Melville, NY) with bovine serum albumin as a standard.

3.1.6. Lipoprotein peroxidation

Samples which fell within literature ranges for LDL densities were pooled for each animal and dialyzed with a dialysis tubing cut-off molecular weight of 12,000-14,000 (Spectrum Medical Industries, INC., Los Angeles) against a 200-fold volume of

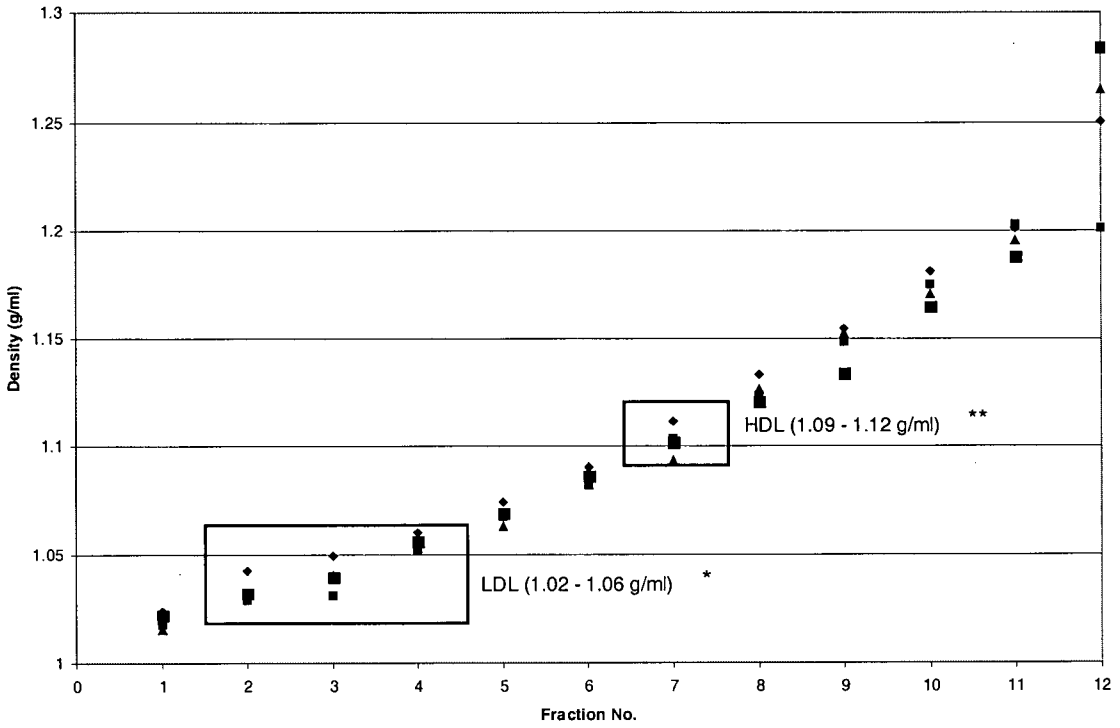

Figure 1. Density fractions of plasma after 24 hours of ultracentrifugation.

◆ CMLPO = casein magnesium deplete 0% dietary phytate added; ■ CMHPO = casein magnesium replete 0% dietary phytate added; ▲ CMLP5 = casein magnesium deplete 0.5% dietary phytate added; ● CMHP5 = casein magnesium replete 0.5% dietary phytate added. *Fractions 2, 3 and 4 are pooled for LDL peroxidation. **Fraction 7 of each group was used for HDL cholesterol analysis.

phosphate-buffered saline (PBS 0.01 M phosphate, 0.15 M NaCl, pH 7.4). This procedure was conducted at 4°C for 24 hours under nitrogen bubbling; the dialysis buffer was changed twice every 8 hours (Scaccini et al., 1992). Samples were stored under nitrogen in the dark at 4°C and used within 2 weeks. Suitable volumes from the dialyzed solutions were diluted with PBS to obtain a final lipoprotein concentration of 0.02 mg/ml. Plasma and lipoprotein fractions were assayed for protein content using the micro-Biorad protein assay using bovine serum albumin fraction (V) cold, precipitated, (FisherBiotech, Fisher Scientific, Fair Lawn) as a standard. Fractions were pooled according to their respective densities.

Samples were separated into 10 fractionated samples of 1.5 ml each and placed in separate 10 ml storage tubes. Lipoprotein oxidation was initiated using 50 μM copper sulfate added to LDL aliquots and incubated in a water bath at 37°C under gentle agitation. At prefixed intervals of time, (every 20 minutes over the first 60 minutes and every 30 minutes over the next 120 minutes and every 60 minutes over the next 120 minutes for a total of 300 minutes), samples were quenched with a final concentration of 1.5 mg/ml EDTA (Cominacini et al., 1991) and relative fluorescence values were measured.

Fluorescence measurements of quenched samples were taken after diluting samples 3 times with PBS buffer. The emission was measured at 430nm with excitation at 360nm using a Shimadzu spectrofluorimeter RF-540 (Shimadzu Corporation, spectrophotometric instruments plant, analytical instruments division, Kyoto, Japan). The excitation and emission slit widths were both maintained at 5nm. The fluorescence

was corrected daily for intensity of the fluorescent bulb using a standard solution of cyclohexane (Cominacini et al., 1991).

3.1.7. Liver tissue antioxidant analyses

Liver tissue preparation

Liver tissue used for biochemical analyses was collected in chilled homogenizing buffer (50mM Tris, 0.1 mM EDTA, pH 7.6). A portion of the hepatic tissue was blotted dry, weighed, and prepared as a 10% homogenate in fresh, chilled homogenizing buffer, using a Polytron homogenizer (Brinkmann Instruments Inc., Westbury, N.Y.) at 55% Maximum speed (850 rpm), for 30 seconds (2 x 15 s) (Kitts et al., 1998).

Susceptibility to in vitro forced peroxidation

The production of 2-thiobarbituric acid reactive substances (TBARS) in liver tissue homogenates was measured following incubation with an equal volume of eight different concentrations of hydrogen peroxide H_2O_2 (0-40mM in 0.9% NaCl containing 2 mM sodium azide (NaN_3) for 30 minutes at 37°C. The reaction was terminated with the addition of 100 µL cold 25% trichloroacetic acid (TCA) containing 0.1 M sodium arsenite, followed by centrifugation at 12,000 x g at 4°C for 5 min. An aliquot of the supernatant was mixed with 0.5% 2-thiobarbituric acid (TBA; Sigma, St. Louis, Mo.) and boiled for 15 minutes, and absorbance was measured at 532 nm (Kitts et al., 1998).

3.1.8. Statistics

The differences among dietary magnesium and calcium levels were analyzed using the one-way analysis of variance. Differences in plasma lipid analysis as well as magnesium and calcium concentrations in all organs were analyzed using the two-way analysis of variance (General Linear Model). Significant differences among treatments were identified by the Tukey's test ($P<0.05$) (Minitab for Windows Release 12.1).

3.2. RESULTS: Experiment 1 - Golden Syrian Hamster Fed Casein Based Diets

3.2.1. Assessment of animal growth performance

Hamsters fed the magnesium replete diets on average gained 43% more weight than animals fed the magnesium deplete diets (Table 2). Although a similar trend indicated that phytate supplemented groups showed less overall weight gain than their counterparts, there was no significant difference in body weight gain between phytate and non-phytate supplemented hamsters (Table 2). In addition, the feed efficiency ratio (FER; g of body weight gain / g of food consumption) showed greater ($P<0.05$) ratios for hamsters fed the magnesium replete diets. There was no effect of dietary phytate on animal growth performance in casein fed hamsters. These results indicate that a magnesium restricting diet had a more profound effect on FER than the presence of phytic acid.

Table 2. Body weight of casein fed hamsters during a 4 week study [1]

	Casein protein diets								
	Mg Deplete				Mg Replete				Significant
	Phy 0		Phy 0.5		Phy 0		Phy 0.5		treatment
									effects[2]
Initial Body weight (g)	88.7	± 0.9	89.3	± 1.7	89.6	± 1.6	88.7	± 0.9	---
Final Body Weight (g)	103.8	± 6.0[a]	104.7	± 5.4[a]	128.2	± 4.5[b]	121.1	± 4.6[b]	M
Weight Gained (g)	21.0	± 6.4[a]	18.2	± 6.1[a]	37.2	± 5.4[b]	31.6	± 4.1[b]	M
Dry matter intake[3]	155.8	± 3.7[a]	161.2	± 5.3[a]	186.7	± 2.9[b]	190.9	± 4.5[b]	M
FER[4]	0.128	± 0.004[a]	0.118	± 0.003[a]	0.210	± 0.004[b]	0.201	± 0.004[b]	M

[1]Data are expressed as mean ± SEM

[2]Significant ($p < 0.05$) treatment levels and interactions, where M is magnesium levels (replete vs. deplete in diet), P is phytate levels (0% added vs. 0.5% added in diet)

[3]Cumulative feed intake for 4 weeks

[4]Feed efficiency ratio (gram body weight gain / gram consumed)

[a,b]Means within the same row that do not share the same postscripts are significantly different ($p < 0.05$)

Table 3. Magnesium and calcium balance and apparent absorption in casein fed hamsters [1]

	Casein Protein Diets				Significant
	Mg Deplete		Mg Replete		treatment
	Phy 0	Phy 0.5	Phy 0	Phy 0.5	effects[4]
Mg					
Intake (mg/d)	1.1 ± 0.1[a]	1.1 ± 0.1[a]	4.5 ± 0.7[b]	4.0 ± 0.3[b]	M
Urinary (mg/d)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	---
Fecal (mg/d)	0.2 ± 0.1[a]	0.2 ± 0.1[a]	0.7 ± 0.1[b]	0.7 ± 0.1[b]	M
Balance[2]	0.9 ± 0.1[a]	0.9 ± 0.2[a]	3.5 ± 0.7[b]	3.4 ± 0.3[b]	M
Apparent Absorption[3]	85.4 ± 2.8	82.2 ± 4.2	81.7 ± 2.3	86.4 ± 2.1	---
Ca					
Intake (mg/d)	44.1 ± 6.0[a]	47.8 ± 4.5[a]	68.4 ± 6.7[b]	71.2 ± 5.5[b]	M
Urinary (mg/d)	0.4 ± 0.2	0.4 ± 0.1	0.6 ± 0.6	0.5 ± 0.2	---
Fecal (mg/d)	31.9 ± 3.8	33.9 ± 3.9	28.6 ± 4.5	32.0 ± 4.7	---
Balance[2]	10.1 ± 4.1[a]	9.5 ± 3.5[a]	38.3 ± 8.1[a]	37.4 ± 6.8[a]	M
Apparent Absorption[3]	26.3 ± 5.1[a]	19.8 ± 4.2[a]	57.2 ± 6.5[b]	55.3 ± 5.9[b]	M

[1]Data are expressed as mean ± SEM

[2]Balance (mg/d) = Intake - (Fecal + Urinary)

[3]Apparent Absorption (%) = [(Intake - Fecal) / Intake] x 100

[4]Significant ($p < 0.05$) treatment levels, where M is magnesium levels (replete vs. deplete in diet), P is phytate levels (0% added vs. 0.5% added in diet)

[a,b]Means within the same row that do not share the same postscripts are significantly ($p < 0.05$).

3.2.2. Assessment of magnesium and calcium balance and apparent absorption in casein fed hamsters

Magnesium intake and fecal excretion of magnesium was related to the level of magnesium in the diet ($P<0.05$). Hence, magnesium balance was thus influenced by the dietary magnesium intake level ($P<0.05$). Dietary magnesium levels did not affect the apparent absorption of magnesium. However, dietary magnesium levels significantly affected calcium balance as well as calcium apparent absorption. This result indicated that the levels of magnesium fed was enhancing overall calcium apparent absorption (Table 3). Magnesium balance was associated with intake levels ($P<0.05$) as indicated by higher magnesium intakes yielding a greater magnesium balance. There was no effect of dietary phytate on apparent absorption or balance of magnesium or calcium in casein fed hamsters.

3.2.3. Effect of dietary magnesium on distribution status of plasma and femur magnesium and calcium in casein fed hamsters

This study indicated a positive relationship between dietary magnesium intake and plasma and femur magnesium status (Table 4). Animals fed magnesium deplete diets corresponded to significant lower plasma magnesium concentration (~50% less) than their counterparts ($P<0.05$). Similarly, animals fed magnesium deplete diets also has a significantly diminished femur magnesium concentration (~35% less), compared to counterparts fed a magnesium replete diet ($P<0.05$).

Table 4. Plasma and femur magnesium and calcium content in casein fed hamsters [1]

	Casein Protein Diets				Significant
	Mg Deplete		Mg Replete		treatment
Minerals	Phy 0	Phy 0.5	Phy 0	Phy 0.5	effects[2]
Mg					
Femur (ug/bone)	471.2 ± 41.2[a]	378.4 ± 52.3[a]	694.3 ± 72.6[b]	659.7 ± 91.1[b]	M
Plasma (mg/L)	33.3 ± 4.4[a]	36.8 ± 5.0[a]	72.8 ± 6.4[b]	64.7 ± 1.7[b]	M
Ca					
Femur (mg/bone)	12.9 ± 2.2	12.7 ± 2.3	13.2 ± 4.2	12.5 ± 6.2	---
Plasma (mg/L)	3.3 ± 0.3	2.9 ± 0.3	3.1 ± 0.3	2.9 ± 0.2	---

[1]Data are expressed as mean ± SEM

[2]Significant ($p < 0.05$) treatment levels and interactions, where M is magnesium levels (replete vs. deplete in diet), P is phytate levels (0% added vs. 0.5% added in diet)

[a,b]Means within the same row that do not share same postscript are significantly different ($p < 0.05$)

Table 5. Tissue magnesium and calcium status in casein fed hamsters [1]

	Casein Protein Diets				Significant
	Mg Deplete		Mg Replete		treatment
Tissue Mineral	Phy 0	Phy 0.5	Phy 0	Phy 0.5	effects[2]
Mg[3]					
Heart	1197.7 ± 39.5^{ab}	1143.8 ± 15.3^{a}	1240.1 ± 20.9^{ab}	1268.3 ± 16.5^{b}	M
Liver	658.3 ± 79.5^{a}	714.2 ± 55.8^{a}	852.1 ± 22.1^{b}	674.7 ± 46.5^{a}	M, P, M x P
Kidney	915.5 ± 45.9	997.9 ± 25.5	925.1 ± 59.4	949.4 ± 21.1	---
Brain	833.0 ± 24.6	868.3 ± 27.1	862.9 ± 16.9	834.5 ± 45.9	---
Ca[3]					
Heart	449.1 ± 15.2^{a}	869.6 ± 55.4^{b}	396.8 ± 12.4^{a}	887.1 ± 203.3^{b}	P
Liver	208.2 ± 17.7	234.5 ± 39.7	291.9 ± 24.8	320.6 ± 66.6	---
Kidney	949.3 ± 45.9^{b}	968.1 ± 72.9^{b}	751.9 ± 30.4^{a}	786.5 ± 22.9^{a}	M
Brain	450.3 ± 31.1	489.8 ± 26.3	498.4 ± 63.4	481.3 ± 42.1	---

[1]Data are expressed as mean ± SEM

[2]Significant ($p < 0.05$) treatment levels and interactions, where M is magnesium levels (replete vs. deplete in diet), P is phytate levels (0% added vs. 0.5% added in diet)

[3]Data are expressed as ppm (dry matter)

[a,b]Means within the same row that do not share the same postscripts are significantly ($p < 0.05$).

Dietary magnesium intake did not have an effect on hamster calcium status, as indexed by both the plasma and femur calcium content (Table 4). Furthermore, there was no effect of dietary phytate on femur and plasma magnesium or calcium in the casein fed hamsters.

3.2.4. Dietary magnesium influence on distribution status of tissue magnesium and calcium in casein fed hamsters

Whole animal magnesium balance was found to influence both the heart and liver magnesium status, while only the apparent absorption of magnesium parameter was shown to influence liver magnesium status ($P<0.05$) in casein fed hamsters. This finding indicated that an interaction between dietary magnesium and dietary phytate existed in regard to the uptake of magnesium in liver tissue ($P<0.05$). The kidney and brain magnesium contents were not affected by either magnesium balance or magnesium apparent absorption (Table 5). These results indicated that magnesium status in internal organ systems, is organ specific and does not always reflect a relatively short term assessment of balance or apparent absorption. Magnesium balance on the other hand, was a factor on the kidney calcium status ($P<0.05$). Animals fed the magnesium replete diets showed a decreased kidney calcium pool (~20% less) when compared to counterparts fed the magnesium deplete diets (Table 5). This finding indicated perhaps a competitive retention of magnesium and calcium occurs in the kidney of hamsters. Animals fed the phytate supplemented diets actually had increased amounts of calcium in heart tissue ($P<0.05$). Hamster liver and brain calcium status was not affected by dietary magnesium intake or presence of dietary phytate (Table 5).

3.2.5. Assessment of plasma lipids in casein fed hamsters

Plasma lipids after 4 weeks of feeding the experimental diets are shown in Table 6. Tri-acylglycerol (TG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-chol) levels were measured. There were no significant dietary effects on any of the measured plasma lipid parameters or the fractionized HDL-chol content.

