Effects of Dietary Mg Status and Susceptibility to Hypertension on In-Vitro Lipid Peroxidation in Rats

by

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ABSTRACT

This study was designed to determine the association between dietary Mg (Mg) status on induction of hypertension in both normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). The particular interest was the link between lipoprotein lipid composition and susceptibility to oxidation and development of hypertension caused by Mg status. Six-week old male SHR and WKY were fed a synthetic diet with varying Mg levels (Mg-replete and deplete) for eight weeks. Two experiments were conducted with animals fed either casein (Experiment I) or soy (Experiment II) protein diets. SHR exhibited significantly (P<0.05) lower plasma cholesterol, triglyceride and phospholipid concentrations than WKY rats, regardless of dietary protein source. In Experiment I, Mg deficiency in casein fed rats significantly (P<0.05) increased plasma and lipoproteins (VLDL and LDL) cholesterol concentrations in both WKY and SHR strains. These results indicated that Mg deficiency significantly (P<0.05) increased plasma and lipoprotein cholesterol concentrations in a similar way in both WKY and SHR rats, regardless of differences in lipid metabolism between these two inbred strains of rats. In Experiment II, the hypercholesterolemic effect of Mg deficiency was diminished when rats were fed a soy-protein diet. Mg deficiency significantly increased plasma cholesterol concentrations only in SHR rats, but not in WKY rats. Lipoproteins isolated from SHR produced less TBARs and fluorescence after 180-min Cu^{2+}-induced forced peroxidation, and, thereby, were less susceptible to lipid peroxidation, than those isolated from WKY. SHR exhibited significantly (P<0.05) higher systolic blood pressure than WKY rats, and rats fed the Mg-deficient diet showed significantly (P<0.05) higher systolic blood pressure than counterparts fed the Mg-replete diet in both Experiments I and II. Mg deficiency also significantly (P<0.05) enhanced the genetic predisposition of the SHR to hypertension, regardless of dietary protein
source. It can be concluded from the present study that greater systolic blood pressure observed in Mg-deficient rats was in agreement with an increased lipoprotein susceptibility to forced peroxidation.
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<tr>
<td>AAS</td>
<td>Atomic absorption spectrophotometer</td>
</tr>
<tr>
<td>ACAT</td>
<td>Acyl-coenzyme A cholesterol acyltransferase</td>
</tr>
<tr>
<td>Apo</td>
<td>Apolipoprotein</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>$^{45}$Ca</td>
<td>Calcium isotope with atomic mass of 45</td>
</tr>
<tr>
<td>CEPT</td>
<td>Cholesterol ester transfer protein</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper element</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>EDRF</td>
<td>Endothelium-derived relaxing factor</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>FGF</td>
<td>Fibroblast growth factor</td>
</tr>
<tr>
<td>HB-EGF</td>
<td>Heparin binding epidermal growth factor</td>
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<td>HDL</td>
<td>High density lipoprotein</td>
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<tr>
<td>HMG-CoA reductase</td>
<td>Beta-hydroxy-beta-methylglutaryl CoA reductase</td>
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<tr>
<td>IDL</td>
<td>Intermediate density lipoprotein</td>
</tr>
<tr>
<td>IHD</td>
<td>Ischemic heart disease</td>
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<tr>
<td>IL-1</td>
<td>Interleukin-1-beta</td>
</tr>
<tr>
<td>LCAT</td>
<td>Lecithin cholesterol acyltransferase</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
</tr>
<tr>
<td>LPP</td>
<td>Lipoprotein peroxidation</td>
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<tr>
<td>MCSF</td>
<td>Monocyte colony stimulating factor</td>
</tr>
<tr>
<td>Mg</td>
<td>Mg</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chemotactic protein</td>
</tr>
<tr>
<td>PAF</td>
<td>Platelet-activating factor</td>
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<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
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<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
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<tr>
<td>SHR</td>
<td>Spontaneous hypertensive rats</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>TBARS</td>
<td>2-Thiobarbituric acid reactive substances</td>
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<tr>
<td>TNF alpha</td>
<td>Tumor necrosis factor alpha</td>
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<tr>
<td>TRL</td>
<td>Triacylglycerol-rich lipoprotein</td>
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<tr>
<td>VLDL</td>
<td>Very low density lipoprotein</td>
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<tr>
<td>VSMC</td>
<td>Vascular smooth muscle cell</td>
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<td>WKY</td>
<td>Wistar-Kyoto rats</td>
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Introduction

Cardiovascular disease (CVD) remains the major cause of death in most Western countries. Also, many of these deaths are caused by ischemic heart disease (IHD) associated with atherosclerosis (Heart and Stroke Foundation, 1993). Atherosclerosis is characterized by the accumulation of cholesterol, derived from circulating lipoproteins (predominantly the low-density lipoprotein (LDL)), in the arterial intima. Macrophages and smooth muscle cells are the predominant lipid-loaded cells contributing to lipid modifications and accumulation in the formation of vascular lesions. In order to be taken up by macrophages to form foam cells, LDL particles have to be oxidatively modified (Steinberg et al., 1989). Studies showed that oxidized LDL could be taken up more rapidly by macrophages than native LDL (Steinberg et al., 1989). Lipoprotein peroxidation is a prerequisite step for the development of atherosclerosis.

Certain factors, such as hyperlipidemia, hypertension and Mg deficiency can increase the risk for atherosclerosis. Mg deficiency accelerates development of atherosclerosis by increasing plasma cholesterol concentration and, thereby, enhancing lipid deposition in the intima. Short- or long-term Mg deficiency produces hyperlipidemia in rats, mainly due to increased plasma triglyceride and free cholesterol concentrations (Gueux et al., 1995; Lerma et al., 1995). Increased plasma triglyceride level in Mg deficient rats is a consequence of decreased lipoprotein lipase (LPL) activity and triglyceride clearance (Rayssiguier and Gueux, 1983). Increased plasma cholesterol level in Mg deficient animals is mainly due to decreased lecithin-cholesterol acyl transferase (LCAT) activity (Gueux et al., 1984) and increased β-hydroxy-β-methylglutaryl-Co A (HMG-CoA) reductase activity (Golf et al., 1991). Reduced LCAT
activity in Mg deficient animals decreases cholesteryl ester concentration in HDL and increases plasma circulating cholesterol concentration. Increased activity of HMG-CoA reductase increases cholesterol biosynthesis. Many experimental studies investigated the effects of Mg-deficiency on the lipid metabolism, and also on plasma and lipoproteins lipid concentrations. However, all experiments were conducted only on normotensive animals, such as weanling rats (Rayssiguier and Gueux, 1983) and Wistar rats (Rayssiguier et al., 1993), but not on hypertensive animals. Therefore, the purpose of the present study was to explore whether the effects of dietary Mg deficiency on plasma and lipoproteins lipid concentrations were similar in Wistar Kyoto rats (WKY) and their hypertensive counterparts, spontaneously hypertensive rats (SHR).

It is well known that hypertension participates in atherogenesis by producing structural or functional abnormalities in the blood vessel wall, such as endothelial injury or/and dysfunction, cell membrane abnormalities, vascular cells proliferation and growth abnormalities, and platelet dysfunction (Dzau, 1990). For instance, dysfunctional endothelium becomes an ineffective barrier to retard LDL particles, platelets and monocytes from adhering to the blood vessel wall and migrating into the intima. Therefore, more LDL will be oxidized in the subendothelial space and taken up by macrophages to convert them into foam cells. The importance of lipoprotein oxidation in atherosclerosis development in hypertensive animals is not known. In the present study, susceptibility of lipoproteins, isolated from WKY and SHR rats, to forced peroxidation was examined in order to determine whether lipoproteins isolated from SHR are more susceptible to lipid oxidation than those from WKY. The susceptibility of lipoproteins isolated from Mg-deplete and replete rats was also studied in the present
study.

Genetic predisposition to hypertension, as well as dietary Mg deficiency, increase blood pressure of animals. Experimental studies showed that Mg deficiency increased vascular blood pressure by enhancing activity of circulating constrictors (Altura and Altura, 1981), decreasing synthesis and release of endothelial-derived relaxation factor (Altura et al., 1993), and increasing intracellular Ca$^{2+}$ concentration and vascular contractility (Altura and Altura, 1981). Since the combined effect of Mg-deficiency and genetic predisposition to hypertension on the blood pressure was not investigated in those studies. The purpose of the present study was to investigate whether Mg deficiency would further increase the blood pressure of SHR rats.

Vasdev and his co-workers (2001) reported that excess endogenous aldehydes played a major role in hypertension by binding sulfhydryl groups of membrane proteins, altering Ca$^{2+}$ channels and increasing cytosolic Ca$^{2+}$ concentration and vascular blood pressure. Increased lipoprotein peroxidation might also lead to blood pressure elevation because lipoprotein oxidation generates a broad spectrum of oxidized fatty acid fragments and shorter chain aldehydes (e.g. malondialdehyde and 4-hydroxynonenal) (Steinbrecher, 1987). Since Gueux and his co-workers (1995) showed that Mg deficiency increased lipoprotein susceptibility to lipid peroxidation, increased lipoprotein susceptibility to lipid peroxidation might contribute to induction of hypertension observed in Mg-deficient rats by producing excessive aldehydes. The present study was designed to examine the link between lipoprotein susceptibility to oxidation and development of hypertension caused by Mg status.
**Thesis Hypotheses**

The hypotheses of this thesis were as follows:

1) The effect of Magnesium (Mg) status on relative changes in plasma and lipoprotein lipid concentrations is similar in both normotensive (WKY) and hypertensive (SHR) rats fed different protein sources.

2) Genetic predisposition to hypertension is associated with increased lipoprotein susceptibility to oxidation.

3) Dietary Mg deficiency leading to a reduced Mg balance will further enhance genetic predisposition of the SHR rats to hypertension.

4) The greater susceptibility of rats to Mg deficient-induced hypertensive conditions acts through increased lipoprotein peroxidation.
Thesis Objectives

The objectives of this thesis were as follows:

1) To determine the effects of Mg deficiency on plasma cholesterol, triglyceride and phospholipid concentrations and also on lipoproteins cholesterol concentrations in normotensive (WKY) and hypertensive (SHR) rats by feeding these two inbred strains with either Mg-replete or deplete diets.

2) To determine the effects of genetic strain (WKY and SHR) and Mg status (Mg-replete and deplete) on lipoprotein susceptibility to peroxidation, by comparing the effects of changes in Mg status in both WKY and SHR on the susceptibility of lipoproteins to in-vitro forced peroxidation.

3) To determine the effects of Mg deficiency on systolic blood pressure in WKY and SHR rats, by relating the specific changes in Mg status in WKY and SHR to their systolic blood pressure.
Literature Review

1.1 Pathophysiological Considerations of Atherosclerosis

Atherosclerosis is a disease of medium and large sized muscle arteries (such as coronary and carotid) and elastic arteries (such as aorta and iliac vessels). This disease has its origin early in life and is characterized by a complex multi-factorial process that involves endothelial cell damage or dysfunction, mononuclear cell adhesion, lipid accumulation and myointimal cell proliferation (Petty, 1991).

1.1.1 The Cellular Pathology of Atherosclerosis

The fatty streak is the earliest lesion of atherosclerosis and is characterized by the subendothelial aggregation of foam cells. These foam cells are grossly flat cholesterol rich lesions consisting of mainly macrophages and some smooth muscle cells (Petty, 1991). Accumulation of cholesterol within the arterial wall is an important characteristic of developing atherosclerotic lesions. The cholesterol is derived from plasma lipoproteins, such as low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL), which contain apolipoprotein B (apo-B) (Petty, 1991).

Macrophages, derived from blood monocytes, are the predominant lipid-loaded cells contributing to lipid modifications and accumulations. Subendothelial migration and localization of monocytes/macrophages in the intima are prerequisite of fatty-streak formation. Some cytokines (including: interleukins, e.g. interleukin-1β (IL-1β); chemokines, e.g. monocyte chemotactic protein-1 (MCP-1); interferons, e.g. interferon-γ (IFN-γ); colony-stimulating factors, e.g. monocyte colony-stimulating factors (M-CSF)) and adhesion molecules modulate the processes of monocyte rolling, arrest, attachment,
and direct migration into the intima (Schwartz and Valente, 1994).

Macrophages can also injure neighboring cells by forming toxic substances, including superoxide anion and lysosomal hydrolases. Macrophages could thus injure the overlying endothelium and set the stage for events culminating in the proliferative lesions of atherosclerosis (Fattah and Abdulla, 1991). Macrophages have also been associated with connective tissue proliferation. Some investigations showed that activated macrophages in culture secreted growth factors for fibroblasts, smooth-muscle cells and endothelium. Activated macrophages are capable of synthesizing and releasing growth factors specific for different target cells: platelet derived growth factor (PDGF), for connective tissue cells such as smooth muscle cells and fibroblast; fibroblast growth factor (FGF), for vascular endothelial cells; and epidermal growth factor or epidermal growth factor-like activity (transforming growth factor-alpha), for epithelial cells (Schwartz and Valente, 1994).

In more advanced atherosclerotic lesions, fatty streaks evolve into fatty plaques, which are made up of increased intimal smooth muscle cells surrounded by connective tissue matrix components and variable amounts of intracellular and extracellular lipid. The smooth muscle cell is the major cellular component in the mature atherosclerotic plaque. Modified smooth muscle cells are the major cell type that traps and processes cholesterol-rich lipid, which in turn enters the arterial lumen and is retained in the vessel wall (Petty, 1991). Some smooth muscle cells also synthesize large amounts of connective tissue components to form the dense fibrous cap, which can significantly expand the lesion size in advanced lesions (Petty, 1991).

1 Fibroblast is a connective tissue cell and forms the fibrous tissues in the body.
Migration and proliferation of vascular smooth muscle cells within the intima are important steps in the lesion formation. Smooth muscle cells contain receptors for modified LDL and growth factors. Migration and proliferation of smooth muscle cells are regulated by a number of cytokines and growth factors, including PDGF, FGF, IL-1β, IL-6, tumor necrosis factor α (TNF α), and heparin binding epidermal growth factor (HB-EGF) (Berliner and Heinecke, 1996). For instance, PDGF secreted by macrophages is a chemoattractant and binds to connective tissues at sites of endothelial injury to attract smooth muscle cells from the media into the intima (Petty, 1991).

The necrotic core plays an important role in plaque rupture. The lethality that has been observed may be due to necrosis or apoptosis. These phenomena appear to result from increased Ca flux, which can induce both necrosis and apoptosis (Berliner and Heinecke, 1996). Also, a number of toxic substances, including oxidized forms of cholesterol, peroxides of polyunsaturated fatty acids, and free radical generation within the plaque, may lead to smooth muscle cell necrosis (Petty, 1991). Formation of large thrombi on lesions is a major cause of myocardial infarction. Larger thrombi, formed when plaques rupture, may lead to closure of the blood vessel. Several factors, including endothelial denudation, plaque rupture, fibrin deposition, and platelet aggregation, regulate thrombus formation in atherosclerotic regions of vessels (Berliner and Heinecke, 1996).

2 IL-6 is a lymphokine produced by antigen- or mitogen-activated T cells, fibroblasts, and macrophages that serves as a differentiation factor for B cells and stimulates immunoglobulin production by B cells.

3 Necrosis is the sum of the morphological changes indicative of cell death and caused by the progressive degradative action of enzymes.

4 Apoptosis is fragmentation of a cell into membrane-bound particles that are then eliminated by phagocytosis.
1.1.2 The Lipid Infiltration and the Endothelial Injury Hypotheses

The lipid infiltration and endothelial injury hypotheses are the two well-defined theories that explain the atherosclerotic process. However, there are some associations between these two hypotheses that suggest interactions may provide a unified theory for the multifactorial nature of pathogenesis of atherosclerosis. In order to understand these two hypotheses, studies of the vessel wall structure, lipoprotein oxidation and associated pathophysiology are necessary.

1.1.2.1 Structure of the Vessel Wall

Normal artery consists of three layers: intima, media and adventitia. Intima contains a thin layer of endothelial cells. Glycocalyx, a layer of complex carbohydrate lining the luminal surface of the endothelium, plays a potential role in controlling the permeability of the endothelium. Arterial media is composed of smooth muscle cells. The other layer is the adventitia composed primarily of collagen, fibroblasts, adipocytes, mast cells, lipids and glycoproteins providing mechanical support for the vessel (Ross and Glomset, 1976).

1.1.2.2 Lipoprotein Physiology

Lipoproteins represent complex macromolecules that are main carriers of dietary or stored fat (cholesterol, cholesterol esters, triglycerides and phospholipids) in plasma. There are two main types of lipoproteins: 1) the triglyceride-rich lipoproteins consisting of chylomicrons and VLDL, and 2) the cholesterol-rich lipoproteins consisting of LDL and high density lipoproteins (HDL). The core lipoproteins, which are hydrophobic, are made up of triglycerides and cholesterol, and the envelope surrounding the core is made up of a single layer of phospholipids and free cholesterol with the polar head group.
oriented towards the hydrophilic aqueous environment of plasma. Apolipoproteins (apo), proteins associated with surface lipids, have major roles: coactivators of certain enzymatic reactions, and providing the ligand for specific receptors (Genest, 1990).

Table 1: Characteristics and compositions of lipoproteins (Genest, 1990).

