

# Magnesium dietary intake modulates blood lipid levels and atherogenesis

(hypercholesterolemia/magnesium deficiency/macrophages)

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Communicated by Chandler McC. Brooks¶, January 9, 1989

**ABSTRACT** In this study, we have examined the effects of variation in dietary Mg on the atherogenic process. Oral supplementation of rabbits fed a high cholesterol diet (1% or 2%) with the Mg salt magnesium aspartate hydrochloride (Magnesio-card) (i) lowers the level of serum cholesterol and triglycerides in normal (25–35%) as well as atherosclerotic (20–40%) animals and (ii) attenuates the atherosclerotic process markedly. In addition, we found that dietary deficiency of Mg augments atherogenesis markedly and stimulates (or activates) macrophages of the reticuloendothelial system. Evidence is presented to indicate that the hypercholesterolemic state may cause the loss of Mg from soft tissues to the serum, thereby masking an underlying Mg deficiency.

Hypercholesterolemia has been widely accepted as a high risk factor for development of atherosclerosis and ischemic heart disease (IHD) (1–3), particularly since cholesterol-rich diets lead to deposition of lipids in blood vessel walls and an atherosclerotic-like state in experimental animals (4–6). Increased blood levels of lipoproteins are thought to eventually lead to endothelial cell injury or denudation with concomitant uptake of the former molecules (7, 8), increased permeability to calcium ions ( $\text{Ca}^{2+}$ ) (9), invasion of the arterial wall by macrophages (10–12), and altered smooth muscle cells (7, 8, 13). It is, however, not clear how the lipoproteins and  $\text{Ca}^{2+}$  gain access to the normally and relatively impermeable arterial walls (4–13).

Approximately 20 years ago, it was suggested on the basis of epidemiologic findings that the incidence of IHD was highest in geographic regions with soft drinking water (14). Of the minerals that are deficient in soft water, magnesium (Mg) is the only element that has been found to be consistently lowered in cardiac muscle of IHD victims (for reviews, see refs. 15 and 16). Several lines of evidence exist that indicate that reduced levels of serum Mg play a role in the development of IHD and that elevated levels of Mg can attenuate or improve this condition (15–20). In 1956, Malkiel-Shapiro and co-workers (21) in an open clinical trial reported that use of intramuscular  $\text{MgSO}_4$  in patients with coronary insufficiency lowered serum  $\beta$ -lipoprotein and resulted in striking improvement of the clinical condition. Subsequently, other investigators, using patients with chronic IHD, in open trials confirmed these findings (22–27). Although considerable evidence now exists that  $\text{Mg}^{2+}$  and lipids interact at different levels in the body, such as on absorption, excretion, and metabolism of one another, no clear, consistent relationship between serum Mg and lipid levels has as yet been demonstrated (15, 16, 20, 28).

Recent emphasis on the potential importance of nutritional factors and preventive rather than palliative medicine in the approach to etiology and treatment of cardiovascular disease led us to examine the effects of a possible “hidden” Mg deficiency as well as the potential benefits of Mg supplementation on the development of atherosclerosis induced by high cholesterol diets in rabbits. We report here that dietary deficiency of Mg (compatible with the reduced dietary intake of Mg seen in the adult population of the Western World; see refs. 29–31), which often is not reflected by serum analysis, exacerbates atherogenesis and stimulates (or activates) macrophages of the reticuloendothelial system (RES). In addition, we demonstrate that pretreatment of animals with orally administered magnesium aspartate hydrochloride (Magnesio-card) (i) attenuates the atherosclerotic process markedly and (ii) lowers serum cholesterol and triglycerides in normal as well as in some atherosclerotic animals. Finally, evidence is presented to indicate that a high serum cholesterol seems to obscure the Mg deficiency that is present in the atherosclerotic animal.