3.2.6. Assessment of LDL peroxidation in casein fed hamsters

LDL peroxidation from casein fed hamsters was determined using relative fluorescence. The relative fluorescence curve of LDL peroxidation showed a typical 3-phase oxidation curve: initiation, propagation and termination (Figure 2). In this study dietary magnesium intake was shown to have an effect on the susceptibility of LDL to peroxidation. Initial relative fluorescence values (Table 7) of LDL peroxidation from animals fed the magnesium-depleted diets were greater than the animals fed magnesium-replete diets ($P<0.05$). This finding indicated a decreased ability of magnesium deplete animals to protect LDL from initial peroxidation reactions. In addition, animals fed the magnesium replete diets displayed a longer initiation phase, as shown by a longer lag time prior to LDL peroxidation (Table 7). Lag time was determined using a 3-point (during propagation phase) linear regression intersecting the initial relative fluorescence value (Figure 3). This in-vitro finding verifies that magnesium replete diets increased the capacity of hamsters with positive magnesium status to cope with lipid peroxidation, thus, delaying the onset of the propagation phase. At 60 minutes exposure to H_2O_2, representing when both magnesium replete and deplete animal LDL fractions under-went

Table 6. Plasma lipids in casein fed hamsters [1]

	Casein protein diets				Significant
	Mg Deplete		Mg Replete		treatment
Plasma lipids	Phy 0	Phy 0.5	Phy 0	Phy 0.5	effects
Tri-acylglycerol (mg / dl)	68.8 ± 4.7	75.5 ± 9.2	66.3 ± 5.8	73.4 ± 8.9	---
Total Cholesterol (mg / dl)	66.0 ± 4.6	75.6 ± 6.9	68.9 ± 5.3	76.1 ± 7.0	---
HDL Cholesterol (mg / dl)	12.1 ± 2.2	12.7 ± 1.7	13.8 ± 1.9	13.0 ± 0.7	---

[1]Data are expressed as mean ± SEM; (n=32)

Means within the same row are not significantly different ($p < 0.05$)

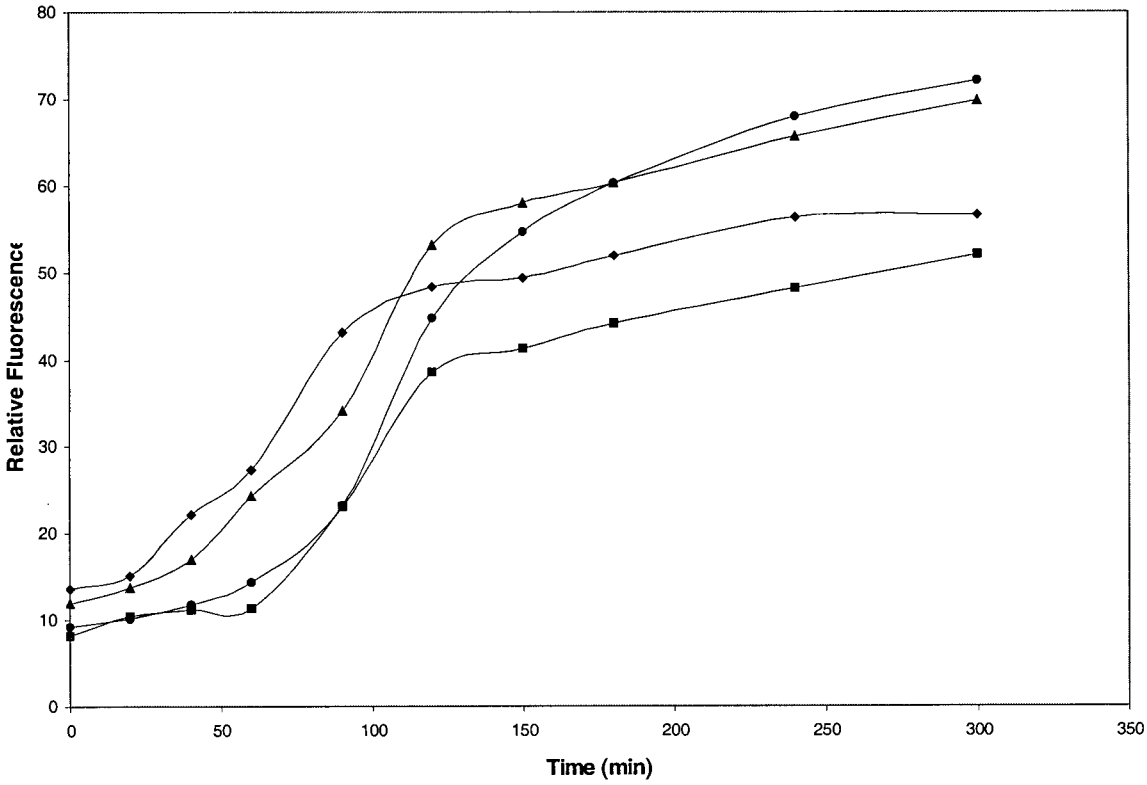

Figure 2. Relative fluorescence curves of time dependant, LDL forced peroxidation in casein fed hamsters. [1]

Lipoprotein oxidation was initiated using 50 μM copper sulfate added to LDL aliquots and incubated in a water bath at 37°C under gentle agitation. At prefixed intervals of time, samples were quenched with a final concentration of 1.5 mg/ml EDTA. ◆ CMLPO = casein magnesium deplete 0% dietary phytate added; ■ CMHPO = casein magnesium replete 0% dietary phytate added; ▲ CMLP5 = casein magnesium deplete 0.5% dietary phytate added; ● CMHP5 = casein magnesium replete 0.5% dietary phytate added.
[1]Values represent means of 8 samples without SEM.

the propagation phase, a significantly lower fluorescence value was observed for LDL from animal fed magnesium replete diets than their counter parts ($P<0.05$). Dietary phytate did not induce a significant effect on hamster in vitro LDL susceptibility to H_2O_2-induced peroxidation.

3.2.7. Assessment of TBARS formation in liver tissue of casein fed hamsters

Forced peroxidation of fresh liver tissue from casein fed hamsters was characterized by sloping curves of TBARS formation as shown in Figure 4. The data indicated that the capacity of liver tissue to resist forced peroxidation was not exclusively determined by the magnesium status. In the absence of dietary phytate, animals fed replete and deplete magnesium diets exhibited a similar capacity to cope with increasing levels of hydrogen peroxide induced oxidation in liver tissue. Conversely, phytate supplemented groups were more susceptible to ex-vivo forced peroxidation of liver tissue. For example, at 0mM of hydrogen peroxide, there was no significant difference in absorbance at 532nm among all different treatment groups. At 40 mM of hydrogen peroxide however, animals fed the non-phytate supplemented diets exhibited reduced liver peroxidation (~65%) than phytate supplemented animals ($P<0.05$). When dietary phytate was absent in the diet, there was no significant difference in hydrogen peroxide induced liver peroxidation at 40mM, between animals fed magnesium-repleted and depleted diets (Figure 5). However, when dietary phytate was introduced, a significantly higher absorbance value (at 40mM of H_2O_2) was observed in animals fed the magnesium-depleted diets ($P<0.05$), when compared to counterparts fed the magnesium-repleted

Table 7. Lag time and relative fluorescence comparisons for LDL peroxidation in casein fed hamsters [1]

	Casein protein diets				Significant
	Mg Deplete		Mg Replete		treatment
	Phy 0	Phy 0.5	Phy 0	Phy 0.5	effects[2]
RF - 0	13.6 ± 1.6[b]	11.9 ± 2.1[b]	8.3 ± 1.4[a]	9.3 ± 1.8[a]	M
RF - 60	27.2 ± 2.7[a]	24.2 ± 1.8[a]	11.3 ± 4.2[b]	14.4 ± 2.2[b]	M
Lag Time (min)	23.2 ± 1.9[a]	24.9 ± 3.2[a]	50.4 ± 4.5[b]	54.1 ± 6.1[b]	M

[1]Data are expressed as mean ± SEM; RF = relative fluorescence measurement; (0,60 = time in min)

[2]Significant ($p < 0.05$) treatment levels and interactions, where M is magnesium levels (replete vs. deplete in diet), P is phytate levels (0% added vs. 0.5% added in diet)

[a,b]Means within the same row that do not share same postscript are significantly different ($p < 0.05$)

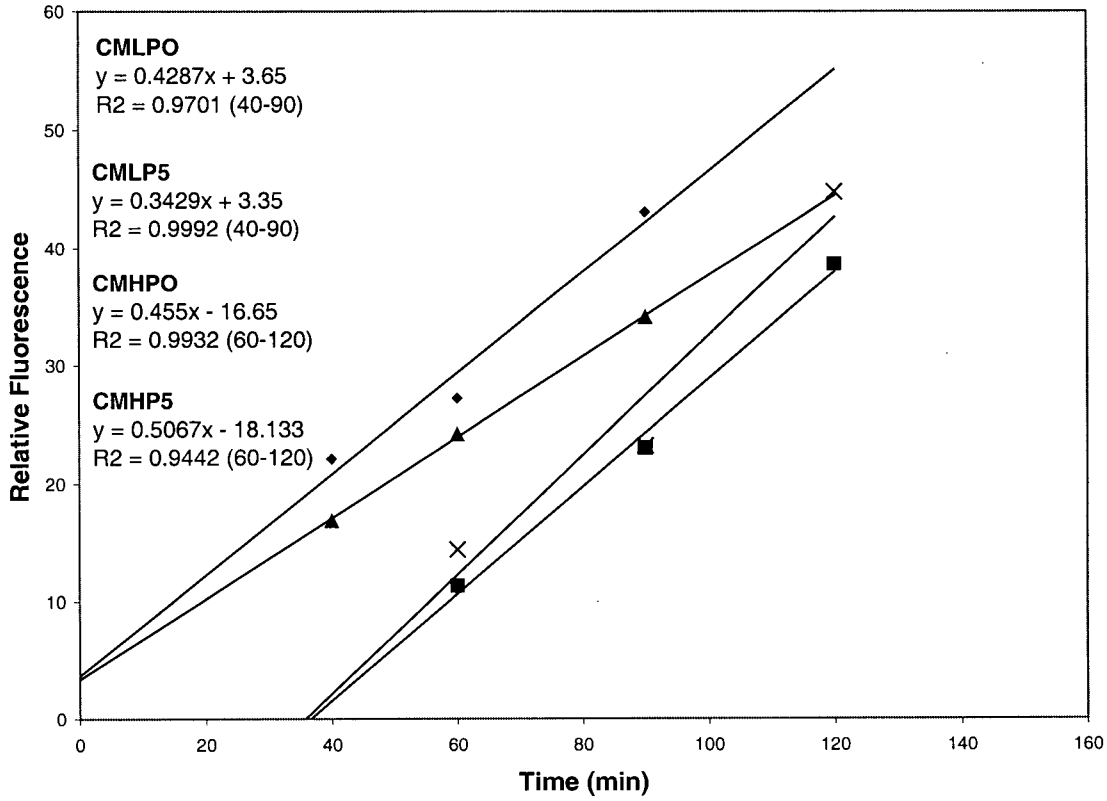

Figure 3. Extrapolation of propagation curves to determine the lag point of time dependant, LDL forced peroxidation in casein fed hamsters. [1]

A 3-point linear regression intersecting initial relative fluorescence value was used to determine lag time in minutes. ◆ CMLPO = casein magnesium deplete 0% dietary phytate added; ■ CMHPO = casein magnesium replete 0% dietary phytate added; ▲ CMLP5 = casein magnesium deplete 0.5% dietary phytate added; × CMHP5 = casein magnesium replete 0.5% dietary phytate added. [1]Values represent means of 8 samples without SEM.

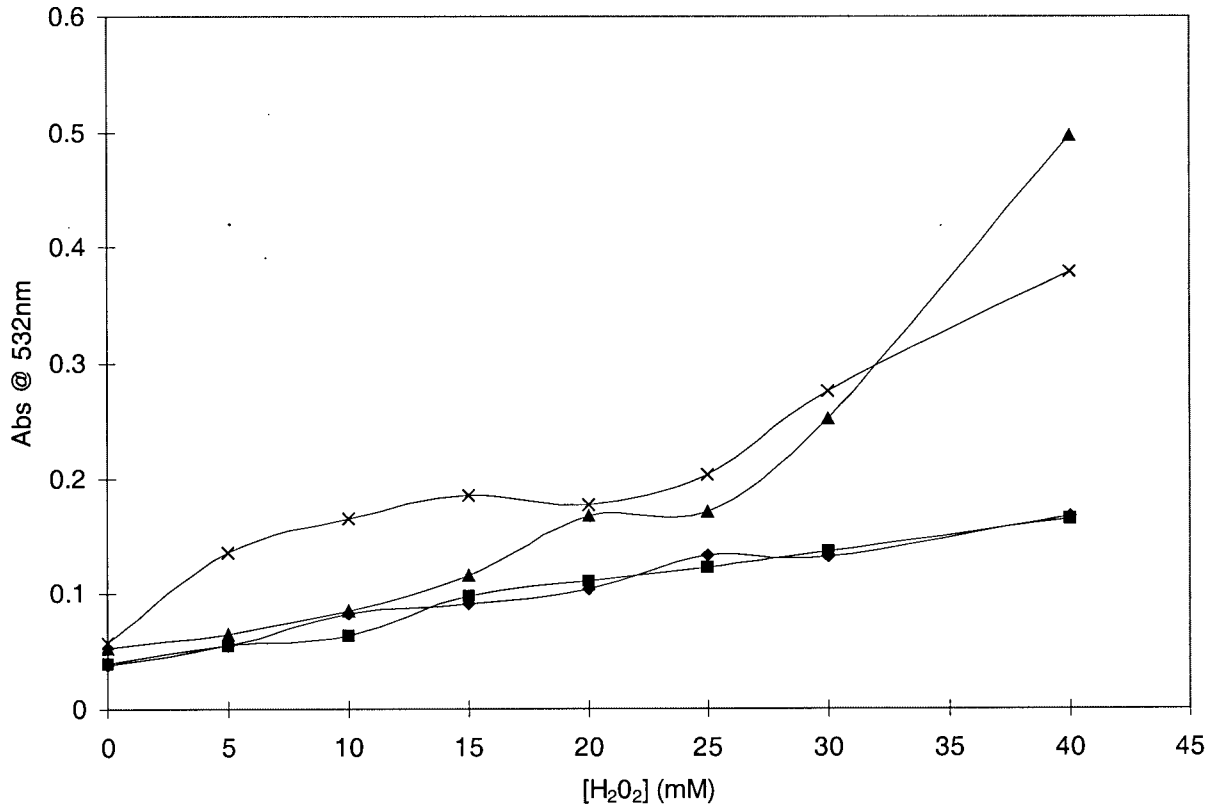

Figure 4. TBARS formation during forced peroxidation of fresh hamster liver from casein fed hamsters. [1]

◆ CMLPO = casein magnesium deplete 0% dietary phytate added; ■ CMHPO = casein magnesium replete 0% dietary phytate added; ▲ CMLP5 = casein magnesium deplete 0.5% dietary phytate added; × CMHP5 = casein magnesium replete 0.5% dietary phytate added. [1]Values represent means of 8 samples without SEM.

diets. Dietary phytate supplementation resulted in an increased absorbance (532nm) value at 40 mM of hydrogen peroxidation of both magnesium deplete and replete diet groups during hydrogen peroxidation (Figure 5). It was also noted that animals fed the magnesium deplete diets with phytate supplemented had the lowest overall ability to cope with forced peroxidation (Figure 5). This result indicated that dietary phytate along with dietary magnesium levels directly affected the ability of liver tissue from hamsters to cope with hydrogen peroxide induced forced peroxidation.

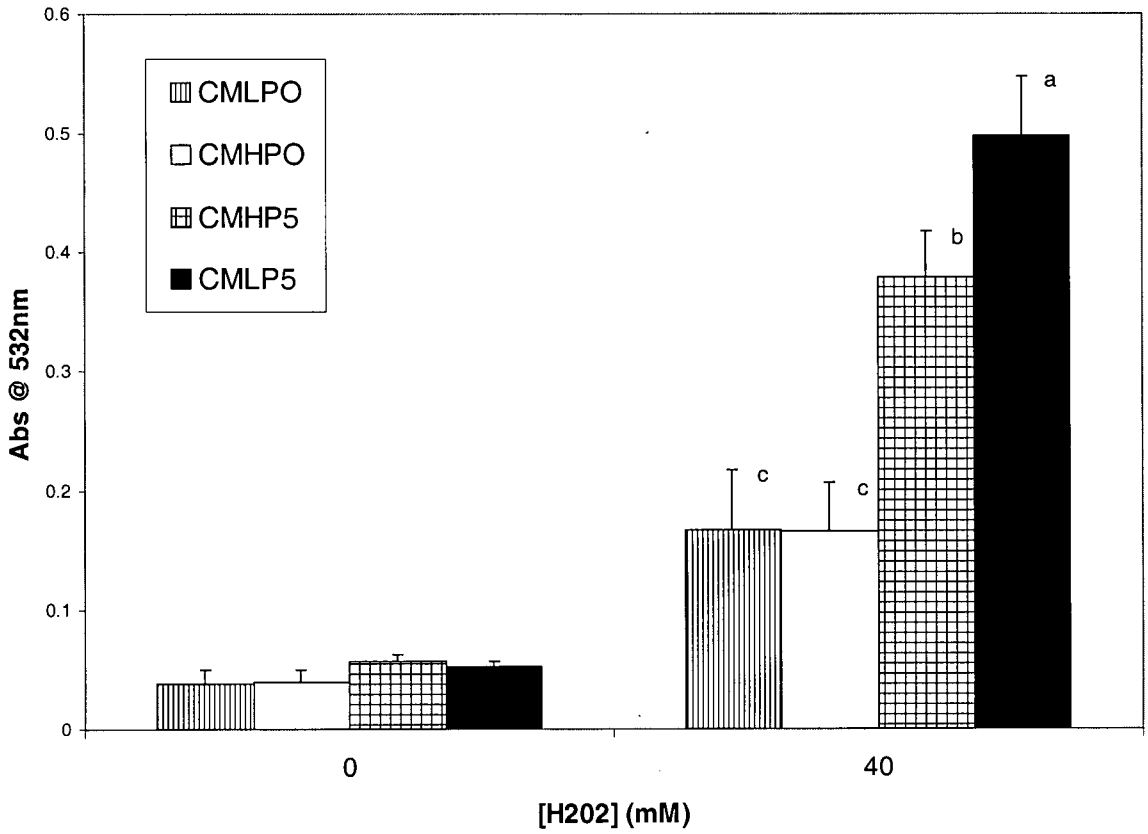

Figure 5. TBARS formation of forced peroxidation fresh hamster liver from casein fed hamsters at 0 and 40 mM of H_2O_2.

CMLPO = casein magnesium deplete 0% dietary phytate added; CMHPO = casein magnesium replete 0% dietary phytate added; CMLP5 = casein magnesium deplete 0.5% dietary phytate added; CMHP5 = casein magnesium replete 0.5% dietary phytate added. [a,b,c] Means with different superscript letters were significantly different ($P<0.05$).

3.3. Discussion: Experiment 1 - Golden Syrian Hamster Fed Casein based Diets

3.3.1. Effect of dietary magnesium and supplemented dietary phytate on magnesium and calcium status

In Experiment 1, magnesium and calcium status of casein fed hamsters was determined by assessing mineral concentrations in the femur, plasma, heart, liver, kidney and brain. Magnesium and calcium balance and bioavailabilty measures were determined from fecal matter and urinary output.

Animals fed the magnesium-depleted casein based diets [185 $\pm$ 3 ppm (dry matter)] had a lower magnesium concentration in femur, heart and plasma,. These findings are consistent with previously reported data. For example, Rimbach and Pallauf (1999) showed that as dietary magnesium was limiting, plasma and femur magnesium of rats were lower than animals fed non-limiting magnesium diets. Under the conditions investigated, magnesium-depleted diets was accompanied by a decline in liver magnesium (~10% less), as indicated by results of Gunther (1981). In the present study with animals fed casein based diets, apparent absorption of magnesium was not affected whereas apparent absorption of calcium was affected by magnesium-depleted diets. This was contradictory to previously reported data by Rimbach and Pallauf (1999) who utilized rats in their study, in contrast to the use of hamsters in the present study. It was noted that perhaps hamsters and rats have sufficiently different digestive systems and hence could also have different apparent absorption systems. As shown by Chiou et al. (1998), the omnivorous rat digested crude fiber significantly poorer than the herbivore

hamster. On the other hand, magnesium-depleted diets had no significant effect on femur and plasma calcium. This was consistent with data reported by Rimbach and Pallauf (1999).

Supplementing dietary phytate at a 0.5% wet weight concentration was shown to have no significant effect on the overall magnesium or calcium status of growing hamsters. This result was contradictory to previously reports by Rimbach and Pallauf (1999) using a much higher dietary phytate level (~10%). In addition, these previous workers used rats as the experimental animal. Furthermore, other researchers (Williams and Taylor 1985) have also shown that hamsters possesses a greater (~30% more) overall phytate digestibility than rats. These findings may explain why dietary-supplemented phytate had no significant effect on hamster calcium status in the present study.