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Density (g/mL)</th>
<th>Protein (%)</th>
<th>Lipid Composition (% weight/weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>weight/weight</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>Chylomicron</td>
<td>&lt;0.93</td>
<td>1-2</td>
<td>85-95</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.93-1.006</td>
<td>6-10</td>
<td>50-65</td>
</tr>
<tr>
<td>LDL</td>
<td>1.019-1.063</td>
<td>18-22</td>
<td>4-8</td>
</tr>
<tr>
<td>HDL</td>
<td>1.063-1.210</td>
<td>45-55</td>
<td>2-7</td>
</tr>
</tbody>
</table>

1.1.2.3 Lipoprotein Metabolism

Lipoprotein metabolism includes three pathway: exogenous pathway; endogenous pathway and the reverse HDL cholesterol pathway (Genest, 1990).

The exogenous pathway handles dietary and intestinal fats, which are composed of triglycerides and cholesterol. Triglycerides are firstly hydrolyzed to fatty acids and monoglycerides by pancreatic lipase in the intestine. Fatty acids and monoglycerides are absorbed in intestinal cells, reassembled as triglycerides and packaged in the triglyceride-rich chylomicrons which are large spherical particles consisting of apo B-48, AI, AII, and AIV. Chylomicrons are then secreted in intestinal lymphatics and enter the systemic circulation via the thoracic duct. In the circulation, they are acquired apo C. Chylomicrons, when circulating in the capillaries of adipose tissue and muscles, bind to lipoprotein lipase (LPL) located on the endothelial cells of adipose tissue and muscle.
LPL, when activated by apo CII on chylomicrons, rapidly hydrolyzes triglycerides into free glycerol, monoglycerides and fatty acids. Fatty acids then rapidly bind to albumin, and are incorporated in adipose tissue for storage or in muscle for energy utilization. After hydrolysis by LPL, chylomicrons, which have lost their surface components and are significantly diminished in size, are now termed a chylomicron remnant. This remnant, which is relatively enriched in cholesterol and phospholipid, consists of only apolipoproteins AI and AII. The remnant particles are then transferred to HDL or form particles consisting of bilayers of phospholipids and apolipoproteins AI. The remnant particles, after transfer to HDL, will acquire apo E from HDL particles and can be rapidly cleared from the circulation by receptor-mediated endocytosis through LDL receptor or a specific apo E receptor (Genest, 1990).

The endogenous pathway metabolizes stored fats. Fatty acids bound to albumin in plasma are rapidly taken up by the liver and packaged in VLDL particles. VLDL particles are secreted continuously from the liver. In contrast to chylomicrons and associated remnants, VLDL is made by the apo-B100 content. There is a single molecule of apo-B per VLDL particle, as well as the apo C and apo E. The secretion rate of VLDL is partly determined by the availability of triglycerides, whereas generation of the apo-B100 proteins is constant. The triglyceride substrate for the VLDL secretory pathway is derived essentially from three sources, all of which are regulated by food intake: 1) free fatty acids generated by lipolysis in adipose tissue through the action of hormone-sensitive lipase provide a major source of liver triglycerides, 2) hepatic uptake of poorly lipolysed remnant particles from either VLDL and chylomicrons also provide triglyceride, and 3) de novo synthesis of triglycerides in
liver (Karpe et al., 1995). Similar to the metabolic fate of chylomicrons, VLDL particles are hydrolyzed to fatty acids and glycerides by LPL, which is activated by apo CII on VLDL. The remnant intermediate density lipoproteins (IDL) are formed as they have acquired apo E and given up apo Al to HDL particles. Then, IDL particles are rapidly taken up via the apo E receptor or LDL receptor in the liver. When IDL core triglycerides are hydrolyzed to fatty acids and glycerides by hepatic LPL, the IDL particles are converted to LDL particles, which contain apo B and no longer have apo E.

When LDL particles circulate in the plasma, they are either taken up by extra hepatic tissues for metabolic needs or return to the liver via the LDL receptor (Genest, 1990). Goldstein and Brown (1982) found that LDL particles, once they were bound to the LDL receptors, were incorporated by receptor-mediated endocytosis in hepatic cells. This endocytotic vesicle containing LDL then fuses with a primary lysosome. The powerful acid hydrolases in the lysosome immediately begin to degrade both the protein and lipid moieties of the internalized LDL. The protein moiety is degraded completely to free amino acids, and these rapidly diffuse out of the lysosome and escape to the surrounding medium. Once apo-B is hydrolyzed, the LDL receptor recirculates to the surface of the cytoplasm. Cholesterol esters of the LDL are hydrolyzed by lysosomal acid cholesterol esterase, and free cholesterol within the LDL particle dissipates in the cytoplasm. Free cholesterol in the cytoplasm has three main effects: 1) it down-regulates production of beta-hydroxy-beta-methylglutaryl CoA reductase (HMG-CoA reductase), the rate limiting enzyme for sterol biosynthesis, thus decreasing the rate of endogenous cholesterol production when exogenous cholesterol is being delivered via LDL; 2) it increases acyl-coenzyme A cholesterol acyltransferase (ACAT) activity which
esterifies free cholesterol, leading to re-esterification and storage in ester form of any excess cholesterol taken up; 3) it down-regulates synthesis of LDL receptors, thereby retarding the rate at which additional LDL cholesterol is delivered to the cell beyond current requirement (Goldstein and Brown, 1982). These events allow hepatic cells to maintain an adequate level of intracellular cholesterol to be obtained from LDL particles or synthesized directly from acetyl-CoA.

The reverse cholesterol (HDL) pathway is a complex process. The initial step involves the net transfer of cholesterol from peripheral cell membranes to the liver. The intestine, chylomicrons, the liver and VLDL are the four different sources of HDL. Free cholesterol is obtained by nascent HDL particles and is then esterified by the plasma enzyme lecithin cholesterol acyl transferase (LCAT), which is activated by apo Al and provides a driving force for the net transfer of cholesterol from peripheral cells into the HDL. The cholesteryl ester formed by LCAT may either be transferred to other lipoproteins by plasma cholesteryl ester transfer protein (CETP) or remain within HDL (Tall, 1995). Cholesterol esters, which are more hydrophobic than free cholesterol, move into the core of a growing HDL particle to form HDL-3. The HDL-3 particles increase in size and transform to HDL-2 particles as further esterification of free cholesterol occurs. Formation of HDL-2 is reversible as HDL-2 particles lose cholesterol esters via the CEPT, which mediates transfer of cholesterol ester from HDL-2 to triglyceride-rich lipoproteins or even LDL. Hepatic triglyceride lipase can convert HDL-2 into HDL-3 particles. HDL particles can acquire apo E and transfer it to triglyceride-rich particles, or can be taken up by the E receptor and catabolized. The
lipid component of HDL particles is preferentially removed by the liver and steroid hormone-producing tissues, such as ovaries and adrenals (Genest, 1990). There are two well-defined pathways for removal of HDL cholesteryl ester from plasma: 1) direct clearance of apoE enriched, cholesteryl ester rich large HDL particles by hepatic LDL receptors, 2) transfer of HDL cholesteryl ester to triglyceride-rich lipoproteins, and subsequent removal by LDL-related receptor protein, or conversion to smaller particles with eventual removal by hepatic LDL related receptor protein or LDL receptors. Both pathways of reverse cholesterol transport result in the return of cholesterol to the liver (Tall, 1995). Apolipoproteins are preferentially removed in the liver. HDL cholesterol is well known as a protective lipoprotein against CVD development (Genest, 1990).

1.1.3 Lipoprotein Oxidation

Accumulation of cholesterol in the arterial intima is an important characteristic of atherosclerotic lesion. The accumulated cholesterol originates primarily from plasma lipoproteins containing apo-B, such as VLDL and LDL. In the pathogenesis of atherosclerosis, LDL peroxidation is the first step in the formation of an atherosclerotic plaque. In the intima, where antioxidant levels are very low, native LDL can be oxidatively modified by endothelial cells, macrophages and smooth muscle cells to generate a broad spectrum of oxidized fatty acid fragments and shorter chain aldehydes (e.g. malondialdehyde and 4-hydroxynonenal). Oxidized fatty acid fragments then attach covalently to and, thereby, mask the lysine ε-amino groups of apo-B present in VLDL and LDL particles (Steinbrecher et al., 1987). Since apo-B is the ligand for the LDL receptor (which is critically important in the removal of native LDL from plasma), the modified forms of apo-B on LDL particles cannot be recognized and removed by
regular LDL receptors. However, the modified LDL particles can be rapidly taken up by the scavenger receptors on monocytes/macrophages. Scavenger receptors on monocytes/macrophages recognize only oxidized LDL, but not native LDL (Goldstein et al., 1979). Following up-take of oxidized LDL, macrophages accumulate the cholesterol derived from oxidized LDL particles in an unregulated manner and, in doing so, form foam cells just beneath the vascular endothelium (Steinberg et al., 1989). Therefore, both LDL oxidation and recruitment of monocytes/macrophages into the intima are prerequisites for formation of fatty streaks.

1.1.4 The Lipid Infiltration and Endothelial Cell Damage Theories

The lipid infiltration hypothesis states that, in the presence of high plasma levels of LDL, LDL concentration in the subendothelial space is increased. More LDL will be oxidatively modified and taken up by macrophages to form foam cells, thereby accounting for the final complicated lesion seen in atherosclerosis.

In addition to faster up-take by macrophages to develop foam cells, oxidatively modified LDL particles are highly cytotoxic and atherogenic. Quinn and co-workers (1987) proposed four effects of oxidized LDL that could favor the atherogenic process:

1. Oxidized LDL can induce functional changes and injuries to the endothelial cells. Endothelial cells form the one cell thick lining of blood vessels, and constitute a dynamic interface between the blood and the rest of the body. Some important roles of the endothelium include: 1) maintenance of vascular tone and permeability; 2) regulation of leukocyte traffic; 3) modulation of homeostasis and thrombosis (Petty, 1991). Endothelial cells also determine the nature of the lipoproteins and other plasma constituents that reach the subendothelial space. Damaged endothelial cells
may promote the following two atherogenic processes: 1) decreasing the significant barrier to lipoprotein accumulation within the vessel wall; 2) initiating smooth muscle cell migration and proliferation within the vessel wall. Haudenschild and Chobanian (1981) found that a dysfunctional endothelium became an ineffective barrier unable to retard platelets and monocytes from adhering to the blood vessel wall. Also, injured endothelium releases excessive PDGFs and lowers growth-inhibiting factors that enhance proliferation and migration of vascular smooth muscle cells from the media to the intima of blood vessel wall (Dzau, 1990).

2. Oxidized LDL itself is actually chemotactic for the circulating monocyte and enhances subsequent recruitment of monocytes into the subendothelial space. In-vitro studies indicated that incubation of endothelial cells with oxidized LDL increased monocyte binding to endothelial cells (Berliner et al., 1990) and production of chemotactic activators, such as MCP-1 (Cushing et al., 1990) and monocyte colony stimulating factor (MCSF) (Rajavasisth et al., 1990) by endothelial cells.

3. Oxidized LDL inhibits motility and exiting of macrophages from the vessel wall. Thus, finally like a vicious cycle, more native LDL in the intima will be oxidized by the endothelial cells and macrophages. The result will be that more oxidized LDL is taken up by macrophages through scavenger receptors to generate a greater number of foam cells, accounting for the final complicated lesion in atherosclerosis (Quinn et al., 1987).

4. Cytotoxicity of oxidized LDL, leading to loss of endothelial integrity, possibly facilitates the entry of LDL or monocytes in the early stages and leads to frank
endothelial denudation later (Quinn et al., 1987).

1.2. Risk Factors for Atherosclerosis

There are certain factors, which can greatly increase the risk for CVD. Major risk factors for CVD include hyperlipidemia, diabetes mellitus, and hypertension. These risk factors act synergistically in enhancing the risk of coronary artery disease. In the following sections, the relationships of hyperlipidemia, Mg deficiency and hypertension with atherosclerosis will be discussed (Anderson et al., 1990).

1.2.1 Hyperlipidemia and Atherosclerosis

The lipid infiltration theory of atheroma formation has provided the rationale for most epidemiological investigations of coronary heart disease. Hyperlipidemia is a major risk factor for atherosclerosis. The increase in plasma LDL cholesterol level is correlated with an increase of cholesterol deposition in the intima forming the foam cells (Fattah and Abdulla, 1991). Also, increased vascular risk parallels increased LDL cholesterol, whereas high cholesterol levels associated with HDL favor cholesterol clearance from the artery. Rising HDL cholesterol levels decreases the risk for coronary heart disease (Genest, 1990).

1.2.2 Mg Deficiency

Mg in food represents the major portion of Mg intake in the general population. Recent surveys of the food intake of individuals have revealed that a major portion of the population have dietary Mg intake lower than recommended dietary allowances (Marx and Neutra, 1997). Several common clinical conditions, such as diabetes mellitus and chronic alcoholism, cause Mg deficiency (Altura and Altura, 1995). Chronic alcoholism has long been known to be associated with a serum and myocardial tissue
loss of Mg. In addition, 40-80% of chronic alcoholics develop high blood pressure. States of chronic diabetes mellitus are also associated with a tissue and serum hypomagnesemia. Despite the use of insulin, the incidence of high blood pressure in long-term diabetics is, again, between 40 and 80% (Altura and Altura, 1999). Mg deficiency is considered a risk factor for hypertension and coronary heart diseases (Altura and Altura, 1995).

Recent experimental investigations showed that Mg deficiency in rats increased lipoprotein susceptibility to peroxidation (Rayssiguier et al., 1993). Also, the important characteristic of hyperlipidemia associated with Mg deficiency in rats is the postprandial accumulation of triglyceride-rich lipoproteins. These lipoproteins isolated from Mg-deficient rats have increased susceptibility to peroxidation and thus might be potentially atherogenic (Gueux et al., 1995). In the following sections, the effects of Mg deficiency on the plasma lipid concentrations, proliferation of vascular smooth muscle cells, and blood pressure will be discussed.

1.2.2.1 Mg Deficiency and Hyperlipidemia

Short- or long-term Mg deficiency is associated with hyperlipidemia, mainly due to increased plasma concentrations of triglyceride and free cholesterol (Gueux et al., 1995; Lerma et al., 1995). Mg deficiency also produces a marked effect on the cholesterol distribution among the lipoprotein fractions, as shown by the increased cholesterol concentration in VLDL and LDL fractions and the decreased cholesterol concentration in HDL fraction (Rayssiguier et al., 1981). Mg deficient animals are prone to atherosclerosis since an increase of LDL/HDL cholesterol concentration increases the risk for lipid deposition in the intima.
An elevated plasma triglyceride level may occur from either increased secretion or impaired removal of triglyceride rich lipoproteins. Rayssiguier and Gueux (1983) demonstrated that hyperlipidemia induced by Mg deficiency was not due to the excessive production of liver lipids, but was due to the decreased clearance of circulating triglycerides. Hydrolysis of triglycerides, which results in free glycerols and free molecules of fatty acids, is catalyzed by LPL and represents a major step in the removal of plasma circulating triglycerides. Experimental studies showed that a significant increase in circulating triglyceride level in Mg deficient rat was a consequence of the decrease in LPL activity (Rayssiguier and Gueux, 1983).

The increased concentration of free cholesterol in Mg deficient animals is due to the impaired LCAT activity (Lerma et al., 1995). LCAT plays an important role in the reverse cholesterol transport, which is responsible for transporting excess cholesterol in peripheral tissues back to the liver for excretion. It is secreted by the liver and catalyzes formation of cholesteryl esters in HDL. Its role is to prevent unesterified cholesterol from accumulating in plasma. LCAT esterifies free cholesterol within HDL; the cholesteryl ester formed is then transferred from HDL to lower density lipoproteins by the action of cholesteryl ester transfer protein (CEPT). The cholesterol is removed from the plasma by hepatic clearance of the plasma lipoproteins (Genest, 1990). Thus, decreased LCAT activity can lead to increased circulating concentrations of free cholesterol together with a decreased concentration of cholesteryl esters (Rayssiguier, 1983).

Mg deficiency can also alter activities of other enzymes, such as HMG CoA reductase. HMG CoA reductase catalyzes reduction of 3-hydroxy-3-methylglutaryl CoA
to mevalonate, using NADPH as an electron donor. The reaction is a key rate-limiting step in the biosynthesis of cholesterol. Golf and his coworkers (1991) reported that Mg deficiency kept HMG CoA reductase in an activated state, which favors cholesterol biosynthesis, resulting in hypercholesterolemia in Mg deficient animals.

1.2.2.2 Role of Mg Deficiency in Vascular Smooth Muscle Cell Proliferation

As discussed earlier, vascular smooth muscle cell proliferation is well accepted as one of the primary components in the pathophysiology of atherosclerosis. Vascular smooth muscle cells are the predominant cells in arteries and are essential for the structural and functional integrity of blood vessels. In atherosclerosis, these cells undergo extensive proliferation and are transformed into foam cells, which accumulate lipids and become a major component of the atherosclerotic lesion.

Bussiere and his co-workers (1994) reported that serum from Mg deficient rats, in comparison with control rats, was shown to enhance cultured vascular smooth muscle cell proliferation. Weglicki and his co-workers (1992) also found that Mg deficiency activated macrophages to produce a systemic pro-inflammatory state. Elevated levels of macrophage-derived cytokines, IL-1, IL-6, and TNF-α, were observed in the plasma of Mg-deficient animals compared to pair-fed controls (Weglicki et al., 1992). Since cytokines can induce vascular smooth muscle cell proliferation, Mg-deficiency activated production of macrophage-derived cytokines is one of the causes of increased vascular smooth muscle cell proliferation observed in rats.