## METHODS

**Animals and Diets.** Young, New Zealand White male rabbits (1.3–1.6 kg) were kept in separate stainless steel cages and accustomed to synthetic Purina Rabbit Chow for 1 week. Purina normal synthetic Rabbit Chow (NSRC; no. 5321) of the following composition was utilized: protein,  $\approx 16.2\%$ ; fat,  $\approx 2.5\%$ ; cholesterol,  $\approx 61.0$  ppm; fiber (crude),  $\approx 13.0\%$ ; total digestible nutrients,  $\approx 66\%$ ; nitrogen-free extract (by difference),  $\approx 51.0\%$ ; ash,  $\approx 7.3\%$ ; all necessary minerals and vitamins, including calcium (0.95%) and magnesium (0.233%). After this time, blood samples were taken from an ear vein for serum chemical analyses. The rabbits were then assigned, randomly, to different subgroups [NSRC containing 0.233% Mg; low Mg (35% normal) NSRC; NSRC plus 1% cholesterol; NSRC plus 2% cholesterol; low Mg NSRC plus 1% cholesterol; low Mg NSRC plus 2% cholesterol; high Mg NSRC; high Mg NSRC plus 1% cholesterol; high Mg NSRC plus 2% cholesterol]. Distilled water ( $\text{Mg}^{2+} < 1 \mu\text{M}$ ) or water containing 17.8 g of magnesium aspartate hydrochloride (Magnesio-card) per liter (high Mg groups) was given ad libitum. After 4 and 8 weeks on the respective diets, additional blood samples were drawn for serum chemical analyses. Diet consumption was monitored every 2–3 days and water consumption was monitored daily.

**RES–Macrophage Function and Histology.** At the end of 8 weeks, global RES phagocytic function (K values) was assessed by means of the colloid carbon clearance technique (32) using 20 mg of colloidal carbon per kg (1431a/Pellikan

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Abbreviations: IHD, ischemic heart disease; RES, reticuloendothelial system; NSRC, normal synthetic rabbit chow.

¶Deceased November 29, 1989.

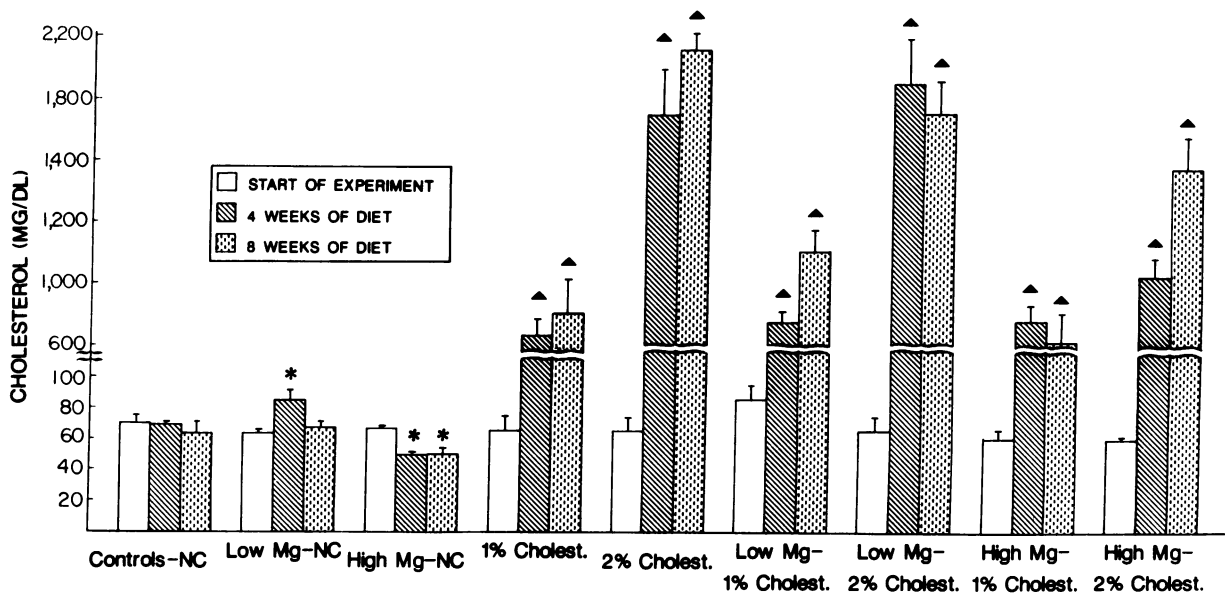


FIG. 1. Comparative effects of high cholesterol intake (1% or 2%) and low vs. high dietary intake of Mg on serum cholesterol levels in rabbits. NC, normal cholesterol. Values are means  $\pm$  SEM ( $n = 4-10$  each). Asterisks signify mean values that are significantly different from controls (0 time;  $P < 0.02$ , paired  $t$  test). Triangles signify mean values that are significantly different from control (0 time;  $P < 0.001$ ).