3.3.2. Effect of dietary magnesium and supplemented dietary phytate on plasma lipids

In this study, plasma lipids such as tri-acylglycerol, total cholesterol and HDL cholesterol were measured as possible indirect indicators of magnesium deficiency. Our results clearly indicated that animals fed magnesium-depleted diets showed an increase in lipid peroxidation; however, plasma lipids were not significantly different among the animals. Previously reported data has indicated that greater levels of lipid peroxides in-fact occur in magnesium deficient animals (Mahfouz and Kummerow, 1989); however, the lipid components where change occured were primarily in the lower density lipoproteins (LDL) such as the very low density lipoprotein (VLDL) and low density lipoprotein (Rayssiguier et al. 1993). Since these parameters were not measured in this

study, it can only be concluded that no significant difference in total plasma lipid content and HDL cholesterol resulted from animals fed magnesium-depleted or repleted diets. A similar result has been reported by Terpstra et al. (1981) where rabbits fed a high casein diet (~40%) resulted in a higher serum cholesterol level as compared to rabbits fed a low casein diet (~10%). The rise in serum cholesterol was mainly reflected in the LDL and VLDL fractions, whereas HDL fractions were relatively unchanged as compared to lower density lipoproteins. Kazunari et al. in 1984 also concluded that rats fed casein based diets did not exhibit a change in serum cholesterol levels. Further duplicated by Jacques et al. in 1986, it was found that rats fed casein protein were observed to have no significant changes in serum cholesterol when diets were not cholesterol-enriched. This study, using 24% casein in diets, showed that total plasma content and HDL cholesterol of animals fed magnesium-depleted and repleted diets were not significantly different.

3.3.3. Effect of dietary magnesium and supplemented dietary phytate on LDL and liver peroxidation

Animals fed magnesium-depleted diets showed a decreased ability to cope with ex-vivo forced peroxidation of LDL. This finding was consistent with previous data reported by Rayssiguier et al. (1993), where rats fed a depleted magnesium diet yielded higher peroxidation values during incubated LDL peroxidation. This current study also concluded that rats fed magnesium-depleted diets was less likely to cope with liver peroxidation. Agreeing with this study, Gunther et al. (1995) and Rayssiguier et al. (1993) also reported that lipid peroxidation in rat hepatocytes was greater in animals fed magnesium depleted diets. These results demonstrated that depleted magnesium diets

reduced magnesium status or balance and hence, was associated with an increase in susceptibility towards lipoprotein and liver tissue lipid peroxidation.

During oxidation of LDL, lipids are converted to lipid peroxides and unsaturated aldehydes (Steinber et al., 1989). These aldehydes react with apoprotein B (apoB) causing a process which generates a fluorescent product with a strong emission maximum at 430nm when excitation is performed at 360nm (Esterbauer et al., 1987). Since LDL from animals fed magnesium deplete diets had a decreased ability to cope with forced peroxidation as compared to their counterparts, it can be said that magnesium has an indirect effect on unsaturated aldehydes reacting with apoB, and thus also an indirect effect on LDL peroxidation of magnesium deplete casein-fed hamsters.

Supplemented dietary phytate also played a role in liver peroxidation in the hamsters. Phytate is a known sequestering agent to the metal-ions such as iron, copper (Lopez et. Al, 1998) and zinc (Zhou et al., 1992) and should reduce magnesium bioavailability. This study showed that animals fed phytate supplemented diets exhibited greater tendency for forced peroxidation in liver tissues as compared to their counterparts. A similar result has been reported by Rimbach and Pallauf (1999) where growing rats fed a high phytate diet showed significant increased hepatic susceptibility to forced peroxidation. A possible explanation for this increase in liver peroxidation is that phytate has been shown to reduce casein digestibility (Lathia et al., 1987). Casein digestibility was significantly reduced when phytate was present during in vitro digestion. Decreased digestion of casein protein can lead to less bioavailable dipeptides and amino acids for absorption. This in turn can have an indirect effect on the liver's ability to cope with forced peroxidation.

4. Experiment 2: Golden Syrian Hamster Fed Soy Based Diets

4.1. Materials and Methods

4.1.1. Composition of soy diets

The composition of the four experimental soy diets are presented in Table 8. They consists of 43 % sucrose, 24 % soybean protein isolate, 20 % corn starch (0.0 % exogenous phytate diet groups) or 19.5% corn starch (0.5% exogenous phytate diet groups), 5 % corn oil, 3.5 % mineral mixture AIN -76 (magnesium replete group) or 3.5 % modified mineral mixture containing no magnesium (magnesium deplete group), 3 % alphacel, 1 % vitamin mixture AIN-76, 0.5 % sodium phytate (0.5% exogenous phytate diet groups) or 0.0 % sodium phytate (0.0 % exogenous phytate diet groups), 0.3 % L-cystein, and 0.2 % choline bitartrate. All experimental diets were iso-nitrogenous and iso-caloric. The magnesium level varied in diets with the replete magnesium diets [761 $\pm$ 6 ppm (dry matter)], containing more magnesium than the deplete magnesium diets [191 $\pm$ 2 ppm (dry matter)]. Diets were prepared and stored at -18°C in a dark environment.

4.1.2. Experimental animals and housing

Thirty-two male Syrian hamsters (Charles River Breeding Laboratories, Montreal) at twenty-one-day of age weighing 90.7 + 1.9g (mean + SEM) were individually housed in wire bottom stainless cages under controlled lighting (12 h light: 12 h dark cycle) and temperature (23°C) conditions. The animals were randomly divided into eight groups of

eight animals (n = 8 hamsters/group) and fed for 4 weeks on the specific experimental diets. All animals were given a 1 week adaptation period to the new environment prior to being introduced to experimental diets. All feed containers and water bottles were thoroughly washed and treated with double-deionized water prior to use. All hamsters had free access to feed and water.

Sacrificing procedures and blood collection protocols were identical to that used in Experiment 1. For complete sacrificing procedures and blood collection protocols, please refer to Section 3.1.2 of Experiment 1.

4.1.3. Mineral Analysis

Magnesium and calcium concentrations in feed, plasma, feces, urine, bone, liver, brain, and kidney were determined using a Perkin-Elmer series 400 atomic absorption spectrophotometer (AAS) (Perkin-Elmer, Norwalk, CT).

Mineral analysis protocols were identical to that used in Experiment 1. For complete mineral analysis protocols, please refer to Section 3.1.3 of Experiment 1.

4.1.4. Plasma Lipid Analysis

Plasma isolation was identical to procedures used in Experiment 1. For complete details, please refer to Section 3.1.4. of Experiment 1.

Table 8. Composition of diets used in Experiment 2.

	Soy Protein Diets			
	Mg Deplete		Mg Replete	
Dietary Constituents[1]	Phy 0	Phy 0.5	Phy 0	Phy 0.5
Soy Protein Isolate	24	24	24	24
Sucrose	43	43	43	43
Corn Starch	20	19.5	20	19.5
Corn Oil	5	5	5	5
Mineral Mixture AIN-76	-	-	3.5	3.5
Modified Mineral Mix (Mg free)	3.5	3.5	-	-
Alphacel	3	3	3	3
Vitamin Mixture AIN-76	1	1	1	1
Sodium Phytate	-	0.5	-	0.5
Cystein	0.3	0.3	0.3	0.3
Choline	0.2	0.2	0.2	0.2
Total Percentage	100	100	100	100
Total Energy[2]	4692	4642	4630	4603
Mg[3]	190[a]	192[a]	770[b]	756[b]
Ca[3]	8643	8340	8616	8158

[a,b]Means within the same row sharing the same postscript are not significantly different ($p < 0.05$).

[1]All constituents are listed as % diet: Phy 0 = 0% phytate added; Phy 0.5 = 0.5% added

[2]cal/g dry matter (bomb calorimetry: calculation performed as dry matter basis)

[3]ppm (dry matter); Magnesium and Calcium content in experimental diets

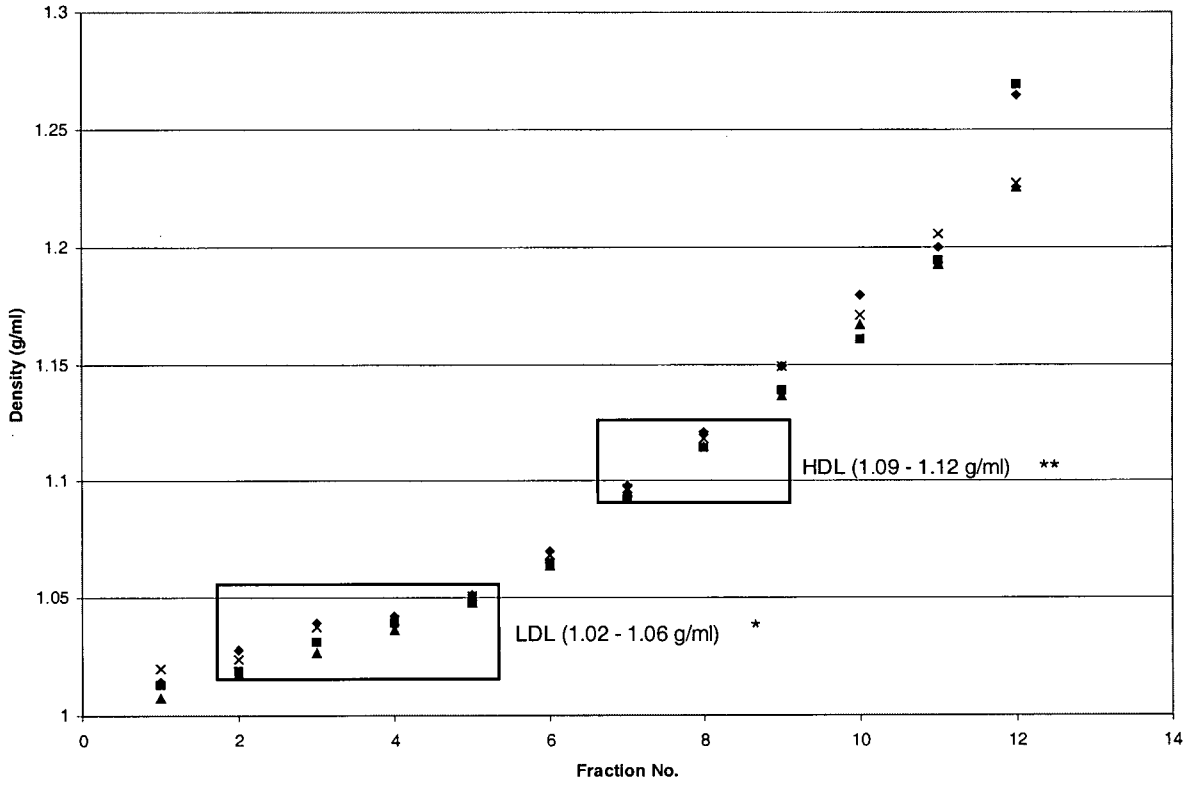

Figure 6. Density fractions of plasma after 24 hours of ultracentrifugation.

◆ SMLPO = soy magnesium deplete 0% dietary phytate added; ■ SMHPO = soy magnesium replete 0% dietary phytate added; ▲ SMLP5 = soy magnesium deplete 0.5% dietary phytate added; • SMHP5 = soy magnesium replete 0.5% dietary phytate added. *Fractions 2,3,4 and 5 of each group were pooled for LDL peroxidation. ** Fractions 7 and 8 of each group were pooled for HDL cholesterol analysis.

4.1.5. LDL isolation

Lipoprotein fractions were isolated by ultracentrifugation in a Beckman Model L2-65B ultracentrifugation (Beckman Instrument, Palo Alto, CA, USA) using a SW-41Ti swinging bucket rotor (Beckman Instrument, Palo Alto, CA, USA).

LDL isolation procedures were identical to procedures used in Experiment 1. For complete details, please refer to Section 3.1.5. of Experiment 1.

4.1.6. Lipoprotein peroxidation

Samples which fell within literature ranges for LDL densities were pooled for each animal and dialyzed with a dialysis tubing cut-off molecular weight of 12,000-14,000 (Spectrum Medical Industries, INC., Los Angeles) against a 200-fold volume of phosphate-buffered saline (PBS 0.01 M phosphate, 0.15 M NaCl, pH 7.4). This procedure was conducted using identical measures to those of Experiment 1. For complete details of lipoprotein peroxidation, please refer to Section 3.1.6. of Experiment 1.

4.1.7. Liver tissue antioxidant analyses

Liver tissue preparation

Liver tissue used for biochemical analyses was collected in chilled homogenizing buffer (50mM Tris, 0.1 mM EDTA, pH 7.6). A portion of the hepatic tissue was blotted dry, weighed, and prepared as a 10% homogenate in fresh, chilled homogenizing buffer,

using a Polytron homogenizer (Brinkmann Instruments Inc., Westbury, N.Y.) at 55% Maximum speed (850 rpm), for 30 seconds (2 x 15 s) (Kitts et al., 1998).

Susceptibility to in vitro forced peroxidation

The production of 2-thiobarbituric acid reactive substances (TBARS) in liver tissue homogenates was measured following incubation with an equal volume of eight different concentrations of H_2O_2 (0-40 mM in 0.9% NaCl containing 2 mM NaN_3) for 30 minutes at 37°C. The reaction was terminated with the addition of 100 µL cold 25% trichloroacetic acid (TCA) containing 0.1 M sodium arsenite, followed by centrifugation at 12,000 x g at 4°C for 5 min. An aliquot of the supernatent was mixed with 0.5% 2-thiobarbituric acid (TBA; Sigma, St. Louis, Mo.) and boiled for 15 minutes, and absorbance was measured at 532 nm (Kitts et al., 1998).

4.1.8. Statistics

The differences among dietary magnesium and calcium levels were analyzed using the one-way analysis of variance. Differences in plasma lipid analysis as well as magnesium and calcium concentrations in all organs were analyzed using the two-way analysis of variance (General Linear Model). Significant differences among treatments were identified by Students T-Test ($P<0.05$) (Minitab for Windows Release 12.1).

4.2. RESULTS: Experiment 2 - Golden Syrian Hamster Fed Soy Based Diets

4.2.1. Assessment of animal growth performance

Hamsters fed magnesium replete diets gained on average ~60% more weight (Table 9) than animals fed magnesium delete diets ($P<0.05$). The feed efficiency ratio (FER; g body weight gain / g of food consumption) was greater in hamsters fed the magnesium replete diets ($P<0.05$). This difference was attributed to the level of dietary magnesium intake which influenced amount of weight gained (Table 9).

There was no effect of supplemented dietary phytate on animal performance in soy fed hamsters.

4.2.2. Assessment of magnesium and calcium balance and apparent absorption in soy fed hamsters

Magnesium intake and subsequent fecal excretion of magnesium reflected the level of magnesium in the diet (Table 10). Hence, magnesium balance was associated with the level of dietary magnesium intake ($P<0.05$). Magnesium apparent absorption, however, was not affected by the level of dietary magnesium intake. Overall calcium intake and fecal excretion of calcium was significantly different ($P<0.05$), and the percentage of absorbed calcium was significantly different among the different animals groups. Subsequently, there was a also significant differences in calcium balance and calcium apparent absorption in soy fed hamsters. Animals fed magnesium-depleted diets showed significant lower magnesium and calcium intakes (Table 10) and subsequent fecal content but no significant difference in magnesium apparent absorption. These

Table 9. Body weight of soy fed hamsters during a 4 week study [1]

	Soy protein diets								
	Mg Deplete				Mg Replete				Significant
	Phy 0		Phy 0.5		Phy 0		Phy 0.5		treatment
									effects[2]
Initial Body weight (g)	90.6	± 0.6	89.3	± 1.2	87.8	± 1.2	88.7	± 0.9	---
Final Body Weight (g)	103.2	± 3.1[a]	106.4	± 4.9[a]	124.5	± 4.1[b]	130.0	± 3.7[b]	M
Weight Gained	19.6	± 1.9[a]	18.0	± 4.7[a]	51.0	± 3.5[b]	49.1	± 3.4[b]	M
Dry matter intake[3]	145.2	± 4.5[a]	144.1	± 5.2[a]	199.1	± 3.5[b]	196.5	± 3.7[b]	M
FER[4]	0.131	± 0.003[a]	0.126	± 0.003[a]	0.251	± 0.005[b]	0.258	± 0.004[b]	M

[1]Data are expressed as mean ± SEM

[2]Significant ($p < 0.05$) treatment levels and interactions, where M is magnesium levels (replete vs. deplete in diet), P is phytate levels (0% added vs. 0.5% added in diet)

[3]Cumulative feed intake for 4 weeks

[4]Feed efficiency ratio (gram body weight gain / gram consumed)

[a,b]Means within the same row that do not share the same postscripts are significantly different ($p < 0.05$)

Table 10. Magnesium and calcium balance and apparent absorption in soy fed hamsters [1]

	Soy Protein Diets				Significant
	Mg Deplete		Mg Replete		treatment
	Phy 0	Phy 0.5	Phy 0	Phy 0.5	effects[4]
Mg					
Intake (mg/d)	1.3 ± 0.2[a]	1.5 ± 0.1[a]	6.3 ± 0.3[b]	6.7 ± 0.5[b]	M
Urinary (mg/d)	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	---
Fecal (mg/d)	0.2 ± 0.1[a]	0.1 ± 0.1[a]	0.7 ± 0.1[b]	0.7 ± 0.1[b]	M
Balance[2]	0.9 ± 0.2[a]	1.3 ± 0.1[a]	5.5 ± 0.3[b]	5.9 ± 0.5[b]	M
Apparent Absorption[3]	87.0 ± 1.8	90.1 ± 1.8	88.8 ± 1.0	89.2 ± 0.9	---
Ca					
Intake (mg/d)	59.9 ± 7.2[ab]	56.4 ± 2.2[a]	76.1 ± 3.3[bc]	79.4 ± 4.7[c]	M
Urinary (mg/d)	1.9 ± 0.6	1.9 ± 0.2	1.4 ± 0.4	1.8 ± 0.1	---
Fecal (mg/d)	52.1 ± 9.6[a]	51.4 ± 6.3[a]	70.5 ± 7.6[b]	80.1 ± 8.1[b]	M
Balance[2]	6.6 ± 2.1[a]	4.7 ± 2.5[a]	4.6 ± 2.1[a]	-2.4 ± 4.1[b]	M
Apparent Absorption[3]	13.6 ± 3.5[a]	9.1 ± 3.4[a]	7.3 ± 3.1[a]	0.9 ± 3.3[b]	M

[1]Data are expressed as mean ± SEM

[2]Balance (mg/d) = Intake - (Fecal + Urinary)

[3]Apparent Absorption (%) = [(Intake - Fecal) / Intake] x 100

[4]Significant ($p < 0.05$) treatment levels and interactions, where M is magnesium levels (replete vs. deplete in diet), P is phytate levels (0% added vs. 0.5% added in diet)

[a,b,c]Means within the same row that do not share the same postscripts are significantly different ($p < 0.05$).

results indicate that overall magnesium balance was lower in magnesium-depleted fed animals but that the percentage of magnesium absorption was not significantly different among the different hamster treatment groups. There was no effect of supplementing dietary phytate on apparent absorption, or balance of magnesium and calcium in soy fed hamsters.