Inflammatory response observed in Mg deficient animals also plays an important role in development of hypertriglyceridemia, because several cytokines also cause a
rapid increase in serum triglycerides by stimulating liver lipogenesis\(^5\) and by decreasing LPL activity (Bussiere \textit{et al.}, 1994). In general, serum from Mg deficient animals contains factors that stimulate the proliferation of arterial medial cells, and hyperlipidemia associated with Mg deficiency causes lipid accumulation in vascular cells (Bussiere \textit{et al.}, 1994).

1.2.2.3 Mg deficiency-induced Hypertension

Acute and chronic hypomagnesemia in animals and humans are often associated with rises in blood pressure and elevations in peripheral vascular resistance in a variety of regions in the circulatory system (Altura and Altura, 1981). Altura and his coworkers (1984) found that rats fed moderately or more severely Mg deficient diets for 12 weeks showed significantly elevated arterial blood pressure and decreased lumen size, compared to control animals fed Mg-enriched diet. Mg deficient animals also showed greater constrictor responses to neurohumoral agents, such as adrenergic amines and vasopressin (Altura \textit{et al.}, 1984). Reductions in serum [Mg\(^{2+}\)] could narrow arterial and arteriolar lumen sizes by enhancing activity of circulating constrictors, thus possibly accounting for elevations in peripheral vascular resistance and changes in systemic blood pressure (Altura and Altura, 1981).

Extracellular Mg\(^{2+}\) exerts important actions on membrane permeability to Ca\(^{2+}\), as well as on binding and translocation of Ca\(^{2+}\). Mg\(^{2+}\) is an activator of adenylate cyclase, an enzyme involved in the synthesis of cyclic adenosine monophosphate\(^6\) (cAMP). Increased and decreased cAMP concentrations participate in vasodilation and

\(^5\) Lipogenesis is the formation of fat or the transformation of nonfat food materials into body fat.
\(^6\) Cyclic adenosine monophosphate is a cyclic nucleotide, adenosine 3', 5'-cyclic monophosphate, that serves as an intracellular and, in some cases, extracellular "second messenger" mediating the action of many peptide or amine
vasoconstriction, respectively. A decrease in cAMP in the absence of extracellular [Mg$^{2+}$] could result in an increased [Ca$^{2+}$] within the cytoplasm because there would be less cAMP-mediated Ca$^{2+}$ sequestration (Altura and Altura, 1981). Since a change in intracellular [Ca$^{2+}$] is a very important second messenger in facilitation of contraction or relaxation of vascular smooth muscle cells, a rise of intracellular [Ca$^{2+}$] modulated by a decrease of extracellular [Mg$^{2+}$] results in the elevated vascular tone and contractility. Thus, such a mechanism could, in part, be responsible for the Ca loading in heart and blood vessels, and the increased vascular tone and reactivity, which occurs in Mg deficient animals. An alternative and contributing mechanism is that the decrease of extracellular [Mg$^{2+}$] leads to inhibition of a Ca$^{2+}$-dependent APTase$^7$ at the membrane, which is Mg$^{2+}$-dependent, and that presumably extrudes Ca$^{2+}$ (Altura et al., 1993).

Mg$^{2+}$ also regulates the vascular blood pressure by controlling the synthesis and release of endothelial-derived relaxant factors. Endothelial cell layer plays an active and important role in modulating vascular tone by synthesizing and releasing a variety of endothelial-derived relaxant factors, which are controlled by the extracellular Mg$^{2+}$ concentration. For instance, an increase in intracellular [Ca$^{2+}$], resulting from the decrease of extracellular [Mg$^{2+}$], leads to the decrease of synthesis and release of prostacyclin (PGI$_2$), endothelial-derived relaxation factor (Altura et al., 1993). Laurent et al. (1999a) also reported that Mg deficiency is accompanied by an increase in plasma nitric oxide (NO)$^8$ level, which could result in vasodilation. However, since a low Mg concentration activates macrophages to produce a systemic pro-inflammatory state, the

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$^7$ Ca$^{2+}$-APTase is a membrane-bound enzyme that hydrolyzes ATP to provide the energy necessary to drive the cellular Ca pump.

$^8$ Nitric oxide (NO) is a gas that acts as a signaling molecule in the body, playing a crucial role in the regulation of blood flow and blood pressure.
resulting inflammatory cytokines activate polymorphonuclear cells (PMNs). When activated, PMNs (such as neutrophils and eosinophils) release superoxide anion and other free-radicals (Weglicki et al., 1992), which, in turn, inactivate endothelium-derived relaxing factors and increase degradation of NO. Therefore, such a mechanism could play a role in the modification of arterial contractile response observed in Mg-deficient animals.

1.2.3 Hypertension

It is well known that hypertension, even when relatively mild, can increase the incidence of cardiac events such as coronary heart disease and sudden death, and most patients with hypertension have significant coronary atherosclerosis. Hypertension participates in atherogenesis by producing structural or functional abnormalities in the blood vessel wall biology (i.e. the increase in the mass of the blood vessel wall, endothelial injury) and exhibiting insulin resistance (Dzau, 1990).

1.2.3.1 Vascular Hypertrophy

Elevated blood pressure increases shear or stress on the blood vessel wall. This increased shear force can induce injury to the blood vessel wall and elicit a proliferation response. In order to reduce vessel wall tension and shear in blood vessels, the blood vessel wall undergoes structural changes. One of the major structural changes is the increase in blood vessel wall mass that is commonly observed in vessels of all sizes ranging from large arteries to resistance arterioles in chronic hypertension. The increase in total mass of the smooth muscle cells and connective tissues components (e.g. collagen, elastin and glycogen) are the major contributors to the increased vessel wall

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 Nitric oxide (NO) is a major regulator of vascular tone in muscular arteries
thickness (Dzau and Gibbons, 1988). Therefore, the increase in intimal smooth muscle cells can accelerate formation of fatty plaques to the more advanced atherosclerosis. The increased number of smooth muscle cells can trap more cholesterol and synthesize large amounts of connective tissues to form the fibrous cap.

1.2.3.2 Vascular Wall Changes in Hypertension

Hypertension participates in atherogenesis by producing structural or functional abnormalities in the vessel wall, such as endothelial injury or/and dysfunction, cell membrane abnormalities, proliferation and growth of vascular cells, and platelet dysfunction (Dzau, 1990).

Endothelial cells determine the nature of lipoproteins and other plasma constituents that reach the subendothelial space. These cells bind LDL through specific high-affinity receptors and modify LDL so that LDL can be recognized and ingested by cells such as macrophages. Hypertension produces endothelial dysfunction (Dzau, 1990). A dysfunctional endothelium becomes an ineffective barrier unable to retard the transport of lipoprotein into the intima, and greatly enhances lipoprotein accumulation within the vessel wall and atherosclerotic lesion formation (Chobanian et al., 1986).

Abnormal platelet adhesion to dysfunctional endothelium enhances the release of PDGF from platelets. Ross (1986) reported that the interaction of platelets with the injured vessel wall stimulated smooth muscle cell proliferation in atherosclerosis (Ross, 1986). Other experimental studies also showed that abnormal endothelium released excessive endothelial derived growth-stimulating factors and produced less growth-inhibiting factors. For instance, injured endothelial cells begin to secrete PDGF, which attracts smooth muscle cells to migrate from the medial layer to the intima (Sarzani et
Similar results were observed by DiCorleto and Bowen-Pope (1983) who found that arterial endothelial cells growing in a plastic culture dish were in an abnormal or injured state and secreted significantly more growth factors, including PDGF, than endothelial cells in vivo. Moreover, the injured endothelial cells begin to make surface proteins, which cause circulating monocytes to stick and ultimately to dive between endothelial cells into the intima. Then, these monocytes secrete more PDGF, which will bring even more smooth muscle cells from the media into the intima. All these smooth muscle cells and monocytes begin to thicken the intimal space both with cells and extracellular matrix materials. These effects will enhance smooth muscle cell proliferation and migration from the media to the intima of the blood vessel wall, and thereby result in increased infiltration of lipids into the blood vessel wall and a consequent rapid progression of the atherosclerosis (Tobian, 1992). The atherosclerotic process can further decrease the capacity of overlying endothelial cells to secret NO, as well as prostacylin (Sugimoto et al., 1988). When endothelium-derived relaxing factor and prostacyclin are diminished, platelets tend to stick to endothelial cells and to one other which can be the start of a thrombus formation blocking the coronary artery (Tobian, 1992).

1.2.3.3 Altered Calcium Status in Hypertensive Animals

Hypertension is characterized intracellularly by excessive level of free Ca and by suppressed level of free Mg. Tissues Ca levels are increased in hypertensive rats (Spieker et al., 1986), as well as in hypertensive patients (Erne et al., 1984). Altura and Altura (1983) showed that SHR rats exhibited elevated serum Ca/Mg ratios, and that vascular smooth muscle cells from SHR rats were found to have decreased membrane
permeability to extracellular Mg$^{2+}$ and intracellular [Mg$^{2+}$], and altered Ca$^{2+}$-Mg$^{2+}$ exchange and membrane binding sites. Tissues, such as heart, lungs kidney, and bone, from SHR rats also demonstrate significantly lowered levels of Mg. Ca$^{2+}$ channels are distributed throughout the circulatory system. Smooth muscle tension and arterial tone are regulated by cytosolic [Ca$^{2+}$]. Vasdev and coworkers (2001) reported that elevated aldehyde level was the cause of elevated blood pressure in hypertensive rats. Aldehydes, which react nonenzymatically with sulfhydryl and amino groups of membrane proteins, alter Ca$^{2+}$ channels and lead to increased cytosolic free Ca$^{2+}$ (Vasdev et al., 2001). Increased intracellular Ca$^{2+}$ concentration results in vascular smooth muscle contraction and increases vascular tone. Increasing vascular tone contributes to a decrease in the lumen diameter and high blood pressure (Laurant et al., 1999a).

1.2.4 Effects of Different Dietary Protein Sources on Mineral Balance and Atherosclerosis

The incidence of atherosclerosis can be modified by diet. Plant-derived proteins have a beneficial effect, but the underlying mechanisms remain unclear. Compared with soybean protein, dietary casein protein produces an increase in serum total cholesterol in which the excess cholesterol is located in the LDL fraction (Madani et al., 2000). Carroll and Kurowska (1995) found that a casein-amino acid mixture produced hypercholesterolemia similar to that of casein protein. This appears to be mainly due to lysine and methionine, although other essential amino acids probably contribute to the effect. Arginine appeared to counteract the hypercholesterolemic effects of other essential amino acids. Soy protein produced a lower level of serum cholesterol in rabbits than did a soy protein-amino acid mixture, suggesting the presence of factors in
soy protein that counteracted the effects of hypercholesterolemic amino acids (Carroll and Kurowska, 1995). Soy protein is also more hypocholesterolemic than casein protein in other animal species, particularly when the diet contains cholesterol, and substitution of soy protein for animal protein in the diet decreases the concentration of serum cholesterol in humans (Carroll and Kurowska, 1995).

Recent studies showed that Wistar Kyoto rats (WKY) exhibit greater plasma total cholesterol and triglyceride concentrations than spontaneously hypertensive rats (SHR) (Yuan et al., 1996). The differences in plasma lipid levels observed in WKY and SHR rats likely represent differences in lipid metabolism between these two inbred strains of rats (Yuan et al., 1996). However, the effects of dietary Mg deficiency on the lipid metabolism were examined only in normotensive animals, but not in hypertensive rats. It would be interesting to study the effects of dietary Mg deficiency on the lipid metabolism in SHR rats, and to examine whether a Mg deficient diet would increase the plasma and lipoproteins lipid concentrations in a similar way in both WKY and SHR rats.

It is well known that hypertension participates in atherogenesis by producing structural or functional abnormalities in the blood vessel wall. However, it is not known whether atherogenesis developed in hypertensive animals is also related to increased lipoprotein oxidation. The present study was designed to study the susceptibility of lipoproteins, isolated from SHR and WKY, to forced peroxidation, and to determine whether hypertension developed in SHR rats was associated with increased lipoprotein susceptibility to peroxidation.

Genetic predisposition to hypertension, as well as reduced Mg balance increases
the blood pressure of animals. It would be interesting to examine whether the above two factors can act synergistically to increase the blood pressure of animals. The purpose of the present study was to investigate whether Mg deficiency would further increase the blood pressure of SHR rats.

Long term Mg-deficiency increases blood pressure and induces sustained hypertension. Mg deficiency-induced hypertension in rats is associated with sustained vasospasm and potentiation of vasoconstrictor activity in most types of large and small blood vessels. This results in increased vascular tone, increased vascular reactivity and decreased peripheral blood flow (Laurant et al., 1999b). The increased blood pressure observed in Mg-deficient animals might also relate to increased lipoproteins and tissue susceptibility to oxidation, which leads to increased production of endogenous aldehydes. Excess endogenous aldehydes play a major role in hypertension by binding sulfhydryl groups of membrane proteins, altering Ca\(^{2+}\) channels, increasing cytosolic free Ca\(^{2+}\) concentration, peripheral vascular resistance and blood pressure (Vasdev et al., 2001).
Materials and Methods

2.1 Rats and Diets

SHR\(^9\) and WKY rats (6-week old male rats), six rats per group for a total of forty-eight rats, were purchased from Charles River (Montreal). The initial body weight of each rat was recorded on arrival. Twenty-four WKY rats and twenty-four SHR rats were randomly divided into either the casein protein group or the soy protein group. In Experiment I, rats were fed the casein diet, whereas, in Experiment II, rats were fed the soy diet. Rats were fed a synthetic diet containing either a Mg-replete or -deplete level for eight weeks (6 rats per group). Since rats fed a casein-based diet were severely Mg-deficient and rats fed a soy-based diet were mildly Mg-deficient, studies with rats fed casein and soy protein diets were separated into two experiments and comparative analysis was not made.

The composition (w/w) of the synthetic diet is shown in Table 2. Synthetic diet was mixed for 40-minutes at speed 2 in a Hobart mixer. Diets were vacuum-packed in polyethylene bags and kept in a freezer (-20°C) until usage. The synthetic diets met the nutrient requirements of laboratory rats (National Academy of Sciences, 1978). All rats were individually housed in stainless-steel cages under controlled temperature (25°C) and lighting (12hrs light: 12hrs dark cycle). Rats were trained to consume the respective diets within a 6-hr period daily (09:00 hours to 15:00 hours) over a 1-week training period that would minimize changes in blood pressure caused by

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\(^9\)SHR is originally derived from Wistar Kyoto (WKY) stock from the NIH Animal Genetic Resource colony and is a non-obese genetic animal model for hypertension.
environmental changes. Distilled deionized water was provided ad libitum. Feed intakes of rats were measured daily, and the body weight gain of rats was recorded weekly throughout the experimental period. Rats were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Rats, Vol.2, of the Canadian Council of Animal Care (1984), as approved by The University of British Columbia Animal Care Committee. The feed efficiency of each animal was determined using equation 1.

\[ \text{Feed Efficiency} = \left( \frac{\text{Body Weight Gain}}{\text{Food Intake}} \right) \times 100 \]  

(Equation 1)

2.2 Balance study

A balance study was conducted for three consecutive days, four-weeks before slaughter. Rats from each group were placed in individual metabolic cages for acclimatization 3 days before sample collection. Metabolic cages (Nalgene, Rochester, NY) for 150-300g rats were equipped with collection funnels and separation cones to separate urine and feces and to eliminate urine washover and contamination of feces. Feed intake, urinary and fecal excretions were weighed everyday. Feces and urine, which were collected daily, were immediately stored in separated 50-mL high density polyethylene containers with caps and kept in a freezer (-20°C) until analysis. Mg and Ca balance were calculated as the followings:
Mg Balance = Mg Intake - (Fecal Mg + Urinary Mg)  \hspace{1cm} \text{(Equation 2)}

Ca Balance = Ca Intake - (Fecal Ca + Urinary Ca)  \hspace{1cm} \text{(Equation 3)}

Table 2: Compositions of the casein and soy protein isolate diets.