Werke, Hanover, F.R.G.). After the latter procedure, the animals were sacrificed, and RES organs (liver, spleen, lungs, kidneys, adrenals) and other select organs (e.g., heart) were excised and weighed. Hematoxylin/eosin-stained sections of these organs were examined microscopically. Aortas (from aortic arch to renal arteries) were also excised, opened, pinned on boards, and fixed in formaldehyde for 24 hr; this was followed by staining with sudan IV (33) and photography. Each stained aorta was assigned a random number and two independent observers determined, in a blind manner, by grid-planimetry, the percentage of the intimal surface containing sudanophilic material; the difference between these two observers was insignificant. Maximal intimal thickness and thickness of the media were determined, by direct measurement, using a microscope equipped with a calibrated ocular reticule. The same three sections of aorta were always selected for the latter: (i) just above the aortic valve, (ii) just below the superior mesenteric artery, and (iii) just above the renal artery. From these locations, an average of the thickest and thinnest areas was taken and is reported herein. Serum cholesterol, total protein, albumin, and triglycerides were measured on Kodak D600 and D700 Ektachem analyzers. Serum sodium, potassium, chloride, calcium, blood urea nitrogen, glucose, and creatinine were measured on a Beckman Astra-8 Analyzer. Serum Mg was assessed on a Centrifichem analyzer (Union Carbide) using calmagite (34). Select serum enzymes—e.g., serum glutamate pyruvate transaminase, serum glutamic-oxaloacetic transaminase, lactic acid dehydrogenase, and alkaline phosphatase—were also analyzed on a Centrifichem analyzer. Where appropriate, statistical analyses were performed using Student's  $t$  test, paired  $t$  test, and analysis of variance with Scheffe's contrast test. A  $P$  value  $< 0.05$  was considered significant.

**RESULTS**

**Effects of Dietary Cholesterol and Mg Content on Serum Biochemical Parameters.** Despite the fact that neither the

growth rate (Table 1), the food intake (Table 1), nor the physiologic behavior<sup>¶</sup> differed significantly among the various dietary subgroups, significant alterations in a number of serum chemistries were found over the 8-week study period. For example, low dietary intake of Mg (22–28 mg/kg of body weight per day) in animals fed a normal cholesterol diet, resulted in a 35% elevation in serum cholesterol (Fig. 1), a 20% increase in triglycerides (Table 2), and a 20% reduction in total serum Mg (Table 3) at the end of 4 weeks compared to the normal dietary intake of Mg (75–90 mg/kg per day). High dietary intake of Mg (225–275 mg/kg per day) resulted in a 24% decrease in serum cholesterol, a 33% decrease in triglycerides, and a 15% elevation in total Mg (Table 3) as well as calcium (Ca) [ $10.8 \pm 0.39$  vs.  $12.4 \pm 0.43$  mg/dl (mean  $\pm$  SEM)] at the end of 4 weeks. By 8 weeks, animals fed high cholesterol diets (1% or 2%) with different, concomitant amounts of Mg exhibited differences in serum triglycerides, cholesterol, Mg, and Ca (Fig. 1, Tables 2 and 3). Cholesterol feeding clearly produced elevations in serum Mg, regardless of the dietary Mg level compared to their respective controls (Table 3). A low Mg, high cholesterol diet (1% or 2%) failed

Table 1. Influence of low vs. high dietary intake of cholesterol on weight gain, average food intake, and water consumed per day