4.2.3. Dietary magnesium influence on distribution status of plasma and femur magnesium and calcium in soy fed hamsters

Dietary mineral intake and plasma and femur magnesium status were related. Animals fed magnesium-depleted diets had a significantly diminished femur magnesium concentration (~45% less) and reduced plasma magnesium concentration (~50% less) compared to counterparts fed the magnesium-replete diets ($P<0.05$). Since the femur can be seen as a storage compartment and the plasma as a transport system of minerals, these findings indicate that dietary magnesium plays an important role in determining storage as well as transport of magnesium within the body.

Dietary magnesium did not play a role on calcium status as indexed by the plasma and femur calcium content (Table 11). In addition, there was no effect of supplemented dietary phytate on plasma and femur magnesium or calcium in soy fed hamsters. This result was similar to that from the previous experiment with casein fed hamsters.

Table 11. Plasma and femur magnesium and calcium status in soy fed hamsters [1]

	Soy Protein Diets				Significant
	Mg Deplete		Mg Replete		treatment
Minerals	Phy 0	Phy 0.5	Phy 0	Phy 0.5	effects[2]
Mg					
Femur (ug/bone)	321.4 ± 51.3[a]	349.7 ± 80.2[a]	651.3 ± 94.3[b]	663.2 ± 84.3[b]	M
Plasma (mg/L)	27.2 ± 2.8[a]	29.9 ± 4.2[a]	62.5 ± 5.1[b]	57.0 ± 4.6[b]	M
Ca					
Femur (mg/bone)	12.9 ± 3.6	12.2 ± 2.9	12.9 ± 3.1	13.1 ± 4.2	---
Plasma (mg/L)	2.20 ± 0.4	2.20 ± 0.3	2.10 ± 0.1	2.20 ± 0.2	---

[1]Data are expressed as mean ± SEM

[2]Significant ($p < 0.05$) treatment levels and interactions, where M is magnesium levels (replete vs. deplete in diet), P is phytate levels (0% added vs. 0.5% added in diet)

[a,b]Means within the same row that do not share same postscript are significantly different ($p < 0.05$)

Table 12. Tissue magnesium and calcium status in soy fed hamsters [1]

	Soy Protein Diets				Significant
	Mg Deplete		Mg Replete		treatment
Tissue Minerals	Phy 0	Phy 0.5	Phy 0	Phy 0.5	effects[2]
Mg[3]					
Heart	977.3 ± 17.3	952.3 ± 30.5	987.6 ± 18.3	965.1 ± 17.4	---
Liver	736.4 ± 23.7	851.3 ± 42.8	844.4 ± 40.2	844.5 ± 61.4	---
Kidney	959.6 ± 18.3	962.5 ± 36.4	987.1 ± 27.1	948.7 ± 17.3	---
Brain	751.3 ± 12.3[a]	770.6 ± 12.6[a]	837.0 ± 19.6[b]	835.4 ± 13.2[b]	M
Ca[3]					
Heart	586.9 ± 88.3	578.6 ± 21.4	533.6 ± 34.3	544.5 ± 17.5	---
Liver	313.4 ± 13.5	284.1 ± 48.4	270.3 ± 40.4	262.0 ± 33.5	---
Kidney	1031.7 ± 51.8[b]	1000.8 ± 77.6[b]	817.1 ± 38.1[a]	717.3 ± 21.1[a]	M
Brain	510.6 ± 39.4	465.5 ± 20.5	514.1 ± 40.8	523.1 ± 91.9	---

[1]Data are expressed as mean ± SEM

[2]Significant ($p < 0.05$) treatment levels and interactions, where M is magnesium levels (replete vs. deplete in diet), P is phytate levels (0% added vs. 0.5% added in diet)

[3]Data are expressed as ppm (dry matter)

[a,b]Means within the same row sharing the same postscript are not significantly different ($p < 0.05$).

4.2.4. Dietary magnesium influence on distribution status of tissue magnesium and calcium in soy fed hamsters

Animals fed the magnesium deplete diets had lower (~10%) brain magnesium concentrations than counterparts on the replete diets. This finding was attributed to a reduced magnesium balance ($P<0.05$) which resuled in lower magnesium levels in brain tissue. In addition, the magnesium balance in these animals also influenced kidney calcium content, inversely ($P<0.05$). For example, animals fed the magnesium-repleted diets had less (~25% less) calcium in the kidney tissues than counterparts fed the magnesium-depleted diets (Table 12). A low magnesium balance in magnesium deplete animals did not influence magnesium status in heart and liver of hamsters. Apparent absorption and supplemented dietary phytate did not play a role in determining magnesium or calcium status in the hamster heart, liver, kidney or brain.

4.2.5. Assessment of plasma lipids in soy fed hamsters

Plasma lipids after 4 weeks of feeding hamsters the experimental diets are shown in Table 13. There were no significant dietary effects associated with magnesium intake or bioavailability of magnesium from phytate on triacylglycerol, total cholesterol and high density lipoprotein cholesterol levels. In accordance with the plasma lipid data, there were no significant differences in the concentration of plasma lipids measured after ultracentrifugation.

4.2.6. Assessment of LDL peroxidation in soy fed hamsters

LDL peroxidation from soy fed hamsters was determined using a relative fluorescence (RF) measure from samples treated with hydrogen peroxide overtime. The RF curve of LDL peroxidation showed a typical 3-phase oxidation pattern: with initiation, propagation and termination stages clearly defined (Figure 7).

The results from this study showed that exogenous dietary phytate supplementation was a significant factor on the susceptibility of hamster LDL

Table 13. Plasma lipids in soy fed hamsters [1]

	Soy protein diets								Significant
	Mg Deplete				Mg Replete				treatment
	Phy 0		Phy 0.5		Phy 0		Phy 0.5		effects
Tri-acylglycerol (mg / dl)	86.8	± 8.0	79.0	± 3.7	88.6	± 9.2	74.7	± 9.1	---
Total Cholesterol (mg / dl)	76.8	± 5.8	77.5	± 8.0	85.4	± 3.3	85.2	± 4.1	---
HDL Cholesterol (mg / dl)	12.2	± 1.2	13.5	± 1.5	13.6	± 2.2	12.9	± 1.6	---

[1]Data are expressed as mean ± SEM

Means within the same row are not significantly different ($p < 0.05$)

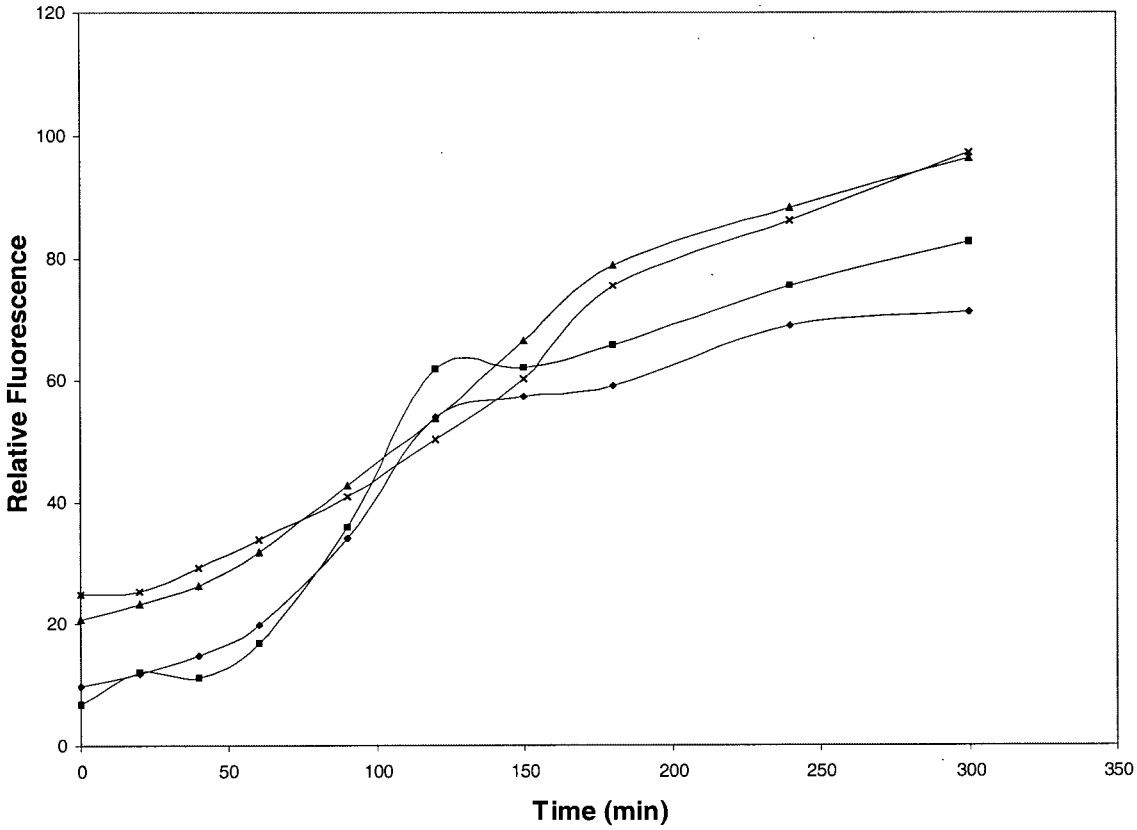

Figure 7. Relative fluorescence curves of time dependant, LDL forced peroxidation in casein fed hamsters. [1]

Lipoprotein oxidation was initiated using 50 μM copper sulfate added to LDL aliquots and incubated in a water bath at 37°C under gentle agitation. At prefixed intervals of time, (every 20 minutes over the first 60 minutes and every 30 minutes over the next 120 minutes and every 60 minutes over the next 120 minutes for a total of 300 minutes), samples were quenched with a final concentration of 1.5 mg/ml EDTA. ◆ SMLPO = soy magnesium deplete 0% dietary phytate added; ■ SMHPO = soy magnesium replete 0% dietary phytate added; ▲ SMLP5 = soy magnesium deplete 0.5% dietary phytate added; × SMHP5 = soy magnesium replete 0.5% dietary phytate added. [1]Values represent means of 8 samples without SEM.

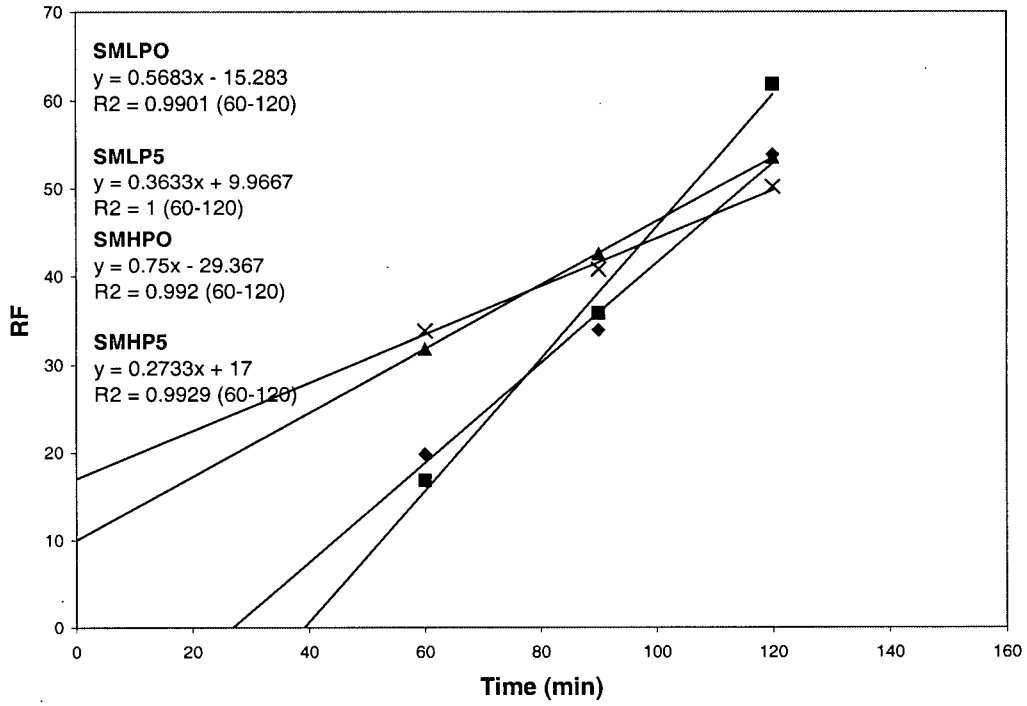

Figure 8. Extrapolation of propagation curves to determine the lag point of time dependant, LDL forced peroxidation in soy fed hamsters. [1]

A 3-point linear regression intersecting initial relative fluorescence value was used to determine lag time in minutes. ◆ SMLPO = soy magnesium deplete 0% dietary phytate added; ■ SMHPO = soy magnesium replete 0% dietary phytate added; ▲ SMLP5 = soy magnesium deplete 0.5% dietary phytate added; × SMHP5 = soy magnesium replete 0.5% dietary phytate added. [1]Values represent means of 8 samples without SEM.

Table 14. Lag time and RF comparisons of LDL peroxidation in soy fed hamsters [1]

	Soy protein diets				Significant
	Mg Deplete		Mg Replete		treatment
	Phy 0	Phy 0.5	Phy 0	Phy 0.5	effects[2]
RF at 0 min	9.8 ± 1.1[a]	20.8 ± 3.1[b]	6.9 ± 2.3[a]	24.8 ± 3.5[b]	P
RF at 60 min	19.8 ± 3.1[a]	31.8 ± 5.3[b]	16.8 ± 2.2[a]	33.8 ± 4.9[b]	P
Lag Time (min)	44.1 ± 2.8[b]	29.8 ± 3.2[a]	48.4 ± 2.5[b]	28.5 ± 4.1[a]	P

[1]Data are expressed as mean ± SEM; RF = relative fluorescence measurement; (0,60 = time in min)

[2]Significant ($p < 0.05$) treatment levels and interactions, where M is magnesium levels (replete vs. deplete in diet), P is phytate levels (0% added vs. 0.5% added in diet)

[a,b]Means within the same row that do not share same postscript are significantly different ($p < 0.05$)

peroxidation (Table 14). Initial RF values at 0 minutes (Table 14) of LDL peroxidation in phytate supplemented animals were significantly greater (~ 150% more) than non-phytate supplemented groups ($P<0.05$), thus indicating that hamsters fed the phytate supplemented diet exhibited greater levels of LDL peroxidation prior to the forced peroxidation. After 60 minute exposure to hydrogen peroxide, the propagation phase of LDL lipid peroxidation from phytate supplemented animals also reflected a reduced capacity to withstand LDL peroxidation. RF values for oxidized LDL at 60 min (Table 14) from animals fed the phytate supplemented diet were greater (~80% more) than control counterparts ($P<0.05$). These results indicated that hamsters fed phytate-supplemented soy diets decreased in abilities to protect against forced-lipid peroxidation.

Lag time was determined using a 3-point (during propagation phase) linear regression intersecting the initial relative fluorescence value (Figure 8) within the initial phase of the curve. Of particular importance, was the finding that lag times for the initiation phase of LDL forced-lipid peroxidation in hamsters fed dietary phytate was also affected. Animals fed both the magnesium replete and deplete diets without phytate had increased forced peroxidation lag times (Table 14) of LDL (~60% more) during the initial phase ($P<0.05$) than counterparts. This study also indicated that dietary phytate supplementation significantly decreased (~60% less) the ability of the hamster to cope with initial LDL lipid peroxidation (Table 14). It was also noted that at 300 minutes after of LDL forced peroxidation, which represented the termination phase, oxidation RF values from animals fed the phytate-supplemented diets were greater than non-phytate control animals. This finding reinforces the conclusion that RF values specific for lag time indication were reduced in hamsters fed phytate supplementated diets. Magnesium

status, on other hand, had no significant effect in determining lag time, initial RF value or RF value at 60 minutes of hamster LDL peroxidation.

4.2.7. Assessment of TBARs formation in liver tissue in soy fed hamsters

TBARs formation during forced peroxidation of fresh liver tissue from soy fed hamsters yielded characteristic curves that are shown in Figure 9. The resistance of liver tissue against forced peroxidation was determined to be sensitive to both dietary magnesium intake and the level of phytate added to the diet. Thus, magnesium status which included bioavailability of magnesium appeared to be important in regulating liver peroxidation. Hamsters fed the magnesium depleted diets showed a greater overall susceptibility to forced peroxidation with increasing amounts of hydrogen peroxide, as indexed by the rate of the reaction (Figure 9). It was noted that there was no significant difference in absorbance values at 532nm when fresh liver tissue was not exposed to hydrogen peroxide in the different animal-fed groups. However, the addition of 40mM hydrogen peroxide resulted in greater susceptibility (~3 fold) to forced peroxidation of liver tissue ($P<0.05$) in the magnesium deplete animals (Figure 10). In addition, animals fed the phytate supplemented diets also showed a significant ($P<0.05$) increase in general susceptibility to forced peroxidation when compared to control counterparts (Figure 10). Indications from the data suggested that lack of dietary magnesium as well as the presence of phytate, both contributed to the reduced capacity of liver tissue to resist forced peroxidation in soy-fed hamsters.

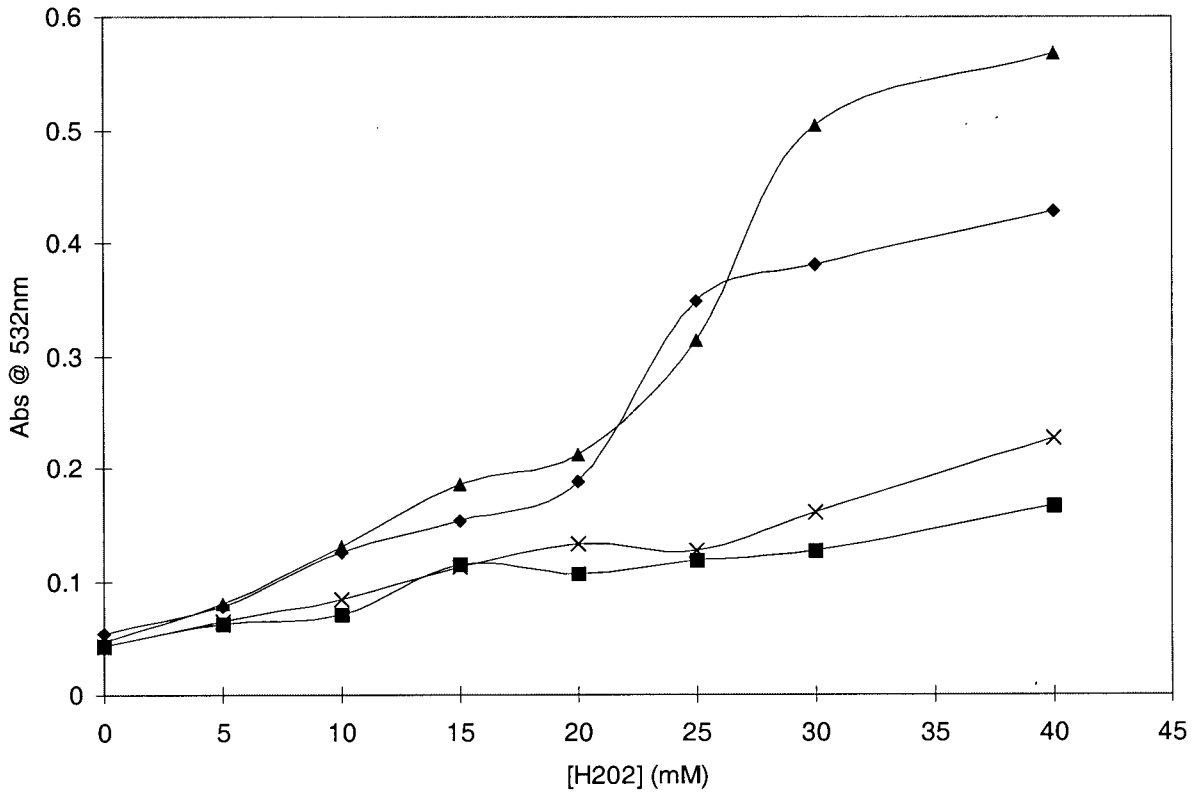

Figure 9. TBARs formation during forced peroxidation of fresh hamster liver from soy fed hamsters.