<table>
<thead>
<tr>
<th></th>
<th>Casein Protein Diet</th>
<th>Soy Protein Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Starch(^1)</td>
<td>25%</td>
<td>25%</td>
</tr>
<tr>
<td>Protein(^2)</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>Sucrose(^3)</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>Alphacel(^3)</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>D,L-methionine(^4)</td>
<td>0.3%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Choline bitartrate(^5)</td>
<td>0.2%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Butter(^6) (non-salted)</td>
<td>3.3%</td>
<td>3.3%</td>
</tr>
<tr>
<td>Beef Tallow(^7)</td>
<td>7.5%</td>
<td>7.5%</td>
</tr>
<tr>
<td>Canola oil(^1)</td>
<td>4.2%</td>
<td>4.2%</td>
</tr>
<tr>
<td>Vitamin mixture AIN-76(^8)</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>Mg-replete diet</td>
<td>3.5% (0.56 mg Mg/g dried diet)</td>
<td>3.5% (5.3 mg Ca/g dried diet)</td>
</tr>
<tr>
<td>Mg-deplete diet</td>
<td>3.5% (0.078 mg Mg/g dried diet)</td>
<td>3.5% (4.8 mg Ca/g dried diet)</td>
</tr>
<tr>
<td>Custom Modified AIN-76 Mineral Mix Free of Mg(^8)</td>
<td>3.5% (0.18 mg Mg/g dried diet)</td>
<td>3.5% (5.5 mg Ca/g dried diet)</td>
</tr>
</tbody>
</table>

\(^1\) Neptune Food Services, Richmond, BC.
\(^2\) Casein, Soy Protein Isolate (SPI): approximately min 90% protein; max 6% moisture; max 5.5% fat; max 0.2% potassium; max 1.2% sodium, ICN Biochemicals Inc., Cleveland, OH, USA.
\(^3\) Nondigestible fiber, ICN Biochemicals Inc., Cleveland, OH, USA.
\(^4\) United States Biochemical, Cleveland, OH.
\(^5\) Van Waters & Rogers, Abbotsford, BC.
\(^6\) Dairyworld Foods, Burnaby, BC.
\(^7\) Cargill Foods, High River, BC.
\(^8\) ICN Biochemicals Inc., Cleveland, OH.
2.3 Blood Pressure Measurement

To confirm the presence of hypertension in the SHR, systolic blood pressure (SBP) recordings were obtained on WKY and SHR the day before slaughter after a 3-days training period. SBP measurements were taken between 15:00 and 17:00 in conscious rats, using an indirect tail-cuff method (Harvard Apparatus Ltd., South Natick, MA). Each recorded value represented the mean of three successive determinations over a period of 10-15 min (Kitts et al., 1992).

2.4 Oxidative Stress Measurements

2.4.1 Lipoproteins Isolation and Collection

At the end of the eight-week experiment, rats were killed after an overnight fast by exsanguination under halothane anesthesia. Femurs were removed and stored 25-mL high density polyethylene containers with caps. Femurs were kept in a freezer at -20°C until analysis. Whole blood was collected by cardiac puncture into heparinized syringe, placed in ice-chilled heparinized 15-mL glass test tubes with caps, and centrifuged immediately at 1300g with an International clinical centrifuge (International Equipment Co., Boston, MA) for 20-min at 4 °C to separate plasma from red blood cells. Plasma was stored in a 25-mL conical-bottom plastic tube with a plug seal closure providing a tight and secure seal, and kept in a freezer at -20°C until analysis.

Plasma lipoproteins were separated into density classes by gradient sucrose gradient ultracentrifugation (Terpstra et al., 1981). Ultracentrifugation was performed at 4 °C in a Beckman model L3-50 ultracentrifuge (Beckman Instruments, Palo Alto, CA, USA) using a Beckman SW 41 Ti rotor (Beckman Canada, Mississauga, ON) at 272,000 g for 22 hrs. The serum density gradient was made with potassium bromide
(KBr, 0.770g) and sucrose (0.050g) added to a centrifuge tube. Subsequently, 2 ml of serum was pipetted into a 12-mL centrifuge tube. With a background density of \( \rho_{20} = 1.25 \) g/ml, 2 ml of a salt solution of \( \rho_{20} = 1.225 \) g/ml (11.42 mg NaCl and 315.54 mg KBr/ml), 4 ml of a salt solution of \( \rho_{20} = 1.10 \) g/ml (11.42 mg NaCl and 133.48 mg KBr/ml), and 4 ml of distilled water were overlayered sequentially over the plasma. All solutions contained \( 10^{-4} \) g/ml ethylenediaminetetraacetic acid (EDTA, disodium salt).

After centrifugation, plasma lipoproteins were collected by pipetting 1-ml fractions starting from the top of the tube. Each 1-ml aliquot was then weighed to calculate the density and stored in a 1.5-mL microcentrifuge tube. Protein concentrations of each 1-ml fraction were determined by the micro-BioRad protein assay, using bovine serum albumin fraction (V), cold alcohol precipitated, (FisherBiotech, Fisher Scientific, Fair Lawn, NJ) as a standard. Lipoprotein fractions were pooled according to relative densities (chylomicron: \(<0.94\) g/ml, VLDL: 0.94-1.006 g/ml, LDL: 1.019-1.063 g/ml, HDL: 1.063-1.210 g/ml) (Genest, 1990) and protein concentrations (VLDL: 0.13-0.20 mg/ml, LDL: 0.10-0.30 mg/ml, HDL: 0.30-0.80 mg/ml, VHDL: 40-50 mg/ml).

2.4.2 Measurement of Lipoprotein Oxidation

Since both LDL and VLDL have been found to undergo lipid peroxidation (Hessler et al., 1983; Morel et al., 1983) and since rats have a relatively small amount of LDL (Chapman, 1980), lipoprotein fractions containing both LDL and VLDL were pooled together to enable the investigation of in-vitro oxidation. Before forced peroxidation, purified lipoprotein fractions were dialyzed in dialysis tubing with a 12,000-14,000 molecular weight cut-off (Spectrum Medical Industries, INC., Los
Angeles, CA), against a 200-fold volume of 0.01 M phosphate-buffered saline (PBS, pH 7.4) (202.5 mL of 0.02M sodium dihydrogen phosphate, 47.5mL of 0.02M disodium hydrogen phosphate, 4.4g of NaCl, and distilled deionized water to a final volume of 500 mL) at 4 °C in the dark for 24 hrs under nitrogen gas (Scaccini et al., 1992).

After dialysis, VLDL and LDL were diluted to a final concentration of 0.010 mg protein/ml and HDL to 0.025 mg protein/ml with 0.01M PBS. Oxidation was initiated using freshly prepared CuSO₄ solution (50μM final concentration) in a 25-mL conical-bottom plastic tube with a plug seal closure providing a tight and secure seal at 37 °C. At prefixed time intervals (0, 15, 30, 60, 90, 120, 150, 180-minutes), a 2.3 ml aliquot was withdrawn and collected into a 10-mL test tube containing EDTA (final concentration: 1.5 mg/ml) to quench the reaction (Cominacini et al., 1991).

2.4.3 Lipid Peroxidation Assays

2.4.3.1 Measurement of Thiobarbituric Acid-Reactive Substances

A 0.35 ml aliquot of the quenched sample was added to 0.70 ml of thiobarbituric acid reagent (0.375 g 2-thiobarbituric acid, 2.08 ml 12 N HCl, 15 ml 100 % trichloroacetic acid, and distilled water to a final volume of 100 ml) in a 10-mL test tube. The reactants in test tube with cap were heated in a boiling water bath at 100°C for 15-minutes. After cooling to room temperature, samples were transferred to a 1.5mL-microcentrifuge tube and centrifuged at 12,800 g for 10-minutes at 4°C, using a Eppendorf 5402 centrifuge. The clear supernatants were analyzed spectrophotometrically at 532 nm using a Shimadzu UV-VIS recording spectrophotometer UV-160 (Shimadzu Corporation, Kyoto, Japan) against a solvent blank (0.35 mL aliquot of distilled deionized water and 0.70 mL of thiobarbituric acid
reagent heated at 100°C for 15-minutes). Using an extinction coefficient of $1.56 \times 10^{5}$ M$^{-1}$·cm$^{-1}$, the results were expressed as nanomoles thiobarbituric acid-reactive substances (TBARS) per milligram protein (Balla et al., 1991).

### 2.4.3.2 Fluorescence Measurement

A 1.2 ml aliquot of quenched samples was diluted 3 times with 0.1 M PBS (pH 7.4) in a 10-mL test tube. Fluorescence measurements were taken at an emission wavelength of 430 nm and excitation wavelength of 360 nm by using a Shimadzu spectrofluorophotometer RF-540 (Shimadzu Corporation, Kyoto, Japan). Both emission and excitation slit widths were set at 5 nm. The drift of sensitivity of the spectrophotofluorometer was checked by measuring the intensity of Raman band of cyclohexane by excitation at 360 nm. The fluorescence was corrected daily for intensity of the Raman band of cyclohexane (≥99.0%) (FisherBiotech, Fisher Scientific, Fair Lawn, NJ) (Cominacini et al., 1991).

### 2.5 Lipid Analysis

Total cholesterol, phospholipid, and triglyceride concentrations of crude plasma and the cholesterol concentrations of pooled lipoprotein fractions (VLDL, LDL and HDL) were determined using enzymatic kits (Boehringer Mannheim GmbH, Mannheim, Germany). Sample solutions were analyzed spectrophotometrically at 500 nm using a Shimadzu UV-VIS recording spectrophotometer UV-160 (Shimadzu Corporation, Kyoto, Japan) against a solvent blank (reagent solution).

### 2.6 Mineral Analysis

Mg and Ca levels in plasma, femur, urine and feces were determined using a Perkin-Elmer series 400 atomic absorption spectrophotometer (AAS) (Perkin-Elmer,
Norwalk, CT). Five standards, listed in the Table 3, were prepared from Ca and Mg references standard solutions (1000 ppm±1%) (FisherBiotech, Fisher Scientific, Fair Lawn, NJ) by diluting specific amounts of Ca and Mg reference solutions with distilled deionized water in volumetric flasks (all glassware was soaked in concentrated hydrochloric acid overnight and rinsed with distilled deionized water before use).

Table 3: The Mg and Ca Concentrations in the five standards.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Mg Concentration (ppm)</th>
<th>Ca Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>0.4</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>10.0</td>
</tr>
<tr>
<td>5</td>
<td>2.4</td>
<td>20.0</td>
</tr>
</tbody>
</table>

2.6.1 Measurement of Plasma Mg and Ca Concentrations

A 0.200 mL aliquot of plasma was removed and added to 4.00 mL of 0.5% lanthanum chloride solution (LaCl₃) in a 10-mL test tube⁹. Plasma total Mg and Ca concentrations were measured directly after dilution in a lanthanum chloride solution, 0.5% LaCl₃, (Rock et al., 1995) with AAS.

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⁹ All test tubes were soaked with hydrochloric acid overnight and rinsed with distilled deionized water before usage.
2.6.2 Measurement of Mg and Ca Concentrations in Feces and Femur

Drying

Aluminum dishes were placed in a vacuum oven (Fisher brand, equipped with a pump, General Electric Motor) overnight at 100°C and cooled in a desiccator. Sample (2-3g fecal sample; a whole solid piece of femur, which was cleansed of adhering soft tissue) was placed into an aluminum dish at 95-100°C for 24 hours in the oven. After drying, samples were kept in a desiccator for cooling (AOAC 7.003, 1984).

Ashing

Empty procelain crucibles and lids were pre-heated in a box-type muffle furnace (Blue M, electric company, Blue Island, IL) at 600°C overnight and were cooled in a desiccator with a cap partly open. Each dried sample was placed into a pre-heated and pre-weighed crucible. The samples in crucible were re-weighed and, then, ashed in a muffle furnace at 550°C for 24 hours or until a white ash was obtained. The ash was dissolved in 12 M hydrochloric acid. A 0.200-mL of solubilized bone or fecal ash were diluted with 4.00-mL of 0.5 % LaCl₃ in 10-mL test tubes for Mg and Ca analyses by AAS (Vormann et al., 1995).

2.6.3 Measurement of Urinary Mg and Ca Concentrations

Frozen urine was thawed at room temperature, 0.060 mL of concentrated hydrochloric acid was added to an aliquot of 1.0-mL urine sample in a 1.5-mL microcentrifuge tube. Concentrated hydrochloric acid (12M) was used to digest and precipitate the organic matter. The mixture was centrifuged at 1380 g for 20-min at 6-10 °C. A 0.200 mL aliquot of clearly separated urine-supernatant was removed and added to 4.00 mL of 0.5 % LaCl₃ in a 10-mL test tube, before analysis of Mg and Ca by
2.7 Statistics

All data were expressed as means with their standard deviations. The statistically significant interactions of dietary Mg level and genetic strain were assessed using two-way analysis of variance (ANOVA). The statistically significant differences of the factors were determined individually using 2-Sample t test of the MINITAB Statistical Software (MINITAB Version 12, PA, 1998). The effects of each factor on the TBARs and fluorescence formation at each time (0, 15, 30, 60, 90, 120, 150, and 180-min, respectively) was analyzed using 2-Sample t test. For all of the above statistical analyses, significance level was set at $p < 0.05$. 
Results

3.1 Experiment 1: Casein Diet

3.1.1 Initial Body Weight, Final Body Weight and Body Weight Gain

The initial body weight of 6-week-old rats, the final body weight of 14-week-old rats, and the body weight gain over the eight-week experiment are summarized in Table 4.

Initial Body Weight

The initial body weights of all rats were similar and were not significantly different (P(genic)=0.501 and P(diet)=0.467).

Final Body Weight

Animal genetic strain was a significant (P<0.001) factor influencing the final body weight of animal. The final body weight of WKY was significantly (P<0.05) greater than that of SHR, for both dietary treatments. Also, differences in dietary Mg intake contributed a significant (P=0.002) effect on the final body weight of animal after eight-week experiment. Rats fed the Mg-replete diet exhibited a significantly (P<0.05) greater final body weight than counterparts fed the Mg deficient diet.

Body Weight Gain

The body weight gain of rats over the eight-week experiment was significantly affected by differences in genetic strain (P<0.001) and dietary Mg level (P=0.001). WKY rats exhibited significantly (P<0.05) greater total body weight gain than SHR rats over the eight-week experiment. Also, rats of both genetic strains fed the Mg replete diet showed significantly (P<0.05) greater total body weight gain than counterparts fed the Mg deplete diet.
Table 4: The initial body weight of 6-week-old rats, and the final body weight of 14-week-old rats and the total body weight gain of WKY and SHR rats fed on a casein based diet of varying Mg level over the eight-week experiment$^{1-3}$.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Initial Body Weight (g)</th>
<th>Final Body Weight (g)</th>
<th>Body Weight Gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
</tr>
<tr>
<td>Mg-R</td>
<td>185.7±8.9</td>
<td>189.5±9.1</td>
<td>393.8±14.9</td>
</tr>
<tr>
<td>Mg-D</td>
<td>185.3±4.5</td>
<td>185.5±4.8</td>
<td>371.0±22.7</td>
</tr>
<tr>
<td>P(Genetic)=0.501</td>
<td>P(Genetic)=0.000</td>
<td>P(Genetic)=0.000</td>
<td></td>
</tr>
<tr>
<td>P(Diet)=0.467</td>
<td>P(Diet)=0.002</td>
<td>P(Diet)=0.001</td>
<td></td>
</tr>
<tr>
<td>P(GeneticxDiet)=0.537</td>
<td>P(GeneticxDiet)=0.979</td>
<td>P(GeneticxDiet)=0.705</td>
<td></td>
</tr>
</tbody>
</table>

$^{1}$All data are reported as mean values ± standard deviation for 6 rats per group

$^{2}$Mg-R = Mg replete diet, Mg-D = Mg deplete diet

$^{3}$SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
3.1.2 **Total Feed Intake, Feed Efficiency and Systolic Blood Pressure.**

Total feed intake and feed efficiency of rats over the eight-week experiment, and the systolic blood pressure measured at 14-week old are summarized in Table 5.

**Total Feed Intake over the eight-weeks of Experiment**

Animal genetic strain was not a significant ($P=0.283$) effect on the total feed intake of rats over the eight-week dietary experiment. The total feed intake of rats was significantly ($P=0.045$) affected by the differences in dietary Mg level. Rats fed the Mg-replete diet exhibited a significantly ($P<0.05$) greater total feed intake than counterparts fed the Mg-deplete diet for both WKY and SHR.

**Feed Efficiency over the eight-weeks of Experiment**

Feed efficiency was significantly ($P<0.001$) affected by differences in genetic strain. WKY showed a significantly ($P<0.05$) greater feed efficiency than SHR for both Mg replete and deplete diets. Differences in dietary Mg level also contributed a significant ($P=0.007$) effect on the feed efficiency of rats, with rats fed the Mg replete diet exhibiting significantly ($P<0.05$) greater feed efficiency than counterparts fed the Mg deplete diet.

**Systolic Blood Pressure**

There was a significant ($P=0.038$) interaction between the differences in genetic strain and dietary Mg level for the systolic blood pressure. Mg deficient diet significantly ($P<0.05$) increased the systolic blood pressure more in SHR than in WKY.
Table 5: The total feed intake and feed efficiency of WKY and SHR rats fed either Mg replete or deplete casein based diets over the eight-week experiment, and the systolic blood pressure of rats at 14-weeks of age, \(^1\-\(^3\).

<table>
<thead>
<tr>
<th>Diets</th>
<th>Total Feed Intake (g)</th>
<th>Feed Efficiency (%)</th>
<th>Systolic Blood Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
</tr>
<tr>
<td>Mg-R</td>
<td>993.2±43.4</td>
<td>952.7±62.7</td>
<td>21.0±0.6</td>
</tr>
<tr>
<td>Mg-D</td>
<td>928.2±48.4</td>
<td>916.6±72.6</td>
<td>20.0±1.3</td>
</tr>
</tbody>
</table>

\[ P(\text{Genetic})=0.283 \]
\[ P(\text{Diet})=0.045 \]
\[ P(\text{Genetic} \times \text{Diet})=0.548 \]

\[ P(\text{Genetic})=0.000 \]
\[ P(\text{Diet})=0.007 \]
\[ P(\text{Genetic} \times \text{Diet})=0.612 \]

\[ P(\text{Genetic})=0.001 \]
\[ P(\text{Diet})=0.025 \]
\[ P(\text{Genetic} \times \text{Diet})=0.038 \]

\(^1\)All data are reported as mean values ± standard deviation for 6 rats per group

\(^a,b\) = significant (P<0.050) difference between treatments means in rows (WKY and SHR)

\(^x,y\) = significant (P<0.050) difference between treatment means in columns (Mg-replete and Mg-deplete diets)

\(^2\)Mg-R = Mg replete diet, Mg-D = Mg deplete diet

\(^3\)SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
3.1.3 Total food intake, Total Mg Intake, Fecal and Urinary Mg Excretion, and Mg Balance

The total food intake, total Mg intake, fecal and urinary Mg excretion, and Mg balance of rats over a three consecutive day balance study are presented in Table 6.