Group	Weight gain after 8 weeks, kg	Food intake per day, g	Water consumed per day, ml
Control	$2.09 \pm 0.11$	$102.5 \pm 7.99$	$231.3 \pm 18.59$
Low Mg, NC	$2.29 \pm 0.08$	$101.84 \pm 5.62$	$217.5 \pm 12.33$
High Mg, NC	$2.01 \pm 0.08$	$102.06 \pm 6.05$	$204.6 \pm 14.92$
Chol			
1%	$2.10 \pm 0.24$	$89.87 \pm 7.98$	$172.6 \pm 22.17$
2%	$1.75 \pm 0.16$	$78.77 \pm 6.9$	$237.0 \pm 36.53$
Low Mg + Chol			
1%	$2.06 \pm 0.06$	$99.49 \pm 6.95$	$194.8 \pm 17.23$
2%	$1.85 \pm 0.05$	$75.92 \pm 3.99$	$192.2 \pm 12.85$
High Mg + Chol			
1%	$1.99 \pm 0.17$	$91.7 \pm 12.98$	$253.3 \pm 38.10$
2%	$1.77 \pm 0.11$	$88.3 \pm 7.59$	$263.9 \pm 24.15$

Each group comprised 4–10 animals. The data are given as means  $\pm$  SEM. NC, normal cholesterol; Chol, cholesterol. Rabbits consumed between 30 and 38 g of food per kg per day over the 8-week study period.

<sup>¶</sup>The appearance and behavior of all experimental animals remained normal over the entire 8-week study period. At no time did any of the animals placed on either low Mg or high cholesterol diets become hyperirritable, exhibit hyperemia of the skin, or show signs of hair loss.

Table 2. Influence of low vs. high dietary intake of Mg and high dietary intake of cholesterol on serum triglycerides over an 8-week period in rabbits

Group	Serum triglycerides, mg/dl		
	0 weeks	4 weeks	8 weeks
Control	131.4 ± 20.3	95.8 ± 10.4	116.6 ± 7.5
Low Mg, NC	113.3 ± 8.8	136.5 ± 10.4*	116.2 ± 10.5
High Mg, NC	151.2 ± 10.8	101.2 ± 6.9†	97.1 ± 3.2†
Chol			
1%	129 ± 18.4	89 ± 13.6	100 ± 15.2
2%	147.8 ± 42.1	129.8 ± 30.9	98.8 ± 21.3
Low Mg + Chol			
1%	211 ± 44.6	174 ± 21.7	143 ± 7.9
2%	149.3 ± 26.4	157.8 ± 60.7	163 ± 40.2
High Mg + Chol			
1%	148 ± 16.4	104 ± 10.6*	108 ± 10.3*
2%	153.8 ± 19.8	84.5 ± 10.01†	90.8 ± 9.2†

The data (means ± SEM) were analyzed by paired *t* tests. Each group comprised 4–10 animals. NC, normal cholesterol; Chol, cholesterol.

\*Significantly different from 0 time (*P* < 0.02).

†Significantly different from 0 time (*P* < 0.01).

to raise serum Ca, as is the case with a low Mg, normal cholesterol diet (serum Ca was 13.8 ± 0.38 mg/dl on the control diet vs. 15.6 ± 0.41 mg/dl on the low Mg diet for 8 weeks) or a high Mg, high cholesterol diet (serum Ca levels rose here at 8 weeks 1.4–1.6 ± 0.26–0.32 mg/dl). Serum Na, K, chloride, blood urea nitrogen, glucose, creatinine, total protein, albumin, serum glutamic-oxaloacetic transaminase, serum glutamate pyruvate transaminase, lactic acid dehydrogenase, and alkaline phosphatase did not differ, significantly, among the subgroups (*P* > 0.05).

**Effects of Mg on Atherosclerotic Lesions.** None of the animals fed a normal cholesterol diet, regardless of Mg intake, showed any areas of intimal sudanophilia in their aortas. The animals fed 2% cholesterol and normal Mg, on the average, showed sudanophilic intimal lesions over 60% of the aortic surface, whereas animals fed 1% cholesterol exhibited lesions over 30% of the aortic intimal surface (Fig. 2). The rabbits fed low Mg, high cholesterol diets showed the greatest amount of sudanophilic intimal lesions (Fig. 2) and the greatest amount of atherosclerotic intimal thickening (Fig. 3). However, in animals fed the high Mg, high cholesterol diets only a small proportion of the aortic intima contained sudanophilic material (Fig. 2) and exhibited minimal intimal thickening (Fig. 3).