◆ SMLPO = soy magnesium deplete 0% dietary phytate added; ■ SMHPO = soy magnesium replete 0% dietary phytate added; ▲ SMLP5 = soy magnesium deplete 0.5% dietary phytate added; × SMHP5 = soy magnesium replete 0.5% dietary phytate added.
[1]Values represent means of 8 samples without SEM.

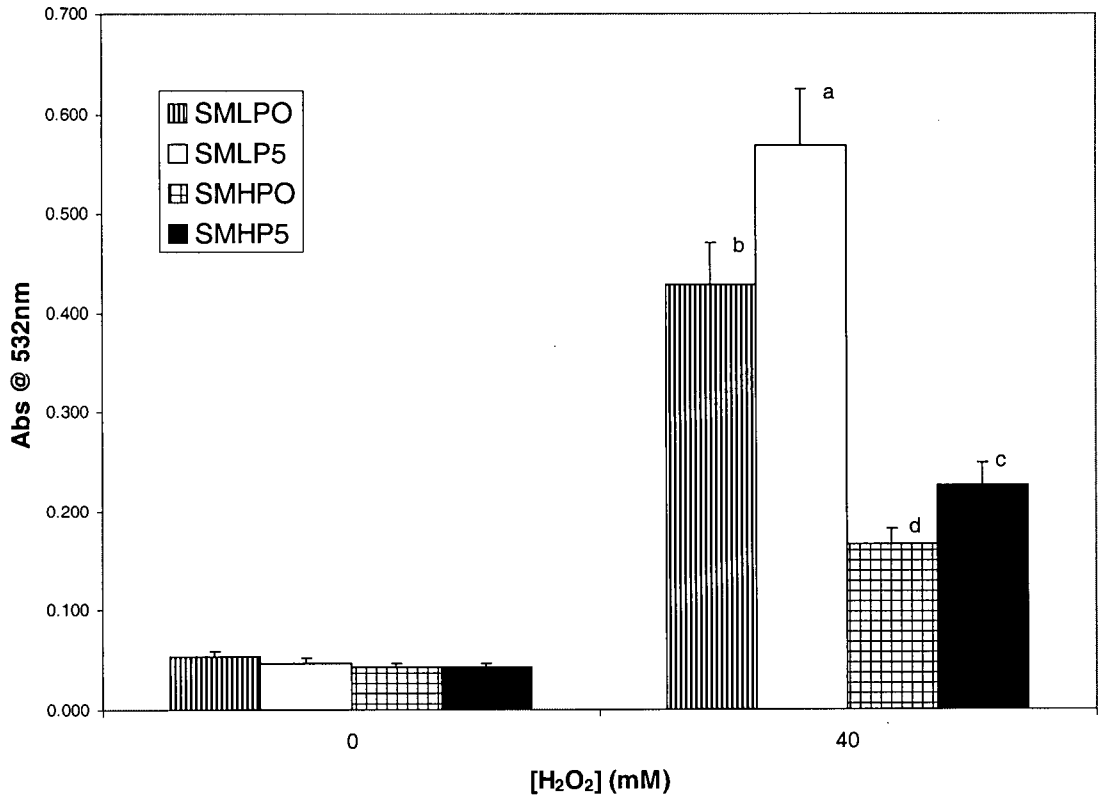

Figure 10. TBARS formation of forced peroxidation fresh hamster liver from soy fed hamsters at 0 and 40 mM of H_2O_2.

SMLPO = soy magnesium deplete 0% dietary phytate added; SMHPO = soy magnesium replete 0% dietary phytate added; SMLP5 = soy magnesium deplete 0.5% dietary phytate added; SMHP5 = soy magnesium replete 0.5% dietary phytate added. [1]Values represent means of 8 samples without SEM. [a,b,c,d] Means with different superscript letters were significantly different ($P<0.05$).

4.3. Discussion of experiment 2 - Golden Syrian Hamster Fed Soy Based Diets

4.3.1. Effect of dietary magnesium and supplemented dietary phytate on magnesium and calcium status

In Experiment 2 of this study, magnesium and calcium status of hamsters was determined by whole body mineral balance and specific concentrations in the femur, plasma, heart, liver, kidney and brain.

Animals fed the magnesium-depleted diets [191 $\pm$ 2 ppm (dry matter)] exhibited a lower magnesium concentration in femur, plasma and brain tissue, which corresponded to a lower magnesium balance. These findings are consistent with previously reported data from Rayssiguier et al. (1993), who showed that animals fed a magnesium deficient diet (35 ppm) had significantly lower plasma magnesium. In addition, Rimbach and Pallauf (1999) also showed a similar trend for reduced femur and plasma magnesium concentration in animals with a lower magnesium balance. Reduced fecal magnesium excretion was also observed from animals fed low dietary intakes of magnesium. In the present study, hamsters fed the magnesium-depleted diets did not exhibit any difference in magnesium apparent absorption, although significantly lower excretion of this mineral content was found in the feces. This result indicated that although the overall amount of magnesium absorption was decreased in animals fed magnesium-depleted diets, the actual percentage of magnesium absorption did not change significantly among the hamsters.

The supplementation of soy protein diets with phytate (~0.5%) had no significant effect on the overall magnesium or calcium status of hamsters. This result was supported by a similar finding in Experiment 1, with casein fed animals. The finding is not

consistent with previously reports (Rimbach and Pallauf 1999). One possible explanation is that the level of dietary phytate supplementation (0.5%) in this study was not sufficiently high enough to induce a significant difference in magnesium bioavailability and thus status in hamsters. As mentioned earlier, a species variation could also be a factor for different results reported herein. For example, previous studies have utilized omnivore rats as the animal model, whereas, in this study we used herbivore hamsters, which have a greater ability to digest dietary phytate (Chiou et al. 1998).

4.3.2. Effect of dietary magnesium and supplemented dietary phytate on plasma lipids

In this study, plasma lipids such as tri-acylglycerol, total cholesterol and HDL cholesterol content was measured as possible indicators of magnesium deficiency. This study showed no significant dietary effects on any of the measured parameters. Our results clearly indicated that animals fed magnesium-depleted diets showed an increase in LDL lipid peroxidation which was independent to the total plasma lipid levels among the animals. As explained in Experiment 1, this finding may be due to the absence of key data in the measured variables. For example, Mahfouz and Kummerow in 1989 showed that enhanced lipid peroxides found in magnesium deficient animals are primarily in the lower density lipoproteins. Since the lipid profile of LDL was not a measured parameter in this study, no significant differences was observed between animals fed magnesium-depleted and repleted diets.

This study showed no significant changes in serum cholesterol of hamsters fed soy based diets. This finding corresponds to earlier reports concluding that rats fed low fat,

cholesterol-free semi purified diets containing soy protein did not exhibit a change in serum cholesterol levels (Kazunari et al., 1984). Also, Jacques et al., 1986 also duplicated our findings when rats fed soy protein were observed to have no significant changes in serum cholesterol when diets were not cholesterol-enriched. Our finding differed from those of Michiro et al. in 1990, where hypocholesterolemic action of soybean protein was shown to occur in rats. These researchers found a significant decrease in serum cholesterol when rats were fed soy protein along with a 0.5% cholesterol diet. This study found no significant changes in serum cholesterol between soy fed and casein fed hamsters. Reasons could be species differentiation of hamsters versus rats and that our study did not supplement exogenous cholesterol into the experimental diets.

4.3.2. Effect of dietary magnesium and supplemented dietary phytate on LDL and liver peroxidation

Animals fed magnesium-depleted diets showed a decreased ability to cope with LDL forced peroxidation. This finding was consistent with previous data reported by Rayssiguier et al. (1993) where animals fed a depleted magnesium diet yielded higher peroxidation values during incubated LDL peroxidation. Findings in this study also included that animals fed magnesium-depleted diets also showed a decreased ability to cope with liver peroxidation. Previous researchers (Rayssiguier et al., 1993; Gunther et al., 1995) also reported this conclusion. These results clearly demonstrated that depleted magnesium diets increased the susceptibility of hamsters to lipid peroxidation.

During oxidation of LDL, lipids are converted to lipid peroxides and unsaturated aldehydes (Steinber et al., 1989). It is these aldehydes that can potentially react with apoprotein B (apoB) on the LDL molecule, thus causing a process which generates a fluorescent product with a strong emission maximum at 430nm when excitation is performed at 360nm (Esterbauer et al., 1987). Since LDL from animals fed phytate supplemented diets had a decreased ability to cope with forced peroxidation as compared to their counterparts, it can be said that exogenous dietary phytate had an indirect effect on unsaturated aldehydes reacting with apoB, and thus also indirectly effecting LDL peroxidation of phytate supplemented soy-fed hamsters.

Supplementary dietary phytate also played a role in liver peroxidation in the hamster. Findings from this study showed that animals fed phytate supplemented diets performed poorly during liver peroxidation as compared to their counterparts. Rimbach and Pallauf (1999) also reported similar findings where animals fed a high phytate diet showed significant increased hepatic susceptibility to forced peroxidation. Although soy protein has been shown to have protective effects on the peroxidization of LDL (Kanazawa et cl., 1995), soy protein is known to be deficient in sulfur-containing amino acids, cysteine and methionine (Moundras et al., 1995). These researchers also reported that the supplementation of dietary methionine led to partially recovery of LDL resistance to peroxidation in rats fed a soy protein diet. Hence, it can be postulated that lack of sulfur-containing amino acids can lead to decreased levels of effective glutathione in the liver, since glutathione requires cysteine and methionine disulfide bonds to maintain its antioxidant capacity (Yuen and Kitts, 1996). Moreover, soy protein is also known to contain trypsin inhibitors (Anderson and Wolf, 1995). These antinutritional compounds

can further lead to decreased bioavalability of amino acids. These findings indicate that amino acid imbalance could indirectly lead to increased liver peroxidation due to decreased antioxidant activity from hepatic glutathione and other antioxidant enzymes.

5. OVERALL CONCLUSION AND FUTURE DIRECTIONS

Magnesium and calcium status, as shown by their respective mineral concentrations in the femur, plasma, liver, kidney brain, heart, feces and urine, was partly affected by dietary magnesium in hamsters. Balance of magnesium calcium in the hamster was significantly affected by dietary magnesium. Apparent absorption of magnesium was not affected by dietary magnesium, but on the other hand, apparent absorption of calcium was significantly affected by dietary magnesium. Dietary phytate supplementation at 0.5% in the diet did not affect magnesium or calcium status in hamsters. No significant difference was seen in balance and apparent absorption of magnesium or calcium by supplementation of 0.5% phytate in the diet.

One method chosen to test the hamster's ability to cope with oxidative stress was peroxide values measurement during LDL peroxidation. The data showed that animals fed magnesium-delete diets presented a decreased ability to cope with LDL peroxidation. Animals fed magnesium-repleted diets performed better overall during a 300-minute LDL peroxidation analysis. Furthermore, supplemented dietary phytate also played a role in determining the hamster's ability to cope with oxidative stress. Animals fed magnesium-depleted diets showed a significant decrease in ability to cope with lipid peroxidation. Hence, both dietary magnesium and supplemented phytate were determining factors in the hamster's ability to cope with LDL peroxidation.

Another method used to test for oxidative stress in the hamsters was the TBARS value measurement during liver peroxidation. Data from this study indicated that dietary

magnesium alongside supplemented dietary phytate had a significant effect in determining hepatocyte peroxidation.

In the present study, Golden Syrian herbivore hamsters were utilized to study the effect of dietary magnesium and phytate on magnesium and calcium status. Since no significant effect on mineral status and absorption was seen in diets with 0.5% of supplemented phytate, future studies perhaps can investigate these parameters in further detail utilizing a 10% phytate diet. This higher phytate content would be more similar to that of vegetarian diets. In addition, a recent discovery by Kamao et al. (2000) has made it possible to easily remove phytate from soybean protein isolates. Hence, hamster balance studies could also be used in the future to compare the effect of phytate-free products versus their counterpart on mineral absorption. Moreover, since rats and humans are both omnivores, it would also be interesting to compare the hamster versus rat model for mineral absorption in future research.

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Appendix I -
Lipoprotein Fractions Isolated by Ultracentrifugation

Table I-1. Protein concentration of individual fractions of hamster plasma from magnesium deplete / no phytate added casein diet

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
A1	0.536	0.157	0.082	0.060	0.381	0.715	0.260	0.185	0.268	0.719	1.118	1.665
A1	0.597	0.208	0.099	0.062	0.624	0.877	0.354	0.135	0.268	0.754	1.154	1.675
A1	0.635	0.261	0.139	0.083	0.468	0.831	0.369	0.166	0.233	0.692	1.092	1.785
A1	0.589	0.209	0.107	0.068	0.491	0.808	0.328	0.162	0.256	0.722	1.121	1.708
[Protein] (mg/ml)	267.726	69.462	16.337	-3.628	216.510	381.441	131.441	45.156	94.288	336.649	544.809	850.538
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
A1	0.140	0.078	0.029	0.017	0.191	0.299	0.041	0.040	0.007	0.151	0.221	1.408
A1	0.131	0.059	0.015	0.020	0.134	0.267	0.037	0.032	0.006	0.140	0.194	1.348
A1	0.155	0.089	0.044	0.017	0.180	0.381	0.048	0.029	0.015	0.130	0.212	1.222
A1	0.142	0.075	0.029	0.018	0.168	0.316	0.042	0.034	0.009	0.140	0.209	1.326
[Protein] (mg/ml)	347.396	0.174	-239.410	-298.438	484.549	1251.910	-173.438	-216.840	-343.576	338.715	696.354	6514.063

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
A2	0.251	0.094	0.011	0.076	0.326	0.513	0.493	0.191	0.253	0.868	1.631	1.921
A2	0.268	0.096	0.023	0.076	0.416	0.677	0.520	0.212	0.274	0.947	1.635	2.313
A2	0.257	0.091	0.026	0.076	0.425	0.625	0.518	0.215	0.306	0.948	1.690	2.573
A2	0.259	0.094	0.020	0.076	0.389	0.605	0.510	0.206	0.278	0.921	1.652	2.269
[Protein] (mg/ml)	95.503	-0.549	-1.195	-0.704	2.043	3.939	3.108	0.437	1.066	6.713	13.129	18.544
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
A2	0.045	0.013	0.000	0.004	0.067	0.187	0.114	0.032	0.071	0.442	1.189	2.072
A2	0.073	0.021	0.000	0.014	0.075	0.157	0.131	0.034	0.064	0.520	1.445	2.352
A2	0.049	0.006	0.001	0.002	0.065	0.191	0.112	0.026	0.067	0.508	1.357	1.664
A2	0.056	0.013	0.000	0.007	0.069	0.178	0.119	0.031	0.067	0.490	1.330	2.029
[Protein] (mg/ml)	-102.257	-12.539	-13.680	-13.125	-7.654	1.943	-3.265	-11.018	-7.800	29.298	103.053	164.405

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
Ap	0.123	0.007	0.031	0.144	0.420	0.760	0.867	0.812	0.345	0.526	1.328	1.818
Ap	0.131	0.017	0.040	0.223	0.437	0.774	0.908	0.824	0.348	0.538	1.333	1.568
Ap	0.131	0.012	0.035	0.158	0.432	0.750	0.895	0.808	0.353	0.542	1.336	1.901
Ap	0.128	0.012	0.035	0.175	0.430	0.761	0.890	0.815	0.349	0.535	1.332	1.762
[Protein] (mg/ml)	27.622	-1.266	-1.061	0.165	2.400	5.311	6.441	5.779	1.689	3.328	10.323	14.097
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
Ap	0.000	0.003	0.001	0.005	0.063	0.133	0.382	0.170	0.060	0.138	1.252	1.566
Ap	0.001	0.000	0.001	0.003	0.057	0.124	0.378	0.167	0.068	0.146	1.238	1.668
Ap	0.000	0.000	0.005	0.016	0.058	0.129	0.364	0.154	0.070	0.150	1.219	1.743
Ap	0.000	0.001	0.002	0.008	0.059	0.129	0.375	0.164	0.066	0.145	1.236	1.659
[Protein] (mg/ml)	-390.451	-13.622	-13.505	-13.007	-8.502	-2.417	19.175	0.655	-7.917	-1.012	94.803	131.900