Total Food Intake

The total food intake of rats over the three consecutive day balance study was significantly influenced by the differences in genetic strain (P=0.002) and dietary Mg level (P<0.001). WKY rats exhibited significantly (P<0.05) greater total food intake than counterparts, SHR rats. Also, rats fed the Mg-replete diet exhibited significantly (P<0.05) lower total food intake than counterparts fed the Mg-deplete diet.

Total Mg Intake

Animal genetic strain was not a significant (P=0.158) factor influencing the Mg intake of rats over the three consecutive day balance study. However, dietary Mg level significantly (P<0.001) affected Mg intake, with rats fed the Mg-replete diet exhibiting significantly (P<0.05) greater Mg intake than counterparts fed the Mg-deplete diet.

Fecal Mg Excretion

Animal genetic strain was not a significant (P=0.834) factor influencing the total fecal Mg excretion by rats over the three consecutive day balance study. However, dietary Mg level was a significant (P<0.001) factor affecting the fecal Mg excretion, with rats fed the Mg replete diet excreting significantly (P<0.05) greater total fecal Mg than counterparts fed the Mg deplete diet.

Urinary Mg Excretion

Total urinary Mg excreted by rats was significantly affected by the differences in
genetic strain (P<0.001) and dietary Mg level (P<0.001). WKY rats excreted a significantly (P<0.05) greater urinary Mg level than counterparts, SHR. Also, rats fed the Mg replete diet excreted a significantly (P<0.05) greater urinary Mg level than counterparts fed the Mg deplete diet.

**Mg Balance**

Animal genetic strain was not a significant (P=0.263) factor influencing the Mg balance. Differences in dietary Mg level significantly (P<0.001) affected the Mg balance of rats. Rats fed the Mg-replete diet exhibited a significantly (P<0.05) greater Mg balance than counterparts fed the Mg deficient diet.
Table 6: Total food intake, Mg intake, fecal and urinary Mg excretion, and Mg balance of WKY and SHR rats fed either Mg replete or deplete casein based diets over the 3 consecutive day balance study.$^1$^3$

<table>
<thead>
<tr>
<th>Diets</th>
<th>Food Intake (g/3days)</th>
<th>Mg Intake (mg/3days)</th>
<th>Fecal Mg (mg/3days)</th>
<th>Urinary Mg (mg/3 days)</th>
<th>Mg Balance (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
</tr>
<tr>
<td>Mg-R</td>
<td>35.7±2.1</td>
<td>33.77±4.28</td>
<td>20.1±1.2</td>
<td>19.0±2.4</td>
<td>6.1±0.5</td>
</tr>
<tr>
<td>Mg-D</td>
<td>43.7±2.9</td>
<td>36.9±2.5</td>
<td>3.40±0.22</td>
<td>2.87±0.19</td>
<td>0.91±0.11</td>
</tr>
<tr>
<td></td>
<td>P(Genetic)=0.002</td>
<td>P(Genetic)=0.158</td>
<td>P(Genetic)=0.834</td>
<td>P(Genetic)=0.000</td>
<td>P(Genetic)=0.263</td>
</tr>
<tr>
<td></td>
<td>P(Diet)=0.000</td>
<td>P(Diet)=0.000</td>
<td>P(Diet)=0.000</td>
<td>P(Diet)=0.000</td>
<td>P(Diet)=0.000</td>
</tr>
<tr>
<td></td>
<td>P(GeneticxDiet)=0.061</td>
<td>P(GeneticxDiet)=0.624</td>
<td>P(GeneticxDiet)=0.828</td>
<td>P(GeneticxDiet)=0.333</td>
<td>P(GeneticxDiet)=0.415</td>
</tr>
</tbody>
</table>

$^1$ All data are reported as mean values ± standard deviation for 6 rats per group

$^2$ Mg-R = Mg replete diet, Mg-D = Mg deplete diet

$^3$ SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
3.1.4 Total Ca Intake, Fecal and Urinary Ca Excretion, and Ca Balance

The total Ca intake, fecal and urinary Ca excretion, and Mg balance of rats over a three consecutive day balance study are presented in Table 7.

Total Ca Intake

Animal genetic strain was a significant (P=0.003) factor influencing the Ca intake of rats over a three consecutive day balance study. SHR showed significantly (P<0.05) lower Ca intake than counterparts, WKY. Differences in dietary Mg level did not significantly (P=0.196) influence the Ca intake of rats.

Fecal Ca Excretion

Animal genetic strain was a significant (P=0.001) factor influencing the fecal Ca excretion by rats, with WKY rats excreting significantly (P<0.05) greater fecal Ca than the SHR counterparts. Dietary Mg level was not a significant (P=0.698) factor influencing the fecal Ca excretion by rats.

Urinary Ca Excretion

Animal genetic strain and dietary Mg level were not significant factors influencing the urinary Ca excretion by rats (P=0.213 and P=0.388, respectively).

Ca Balance

Differences in genetic strain significantly (P=0.020) influenced the Ca balance of rats. WKY exhibited a significantly (P<0.05) greater Ca balance than counterparts, SHR. Dietary Mg level was also a significant (P=0.013) factor for the Ca balance of rats, with rats fed the Mg replete diet exhibiting significantly (P<0.05) lower Ca balance than counterparts fed the Mg deplete diet.
Table 7: Total Ca intake, fecal and urinary Ca excretion, and Ca balance of WKY and SHR rats fed either Mg replete or deplete casein based diets over the 3 consecutive day balance study\textsuperscript{1,3}.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Ca Intake (mg/3days)</th>
<th>Fecal Ca (mg/3days)</th>
<th>Urinary Ca (mg/3days)</th>
<th>Ca Balance (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Mg-R</td>
<td>189±11</td>
<td>179±23</td>
<td>110±7</td>
<td>103±13</td>
</tr>
<tr>
<td>Mg-D</td>
<td>209±14</td>
<td>176±12</td>
<td>118±9</td>
<td>96.9±8.5</td>
</tr>
<tr>
<td>P(Genetic)=0.003</td>
<td>P(Genetic)=0.001</td>
<td>P(Genetic)=0.388</td>
<td>P(Genetic)=0.020</td>
<td></td>
</tr>
<tr>
<td>P(Diet)=0.196</td>
<td>P(Diet)=0.698</td>
<td>P(Diet)=0.213</td>
<td>P(Diet)=0.013</td>
<td></td>
</tr>
<tr>
<td>P(GeneticxDiet)=0.089</td>
<td>P(GeneticxDiet)=0.077</td>
<td>P(GeneticxDiet)=0.104</td>
<td>P(GeneticxDiet)=0.205</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1}All data are reported as mean values ± standard deviation for 6 rats per group.

\textsuperscript{2}Mg-R = Mg replete diet, Mg-D = Mg deplete diet.

\textsuperscript{3}SHR = Spontaneously hypertensive rats, WKY = Normotensive rats.
3.1.5 Plasma and Femoral Mg and Ca Concentrations

Plasma and femoral Mg and Ca concentrations of rats fed either Mg replete or deplete casein diets are presented in Table 8.

Plasma Mg Concentration

Animal genetic strain was not a significant (P=0.819) factor influencing the plasma Mg concentration in rats. However, plasma Mg concentration was significantly (P<0.001) affected by varying Mg level in diet. Rats fed the Mg-replete diet exhibited a significantly (P<0.05) greater plasma Mg concentration than counterparts fed the Mg-deplete diet.

Femoral Mg Concentration

Femoral Mg concentration was significantly (P=0.001) affected by the genetic strain factor, with WKY rats exhibiting significantly (P<0.05) greater femoral Mg concentration than SHR counterparts. Differences in dietary Mg intake however significantly (P<0.001) affected the femoral Mg concentration of rats, with rats fed the Mg-replete diet showing significantly (P<0.05) greater femoral Mg concentrations, compared to counterparts fed the Mg-deplete diet.

Plasma Ca Concentration

SHR produced relatively higher plasma Ca concentrations when compared to WKY for both individual diets. However, the differences were not significant (P>0.05), and animal genetic strain was not a significant (P=0.360) factor influencing the plasma Ca concentration in rats. Also, rats fed the Mg-deplete diet showed relatively greater mean value of the plasma Ca concentration than counterparts fed the Mg-replete diet. This was true for both genetic strains, where the differences in plasma Ca concentration were not significant (P>0.05). Dietary Mg level was not a significant (P=0.143) factor for the plasma Ca concentration.

Femoral Ca Concentration
Differences in animal strain, as well as dietary Mg level, were not significant factors influencing the femoral Ca concentration (P=0.897 and P=0.097, respectively).
Table 8: Plasma and femoral Mg and Ca concentrations of WKY and SHR rats fed either Mg replete or deplete casein based diet over the eight-week experiment\(^1\) to \(^3\).

<table>
<thead>
<tr>
<th>Diets</th>
<th>Plasma Mg Concentration (mmol/L)</th>
<th>Femoral Mg Concentration (mg Mg/g dried femur)</th>
<th>Plasma Ca Concentration (mmol/L)</th>
<th>Femoral Ca Concentration (mg Ca/g dried femur)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Mg-R</td>
<td>0.822±0.069</td>
<td>0.841±0.050</td>
<td>4.36±0.13</td>
<td>3.98±0.38</td>
</tr>
<tr>
<td>Mg-D</td>
<td>0.374±0.047</td>
<td>0.340±0.073</td>
<td>2.79±0.56</td>
<td>2.02±0.31</td>
</tr>
<tr>
<td></td>
<td>P(Genetic)=0.819</td>
<td>P(Genetic)=0.001</td>
<td>P(Genetic)=0.360</td>
<td>P(Genetic)=0.897</td>
</tr>
</tbody>
</table>

\(^1\)All data are reported as mean values ± standard deviation for 6 rats per group

\(^2\)Mg-R = Mg replete diet, Mg-D = Mg deplete diet

\(^3\)SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
3.1.6 Plasma and Lipoproteins Lipid Concentrations

Plasma total cholesterol, triglyceride and phospholipid concentrations are summarized in Table 9. The cholesterol concentrations of the isolated VLDL, LDL and HDL fractions are also summarized in Table 9.

Plasma Total Cholesterol Concentration

The plasma total cholesterol concentration was significantly (P<0.001) affected by animal genetic strain. SHR had significantly (P<0.05) lower plasma total cholesterol concentrations than WKY for both Mg-replete and deplete diets. Dietary Mg level was also a significant (P=0.049) factor on the plasma total cholesterol concentration, with rats fed the Mg deplete diet showing significantly (P<0.05) greater plasma total cholesterol concentration than counterparts fed the Mg deplete diet.

VLDL Cholesterol Concentration

Animal strain differences were observed to have a significant (P=0.048) effect on the VLDL cholesterol concentration. WKY rats showed significantly greater VLDL cholesterol concentration than SHR counterparts. Dietary Mg level exhibited a significant (P=0.048) effect on the VLDL cholesterol concentration. Rats fed a Mg deplete diet exhibited a significantly (P<0.05) greater VLDL cholesterol concentration than counterparts fed a Mg replete diet.

LDL Cholesterol Concentration

Differences in genetic strain (P=0.011) produced significant effects on the LDL cholesterol concentration in rats. WKY rats, fed both Mg-replete and deplete diets, showed significantly (P<0.05) greater LDL cholesterol concentrations than SHR counterparts. Dietary Mg level was also a significant (P=0.025) factor of the VLDL cholesterol concentration in rats.
In the present study, rats fed a Mg deplete diet showed significantly (P<0.05) greater VLDL cholesterol concentrations than counterparts fed a Mg replete diet.

**HDL Cholesterol Concentration**

Animal genetic strain was a significant (P=0.049) factor for the HDL cholesterol concentration in rats. WKY rats showed significantly (P<0.05) greater HDL cholesterol concentration than counterparts, SHR rats. Dietary Mg level had no significant (P=0.144) effect on the HDL cholesterol concentration in rats.

**Plasma Total Triglyceride Concentration**

Animal genetic strain was a significant (P=0.007) factor influencing the plasma total triglyceride concentration. WKY rats exhibited significantly (P<0.05) greater plasma total triglyceride concentration than counterparts, SHR. Dietary Mg level was not a significant (P=0.788) factor influencing the plasma total triglyceride concentration.

**Plasma Total Phospholipid Concentration**

Mg deficient diet significantly (P<0.05) increased the plasma phospholipid concentration in WKY, but had no effect on the plasma phospholipid concentration in SHR. There was a significant (P=0.042) interaction between differences in genetic strain and dietary Mg level for the plasma total phospholipid concentration.
Table 9: Plasma and lipoproteins lipid concentrations in WKY and SHR rats fed either Mg replete or deplete casein based diets over the eight-week experiment\textsuperscript{1,3}.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Plasma Cholesterol Concentration (mmol/L)</th>
<th>VLDL Cholesterol Concentration (mmol/L)</th>
<th>LDL Cholesterol Concentration (mmol/L)</th>
<th>HDL Cholesterol Concentration (mmol/L)</th>
<th>Plasma Triglyceride Concentration (mmol/L)</th>
<th>Plasma Phospholipid Concentration (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Mg-R</td>
<td>2.85±0.27</td>
<td>1.73±0.27</td>
<td>0.15±0.07</td>
<td>0.10±0.05</td>
<td>0.55±0.06</td>
<td>0.45±0.05</td>
</tr>
<tr>
<td>Mg-D</td>
<td>3.24±0.32</td>
<td>2.01±0.18</td>
<td>0.25±0.10</td>
<td>0.15±0.03</td>
<td>0.68±0.06</td>
<td>0.53±0.02</td>
</tr>
<tr>
<td>P(Genetic)=0.000</td>
<td>P(Diet)=0.049</td>
<td>P(Genetic)=0.048</td>
<td>P(Genetic)=0.011</td>
<td>P(Genetic)=0.049</td>
<td>P(Genetic)=0.049</td>
<td>P(Genetic)=0.049</td>
</tr>
<tr>
<td>P(Diet)=0.049</td>
<td>P(Diet)=0.048</td>
<td>P(Diet)=0.025</td>
<td>P(Diet)=0.144</td>
<td>P(Diet)=0.788</td>
<td>P(Diet)=0.087</td>
<td></td>
</tr>
<tr>
<td>P(GeneticxDiet)=0.064</td>
<td>P(GeneticxDiet)=0.088</td>
<td>P(GeneticxDiet)=0.118</td>
<td>P(GeneticxDiet)=0.521</td>
<td>P(GeneticxDiet)=0.170</td>
<td>P(GeneticxDiet)=0.042</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1}All data are reported as mean values ± standard deviation for 6 rats per group.

\textsuperscript{a,b} = significant (P<0.050) difference between treatments means in rows (WKY and SHR)

\textsuperscript{x,y} = significant (P<0.050) difference between treatment means in columns (Mg-replete and Mg-deplete diets)

\textsuperscript{2}Mg-R = Mg replete diet, Mg-D = Mg deplete diet

\textsuperscript{3}SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
3.1.7 Lipoprotein Peroxidation

The Formation Rate of TBARs in Combined Fractions of Isolated VLDL and LDL During the 180-min Forced Peroxidation

The rate of TBARS formation measured in the combined fractions of isolated LDL and VLDL collected from WKY and SHR rats fed either a Mg-replete or -deplete diet is shown in Figure 1. Lipoprotein peroxidation (LPP) in vivo is roughly divided into three consecutive phases: lag phase, propagation and a decomposition period (Esterbauer et al., 1992). After 90-min incubation in the presence of 50 μM of CuSO₄, there was a trend, but not at a statistically significant level, that LPP of the combined VLDL and LDL fractions collected from Mg-deficient rats produced relatively greater TBARS than LPP of combined VLDL and LDL fractions collected from Mg-replete rats.
Figure 1: Temporal pattern of TBARs formation following forced peroxidation in the combined fractions of VLDL and LDL collected from SHR and WKY rats fed casein diets varying in Mg level$^{1,2}$

$^1$SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
$^2$Values expressed as means for 6 rats per group, SEM was not shown

Analysis of variance (ANOVA) was used to test for differences between experimental dietary treatments (Mg replete and deplete diets) and genetic strains (normotensive and spontaneously hypertensive), respectively, at 15, 30, 60, 90, 120, 150, and 180-min.
The Formation Rate of TBARS in the Isolated HDL during the 180-min Forced Peroxidation

The rate of TBARS formation in isolated HDL fractions collected from WKY and SHR, fed either Mg-replete or -deficient diet is shown in Figure 2. There was a trend, but not at a statistically significant level, that HDL fractions isolated from WKY rats generated relatively more TBARS than that isolated from SHR rats after 60-min incubation in the presence of 50 μM of CuSO₄. Also, LPP of HDL fractions collected from WKY rats generated significantly (P=0.049) more TBARS than that of serum samples collected from SHR counterparts at the end of the 180-min incubation in the presence of 50 μM of CuSO₄.