Table 3. Influence of low vs. high dietary intake of Mg and high dietary intake of cholesterol on serum Mg levels in rabbits

Group	Serum Mg, mg/dl		
	0 weeks	4 weeks	8 weeks
Control, NC	2.75 ± 0.18	2.63 ± 0.26	2.70 ± 0.08
Low Mg, NC	3.05 ± 0.07	2.43 ± 0.05*	2.25 ± 0.16*
High Mg, NC	2.73 ± 0.04	3.15 ± 0.14*	3.48 ± 0.24*
Chol			
1%	2.90 ± 0.08	3.53 ± 0.47	3.25 ± 0.31
2%	2.78 ± 0.17	3.82 ± 0.41*	3.85 ± 0.31*
Low Mg + Chol			
1%	2.62 ± 0.13	2.57 ± 0.14	2.52 ± 0.22
2%	2.42 ± 0.17	3.37 ± 0.31*	2.62 ± 0.12
High Mg + Chol			
1%	2.84 ± 0.12	4.48 ± 0.16*	4.14 ± 0.12*
2%	2.75 ± 0.18	5.25 ± 0.44*	3.52 ± 0.26*

The data (means ± SEM) were analyzed by paired *t* tests. Each group comprised 4–10 animals. NC, normal cholesterol; Chol, cholesterol.

\*Significantly different from 0 time (*P* < 0.02).

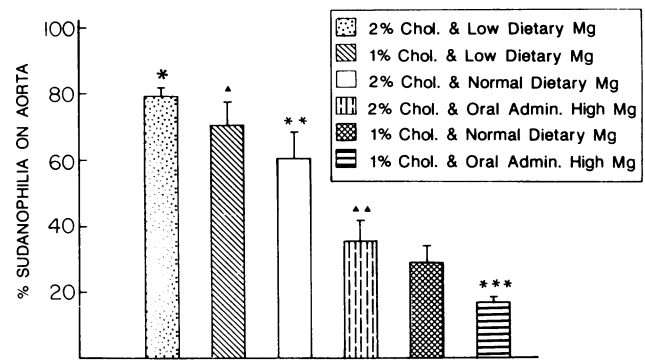


FIG. 2. Comparative effects of low vs. high dietary intake of Mg on development of atherosclerotic lesions in aortas of cholesterol-fed rabbits. Values are means ± SEM (*n* = 4–6 each). \*, Significantly different from 2% cholesterol plus normal Mg and 2% cholesterol plus high Mg (*P* < 0.05, Student's *t* test); ▲, significantly different from all other values, except for low Mg plus 2% cholesterol and 2% cholesterol (*P* < 0.001, analysis of variance, Scheffe's contrast test); \*\*, significantly different from all other values, except for low Mg plus 2% cholesterol and low Mg plus 1% cholesterol (*P* < 0.01, analysis of variance); ▲▲, significantly different from all values, except for 1% cholesterol plus normal Mg (*P* < 0.01, analysis of variance); \*\*\*, significantly different from all other values (*P* < 0.01, analysis of variance).

**RES-Macrophage Studies.** In view of the known link between macrophages and the atherogenic process (8, 10, 11) and the well-known importance of RES-macrophages to host defense (see ref. 35 for review), studies were undertaken to assess RES-macrophage function in animals fed the diverse diets. The RES phagocytosis studies revealed no significant differences in RES phagocytic index (K values) between the normal Mg-, high Mg-, and low Mg-consuming groups on normal cholesterol diets (Table 4). However, in the presence of 1% and 2% cholesterol, the different Mg-consuming groups exhibited K values that were differentially and significantly affected. For example, the high Mg groups had significantly lower phagocytic indices (12–20% depressed, *P* < 0.02) than the normal Mg group, whereas the low Mg groups were significantly higher (39–57% stimulated, *P* < 0.01) than