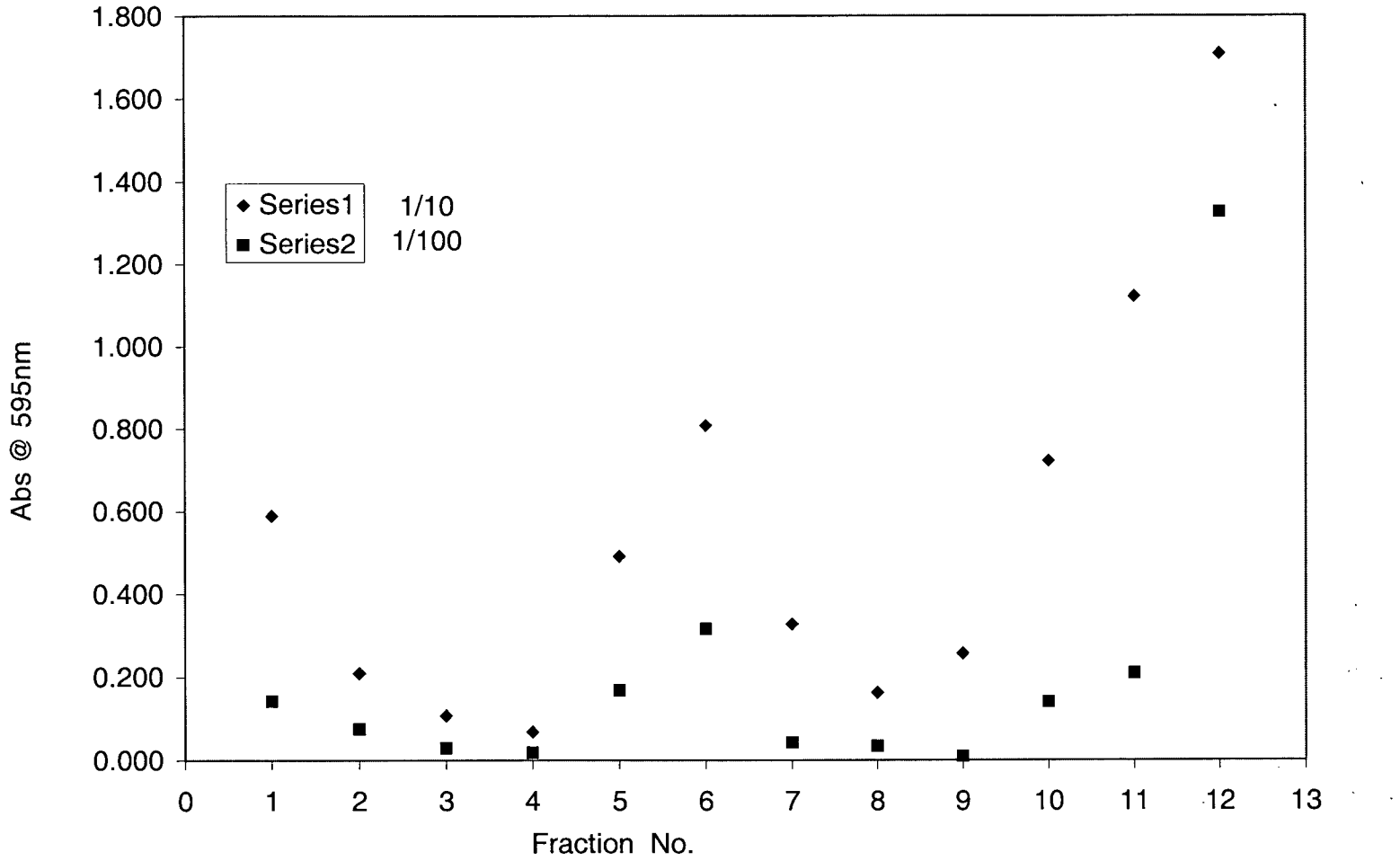

Figure I-1. Absorbance of hamster lipoprotein from magnesium deplete / no phytate added casein diet

Table I-2. Protein concentration of individual fractions of hamster plasma from magnesium replete / no phytate added casein diet

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
B1	0.111	0.090	0.229	0.069	0.236	0.370	0.323	0.124	0.148	0.438	0.911	1.483
B1	0.148	0.108	0.215	0.094	0.259	0.391	0.344	0.135	0.145	0.493	1.316	1.795
B1	0.165	0.139	0.122	0.097	0.193	0.361	0.222	0.164	0.137	0.446	1.146	1.655
B1	0.141	0.112	0.189	0.087	0.229	0.374	0.296	0.141	0.143	0.459	1.124	1.644
[Protein] (mg/ml)	34.392	19.288	59.045	5.920	80.226	155.573	115.122	34.219	35.434	199.844	546.372	817.205
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
B1	0.045	0.034	0.041	0.008	0.096	0.104	0.022	0.006	0.007	0.036	0.174	1.341
B1	0.039	0.025	0.037	0.000	0.064	0.047	0.029	0.008	0.009	0.014	0.187	1.219
B1	0.034	0.040	0.035	0.015	0.067	0.082	0.016	0.009	0.004	0.039	0.198	1.213
B1	0.039	0.033	0.038	0.008	0.076	0.078	0.022	0.008	0.007	0.030	0.186	1.258
[Protein] (mg/ml)	-187.326	-220.313	-196.007	-352.257	1.910	12.326	-275.868	-352.257	-357.465	-237.674	578.299	6158.160

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
B2	0.170	0.115	0.076	0.281	0.633	0.685	0.451	0.162	0.280	1.116	1.685	1.933
B2	0.183	0.223	0.089	0.296	0.626	0.715	0.483	0.181	0.294	1.147	1.674	2.046
B2	0.170	0.100	0.082	0.278	0.635	0.705	0.452	0.169	0.272	1.163	1.732	2.193
B2	0.174	0.146	0.082	0.285	0.631	0.702	0.462	0.171	0.282	1.142	1.697	2.057
[Protein] (mg/ml)	51.580	36.823	3.663	109.219	289.601	326.233	201.406	49.670	107.656	555.573	844.635	1032.309
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
B2	0.014	0.014	0.007	0.039	0.166	0.311	0.101	0.000	0.065	0.593	1.488	1.634
B2	0.031	0.024	0.020	0.052	0.189	0.339	0.140	0.000	0.067	0.583	1.481	1.761
B2	0.008	0.018	0.005	0.029	0.150	0.315	0.100	0.000	0.054	0.577	1.741	1.999
B2	0.018	0.019	0.011	0.040	0.168	0.322	0.114	0.000	0.062	0.584	1.570	1.798
[Protein] (mg/ml)	-300.174	-294.965	-336.632	-183.854	484.549	1283.160	199.826	-392.188	-69.271	2651.215	7784.896	8972.396

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
Bp	0.022	0.000	0.007	0.138	0.418	0.680	0.808	0.798	0.464	0.444	1.310	1.635
Bp	0.025	0.000	0.006	0.150	0.425	0.684	0.817	0.801	0.476	0.483	1.346	1.841
Bp	0.019	0.000	0.007	0.127	0.407	0.675	0.796	0.785	0.470	0.491	1.394	1.658
BP	0.022	0.000	0.007	0.138	0.417	0.680	0.807	0.795	0.470	0.473	1.350	1.711
[Protein] (mg/ml)	-27.760	-39.219	-35.747	32.830	177.795	314.774	381.094	374.670	205.573	206.962	663.906	852.101
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
Bp	0.000	0.000	0.000	0.000	0.033	0.148	0.119	0.324	0.028	0.219	1.142	1.493
Bp	0.003	0.000	0.000	0.000	0.034	0.150	0.132	0.338	0.031	0.229	1.147	1.606
Bp	0.009	0.013	0.010	0.016	0.051	0.157	0.131	0.318	0.018	0.199	1.059	1.421
Bp	0.004	0.004	0.003	0.005	0.039	#REF!	0.152	0.127	0.327	0.026	0.216	1.507
[Protein] (mg/ml)	-371.354	-369.618	-374.826	-364.410	-187.326	#REF!	397.743	271.007	1309.201	-258.507	731.076	7455.035

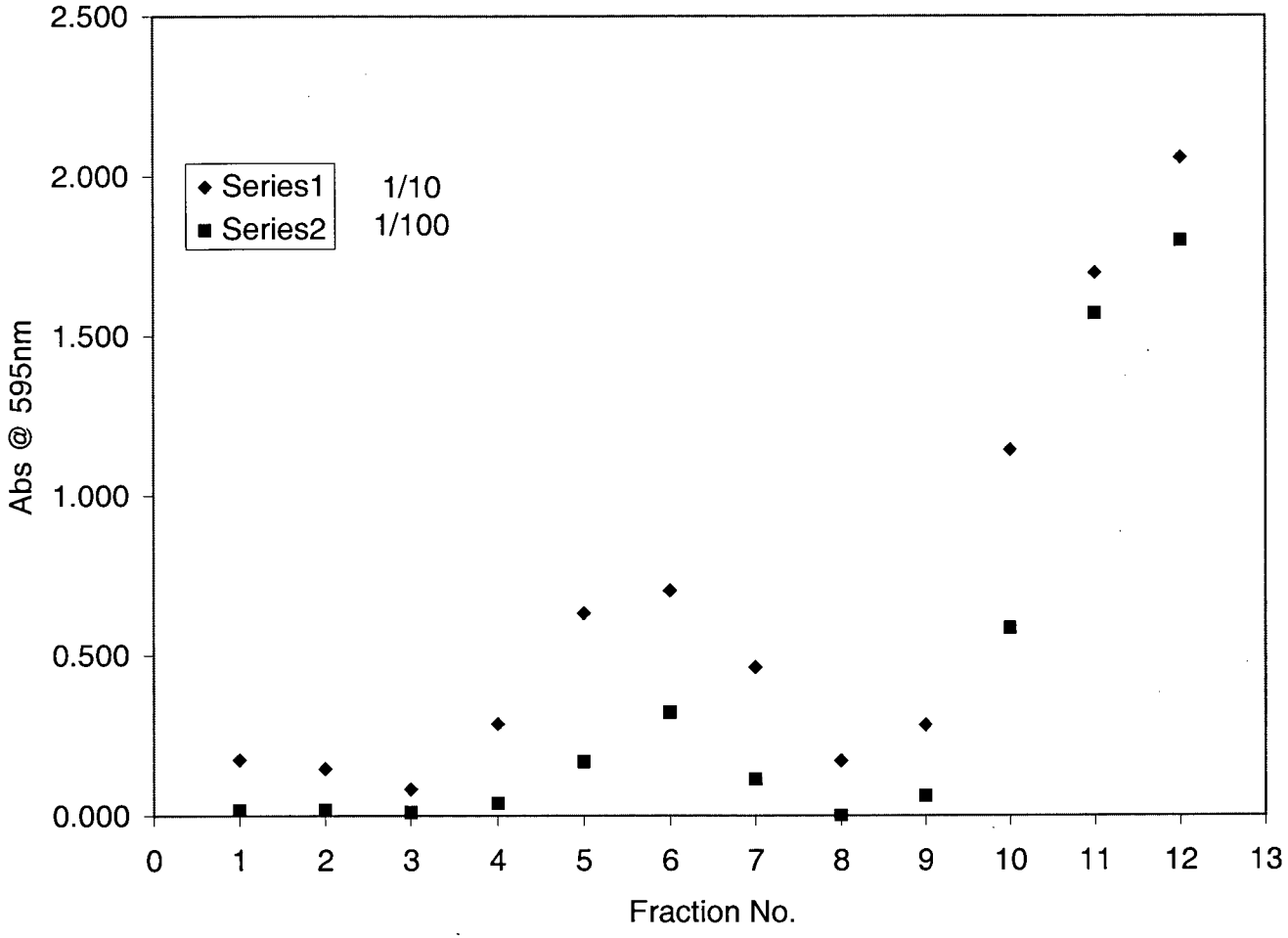

Figure I-2. Absorbance of hamster lipoprotein from magnesium replete / no phytate added casein diet

Table I-3. Protein concentration of individual fractions of hamster plasma from magnesium deplete / 0.5% phytate added casein diet

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
C1	0.370	0.228	0.121	0.099	0.268	0.359	0.308	0.183	0.187	0.290	0.446	0.990
C1	0.349	0.234	0.083	0.101	0.246	0.401	0.350	0.226	0.184	0.248	0.418	0.842
C1	0.376	0.163	0.068	0.056	0.185	0.327	0.277	0.121	0.186	0.258	0.439	0.899
C1	0.365	0.208	0.091	0.085	0.233	0.362	0.312	0.177	0.186	0.265	0.434	0.910
[Protein]	150.885	69.288	8.003	5.226	82.135	149.497	123.108	52.795	57.483	98.976	186.997	434.913
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
C1	0.180	0.035	0.016	0.002	0.067	0.061	0.110	0.018	0.018	0.158	0.432	0.659
C1	0.168	0.034	0.017	0.007	0.090	0.055	0.128	0.015	0.000	0.121	0.453	0.508
C1	0.082	0.017	0.004	0.002	0.026	0.028	0.131	0.027	0.008	0.121	0.452	0.663
C1	0.143	0.029	0.012	0.004	0.061	0.048	0.123	0.020	0.009	0.133	0.446	0.610
[Protein]	354.340	-242.882	-327.951	-373.090	-74.479	-142.188	248.438	-288.021	-347.049	302.257	1928.993	2784.896

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
C2	0.293	0.118	0.050	0.250	0.674	0.752	0.620	0.298	0.370	0.943	1.571	1.946
C2	0.334	0.127	0.068	0.285	0.723	0.817	0.681	0.320	0.405	1.020	1.629	2.032
C2	0.323	0.111	0.046	0.268	0.735	0.832	0.643	0.297	0.419	1.014	1.658	2.018
C2	0.317	0.119	0.055	0.268	0.711	0.800	0.648	0.305	0.398	0.992	1.619	1.999
[Protein]	125.712	22.587	-10.747	100.191	330.920	377.622	298.281	119.635	168.073	477.622	804.184	1001.753
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
C2	0.057	0.015	0.014	0.033	0.142	0.132	0.092	0.068	0.090	0.230	1.051	1.351
C2	0.082	0.019	0.021	0.043	0.198	0.149	0.132	0.095	0.097	0.229	1.130	1.350
C2	0.051	0.013	0.016	0.060	0.141	0.119	0.108	0.068	0.083	0.212	1.074	1.353
C2	0.063	0.016	0.017	0.045	0.160	0.133	0.111	0.077	0.090	0.224	1.085	1.351
[Protein]	-62.326	-310.590	-303.646	-156.076	442.882	302.257	184.201	8.854	76.563	772.743	5258.854	6646.007

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
Cp	0.058	0.023	0.029	0.145	0.429	0.777	0.946	0.764	0.323	0.733	1.437	2.007
Cp	0.084	0.035	0.060	0.208	0.498	0.822	0.997	0.812	0.354	0.799	1.398	2.065
Cp	0.077	0.014	0.058	0.193	0.496	0.829	0.954	0.822	0.293	0.582	1.334	2.238
CP	0.073	0.024	0.049	0.182	0.474	0.809	0.966	0.799	0.323	0.705	1.390	2.103
[Protein]	-1.198	-26.719	-13.698	55.573	207.830	382.309	463.733	377.101	129.115	327.795	684.566	1056.267
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
Cp	0.004	0.005	0.006	0.029	0.036	0.164	0.342	0.271	0.055	0.405	1.247	1.612
Cp	0.028	0.013	0.028	0.049	0.055	0.144	0.356	0.282	0.077	0.453	1.261	1.712
Cp	0.018	0.015	0.021	0.036	0.044	0.175	0.341	0.263	0.067	0.450	1.245	1.615
Cp	0.017	0.011	0.018	0.038	0.045	0.161	0.346	0.272	0.066	0.436	1.251	1.646
[Protein]	-305.382	-334.896	-296.701	-194.271	-157.813	446.354	1411.632	1024.479	-46.701	1878.646	6123.438	8182.465

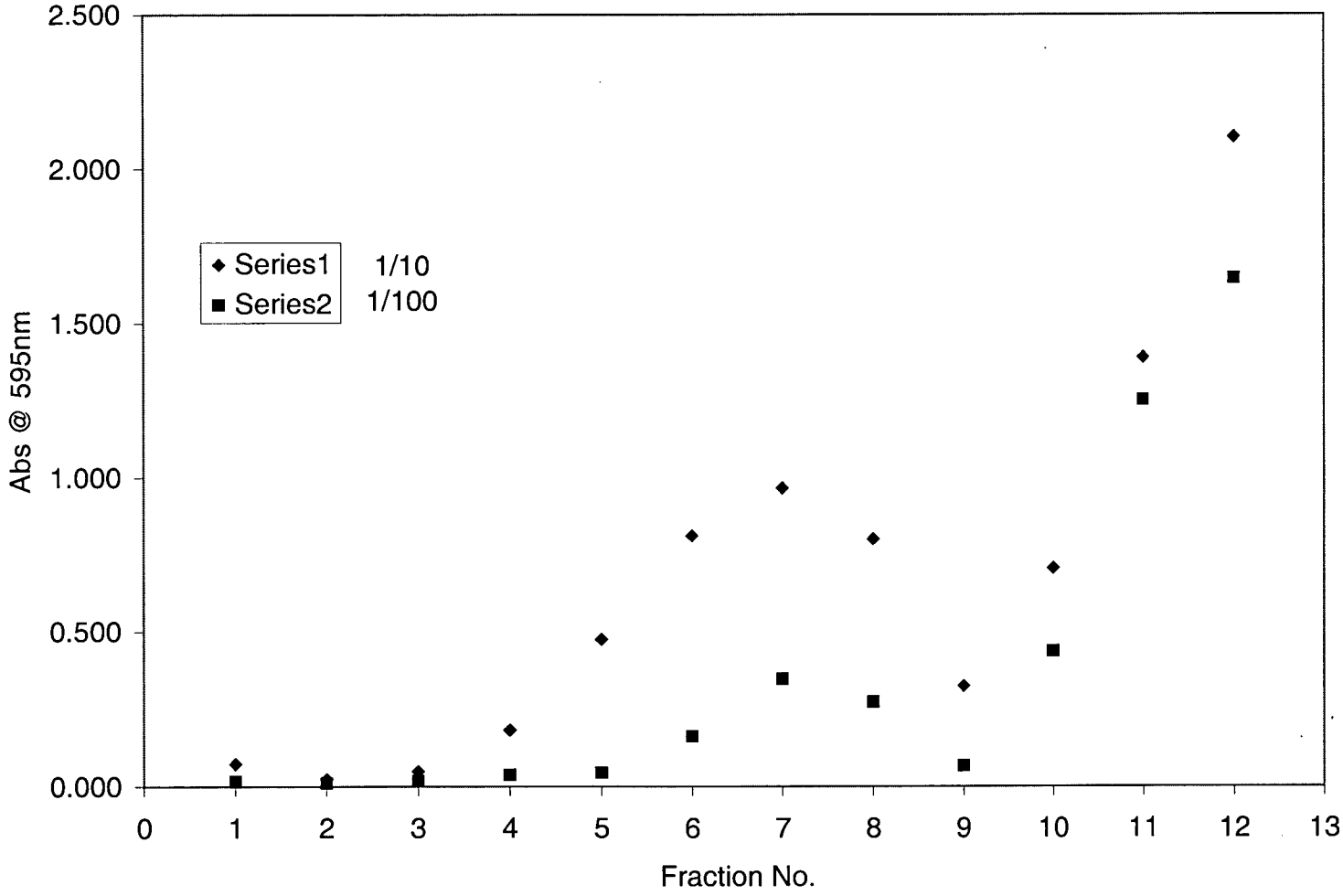

Figure I-3. Absorbance of hamster lipoprotein from magnesium deplete / 0.5% phytate added casein diet

Table I-4. Protein concentration of individual fractions of hamster plasma from magnesium replete / 0.5% phytate added casein diet

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
D1	0.226	0.134	0.071	0.053	0.346	0.554	0.448	0.254	0.251	0.410	0.860	1.469
D1	0.274	0.058	0.033	0.125	0.278	0.483	0.432	0.190	0.297	0.380	0.584	0.988
D1	0.188	0.141	0.069	0.090	0.224	0.410	0.523	0.286	0.261	0.591	1.050	1.846
D1	0.229	0.111	0.058	0.089	0.283	0.482	0.468	0.243	0.270	0.460	0.831	1.434
[Protein] (mg/ml)	**80.226**	**18.594**	**-9.184**	**7.309**	**108.003**	**211.997**	**204.358**	**87.517**	**101.233**	**200.538**	**393.767**	**707.830**
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
D1	0.059	0.025	0.023	0.030	0.018	0.079	0.118	0.059	0.001	0.105	0.161	1.038
D1	0.040	0.017	0.012	0.030	0.005	0.123	0.165	0.063	0.008	0.095	0.206	0.787
D1	0.019	0.003	0.000	0.022	0.017	0.073	0.127	0.061	0.011	0.109	0.278	1.693
D1	0.039	0.015	0.012	0.027	0.013	0.092	0.137	0.061	0.007	0.103	0.215	1.173
[Protein] (mg/ml)	**-187.326**	**-314.063**	**-331.424**	**-249.826**	**-322.743**	**85.243**	**319.618**	**-74.479**	**-357.465**	**144.271**	**727.604**	**5715.451**