LPP of HDL fractions collected from rats fed the Mg replete diet was characterized by producing significantly (P<0.05) less TBARS after 30-min incubation in a 50 μM of CuSO₄ solution, as compared to that of serum samples collected from rats fed the Mg deplete diet. This trend was common to both SHR and WKY rats. Also, HDL fractions collected from rats fed the Mg deplete diet produced significantly (P=0.000) greater TBARS at the end of the 180-min forced peroxidation incubation, than that collected from rats fed the Mg replete diet.
Figure 2: Temporal pattern of TBARs formation following forced peroxidation in isolated HDL fractions collected from SHR and WKY rats fed casein diets varying in Mg level.

1SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
2Values expressed as means for 6 rats per group, SEM was not shown

Analysis of variance (ANOVA) was used to test for differences between experimental dietary treatments (Mg replete and deplete diets) and genetic strains (normotensive and spontaneously hypertensive), respectively, at 15, 30, 60, 90, 120, 150, and 180-min.
The Formation Rate of Fluorescence in the Combined Fractions of Isolated VLDL and LDL during the 180-min Forced Peroxidation

The rate of fluorescence formation in the combined fractions of isolated VLDL and LDL obtained from WKY and SHR rats, fed Mg-replete or deplete diets is presented in Figure 3. There was a trend, but not at a statistically significant level, that combined fractions of isolated VLDL and LDL collected from WKY rats yielded relatively greater fluorescence after 60-min incubation in the presence of 50 μM of CuSO₄.

There was a trend, but not at a statistically significant level, that LPP of the combined isolated VLDL and LDL fractions collected from rats fed the Mg deplete diet was characterized by a relatively greater fluorescence generation after 60-min incubation in the presence of 50 μM of CuSO₄, in comparison to that of serum samples collected from rats fed the Mg replete diet. This result was common to both WKY and SHR rats.
Figure 3: Temporal pattern of fluorescence formation following forced peroxidation in isolated fractions of combined VLDL and LDL, collected from SHR and WKY rats fed casein diets varying in Mg level\(^1\)-\(^2\).

\(^1\)SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
\(^2\)Values expressed as means for 6 rats per group, SEM was not shown

Analysis of variance (ANOVA) was used to test for differences between experimental dietary treatments (Mg replete and deplete diets) and genetic strains (normotensive and spontaneously hypertensive), respectively, at 15, 30, 60, 90, 120, 150, and 180-min.
The Formation Rate of Fluorescence in HDL Fractions during the 180-min Forced Peroxidation

The rate of fluorescence formation in the HDL fractions collected from WKY and SHR rats fed either Mg replete or deficient casein diet during the 180-min Cu$^{2+}$-forced peroxidation is shown in Figure 4. When rats were fed the Mg replete diet, the ex-vivo peroxidation of HDL fractions collected from SHR rats exhibited a significantly [P(15-min)=0.000, P(30-min)=0.004, P(60-min)=0.006, P(90-min)=0.035] greater fluorescence formation than that of serum samples collected from WKY rats, during the first 90-min Cu-forced peroxidation. However, this trend was reversed after 120-min incubation with HDL fractions collected from WKY producing relatively (P>0.05) greater fluorescence on the peroxidation than that of serum samples collected from SHR rats. When rats were fed the Mg deplete diet, there was a trend, but not at a statistically significant level, that HDL fractions obtained from WKY rats produced relatively greater fluorescence on peroxidation than that serum samples collected from SHR rats after 30-min incubation. At the end of 180-min incubation, HDL fractions collected from WKY rats generated significantly (P=0.000) greater fluorescence on the peroxidation than those collected from SHR.

Ex-vivo forced peroxidation of HDL fractions collected from WKY rats fed the Mg deplete diet yielded significantly [P(15-min)=0.014, P(30-min)=0.013, P(60-min)=0.061, and P(90-min)=0.017] greater fluorescence than serum samples collected from SHR counterparts fed the Mg replete diet after 15-min incubation. Also, isolated HDL from rats fed the Mg deplete diet produced significantly (P=0.000) greater total fluorescence at the end of 180-min incubation, compared to those obtained from rats fed the Mg-replete diet.
Figure 4: Temporal pattern of fluorescence formation following forced peroxidation in HDL collected from SHR and WKY rats fed casein diets varying in Mg level\(^1\)\(^2\).

\(^1\)SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
\(^2\)Values expressed as means for 6 rats per group, SEM was not shown

Analysis of variance (ANOVA) was used to test for differences between experimental dietary treatments (Mg replete and deplete diets) and genetic strains (normotensive and spontaneously hypertensive), respectively, at 15, 30, 60, 90, 120, 150, and 180-min.
3.2 Experiment 2: Soy Diet

3.2.1 Initial Body Weight, Final Body Weight and Body Weight Gain

The initial body weight of 6-week-old rats, final body weight of 14-week-old rats, and the total body weight gain of rats over the eight-week experiment are summarized in Table 10.

Initial Body Weight

There was no significant difference (P>0.05) in the initial body weight of all rats, and differences in genetic strain was not a significant factor influencing the initial body weight of rats (P=0.538).

Final Body Weight

The final body weight of rats was significantly affected (P=0.001) by the differences in animal genetic strain. WKY rats showed significantly (P<0.05) greater final body weight than SHR counterparts. The differences in dietary Mg level did not produce a significant (P=0.125) effect on the final body weight of rats.

Body Weight Gain

The total body weight gain of rats over the eight-weeks of experiment was significantly (P<0.001) affected by differences in genetic strain, with WKY rats exhibiting a significantly (P<0.05) greater body weight gain than SHR counterparts. Dietary Mg level was not a significant factor (P=0.268) influencing the body weight gain of rats over the eight-week experiment.
Table 10: Initial body weight of 6-week-old rats, final body weight of 14-week-old rats, and the total body weight gain of WKY and SHR rats fed either Mg replete or deplete soy diets over the 8 week-experiment \textsuperscript{1-3}.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Initial Body Weight (g)</th>
<th>Final Body Weight (g)</th>
<th>Body Weight Gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
</tr>
<tr>
<td>Mg-R</td>
<td>191.7±12.2</td>
<td>198.0±10.2</td>
<td>390.3±24.1</td>
</tr>
<tr>
<td>Mg-D</td>
<td>189.8±6.6</td>
<td>188.2±6.0</td>
<td>379.7±11.8</td>
</tr>
</tbody>
</table>

P(Genetic)=0.538  P(Genetic)=0.001  P(Genetic)=0.000
P(Diet)=0.133     P(Diet)=0.125    P(Diet)=0.268
P(GeneticxDiet)=0.296  P(GeneticxDiet)=0.758  P(GeneticxDiet)=0.830

\textsuperscript{1}All data are reported as mean values ± standard deviation for 6 rats per group

\textsuperscript{2}Mg-R = Mg replete diet, Mg-D = Mg deplete diet

\textsuperscript{3}SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
3.2.2 Total Feed Intake, Feed Efficiency, and Systolic Blood Pressure

The total feed intake and the feed efficiency of rats over the eight-week experimental period and the systolic blood pressure of 14-week-old rats are summarized in Table 11.

Feed Intake

The total feed intake of rats over the eight-week experiment was not significantly affected by differences in animal strain (P=0.498) or dietary Mg treatment (P=0.292).

Feed Efficiency

The feed efficiency of rats was significantly (P<0.001) affected by differences in animal strain, with WKY showing a significantly (P<0.05) greater feed efficiency than SHR. Dietary Mg level did not significantly (P=0.656) affect feed efficiency of rats.

Systolic Blood Pressure

There was significant (P=0.040) interaction between differences in genetic strain and dietary Mg level for the systolic blood pressure. Mg deplete diet significantly (P<0.05) increased the systolic blood pressure more in SHR than in WKY rats.
Table 11: The total feed intake and feed efficiency of WKY and SHR rats fed either Mg replete or deplete soy diets over the eight-week experiment, and the systolic blood pressure of rats at 14-weeks of age\textsuperscript{1-3}.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Feed Intake (g)</th>
<th>Feed Efficiency (%)</th>
<th>Systolic Blood Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
</tr>
<tr>
<td>Mg-R</td>
<td>973.3±84.6</td>
<td>995.0±116.0</td>
<td>20.4±0.5</td>
</tr>
<tr>
<td>Mg-D</td>
<td>943.2±28.0</td>
<td>961.9±29.7</td>
<td>20.1±0.8</td>
</tr>
<tr>
<td></td>
<td>P(Genetic)=0.498</td>
<td>P(Genetic)=0.000</td>
<td>P(genetic)=0.012</td>
</tr>
<tr>
<td></td>
<td>P(Diet)=0.292</td>
<td>P(Diet)=0.656</td>
<td>P(Diet)=0.036</td>
</tr>
<tr>
<td></td>
<td>P(GeneticxDiet)=0.969</td>
<td>P(GeneticxDiet)=0.764</td>
<td>P(GeneticxDiet)=0.040</td>
</tr>
</tbody>
</table>

\textsuperscript{1}All data are reported as mean values ± standard deviation for 6 rats per group

\textsuperscript{a,b} = significant (P<0.050) difference between treatments means in rows (WKY and SHR)

\textsuperscript{x,y} = significant (P<0.050) difference between treatment means in columns (Mg-replete and Mg-deplete diets)

\textsuperscript{2}Mg-R = Mg replete diet, Mg-D = Mg deplete diet

\textsuperscript{3}SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
3.2.3 Total food intake, Total Mg Intake, Fecal and Urinary Mg Excretion, and Mg Balance

The total food intake, total Mg intake, fecal and urinary Mg excretion, and Mg balance of rats over a three consecutive day balance study are presented in Table 12.

**Total Food Intake**

Genetic strain was a significant (P<0.001) factor influencing the total food intake of rats over the three consecutive day balance study. For instance, WKY exhibited significantly (P<0.05) greater total food intake than SHR counterparts. Dietary Mg level exhibited significant (P=0.028) effect on the total food intake of rats. Rats fed the Mg replete diet exhibited significantly (P<0.05) greater total food intake than counterparts fed the Mg deplete diet.

**Total Mg Intake**

Differences in animal genetic strain exhibited significant (P=0.004) effect on the Mg intake of rats over the three consecutive day balance study. WKY rats exhibited a significantly (P<0.05) greater Mg intake than SHR counterparts. Dietary Mg level was a significant (P<0.001) factor influencing the Mg intake of, with rats fed the Mg replete diet showing a significantly (P<0.05) greater Mg intake in comparison to counterparts fed the Mg deplete diet.

**Fecal Mg Excretion**

Genetic strain was not a significant (P=0.288) factor influencing the total fecal Mg excreted by rats over the three consecutive day balance study. Differences in dietary Mg level exhibited significant (P<0.001) effect on the fecal Mg excretion by rats over the three day balance study, with rats fed the Mg replete diet excreting significantly (P<0.05) greater fecal Mg
than counterparts fed the Mg deplete diet.

**Urinary Mg Excretion**

Animal genetic strain and dietary Mg level were significant (P=0.002 and P=0.046, respectively) factors influencing the total urinary Mg level excreted by rats over the three consecutive day balance study. SHR rats excreted significantly (P<0.05) a greater urinary Mg level than WKY counterparts. Also, rats fed the Mg replete diet excreted a significantly (P<0.05) greater urinary Mg level than counterparts fed the Mg deplete diet.

**Mg Balance**

There was a significant (P=0.010) interaction between the differences in genetic strain and dietary Mg level for Mg balance. Mg deficient diet significantly (P<0.05) reduced the Mg balance to a greater extent in SHR rats than WKY counterparts.
Table 12: Total food intake, Mg intake, fecal and urinary Mg excretion, and Mg balance of WKY and SHR rats fed either Mg replete or deplete soy-diets over the 3 consecutive day balance study.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Food Intake (g/3days)</th>
<th>Mg Intake (mg/3days)</th>
<th>Fecal Mg (mg/3days)</th>
<th>Urinary Mg (mg/3 days)</th>
<th>Mg Balance (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
</tr>
<tr>
<td>Mg-R</td>
<td>40.5±2.0</td>
<td>37.4±3.2</td>
<td>26.7±1.3</td>
<td>24.6±2.1</td>
<td>9.19±0.55</td>
</tr>
<tr>
<td>Mg-D</td>
<td>45.6±1.6</td>
<td>38.2±4.5</td>
<td>8.34±0.30</td>
<td>6.99±0.82</td>
<td>2.17±0.25</td>
</tr>
<tr>
<td>P(Genetic)=0.000</td>
<td>P(Genetic)=0.004</td>
<td>P(Genetic)=0.288</td>
<td>P(Genetic)=0.002</td>
<td>P(Genetic)=0.000</td>
<td></td>
</tr>
<tr>
<td>P(Diet)=0.028</td>
<td>P(Diet)=0.000</td>
<td>P(Diet)=0.000</td>
<td>P(Diet)=0.046</td>
<td>P(Diet)=0.000</td>
<td></td>
</tr>
<tr>
<td>P(GeneticxDiet)=0.107</td>
<td>P(GeneticxDiet)=0.496</td>
<td>P(GeneticxDiet)=0.095</td>
<td>P(GeneticxDiet)=0.634</td>
<td>P(GeneticxDiet)=0.010</td>
<td></td>
</tr>
</tbody>
</table>

1All data are reported as mean values ± standard deviation for 6 rats per group

a,b = significant (P<0.050) difference between treatments means in rows (WKY and SHR)

x,y = significant (P<0.050) difference between treatment means in columns (Mg-replete and Mg-deplete diets)

Mg-R = Mg replete diet, Mg-D = Mg deplete diet

SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
3.2.4 Total Ca Intake, Fecal and Urinary Ca Excretion, and Ca Balance

The total Ca intake, fecal and urinary Ca excretion, and Mg balance of rats over a three consecutive day balance study are presented in Table 13.

Total Ca Intake

Animal genetic strain was a significant (P<0.001) factor influencing the Ca intake of rats over the three consecutive day balance study. SHR showed a significantly (P<0.05) lower Ca intake than WKY counterparts. Differences in dietary Mg level did not significantly (P=0.171) affect the Ca intake of rats.

Fecal Ca Excretion

Animal genetic strain was a significant (P=0.001) factor influencing the fecal Ca level excreted by rats over the three consecutive day balance study, with WKY excreting a significantly (P<0.05) greater fecal Ca level than SHR rats. Dietary Mg level was not a significant (P=0.160) factor influencing the level of fecal Ca excretion by rats.

Urinary Ca Excretion

There was a significant (P=0.010) interaction between the differences in genetic strain and dietary Mg level for Mg balance. Mg deficient diet significantly (P<0.05) reduced the Mg balance to a greater extent in SHR rats than WKY counterparts.

Ca Balance

Differences in genetic strain significantly (P=0.035) influenced the Ca balance of rats. WKY rats exhibited a significantly (P<0.05) greater Ca balance than SHR counterparts. Dietary Mg level was also a significant (P<0.001) factor for the Ca balance of rats, with rats fed the Mg replete diet exhibiting significantly (P<0.05) lower...
Ca balance than counterparts fed the Mg deplete diet.
Table 13: Total Ca intake, fecal and urinary Ca excretion, and Ca balance of WKY and SHR rats fed either Mg replete or deplete soy-diets over the 3 consecutive day balance study\(^1\)\(^3\).

<table>
<thead>
<tr>
<th>Diets</th>
<th>Ca Intake</th>
<th>Fecal Ca</th>
<th>Urinary Ca</th>
<th>Ca Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/3days)</td>
<td>(mg/3days)</td>
<td>(mg/3days)</td>
<td>(mg)</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Mg-R</td>
<td>229±12</td>
<td>211±18</td>
<td>141±13</td>
<td>128±16</td>
</tr>
<tr>
<td>Mg-D</td>
<td>250±9</td>
<td>210±25</td>
<td>141±8</td>
<td>114±12</td>
</tr>
<tr>
<td></td>
<td>P(Genetic)=0.000</td>
<td>P(Genetic)=0.001</td>
<td>P(Genetic)=0.000</td>
<td>P(Genetic)=0.035</td>
</tr>
<tr>
<td></td>
<td>P(Diet)=0.171</td>
<td>P(Diet)=0.160</td>
<td>P(Diet)=0.022</td>
<td>P(Diet)=0.000</td>
</tr>
<tr>
<td></td>
<td>P(GeneticxDiet)=0.118</td>
<td>P(GeneticxDiet)=0.199</td>
<td>P(GeneticxDiet)=0.011</td>
<td>P(GeneticxDiet)=0.052</td>
</tr>
</tbody>
</table>

\(^1\)All data are reported as mean values ± standard deviation for 6 rats per group

\(^a,b\) = significant (P<0.050) difference between treatments means in rows (WKY and SHR)

\(^x,y\) = significant (P<0.050) difference between treatment means in columns (Mg-replete and Mg-deplete diets)

\(^2\)Mg-R = Mg replete diet, Mg-D = Mg deplete diet

\(^3\)SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
3.2.5 Plasma and Femoral Mg and Ca Concentrations

Plasma and femoral Mg and Ca concentrations of rats fed either Mg replete or deplete casein diets are presented in Table 14.

Plasma Mg Concentration

There was a significant (P=0.046) interaction between differences in genetic strain and dietary Mg level for the plasma Mg concentration, with Mg deplete diet significantly (P<0.05) lowering the plasma Mg concentration in SHR to a greater extent than in WKY.