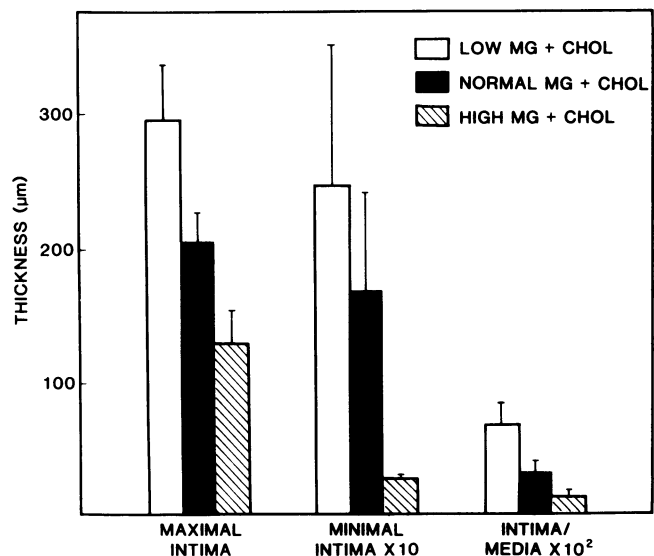


FIG. 3. Influence of varying Mg intake on thickness and intima/media ratio of intimal atheromas in rabbits fed 2% cholesterol. Values are means ± SD (*n* = 4 or 5 each). All high Mg and low Mg values are significantly different from their respective normal Mg values (*P* < 0.02, Student's *t* test).

Table 4. Influence of low vs. high dietary intake of Mg and high dietary intake of cholesterol on RES phagocytic index and RES organ weights after 8 weeks in rabbits

Group	Phagocytic index*	RES organ weight, % body weight	
		Liver	Spleen
Control	0.049 ± 0.003	2.58 ± 0.21	0.028 ± 0.005
Low Mg, NC	0.051 ± 0.003	2.82 ± 0.14	0.032 ± 0.002
High Mg, NC	0.048 ± 0.002	2.67 ± 0.14	0.037 ± 0.14
Chol			
1%	0.045 ± 0.002	3.86 ± 0.20 <sup>†</sup>	0.058 ± 0.010 <sup>‡</sup>
2%	0.060 ± 0.004	5.57 ± 0.36 <sup>†</sup>	0.053 ± 0.008 <sup>‡</sup>
Low Mg + Chol			
1%	0.068 ± 0.009 <sup>†</sup>	4.48 ± 0.38 <sup>†</sup>	0.089 ± 0.015 <sup>‡</sup>
2%	0.077 ± 0.008 <sup>†</sup>	4.34 ± 0.33 <sup>†</sup>	0.070 ± 0.016 <sup>‡</sup>
High Mg + Chol			
1%	0.043 ± 0.001 <sup>†</sup>	3.56 ± 0.24 <sup>†</sup>	0.043 ± 0.004 <sup>‡</sup>
2%	0.039 ± 0.003 <sup>†</sup>	4.02 ± 0.37 <sup>†</sup>	0.049 ± 0.009 <sup>‡</sup>

The data (means ± SEM) were analyzed by *t* tests. Each group comprised 4–10 animals. NC, normal cholesterol; Chol, cholesterol. \*K value (see text).

<sup>†</sup>Significantly different from controls ( $P < 0.02$ ).

<sup>‡</sup>Significantly different from controls ( $P < 0.01$ ).

normal dietary intake of Mg. The organs accounting for 95% of the global phagocytic index in rabbits and mammals, in general, are the liver and spleen (35). When comparing the % body weight of these organs among the various subgroups (Table 4), we observed the following: (i) no differences were noted among the normal cholesterol-fed groups; (ii) the liver weight was increased in all high cholesterol subgroups—e.g., by 38–50%, 68–74%, and 38–56% in normal, low, and high Mg groups, respectively ( $P < 0.02$ ); and (iii) the spleen weight was increased in all high cholesterol subgroups—e.g., by 89–107%, 150–218%, and 54–75%, respectively ( $P < 0.01$ ). It is obvious that the degree of splenomegaly is far greater than the degree of hepatomegaly. Microscopic studies performed on livers and spleens of the cholesterol groups indicated that the increase in liver weight is primarily due to fatty infiltration, whereas the changes in splenic weight are attributed to drastic alterations in the diverse cell types; the most striking changes in spleens were noted in the low Mg, high cholesterol groups wherein the white pulp was reduced but many lipid-laden cells were seen.