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
D2	0.173	0.125	0.136	0.327	0.646	0.707	0.510	0.235	0.316	0.965	1.625	2.532
D2	0.202	0.119	0.141	0.368	0.649	0.735	0.548	0.243	0.342	1.135	1.759	2.269
D2	0.192	0.119	0.127	0.354	0.653	0.764	0.516	0.248	0.329	0.883	1.694	2.114
D2	0.189	0.121	0.135	0.350	0.649	0.735	0.525	0.242	0.329	0.994	1.693	2.305
[Protein] (mg/ml)	**59.219**	**23.802**	**30.920**	**142.899**	**298.976**	**343.767**	**234.045**	**86.823**	**132.135**	**478.663**	**842.378**	**1161.302**
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
D2	0.042	0.043	0.042	0.055	0.214	0.305	0.173	0.079	0.135	0.639	1.144	1.589
D2	0.055	0.035	0.036	0.048	0.204	0.351	0.174	0.065	0.141	0.654	1.104	1.512
D2	0.048	0.041	0.037	0.048	0.190	0.322	0.171	0.076	0.090	0.508	0.977	1.432
D2	0.048	0.040	0.038	0.050	0.203	0.326	0.173	0.073	0.122	0.600	1.075	1.511
[Protein] (mg/ml)	**-140.451**	**-185.590**	**-192.535**	**-130.035**	**663.368**	**1305.729**	**507.118**	**-10.243**	**243.229**	**2734.549**	**5206.771**	**7477.604**

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
Dp	0.033	0.016	0.041	0.145	0.510	0.901	1.009	0.818	0.465	0.630	1.432	2.012
Dp	0.047	0.016	0.060	0.183	0.557	0.991	1.085	0.935	0.465	0.639	1.464	1.864
Dp	0.036	0.016	0.050	0.141	0.510	0.936	1.054	0.917	0.425	0.635	1.465	2.280
Dp	0.039	0.016	0.050	0.156	0.526	0.943	1.049	0.890	0.452	0.635	1.454	2.052
[Protein] (mg/ml)	**-19.080**	**-30.885**	**-13.003**	**42.205**	**234.566**	**451.753**	**507.309**	**424.323**	**196.024**	**291.337**	**717.899**	**1029.531**
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
Dp	0.011	0.009	0.012	0.023	0.066	0.134	0.274	0.164	0.052	0.356	1.282	1.492
Dp	0.017	0.009	0.017	0.025	0.072	0.136	0.329	0.162	0.059	0.375	1.314	1.552
Dp	0.007	0.008	0.010	0.023	0.068	0.136	0.285	0.159	0.042	0.315	1.280	1.513
Dp	0.012	0.009	0.013	0.024	0.069	0.135	0.296	0.162	0.051	0.349	1.292	1.519
[Protein] (mg/ml)	**-331.424**	**-347.049**	**-324.479**	**-268.924**	**-34.549**	**312.674**	**1149.479**	**449.826**	**-126.563**	**1423.785**	**6336.979**	**7519.271**

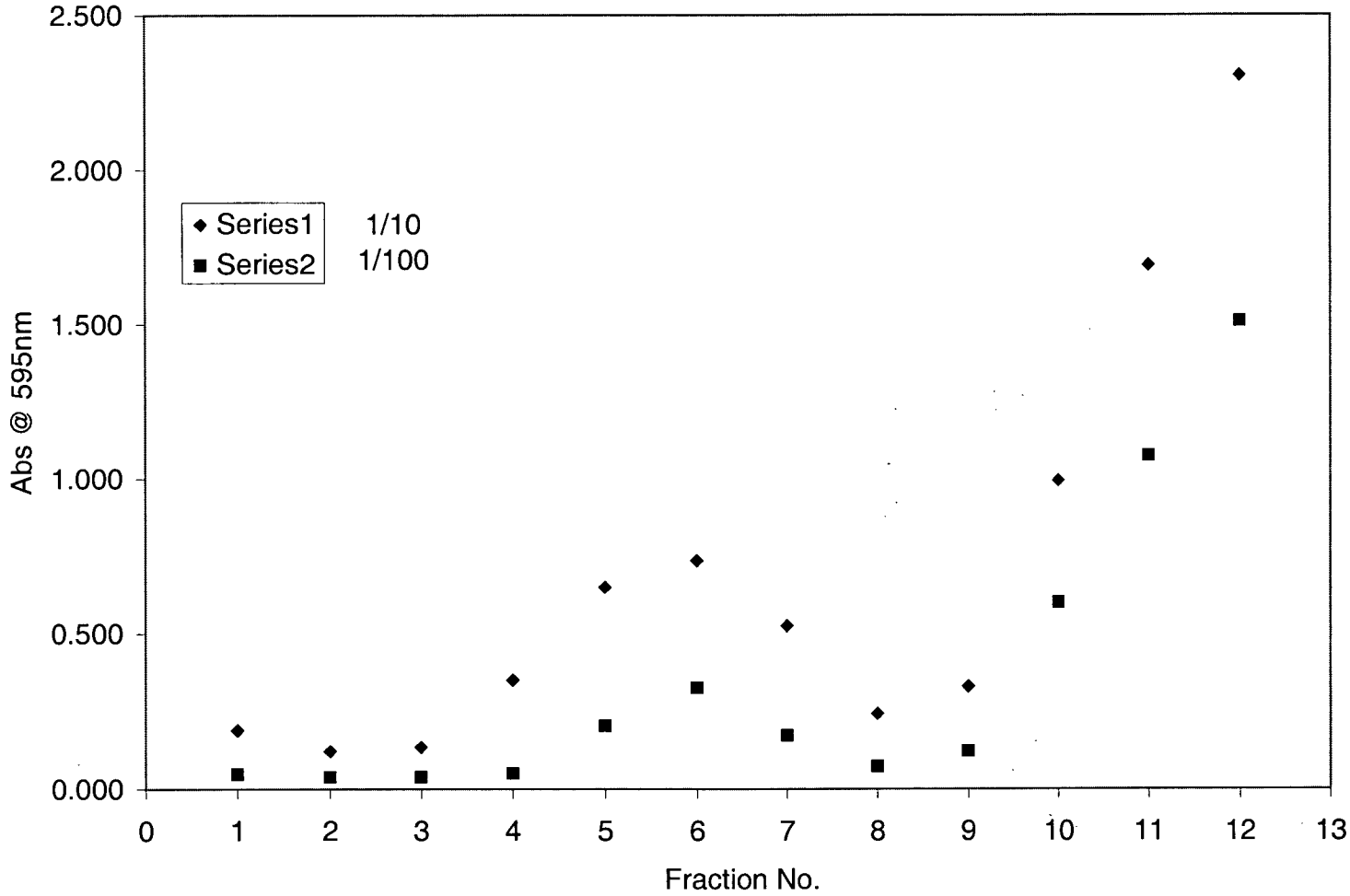

Figure I-4. Absorbance of hamster lipoprotein from magnesium replete / 0.5% phytate added casein diet

Table I-5. Protein concentration of individual fractions of hamster plasma from magnesium deplete / no phytate added soy diet

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
E1	0.176	0.122	0.075	0.072	0.149	0.308	0.492	0.173	0.309	0.521	0.985	1.790
E1	0.265	0.122	0.082	0.089	0.258	0.370	0.341	0.224	0.314	0.621	1.096	1.803
E1	0.319	0.138	0.073	0.085	0.265	0.511	0.466	0.260	0.305	0.552	1.118	1.804
E1	**0.253**	**0.127**	**0.077**	**0.082**	**0.224**	**0.396**	**0.433**	**0.219**	**0.309**	**0.565**	**1.066**	**1.799**
[Protein]	92.726	27.101	0.712	3.490	77.448	167.205	186.302	74.844	121.892	254.878	516.163	897.760
	1/100	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**
E1	0.064	0.014	0.026	0.019	0.081	0.204	0.016	0.105	0.079	0.205	0.666	1.655
E1	0.058	0.009	0.025	0.001	0.075	0.091	0.021	0.089	0.081	0.219	0.746	1.775
E1	0.044	0.016	0.019	0.016	0.052	0.115	0.011	0.085	0.072	0.201	0.733	1.730
E1	**0.055**	**0.013**	**0.023**	**0.012**	**0.069**	**0.137**	**0.016**	**0.093**	**0.077**	**0.208**	**0.715**	**1.720**
[Protein]	-103.993	-324.479	-270.660	-329.688	-31.076	319.618	-308.854	92.188	10.590	692.882	3331.771	8566.146

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
Ep	0.192	0.051	0.090	0.156	0.447	0.805	0.970	0.678	0.311	0.577	1.356	1.777
Ep	0.206	0.056	0.102	0.203	0.519	0.885	1.068	0.802	0.278	0.332	0.915	1.458
Ep	0.169	0.053	0.097	0.161	0.483	0.876	1.029	0.770	0.238	0.561	1.316	1.933
Ep	**0.189**	**0.053**	**0.096**	**0.173**	**0.483**	**0.855**	**1.022**	**0.750**	**0.276**	**0.490**	**1.196**	**1.723**
[Protein]	59.219	-11.441	10.955	51.059	212.344	406.267	493.247	351.406	104.358	215.990	583.524	858.003
	1/100	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**
Ep	0.016	0.145	0.020	0.048	0.049	0.209	0.427	0.218	0.077	0.261	1.239	1.664
Ep	0.014	0.013	0.020	0.031	0.053	0.168	0.391	0.188	0.081	0.230	1.234	1.485
Ep	0.028	0.026	0.074	0.041	0.049	0.165	0.368	0.182	0.057	0.226	0.860	1.701
Ep	**0.019**	**0.061**	**0.038**	**0.040**	**0.050**	**0.181**	**0.395**	**0.196**	**0.072**	**0.239**	**1.111**	**1.617**
[Protein]	-291.493	-72.743	-194.271	-183.854	-130.035	548.785	1666.840	628.646	-18.924	852.604	5394.271	8027.951

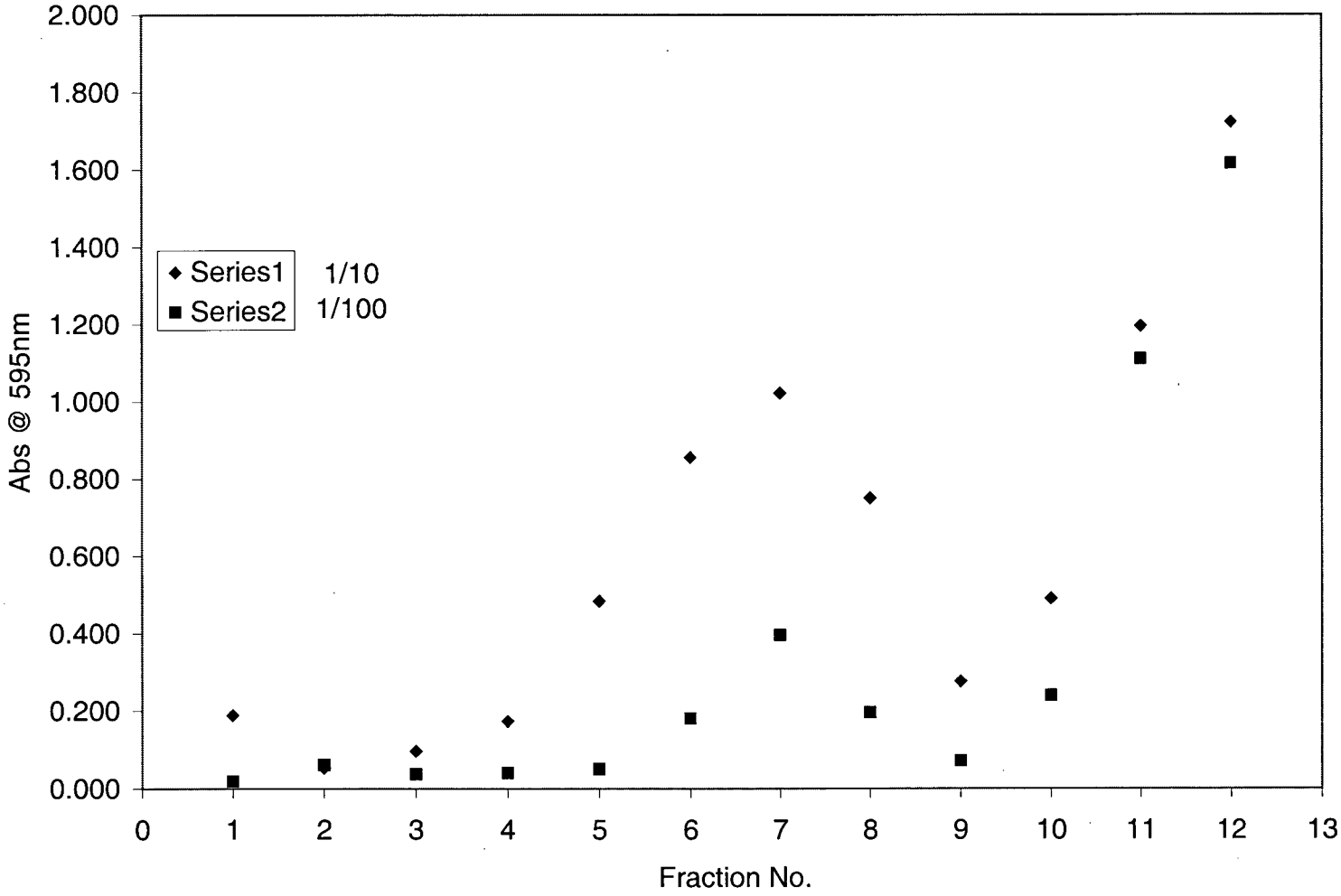

Figure I-5. Absorbance of hamster lipoprotein from magnesium deplete / no phytate added soy diet

Table I-6. Protein concentration of individual fractions of hamster plasma from magnesium replete / no phytate added soy diet

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
F1	0.168	0.158	0.208	0.298	0.305	0.701	0.537	0.249	0.316	0.348	0.547	0.783
F1	0.157	0.167	0.209	0.266	0.428	0.538	0.415	0.345	0.380	0.603	0.809	0.799
F1	0.189	0.151	0.213	0.338	0.614	0.784	0.621	0.343	0.242	0.460	0.932	0.828
F1	**0.171**	**0.159**	**0.210**	**0.301**	**0.449**	**0.674**	**0.524**	**0.312**	**0.313**	**0.470**	**0.763**	**0.803**
[Protein]	50.017	43.420	70.156	117.378	194.635	311.997	233.872	123.455	123.628	205.747	358.003	379.184
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
F1	0.067	0.036	0.035	0.094	0.133	0.135	0.088	0.026	0.034	0.181	0.336	0.561
F1	0.066	0.037	0.035	0.097	0.135	0.157	0.107	0.036	0.088	0.315	0.909	1.432
F1	0.046	0.036	0.049	0.092	0.200	0.251	0.177	0.026	0.076	0.314	0.906	1.541
F1	**0.060**	**0.036**	**0.040**	**0.094**	**0.156**	**0.181**	**0.124**	**0.029**	**0.066**	**0.270**	**0.717**	**1.178**
[Protein]	-81.424	-202.951	-185.590	99.132	420.313	550.521	253.646	-239.410	-48.438	1014.063	3342.188	5743.229

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
F2	0.172	0.137	0.172	0.421	0.729	0.800	0.644	0.295	0.372	1.020	1.558	1.833
F2	0.175	0.129	0.172	0.426	0.755	0.841	0.648	0.308	0.387	1.003	1.546	1.833
F2	0.196	0.136	0.200	0.447	0.755	0.756	0.650	0.334	0.432	1.052	1.541	1.919
F2	**0.181**	**0.134**	**0.181**	**0.431**	**0.746**	**0.799**	**0.647**	**0.312**	**0.397**	**1.025**	**1.548**	**1.862**
[Protein]	55.052	30.573	55.226	185.434	349.497	376.927	297.934	123.455	167.552	494.635	767.205	930.399
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
F2	0.049	0.038	0.049	0.102	0.315	0.417	0.187	0.070	0.115	0.689	1.161	1.685
F2	0.047	0.040	0.041	0.091	0.304	0.422	0.193	0.077	0.135	0.737	1.222	1.735
F2	0.048	0.042	0.054	0.100	0.297	0.338	0.187	0.062	0.132	0.717	1.187	1.671
F2	**0.048**	**0.040**	**0.048**	**0.098**	**0.305**	**0.392**	**0.189**	**0.070**	**0.127**	**0.714**	**1.190**	**1.697**
[Protein]	-142.188	-183.854	-142.188	116.493	1198.090	1651.215	592.188	-29.340	271.007	3328.299	5805.729	8446.354

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
Fp	0.063	0.022	0.020	0.117	0.591	0.954	1.066	0.818	0.350	0.738	1.493	2.441
Fp	0.061	0.019	0.029	0.115	0.617	0.986	1.064	0.853	0.352	0.733	1.493	1.986
Fp	0.065	0.020	0.026	0.104	0.612	0.972	1.086	0.832	0.344	0.740	1.537	2.105
Fp	**0.063**	**0.020**	**0.025**	**0.112**	**0.607**	**0.971**	**1.072**	**0.834**	**0.349**	**0.737**	**1.508**	**2.177**
[Protein]	-6.406	-28.628	-26.198	19.115	276.753	466.337	519.115	395.330	142.378	344.635	746.024	1094.809
	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100	1/100
Fp	0.020	0.019	0.017	0.028	0.137	0.227	0.542	0.218	0.076	0.300	1.378	1.562
Fp	0.031	0.030	0.027	0.033	0.143	0.240	0.566	0.238	0.081	0.317	1.423	1.594
Fp	0.026	0.025	0.027	0.031	0.125	0.218	0.519	0.199	0.075	0.299	1.410	1.580
Fp	**0.026**	**0.025**	**0.024**	**0.031**	**0.135**	**0.228**	**0.542**	**0.218**	**0.077**	**0.305**	**1.404**	**1.579**
[Protein]	-258.507	-263.715	-268.924	-232.465	310.938	797.049	2432.465	744.965	10.590	1198.090	6918.576	7830.035