Femoral Mg concentration

Femoral Mg concentration was not significantly (P=0.627) affected by the animal genetic strain factor, but was significantly (P<0.001) affected by the dietary Mg level. For instance, rats fed the Mg deplete diet exhibited significantly (P<0.05) lower femoral Mg concentration than counterparts fed the Mg replete diet.

Plasma Ca Concentration

Differences in animal genetic strain exhibited significant (P=0.001) effect on the plasma Ca concentration. SHR showed significantly (P<0.05) greater plasma Ca concentration than WKY counterparts. Dietary Mg level was not a significant (P=0.116) factor influencing the plasma Ca concentration.

Femoral Ca Concentration

Animal genetic strain, as well as dietary Mg level, were not significant factors influencing the Femoral Ca Concentration (P=0.746 and P=0.204, respectively).
Table 14: Plasma and femoral Mg and Ca concentrations of WKY and SHR rats fed either Mg replete or deplete soy diets over the eight-week experiment\textsuperscript{1,3}.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Plasma Mg Concentration (mmol/L)</th>
<th>Femoral Mg Concentration (mg Mg/g dried femur)</th>
<th>Plasma Ca Concentration (mmol/L)</th>
<th>Femoral Ca Concentration (mg Ca/g dried femur)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Mg-R</td>
<td>0.64±0.11\textsuperscript{ax}</td>
<td>0.82±0.07\textsuperscript{ax}</td>
<td>4.56±0.17</td>
<td>4.51±0.18</td>
</tr>
<tr>
<td>Mg-D</td>
<td>0.44±0.03\textsuperscript{by}</td>
<td>0.50±0.03\textsuperscript{by}</td>
<td>2.92±0.40</td>
<td>2.80±0.32</td>
</tr>
<tr>
<td>P(Genetic)=0.000</td>
<td>P(Genetic)=0.627</td>
<td>P(Genetic)=0.001</td>
<td>P(Genetic)=0.746</td>
<td></td>
</tr>
<tr>
<td>P(Diet)=0.000</td>
<td>P(Diet)=0.000</td>
<td>P(Diet)=0.116</td>
<td>P(Diet)=0.204</td>
<td></td>
</tr>
<tr>
<td>P(Genetic\times Diet)=0.046</td>
<td>P(Genetic\times Diet)=0.963</td>
<td>P(Genetic\times Diet)=0.924</td>
<td>P(Genetic\times Diet)=0.174</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1}All data are reported as mean values ± standard deviation for 6 rats per group

\textsuperscript{a,b} = significant (P<0.050) difference between treatments means in rows (WKY and SHR)

\textsuperscript{x,y} = significant (P<0.050) difference between treatment means in columns (Mg-replete and Mg-deplete diets)

\textsuperscript{2}Mg-R = Mg replete diet, Mg-D = Mg deplete diet

\textsuperscript{3}SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
3.2.6 Plasma and Lipoprotein Lipid Concentrations

Plasma total cholesterol, triglyceride and phospholipid concentrations are presented in Table 15. The cholesterol concentrations of the isolated VLDL, LDL and HDL fractions are also summarized in Table 15.

Plasma Total Cholesterol Concentration

A significant \( P=0.012 \) interaction between differences in animal strain and dietary Mg treatment existed for plasma total cholesterol concentration. The Mg deplete diet significantly \( P<0.05 \) increased plasma cholesterol concentration in SHR rats, but had no effect on plasma cholesterol concentration in WKY rats.

VLDL Cholesterol Concentration

Differences in animal strain and dietary Mg level produced no significant effect on rat VLDL cholesterol concentration \( (P=0.192 \text{ and } P=0.175, \text{ respectively}) \).

LDL Cholesterol Concentration

Animal genetic strain, as well as dietary Mg level, were not significant \( P=0.068 \text{ and } P=0.580, \text{ respectively}) \) factors for the LDL cholesterol concentration.

HDL Cholesterol Concentration

Differences in animal genetic strain produced a significant \( P=0.038 \) effect on the HDL cholesterol concentration. WKY exhibited a significantly \( P<0.05 \) greater HDL cholesterol concentration than SHR. Dietary Mg level was not a significant \( P=0.107 \) factor influencing the HDL cholesterol concentration.

Plasma Total Triglyceride Concentration

Differences in animal strain displayed a significant \( P<0.001 \) effect on the plasma total triglyceride concentration. WKY had a significantly \( P<0.05 \) greater
plasma triglyceride concentration than SHR. Plasma triglyceride concentration was not significantly (P=0.422) affected by altering the level of Mg in the diet.

**Plasma Total Phospholipid Concentration**

Plasma total phospholipid content were significantly (P<0.001) affected by differences in animal strain. WKY had a significantly (P<0.05) greater plasma total phospholipid concentration than SHR. Differences in Mg level in diet resulted in no significant (P=0.487) influence on the plasma phospholipid concentration.
Table 15: Plasma and lipoproteins lipid concentrations in WKY and SHR rats fed either Mg replete or deplete soy diets over the eight-week experiment\(^1\)

<table>
<thead>
<tr>
<th>Diets</th>
<th>Plasma Cholesterol (mmol/L)</th>
<th>VLDL Cholesterol (mmol/L)</th>
<th>LDL Cholesterol (mmol/L)</th>
<th>HDL Cholesterol (mmol/L)</th>
<th>Plasma Triglyceride (mmol/L)</th>
<th>Plasma Phospholipid (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Mg-R</td>
<td>2.80±0.24(^{ax})</td>
<td>1.95±0.17(^{hx})</td>
<td>0.15±0.05</td>
<td>0.16±0.06</td>
<td>0.23±0.05</td>
<td>0.17±0.05</td>
</tr>
<tr>
<td>Mg-D</td>
<td>2.81±0.14(^{ax})</td>
<td>2.25±0.09(^{by})</td>
<td>0.16±0.06</td>
<td>0.20±0.03</td>
<td>0.22±0.07</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>P(Genetic)=0.000</td>
<td>P(Diet)=0.105</td>
<td>P(Genetic)=0.192</td>
<td>P(Diet)=0.580</td>
<td>P(Genetic)=0.068</td>
<td>P(Diet)=0.107</td>
<td>P(Diet)=0.000</td>
</tr>
</tbody>
</table>

\(^1\)All data are reported as mean values ± Standard deviation for 6 rats per group

\(^{a,b}\) = significant (P<0.050) difference between treatments means in rows (WKY and SHR)

\(^{x,y}\) = significant (P<0.050) difference between treatment means in columns (Mg-replete and Mg-deplete diets)

\(^2\)Mg-R = Mg replete diet, Mg-D = Mg deplete diet

\(^3\)SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
3.2.7 Lipoprotein Oxidation

The Formation Rate of TBARs in the Combined Fractions of VLDL and LDL during the 180-min Forced Peroxidation

The rate of TBARs generation during the 180-min in vitro oxidative stress for the combined fractions of isolated VLDL and LDL is presented in Figure 5. Combined VLDL and LDL fractions isolated obtained from WKY produced significantly ($P(90\text{-min})=0.006$, $P(120\text{-min})=0.030$, $P(180\text{-min})=0.007$) greater TBARs than that from the SHR after 90-min incubation in the presence of 50 μM of CuSO₄. Forced peroxidation of the combined isolated VLDL and LDL fractions from WKY also produced significantly ($P=0.007$) greater total TBARs than that from SHR during the 180-min forced peroxidation. There was a trend, but not at a statistically significant level, that plasma lipoproteins collected from rats fed the Mg deficient diet produced relatively ($P>0.05$) greater TBARs than those from rats fed the Mg replete diet after 60-min forced peroxidation.
Figure 5: Temporal pattern of TBARs formation following forced peroxidation in the combined fractions of VLDL and LDL collected from SHR and WKY rats fed soy diets varying in Mg level.\(^1\)\(^2\).

\(^1\)SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
\(^2\)Values expressed as means for 6 rats per group, Standard deviation was not shown

Analysis of variance (ANOVA) was used to test for differences between experimental dietary treatments (Mg replete and deplete diets) and genetic strains (normotensive and spontaneously hypertensive), respectively, at 15, 30, 60, 90, 120, 150, and 180-min.
The Formation Rate of TBARs in the HDL Fractions during the 180-min Forced Peroxidation

Figure 6 shows the rate of TBARs formation in plasma HDL fractions collected from WKY and SHR fed either Mg replete or deplete soy diet, during the 180-min incubation in the presence of 50 μM of CuSO₄. HDL isolated from WKY produced significantly greater TBARs than SHR at incubation times of 15 (P=0.007), 90 (P=0.006), 120 (P=0.030), 150 (P<0.001) and 180-min (P=0.007). HDL isolated from WKY producing greater TBARs after 15-min forced peroxidation, compared to that isolated from SHR. This trend was common to both dietary treated animal groups (Mg-replete and deplete fed rats). At the end of the 180-min incubation of lipoproteins in the presence of 50 μM of CuSO₄, HDL fractions collected from WKY produced a significantly (P=0.007) greater forced TBARs than that of SHR.
Figure 6: Temporal pattern of TBARs formation following forced peroxidation in HDL fractions collected from SHR and WKY rats fed soy diets varying in Mg level\(^1\).\(^2\).

\(^1\)SHR = Spontaneously hypertensive rats, WKY = Normotensive rats
\(^2\)Values expressed as means for 6 rats per group, SEM was not shown

Analysis of variance (ANOVA) was used to test for differences between experimental dietary treatments (Mg replete and deplete diets) and genetic strains (normotensive and spontaneously hypertensive), respectively, at 15, 30, 60, 90, 120, 150, and 180-min.
The Formation Rate of Fluorescence in the Combined Fractions of VLDL and LDL during the 180-min Forced Peroxidation

The rate of fluorescence formation in the combined VLDL and LDL fractions lipoprotein during the 180-min incubation period in the presence of 50 μM of CuSO₄ is presented in Figure 7. When rats were fed the Mg-deplete diet, there was a trend, but not at a statistically significant level, that combined VLDL and LDL fractions isolated from WKY produced relatively (P>0.05) greater fluorescence than those from SHR after the 90-min incubation. When rats were fed the Mg replete diet, there was a trend, but not at a statistically significant level, that pooled LDL and VLDL lipoproteins collected from WKY produced greater fluorescence than those from SHR after 150-min incubation.

Combined VLDL and LDL isolated from WKY fed the Mg replete diet produced significantly [P(15-min)=0.025, P(30-min)=0.010, P(60-min)=0.006] less TBARs at the first 60-min incubation and relatively (P>0.05) less TBARs from 90-180-min incubation, when compared to corresponding lipoproteins isolated from counterparts fed the Mg-deplete diet.
Figure 7: Temporal pattern of fluorescence formation following forced peroxidation in the combined fractions of VLDL and LDL from SHR and WKY rats fed soy diets varying in Mg level\textsuperscript{1-2}.

\textsuperscript{1}SHR = Spontaneously hypertensive rats, WKY = Normotensive rats

\textsuperscript{2}Values expressed as means for 6 rats per group, SEM was not shown

Analysis of variance (ANOVA) was used to test for differences between experimental dietary treatments (Mg replete and deplete diets) and genetic strains (normotensive and spontaneously hypertensive), respectively, at 15, 30, 60, 90, 120, 150, and 180-min.
The Formation Rate of Fluorescence in HDL Fractions during the 180-min Forced Peroxidation

The rate of fluorescence formation in isolated HDL fractions from WKY and SHR fed both Mg replete and deficient soy diets, during the 180-min oxidative period is shown in Figure 8. When rats were fed the Mg replete diet, LPP of HDL fractions isolated from WKY produced significantly \( P(15\text{-min})<0.001, \ P(30\text{-min})=0.013, \ P(60\text{-min})=0.016, \ P(90\text{min})=0.0016,\ P(120\text{-min})=0.0074, \ P(150\text{-min})=0.0098 \) and \( P(180\text{-min}) =0.0074 \) greater fluorescence than corresponding HDL fractions isolated from SHR after 15-min forced peroxidation. HDL fractions isolated from WKY fed the Mg deplete diet produced significantly \( P(90\text{-min})=0.050, \ P(120\text{-min})=0.018, \ P(150\text{-min})=0.027, \) and \( P(180\text{-min})=0.029 \) greater fluorescence on the forced peroxidation than corresponding HDL fractions isolated from SHR after 90-min incubation. Generally, forced peroxidation of HDL fractions isolated from WKY exhibited significantly \( P(15\text{-min})<0.001, \ P(60\text{-min})=0.028, \ P(90\text{min})<0.001, \ P(120\text{-min})<0.001, \ P(150\text{-min})<0.001, \) and \( P(180\text{-min})<0.001 \) greater fluorescence formation than those isolated from SHR after 15-min incubation.

There was a trend, but not at a statistically significant level, that forced peroxidation of HDL fractions collected from rats fed the Mg deficient soy diet produced a relatively \( P>0.05 \) greater fluorescence than corresponding HDL fractions collected from counterparts fed the Mg replete soy diet after 60-min incubation. This trend was common to animal strains, WKY and SHR.
Figure 8: Temporal pattern of fluorescence formation following forced peroxidation in HDL fractions collected from SHR and WKY rats fed soy diets varying in Mg level\textsuperscript{1,2}. 

\textsuperscript{1}SHR = Spontaneously hypertensive rats, WKY = Normotensive rats

\textsuperscript{2}Values expressed as means for 6 rats per group, SEM was not shown

Analysis of variance (ANOVA) was used to test for differences between experimental dietary treatments (Mg replete and deplete diets) and genetic strains (normotensive and spontaneously hypertensive), respectively, at 15, 30, 60, 90, 120, 150, and 180-min.
Discussion

Initial and Final Body Weight, Body Weight Gain and Feed Efficiency of Rats over the eight-week experimental Period

In the present study, WKY rats fed both Mg-replete and deplete diets exhibited significantly greater final body weight and total body weight gain than SHR rats. Hulman and his coworkers (1993) also reported that WKY gained weight faster than SHR rats and exhibited greater final body weight than SHR rats. In the present study, WKY rats also exhibited significantly greater feed efficiency than SHR counterparts, regardless of the dietary Mg intake level. The uniformly reduced feed efficiency observed in SHR, compared to WKY rats fed experimental diets is consistent with former studies with these genetic strains of rats (Kitts et al., 1992).

In Experiment I, rats of both genetic strains fed a Mg-replete casein diet exhibited significantly greater final body weight and body weight gain over the eight-week experimental period compared to counterparts fed a Mg deplete diet. Similar trends were also observed in rats fed the soy protein diet in Experiment II, but not at a statistically significant level. Similar results were obtained in the other study conducted by Gueux and his coworkers (1995), who reported that Wistar rats fed the control diet (960mg Mg/kg) exhibited significantly greater final body weight than those fed the Mg deplete diet (30mg Mg/kg) after a relatively short 8-day feeding experiment.

Mg and Ca Balance, Plasma and Femoral Mg and Ca Concentrations

In the present study, rats fed the Mg deplete diet showed significantly lower Mg intake and balance than counterparts fed the Mg replete diet in both Experiments I and II. These results confirmed that rats fed the Mg deplete diet were Mg-deficient. In
Experiments I and II, Mg deficient rats exhibited significantly lower plasma and femoral Mg concentrations than Mg-replete rats. These findings corresponded with the lower Mg balance observed in Mg deplete rats than in Mg-replete rats. Results obtained in the present study were consistent with the other study conducted by Creedon and coworkers (1999), who reported that serum and femoral Mg levels were reduced by 70, 38%, respectively, in 13-week-old male Wistar rats fed a Mg-deplete diet (20 mg Mg/kg diet), in comparison to a pair-fed group given a control diet (400 mg Mg/kg diet).

In Experiments I and II, SHR rats exhibited significantly lower Ca balance than WKY rats, corresponding with the significantly lower Ca intake observed in SHR than in WKY counterparts. In Experiment II, regardless of the fact that SHR exhibited significantly lower Ca balance than WKY counterparts, SHR rats showed significantly greater plasma Ca concentration than WKY counterparts.

Plasma and Lipoproteins Lipid Concentrations

In the present study, WKY rats exhibited significantly greater plasma total cholesterol, triglyceride and phospholipid concentrations than SHR counterparts, regardless of the level of dietary Mg intake. These findings possibly reflected specific differences in lipid metabolism between these two inbred strains of normotensive and hypertensive rats. The results reported in the present study were consistent with the former studies (Yuan at el., 1996; Yuan at el., 1998), where SHR rats exhibited significantly lower plasma total cholesterol and triglyceride concentrations. The reduced plasma cholesterol concentration in SHR rats had been reported mainly due to the decreased cholesterol synthesis in the liver (Iritani et al., 1977). Study showed that acetate incorporation into cholesterol in liver slices was markedly decreased in SHR.
(Iritani et al., 1977). Also, the activity of glucose-6-phosphate dehydrogenase\footnote{Glucose-6-phosphate dehydrogenase is an enzyme of the oxidoreductase class that catalyzes the oxidation of glucose-6-phosphate to a lactone, reducing NADP\(^+\) to NADPH. The reduction is the first step in the pentose phosphate pathway of glucose metabolism.} was lower in SHR than in WKY rats (Iritani et al., 1977). The reduced availability of intracellular NADPH, due to the lower activity of glucose-6-phosphate dehydrogenase in the SHR, compared to the WKY rats, represented a limiting factor for cholesterol synthesis(Iritani et al., 1977). Therefore, a lower plasma cholesterol level in SHR rats could be mainly due to a decreased cholesterol synthesis rate in the liver (Iritani et al., 1977).