## DISCUSSION

Results reported here demonstrate that the atherogenic process is significantly affected by the levels of Mg in the diet in rabbits fed a high cholesterol diet. Low levels of dietary Mg enhanced, whereas high levels of dietary Mg retarded, the development of atherogenic lesions. An unexpected and, we believe, significant observation was that animals fed a high cholesterol diet exhibited an increase in serum Mg levels, compared to their respective controls, irrespective of the Mg level in their diet. This is not a reflection of kidney, liver, or intestinal damage since various tissue enzymes and serum components were not altered in the animals fed a high cholesterol diet. The above observation is also not explained by changes in circulating blood proteins or volume as serum total protein and albumin levels were not altered among the groups.

Although not discussed by the authors, a careful review of data presented by Vitale *et al.* (28) provides corroborating evidence supporting the above observation. These authors observed that at a given level of Mg in the diet (12–192 mg/100 g), rats maintained on a diet yielding a higher level of serum cholesterol had Mg levels 11–43% higher than animals fed a diet yielding a low serum cholesterol level. Our observations

and these data (28) showing that a higher level of Mg is present in hypercholesterolemic animals, we believe, suggest that the hypercholesterolemic state results in loss of Mg from soft tissues, thereby generating an underlying Mg deficiency, which, as shown here, augments the atherogenic process. This suggestion is also supported by other results of Vitale *et al.* (28), who showed that the addition of cholesterol and cholic acid to an otherwise adequate Mg diet produced overt, clinical signs of Mg deficiency that could be effectively reversed upon supplementation of Mg in the diet. Several workers have reported inverse relationships between Mg and cholesterol in the serum of humans (21, 27) as well as relationships between Mg and heart disease (14–18, 22), whereas others fail to find any such relationships (36–38).

Overall, our data suggest that (i) the dietary intake, and serum level, of Mg can be very important in modulating the serum level of lipids—i.e., cholesterol and triglycerides—in rabbits; (ii) low Mg intake [comparable to the reduced levels observed in current diets of young adults and the aging population of the Western World (29–31)], which in itself will not produce clinical signs of Mg deficiency in human subjects or in our animal studies, augments the atherogenic process in rabbits and stimulates (or activates) RES—macrophages; (iii) such a Mg deficient diet increases lipid deposition in high cholesterol-fed animals (the greater the degree of concomitant cholesterol intake, the greater is the relative increase in lipid deposition); (iv) high dietary cholesterol, or blood levels of lipids, increases the amount of Mg circulating in the blood; and (v) higher than normal Mg intake appears to be a very useful ameliorative agent in experimental atherogenesis, at least in rabbits.

It has been shown in several studies that divalent cations can increase the excretion of lipids in animals and human subjects (39, 40). Such an effect has been attributed to the ability of these cations to form insoluble salt complexes with fatty acids or form complexes with bile acid derivatives to reduce their absorption (39, 40). Although the serum cholesterol level was indeed significantly lower in some of the high Mg, high cholesterol-fed subgroups (i.e., 2%; Fig. 1), it was still a considerably elevated value in the latter, and it was not affected in the 1% subgroups, except when compared to those animals fed low Mg diets (Fig. 1). So, this explanation is unlikely to be the only reason for our results. A strong correlation is known to exist between dietary cholesterol levels, serum cholesterol levels, and the formation of atherosclerotic plaques (1–8). If Mg was simply varying the intestinal absorption of cholesterol, then one would anticipate a good correlation between serum cholesterol levels and the extent of atherogenic lesions in our experiments. Furthermore, Nakamura *et al.* (41), using radiolabeled cholesterol and rabbits fed low Mg in their diets, reported no increases in radioactivity in liver, heart, or kidney, thus arguing against intestinal absorption as a factor.

A replot of our data shown in Figs. 1 and 2, presented in Fig. 4, illustrates that the extent of atherogenic lesions is, in fact, poorly correlated with the level of serum cholesterol and highly dependent on the level of dietary Mg. The former observation is consistent with the report of Vitale *et al.* (28), who also observed a magnesium-induced “. . . dissociation between (the) degree of hypercholesterolemia and the extent of vascular sudanophilia.” The effect of low dietary Mg on the formation of intimal lesions is striking. Comparison of the extent of intimal involvement in rabbits fed a 1% vs. a 2% cholesterol diet shows that relative to a low Mg diet, a high Mg diet reduces intimal involvement by 52% and 45%, respectively. It is of interest to note that orally supplemented Mg is somewhat more effective in preventing intimal lesions at the lower level of dietary cholesterol, perhaps indicating that its effectiveness is reduced at higher dietary cholesterol levels. It is apparent that a mechanism other than simply