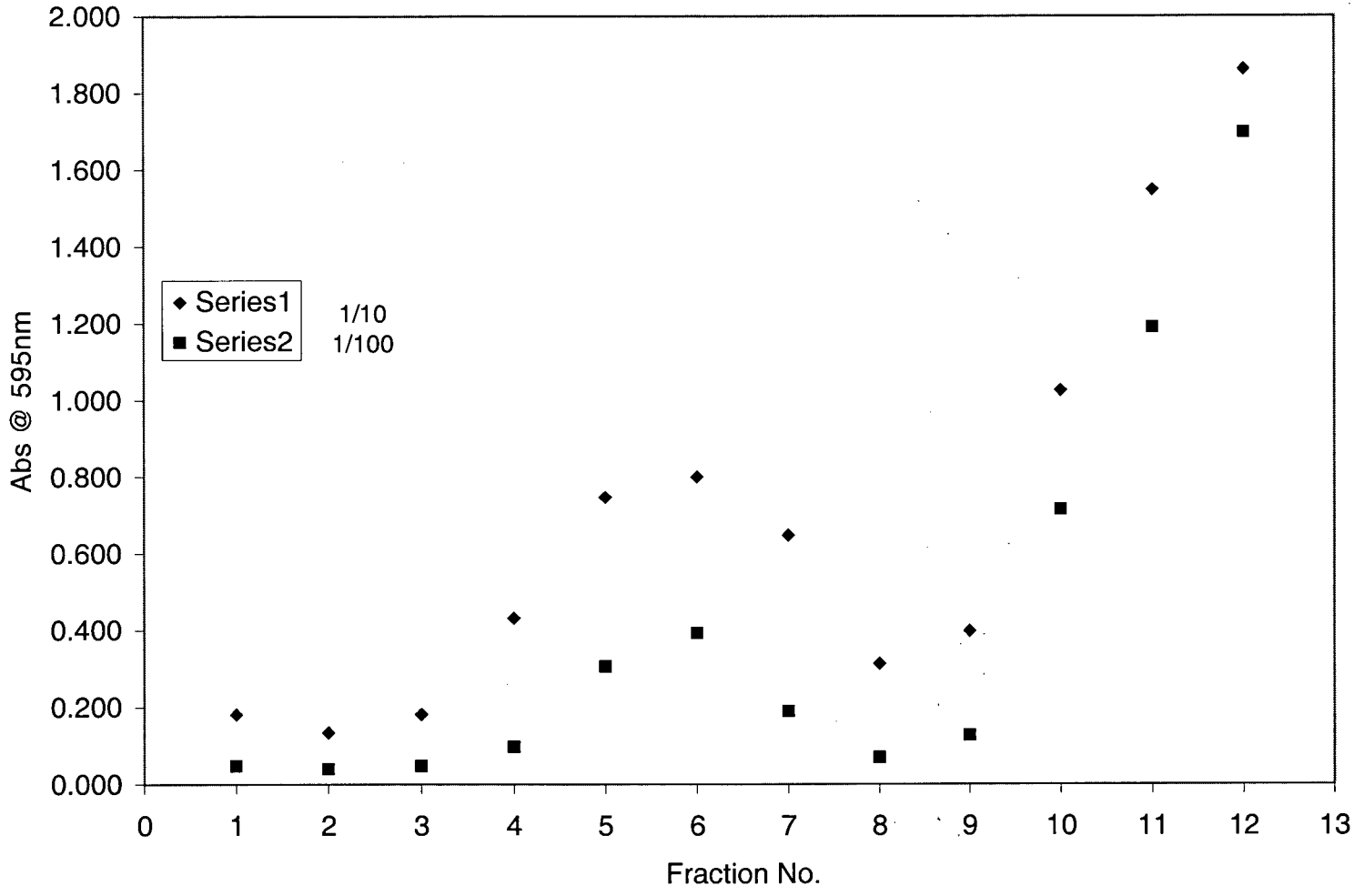

Figure I-6. Absorbance of hamster lipoprotein from magnesium replete / no phytate added soy diet

Table I-7. Protein concentration of individual fractions of hamster plasma from magnesium deplete / 0.5% phytate added soy diet

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
Gp	0.120	0.048	0.041	0.133	0.477	0.917	1.080	0.956	0.393	0.381	1.211	2.028
Gp	0.113	0.034	0.041	0.139	0.483	0.875	1.004	0.989	0.387	0.397	1.302	2.109
Gp	0.099	0.033	0.038	0.115	0.357	0.831	1.047	0.917	0.391	0.383	1.202	2.200
Gp	**0.111**	**0.038**	**0.040**	**0.129**	**0.439**	**0.874**	**1.044**	**0.954**	**0.390**	**0.387**	**1.238**	**2.112**
[Protein]	18.420	-19.253	-18.385	27.969	189.427	416.163	504.358	457.656	164.080	162.344	605.747	1060.955
	1/100	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**
Gp	0.014	0.010	0.007	0.010	0.057	0.198	0.364	0.308	0.077	0.147	1.016	1.511
Gp	0.024	0.014	0.014	0.014	0.055	0.198	0.375	0.296	0.084	0.151	1.008	1.555
Gp	0.029	0.024	0.012	0.017	0.070	0.190	0.317	0.271	0.073	0.154	1.041	1.538
Gp	**0.022**	**0.016**	**0.011**	**0.014**	**0.061**	**0.195**	**0.352**	**0.292**	**0.078**	**0.151**	**1.022**	**1.535**
[Protein]	-275.868	-308.854	-334.896	-321.007	-76.215	625.174	1441.146	1126.910	14.063	392.535	4928.993	7600.868

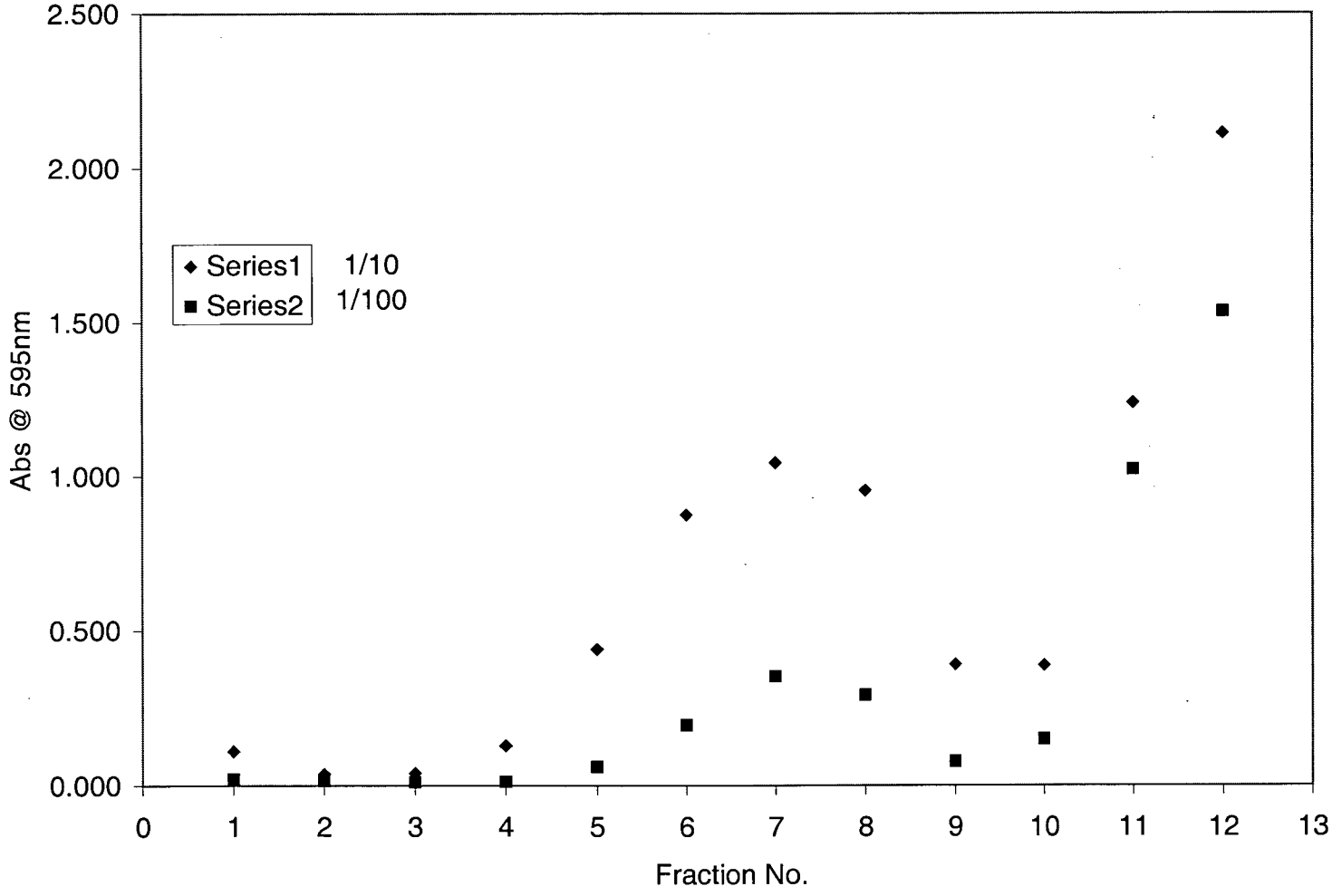

Figure I-7. Absorbance of hamster lipoprotein from magnesium deplete / 0.5% phytate added soy diet

Table I-8. Protein concentration of individual fractions of hamster plasma from magnesium replete / 0.5% phytate added soy diet

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
H2	0.233	0.161	0.338	0.460	0.618	0.656	0.559	0.301	0.373	0.986	1.711	1.915
H2	0.200	0.174	0.281	0.427	0.609	0.682	0.519	0.254	0.386	0.961	1.533	1.994
H2	0.207	0.179	0.325	0.485	0.665	0.703	0.580	0.303	0.373	0.904	1.556	1.679
H2	**0.213**	**0.171**	**0.315**	**0.457**	**0.631**	**0.680**	**0.553**	**0.286**	**0.377**	**0.950**	**1.600**	**1.863**
[Protein]	71.892	50.017	124.670	198.976	289.253	315.122	248.628	109.740	157.309	455.747	794.115	930.920
	1/100	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**
H2	0.006	0.000	0.006	0.037	0.383	0.452	0.203	0.051	0.088	0.581	1.330	1.593
H2	0.007	0.000	0.016	0.031	0.375	0.433	0.199	0.057	0.098	0.615	1.325	1.668
H2	0.000	0.000	0.009	0.031	0.384	0.757	0.205	0.150	0.097	0.533	1.396	1.626
H2	**0.004**	**0.000**	**0.010**	**0.033**	**0.381**	**0.547**	**0.202**	**0.086**	**0.094**	**0.576**	**1.350**	**1.629**
[Protein]	-369.618	-392.188	-338.368	-220.313	1590.451	2458.507	661.632	55.729	99.132	2609.549	6640.799	8092.188

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
Hp	0.090	0.016	0.020	0.166	0.661	0.884	1.024	0.937	0.456	0.713	1.423	2.046
Hp	0.090	0.008	0.025	0.162	0.665	0.910	1.024	0.937	0.482	0.686	1.439	1.897
Hp	0.088	0.004	0.016	0.136	0.651	0.905	1.048	0.962	0.480	0.907	1.449	1.985
Hp	**0.089**	**0.009**	**0.020**	**0.155**	**0.659**	**0.900**	**1.032**	**0.945**	**0.473**	**0.769**	**1.437**	**1.976**
[Protein]	7.309	-34.358	-28.628	41.337	304.010	429.358	498.281	453.142	206.962	361.128	709.219	989.948
	1/100	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**	**1/100**
Hp	0.005	0.002	0.000	0.008	0.112	0.203	0.447	0.323	0.064	0.356	1.336	1.639
Hp	0.016	0.004	0.000	0.015	0.119	0.205	0.445	0.333	0.067	0.366	1.327	1.708
Hp	0.016	0.008	0.002	0.016	0.102	0.181	0.426	0.297	0.053	0.335	1.306	1.772
Hp	**0.012**	**0.005**	**0.001**	**0.013**	**0.111**	**0.196**	**0.439**	**0.318**	**0.061**	**0.352**	**1.323**	**1.706**
[Protein]	-327.951	-367.882	-388.715	-324.479	185.938	630.382	1896.007	1262.326	-72.743	1442.882	6498.438	8494.965

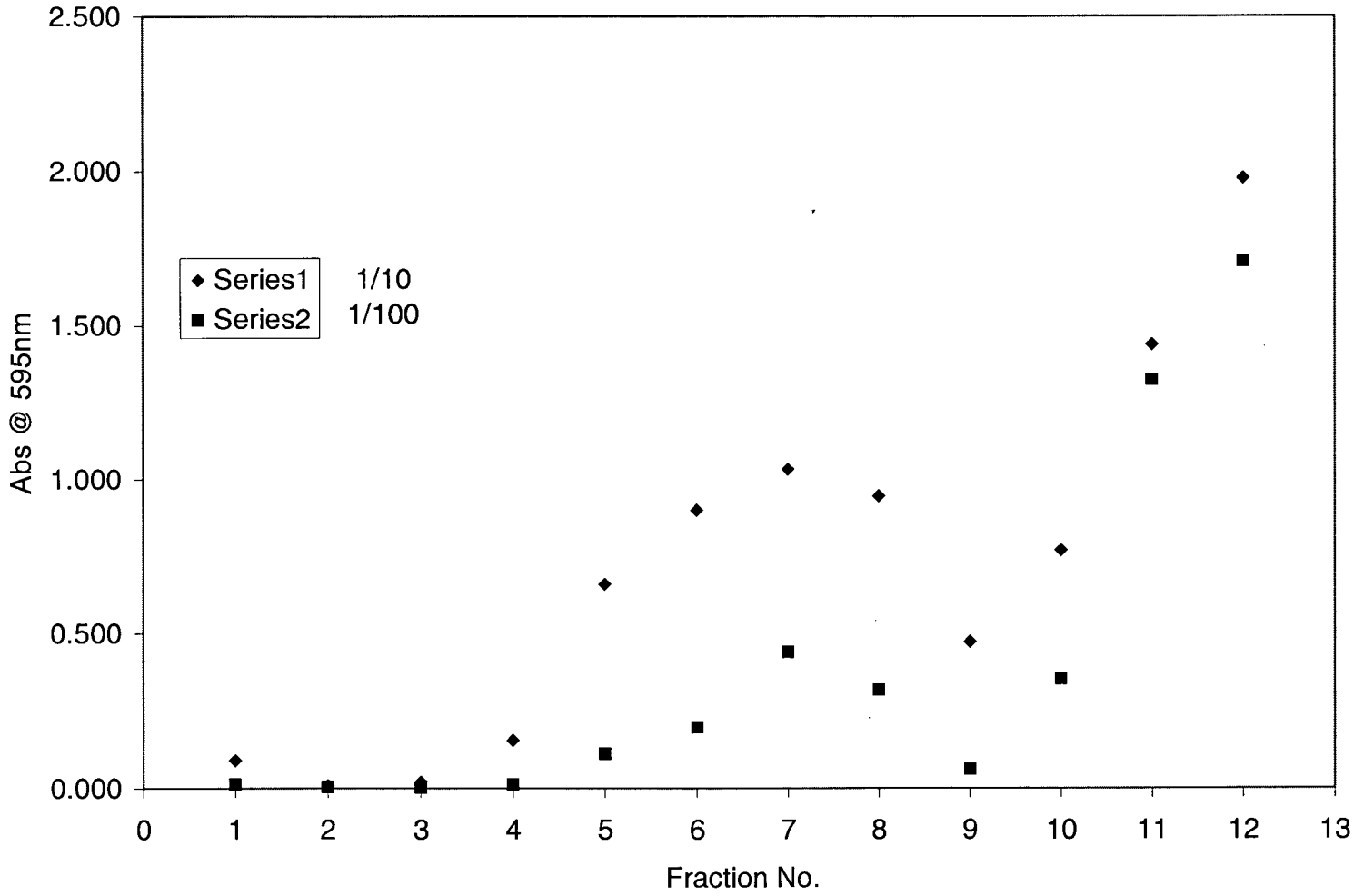

Figure I-8. Absorbance of hamster lipoprotein from magnesium replete / 0.5% phytate added casein diet

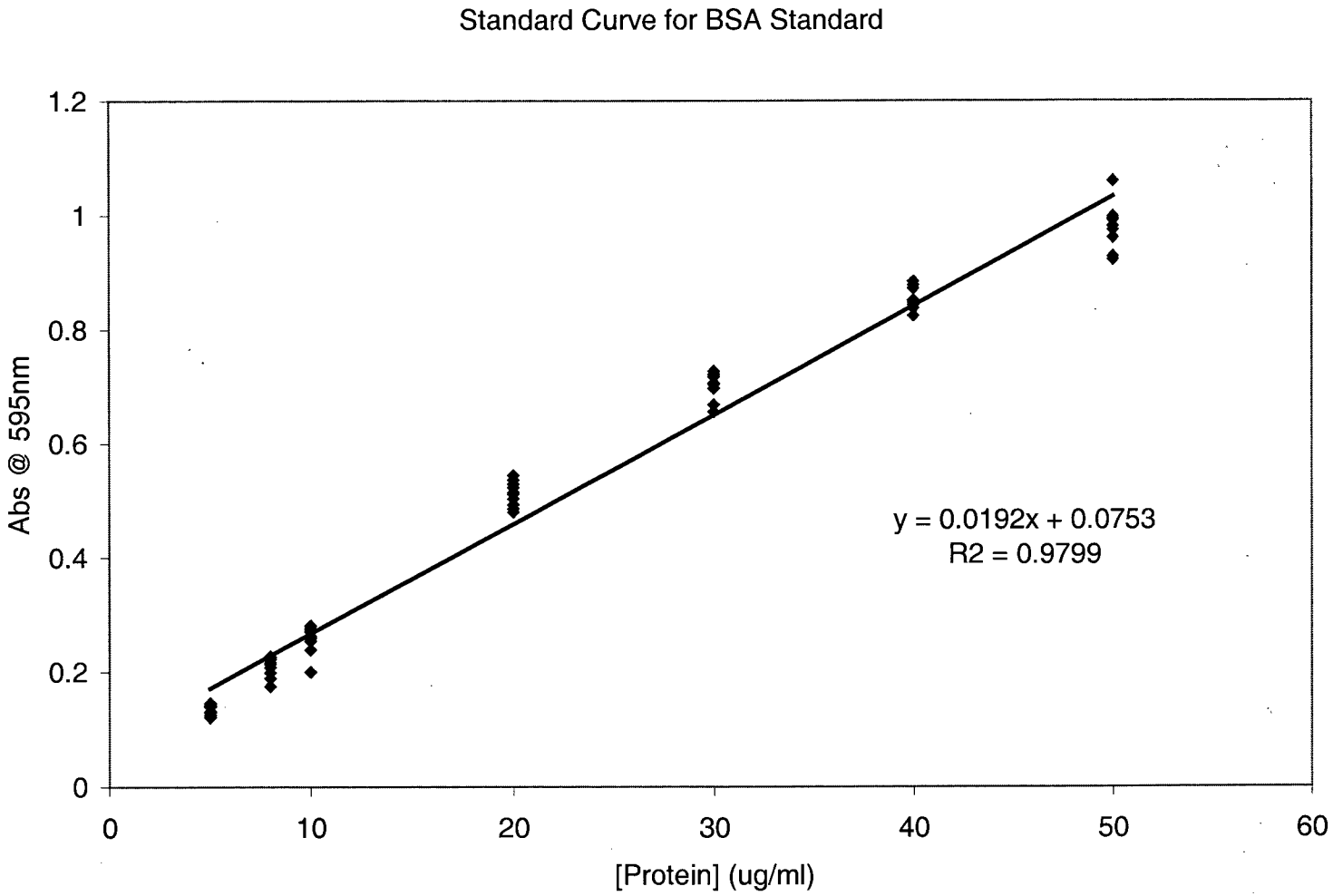

Figure I-9. BSA Standard Curve