A number of experimental studies showed that Mg deficiency participates in hyperlipidemia in normotensive rats. For instance, Rayssiguier and his co-workers (1981) reported that Mg deficiency increased plasma triglyceride and cholesterol concentrations of weanling male Sherman rats. The increase of plasma cholesterol level observed in Mg-deficient rats is mainly due to reduced activities of LCAT and HMG CoA reductase (Rayssiguier, 1983; Golf et al., 1991). LCAT is responsible for transporting excess cholesterol in peripheral tissues back to the liver for excretion. It catalyzes the formation of cholesteryl esters in HDL; the cholesteryl ester formed is then transferred from HDL to lower density lipoproteins by the action of cholesteryl ester transfer protein (CETP). The cholesterol is then removed from the plasma by hepatic clearance of the plasma lipoproteins (Genest, 1990). Thus, decreased LCAT activity can lead to increased concentration of circulating cholesterol together with a reduced clearance of excess cholesterol in peripheral tissues (Rayssiguier, 1983). Golf and his coworkers (1991) also reported that Mg deficiency kept HMG CoA reductase in an
activated state and favored cholesterol biosynthesis, thereby resulting in hypercholesterolemia in Mg deficient rats.

It was consistently observed in the present study that Mg deficiency increased plasma cholesterol concentrations in casein fed rats. In Experiment I, rats of both genetic strains fed the Mg-deplete casein diet exhibited significantly greater plasma total cholesterol concentration than counterparts fed the Mg-replete diet. These results indicated that Mg deficiency increased the plasma cholesterol concentration in a similar way in both WKY and SHR rats, regardless of the specific differences in lipid metabolism between these two inbred strains of rats. However, when rats were fed the soy diet in Experiment II, Mg deficiency was associated with a significant increase in the plasma cholesterol concentration only in SHR rats, but not in WKY rats. The decreased hypercholesterolemic effect of Mg deficiency observed in WKY rats might due to the hypocholesterolemic effect of soy protein. Recent experimental studies reported that substitution of soy protein for animal protein, casein, in the diet decreased serum cholesterol concentration in humans (Carroll and Kurowska, 1995). In the present study, feeding a soy protein diet reduced the hypercholesterolemic effect of Mg deficiency. For instance, when rats were fed the casein protein diet, WKY rats fed the Mg-deplete diet exhibited significantly greater plasma cholesterol concentration than counterparts fed the Mg-replete diet. However, when rats were fed the soy-protein diet, the hypercholesterolemic effect of Mg deficiency was diminished. Furthermore, a Mg deficiency did not significantly influence the plasma cholesterol concentrations in WKY rats. This finding indicated that the hypercholesterolemic effect of Mg deficiency was somehow diminished by the feeding of dietary soy-protein. It was of interest that the hypocholesterolemic effect of dietary soy protein observed in cholesterol-fed rats was
somewhat similar to that observed in Mg-deficient rats. The hypocholesterolemic effect of soy protein, however, was only observed in the WKY rats, but not in the SHR rats. This indicated that there might be specific differences in the lipid metabolism between these two inbred strains of normotensive and hypertensive rats.

Rayssiguier and his co-workers (1981) also reported that Mg deficiency increased cholesterol concentrations in VLDL and LDL lipoprotein fractions. Similar results were observed in the present study. In Experiment I, Mg-deficient rats of both genetic strains exhibited significantly greater VLDL and LDL cholesterol concentrations than Mg-replete rats. These results also indicated that the effects of Mg-deficiency on the lipoprotein cholesterol concentration were similar in both WKY and SHR rats, regardless of the differences in lipid metabolism between these two genetic strains. However, in Experiment II, when rats were fed a soy-based diet, Mg deficiency did not significantly affect the VLDL and LDL cholesterol concentrations. The reduced hypercholesterolemic effect of Mg-deficiency on VLDL and LDL cholesterol concentrations observed in Experiment II indicated that dietary soy protein might play a beneficial role in diminishing the hypercholesterolemic effect of Mg-deficiency.

**Lipoprotein Peroxidation**

Oxidative modification of LDL is an important factor for the development of atherosclerosis (Steinbrecher et al., 1987). Oxidation of LDL is a lipid peroxidation process in which the polyunsaturated fatty acids contained in the LDL lipids are rapidly converted to lipid hydroperoxides and then to some saturated aldehydes (Steinbrecher et al., 1987). Lipoproteins can also be oxidized in-vitro in a cell-free medium containing a sufficiently high concentration of Cu$^{2+}$ or Fe$^{2+}$ (Cominacini et al., 1991). In the present study, incubation of isolated lipoprotein
fractions in the presence of 50 μM of CuSO₄ showed that oxidative modification of lipoproteins isolated from WKY rats produced significantly greater TBARs and fluorescence, and thereby were more susceptible to lipid oxidation than lipoproteins isolated from SHR rats. Similar results were observed in the oxidative modifications of the combined VLDL-LDL fractions and the HDL fractions. Since susceptibility of lipoprotein to in vitro Cu²⁺-induced forced peroxidation represented susceptibility of lipoproteins to similar oxidative stress in vivo, the findings of SHR lipoproteins being less susceptible to forced peroxidation indicated that SHR lipoproteins might not be oxidatively modified easily in-vivo. Lipoprotein peroxidation might play a less important role in the development of atherosclerotic lesions in hypertensive rats; other characteristic factors of hypertension, such as structural or functional abnormalities of the blood vessels in hypertensive rats, might play a more important role in development of atherosclerotic lesions.

Accumulation of cholesterol in the arterial intima is an important characteristic of atherosclerotic lesion, and the accumulated cholesterol originates primarily in plasma lipoproteins containing apo-B, such as VLDL and LDL (Petty, 1991). Hypercholesterolemia is another important factor for development of atherosclerosis (Fattah and Abdulla, 1991). In the present study, when rats were fed the casein diet, SHR rats exhibited significantly lower plasma and lipoproteins (VLDL, LDL and HDL) cholesterol concentrations than WKY counterparts. Similar results were observed in Experiment II, and SHR rats exhibited significantly lower plasma cholesterol concentration than WKY counterparts. Thus, initiation of atherosclerosis as a result of cholesterol deposition into aortic luminal tissue might not be potentiated by hypertension.

Lipoproteins isolated from Mg-deficient rats produced greater TBARs and fluorescence
after the 180-min incubation in the presence of 50 μM of CuSO₄, and were more susceptible to lipid oxidation compared to lipoproteins isolated from Mg-replete rats. These results indicated that Mg deficiency increased susceptibility of lipoprotein to lipid oxidation and, thus, caused more LDL to be oxidized and taken up by macrophages to form foam cells in the intima in-vivo. Also, elevated plasma and lipoproteins cholesterol concentrations observed in Mg-deficient rats, reported in the present study, also reflected that Mg deficient rats might be relatively more susceptible to the formation of fatty streak by depositing and accumulating more cholesterol in the arterial intima. The results reported in the present study agreed with some other in-vivo studies, which reported that Mg deficiency enhanced lipid deposition within the valves of the left ventricle and in the aorta in rats fed cholesterol and cholic acid (Hungerford and Bernick, 1980). The degree of lipid deposition in the aorta of cholesterol-fed rabbits can be similarly enhanced by feeding a Mg-deficient diet (Nakamura et al., 1965). Therefore, increased lipoprotein susceptibility to lipid oxidation, as well as increased plasma and lipoproteins cholesterol concentrations observed in the present study might be the major characteristics of Mg-deficiency in the promotion of cardiovascular pathology associated with Mg deficiency.

**Systolic Blood Pressure**

Ca²⁺ channels are distributed throughout the circulatory system and regulate vascular tone. A rise of cytosolic Ca²⁺ concentration could elevate blood pressure by facilitating vascular smooth muscle contraction (Altura et al., 1993) and decreasing synthesis and release of endothelial-derived relaxation factors (i.e. PGI₂) (Altura et al., 1993). Increasing vascular tone could further thicken vascular wall and reduce lumen size (Laurant et al., 1999b). It has been suggested that the cytosolic Ca²⁺ concentration reflects vascular tone and blood pressure (Laurant et al., 1999a).
In the present study, SHR rats exhibited significantly higher systolic blood pressure than WKY counterparts, regardless of the differences in dietary Mg level and protein source. The significantly higher systolic blood pressure observed in SHR rats, compared to WKY counterparts confirmed that SHR rats were predisposed to hypertension. A recent study showed that elevated aldehyde level was the cause of hypertension in SHR rats (Vasdev et al., 2001). Excess endogenous aldehydes observed in tissues (liver, kidney and heart) in SHR rats bound to sulfhydryl groups of vascular Ca\(^{2+}\) channels and blocked Ca\(^{2+}\) channels, thereby leading to the increased cytosolic Ca\(^{2+}\) concentration and elevated blood pressure in this genetic strain of rat. Altura and Altura (1981) also showed that vascular smooth muscle cells obtained from SHR rats exhibited decreased membrane permeability to extracellular Mg\(^{2+}\), lower intracellular [Mg\(^{2+}\)] and altered Ca\(^{2+}\)–Mg\(^{2+}\) exchange and membrane sites. Elevated extracellular [Mg\(^{2+}\)] exerted very little in the way of relaxant action on the SHR blood vessels, indicating an underlying defect in vascular Mg\(^{2+}\) metabolism and Ca\(^{2+}\) handling at the plasma and intracellular membranes. Altura and Altura (1981) also suggested that these defects were inherited. Further studies were conducted to support this hypothesis. Studies showed that erythrocyte membranes of essential hypertensive patients exhibited decreased permeability to extracellular Mg\(^{2+}\) (Palisso et al., 1987), and red blood cells plasma membranes from hypertensive subjects did not handle Mg\(^{2+}\) as did similar red blood cell membranes from control subjects (Mattingly et al., 1991). A number of studies also showed that SHR rats exhibited elevated serum, bone, kidney, lung and heart Ca concentrations (Wallach and Verch, 1986). Plasma Ca concentration correlated closely with cytosolic Ca\(^{2+}\) concentration, as well as blood pressure in rats. In the present study, aldehyde level and cytosolic Ca\(^{2+}\) concentration were not measured, but plasma Ca concentration was measured instead. In Experiment II, SHR rats exhibited significantly greater plasma Ca
concentrations than WKY rats, even though SHR rats had significantly lower Ca balance than WKY rats. These results indicate that there might be an underlying defect in Ca$^{2+}$ handling at the plasma membranes in SHR rats. The increased plasma Ca concentration observed in SHR rats was in agreement with the increased systolic blood pressure observed in this genetic strain rat in the present study.

In the present study, Mg-deficient rats exhibited significantly higher systolic blood pressure than rats fed the Mg-replete diet. The results obtained in the present study were similar to those obtained in other studies, where rats fed moderately or more severely Mg deficient diets for 12-weeks showed significantly greater arterial blood pressure and smaller lumen size, compared to control rats fed enriched Mg-diet (Altura et al., 1984). A number of studies showed that Mg deficiency could elevate the blood pressure by enhancing circulating constrictors activities (Altura and Altura, 1981), decreasing the synthesis and release of endothelial-derived relaxant factors (Altura et al., 1993), inactivating endothelium-derived relaxing factors, and increasing degradation of NO. In the present study, a significant interaction between differences in Mg deficiency and genetic strain existed for the systolic blood pressure. The Mg deficiency increased systolic blood pressure of rats, and the increase was significantly greater in SHR than in WKY rats. These findings indicated that Mg deficiency further enhanced the genetic predisposition of the SHR rats to hypertension.

One of the hypotheses of this study was that elevated systolic blood pressure observed in Mg-deficient rats might reflect an increased lipoprotein susceptibility to peroxidation. The increased susceptibility of lipoprotein to peroxidation could result in greater generation of aldehydes since, during lipoprotein peroxidation, a broad spectrum of oxidized fatty acid
fragments and shorter chain aldehydes would be produced. The resulting aldehydes, in turn, would block the Ca\(^{2+}\) channels and increase cytosolic Ca\(^{2+}\) concentration. Elevated cytosolic Ca\(^{2+}\) concentration, which correlated closely to the cytosolic Ca\(^{2+}\) concentration (George and Heaton, 1975; Planells et al., 1995), would therefore result in vascular smooth muscle contraction and increased vascular tone. Results from the present study supported the hypothesis that elevated blood pressure observed in Mg-deficient rats might act through an increased susceptibility of lipoproteins to peroxidation. In the present study, Mg deficient rats exhibiting significantly greater systolic blood pressure also exhibited greater lipoprotein susceptibility to forced peroxidation, in comparison with Mg-replete rats. Mg deficient rats also showed relatively greater plasma Ca concentrations than Mg-replete rats. The increased lipoprotein susceptibility to forced peroxidation was in agreement with the relatively greater plasma Ca concentration and significantly higher systolic blood pressure observed in Mg-deficient rats. These findings supported the hypothesis that increased systolic blood pressure induced by Mg-deficiency might act through the increased susceptibility of lipoproteins to oxidation.
General Conclusions

In the present study, animal genetic strain was a significant factor influencing plasma lipid concentrations. SHR rats exhibited significantly lower plasma cholesterol, triglyceride and phospholipid concentrations than WKY counterparts in both Experiments I and II. These findings indicated that there were differences in lipid metabolism between these two inbred strains of normotensive and hypertensive rats.

One of the objectives of the present study was to investigate whether the effect of Mg status on relative changes in plasma and lipoproteins lipid concentrations was similar in both WKY and SHR rats fed different protein sources. In Experiment I, with casein diets, Mg deficiency significantly increased plasma and lipoproteins (VLDL and LDL) cholesterol concentrations in both WKY and SHR rats. These results indicated that Mg deficiency increased plasma and lipoprotein cholesterol concentrations in a similar way in both WKY and SHR rats, regardless of the differences in lipid metabolism between these two inbred strains of rats. In Experiment II, with soy diets, Mg deficiency significantly increased plasma cholesterol concentrations only in SHR rats, but not in WKY rats, and only slightly increased the VLDL and LDL cholesterol concentrations in both genetic strains of rats fed soy protein diet. The hypercholesterolemic effect of Mg deficiency was somehow diminished by dietary soy protein. Also, the hypocholesterolemic effect of dietary soy protein was found only in WKY, but not in SHR rats.

In the present study, lipoproteins isolated from SHR rats produced less TBARs and fluorescence at the end of forced peroxidation, and, in fact, were less susceptible to lipid peroxidation than lipoproteins isolated from WKY rats. Also, lipoproteins isolated from Mg-deficient rats were more susceptible to lipid peroxidation than lipoproteins isolated from Mg-
replete rats. Increased lipoprotein peroxidation and increased plasma and lipoprotein cholesterol concentrations observed in Mg-deficient rats were major characteristics of Mg-deficiency in the promotion of atherosclerosis. These results were in agreement with some other in-vivo studies, which reported that Mg deficiency enhanced lipid deposition within the valves of the left ventricle and in the aorta in rats fed cholesterol (Hungerford and Bernick, 1980).

In the present study, SHR rats exhibited significantly greater systolic blood pressure than WKY counterparts, regardless of the differences in dietary Mg level and protein source. These results confirmed that SHR rats were predisposed to hypertension. Mg deficiency significantly increased systolic blood pressure of rats, and the increase was significantly greater in SHR than in WKY rats. These findings supported the hypothesis made in the present study that Mg deficiency elevated systolic blood pressure of rats and also further enhanced the genetic predisposition of SHR rats to hypertension.

Mg deficiency-induced hypertension observed in rats might act through increased lipoprotein peroxidation. In the present study, lipoproteins isolated from Mg-deficient rats produced greater TBARs and fluorescence at the end of the forced peroxidation, compared to lipoproteins isolated from Mg-replete rats. These results indicated that Mg deficiency enhanced lipoprotein peroxidation. Increased lipoprotein peroxidation observed in Mg-deficient rats might lead to a greater generation of aldehydes, which might block the Ca$^{2+}$ channels, and increase the cytosolic Ca$^{2+}$ concentration and blood pressure. The greater systolic blood pressure observed in Mg-deficient rats was in agreement with the increased lipoprotein susceptibility to forced peroxidation. These findings supported that increased systolic blood pressure induced by Mg-deficiency might act through the increased susceptibility of lipoproteins to oxidation.
Recommendations for Further Work

The findings of lower susceptibility of SHR lipoproteins to forced peroxidation observed in the present study indicated that lipoprotein peroxidation might not be the major factor in the promotion of hypertension. A recent study (Vasdev et al., 2001) showed that supplementation of ascorbic acid produced a significant decrease in blood pressure. However, it was not known whether the supplementation of vitamin C in the diet would also diminish adverse renal vascular changes. It would be of interest to investigate the effects of Vitamin C supplemented diet on lipoprotein oxidation and changes in the blood vessel wall biology in hypertensive rats.

In the present study, Mg deficiency significantly increased the systolic blood pressure in rats and further enhanced the genetic predisposition of SHR rats to hypertension. It would be worthy to study the effects of Mg supplemented diet on the systolic blood pressure and the blood vessel wall biology in rats.

In the present study, dietary soy protein diminished the hypercholesterolemic effects of Mg-deficiency and exhibited a hypocholesterolemic effect on WKY rats. Finally, it would be of interest to further investigate the effects of differences in protein source (soy, casein, or fish proteins) on the plasma lipid concentrations, the susceptibility of lipoproteins to forced peroxidation, and the in-vivo development of atherosclerotic lesions in rats.
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