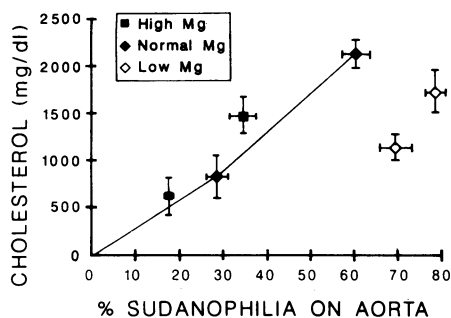


FIG. 4. Relationship between serum cholesterol level, dietary Mg, and atherosclerotic lesions (% sudanophilia on aorta). Replot of data from Figs. 1 and 2. Bars = SEM. The solid line is used to separate the low from the normal and high Mg subgroups.

affecting the intestinal absorption of cholesterol is in effect here.

Our present findings in the normal cholesterol-fed as well as high cholesterol-fed animals demonstrate that dietary intake of Mg can also alter the serum level of triglycerides. Since Mg is known to widely affect protein metabolism, which includes the apolipoproteins involved in lipid transport and metabolism (2, 3), it is possible this increased plasma lipid clearance would thus decrease wall deposition of lipids and atheroma lesions.

Mg is well known to affect Ca uptake into soft tissues [particularly vascular smooth muscle cells (15–17)], which is known to be an important factor in atherosclerosis (9–13, 42). Our finding that dietary Mg and cholesterol can alter serum Ca may be related to these phenomena. The fact that high dietary cholesterol intake results in increased serum levels of Mg (Table 3) may indicate that as Mg is lost from soft tissues, including vascular smooth muscle,  $Ca^{2+}$  thus could gain access to the latter cells to aid the atherosclerotic process. Moreover, Mg affects platelet function and coagulation (16), both thought to be involved in atherogenesis (4–8). Increased  $Mg^{2+}$  prolongs clotting time and decreases aggregation of platelets *in vitro* and *in vivo* (43). In addition, accumulation of platelets that usually occurs at sites of injury in blood vessels can be prevented by excess  $Mg^{2+}$  (44). A number of endothelial cell factors, suggested to play roles in the atherogenic process—e.g., prostacyclin generation, endothelial-derived relaxant factors, platelet-derived growth factor, etc.—all appear to be dependent upon  $[Mg^{2+}]$  (for review, see ref. 45).

Macrophages have been implicated as the prime source of foam cells in atherogenesis (10–12). These phagocytic cells enter the vascular wall and accumulate lipids. It has been well demonstrated that macrophages are part of the lesion produced by atherogenic diets, and they seem to play an important role in the “inception and progression” of the lesion (10–12). Our data demonstrate that such macrophages seem to be increased in abundance by diets low in  $Mg^{2+}$ . The significantly increased size of the spleens and changes in magnitude of white pulp of such animals together with the increased phagocytic index suggest a major role for these cells in deposition of lipids in the arterial walls. According to Zieve *et al.* (46), Mg deficiency can cause a 50% reduction in splenic protein synthesis and a concomitant 350% increase in splenic DNA synthesis in rats; the red pulp of the organ becomes congested with new, young cells. The cellular proliferation seen in the Mg-deficient spleen could be viewed as a causal factor for the exacerbation of atherogenesis in our study.

Another interesting result from our experiments is the fact that the presence of higher than normal cholesterol in the blood seems to somehow shift Mg out of organs into the blood. It is thus not surprising that it has been difficult to find

an inverse relationship between serum Mg and serum lipid levels. The often-referenced findings of elevated serum Mg in some IHD cases as well as the observed elevation in serum Mg after acute myocardial infarctions (for review, see ref. 47) may be a consequence of higher than normal blood lipid levels. Further, the normal to elevated levels of Mg present in hypercholesterolemic animals may indicate that, in humans, determination of normal Mg levels in hypercholesterolemic individuals may mask a “hidden Mg deficiency.” Lastly, it is important to emphasize here that this report demonstrates that orally supplemented Mg is highly effective in preventing the atherogenic process in rabbits.

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