Hypertriglyceridemic serum from magnesium-deficient rats induces proliferation and lipid accumulation in cultured vascular smooth muscle cells

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An important characteristic of hyperlipemia associated with magnesium deficiency in rats is the postprandial accumulation of triglyceride-rich lipoproteins. The present investigation was performed to determine the effect of serum from magnesium-deficient animals on cultured vascular smooth muscle cells (VSMC). Sera were obtained from control and magnesium-deficient rats fed adequate or deficient diets for 8 days. Magnesium-deficient animals were hypertriglyceridemic compared with control rats, but their total cholesterolemia was not significantly modified. Pooled sera from control and magnesium-deficient animals were added to the culture medium at various concentrations. The maximum of proliferation for both control and magnesium-deficient sera was reached when they were added at 6% to the culture medium and on day 4 after the begining of incubation. Medium containing serum from magnesium-deficient rats stimulated the cell proliferation as monitored by cell count and [^{3}H]thymidine incorporation. Staining of VSMC with Oil red O and measuring lipids have shown a marked lipid accumulation (triglycerides) in cells incubated with serum obtained from magnesium-deficient animals compared with serum from control rats. These results indicate that serum from magnesium-deficient rats contains factors that stimulate proliferation of arterial medial cells and that hyperlipemia associated with magnesium-deficiency may cause lipid accumulation in vascular cells. (J. Nutr. Biochem. 5:585–590, 1994.)

Keywords: magnesium; hypertriglyceridemia; vascular smooth muscle cells; atherosclerosis; rat

Introduction

Magnesium (Mg) deficit has been implicated as a risk factor for development of atherosclerosis.[1–3] Experimental Mg deficiency enhances vascular lipid infiltration in rodents fed atherogenic diets, and dietary Mg supplementation can prevent atherosclerosis.[4,5] These lesions, however, are not necessarily correlated with cholesterolemia, and the mechanism causing them has yet to be indentified. An important characteristic of hyperlipemia associated with Mg deficiency in rats is the postprandial accumulation of triglyceride-rich lipoproteins (TGRLP).[6,7] These lipoproteins isolated from Mg-deficient rats have increased susceptibility to peroxidation and thus might be potentially atherogenic.[8,9]

Vascular smooth muscle cell (VSMC) proliferation and lipid accumulation are well accepted as primary components in the pathophysiology of atherosclerosis.[10] VSMC are the predominant cells in arteries and are essential for the structural and functional integrity of blood vessels. In atherosclerosis these cells deviate from their normal state; undergo extensive proliferation; and are transformed into foam cells accumulating lipids, becoming a major component of the atherosclerotic lesion.[10]

Given the current interest in the effect of Mg deficiency on lipid metabolism and cardiovascular disease, the present study was performed to determine whether the serum from Mg-deficient animals could alter the proliferation of cultured VSMC and whether this hyperlipidemic serum causes the lipid accumulation in these cells.

Methods and materials

Animals and diets

Weanling male Wistar rats (IFFA-CREDO, L'Arbresle, France) weighing about 60 g were randomly divided into Mg-deficient and control groups. The institution's guide for the care and use of

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laboratory animals was used. The rats were housed in wire-bottomed cages in a temperature-controlled room (22° C) with a 12-hr dark (20:00–08:00 hr) and 12-hr light period. They were pair fed the appropriate diets for 8 days using an automatic feeding apparatus. Distilled water was provided ad libitum. The synthetic diets contained (g/kg): casein 200, DL-methionine 3, sucrose 650, corn oil 50, choline bitartrate 2, modified AIN-76 mineral mix 35, and AIN-76A vitamin mix 10 (ICN Biomedicals, Orsay, France). MgO was omitted from the AIN mineral mix in Mg-deficient diet. The Mg concentration of diets determined by flame atomic absorption spectrometric analysis (Perkin Elmer 400, Norwalk, CT USA) were 35 mg/kg (deficient) and 980 mg/kg (control). Experiments were performed in nonfasting rats. Animals were anesthetized with sodium pentobarbital (40 mg/kg of body weight i.p.). Blood was collected by exsanguination via abdominal aorta and serum was obtained after clot formation and centrifugation (1,000*g*, 15 min). Six pools from control and Mg-deficient groups, eight animals per group from independent experiments, were constituted. Pooled sera were filtered through a 0.2-μm filter (Millipore, Molsheim, France), decomplemented at 56° C for 30 min, and then stored at −20° C.

Biochemical analyses in serum

Mg in serum was determined by atomic absorption flame spectrometry (Perkin Elmer 400) after dilution in lanthanum chloride solution containing 1 g La/L. Triglycerides (Biotrol, Paris, France) and total cholesterol (BioMérieux, Charbonnières-les-Bains, France) were determined by enzymatic procedures.

VSMC culture

Primary VSMC were prepared according to a modification of the method described by Travo et al.[11] Briefly, 12-week-old male Wistar rats, weighing about 300 g, fed a standard pellet diet (UAR, Villemoisson, France) were killed by cervical dislocation and the aorta was dissected out. After asseptical removal, aorta was skinned with fine metal forceps and incubated in 3 mL of Hank's Balanced Salt Solution (HBSS) (Sigma, St Louis, MO USA) and 3 mL of collagenase (Seromed, Berlin, Germany) (200 U/mL of HBSS) at 37° C for 15 min. The adventitia was then carefully stripped off. The aorta was longitudinally opened and cut into small pieces. Pieces of endothelium-denuded aorta were incubated at 37° C for 20 min in 6 mL of HBSS containing 100 U/mL of collagenase. Then an elastase digestion was performed by incubating pieces of aorta with 3 mL of collagenase solution (200 U/mL of HBSS) and 3 mL of elastase (Biosys, Compiègne, France) solution (17.5 U/mL of HBSS) at 37° C for 40 min with a slow shaking movement. The obtained smooth muscle cell suspension was centrifuged at 180*g* for 2 min. The pellet was resuspended in minimal essential medium (MEM) (Eurobio, Les Ulis, France), and cells were incubated in plastic culture flasks (Corning Inc., Corning, NY USA) with MEM supplemented with 200 mM L-Glutamin (Sigma), Penicillin-Streptomycin solution (Sigma) (10,000 U/mL-10 mg/mL), amino acid concentrated solution (100x) (Sigma) and containing 10% of fetal calf serum (FCS) (Seromed, Berlin, Germany). Cells were incubated in a humidified incubator at 37° C with an atmosphere of 95% air-5% CO_2. The medium was changed 2 days later, and cells were placed in supplemented MEM containing 5% FCS. After that, the medium was changed every 2 days. VSMC were identified according to morphological criteria: the "hill and valley" typical growth pattern of VSMC and immunologically with anti-α actin monoclonal antibody (Sigma) followed by a second fluorescent (FITC) goat anti-mouse IgG antibody (Sigma). The passage number of the cultured cells used in this study ranged from two to six.

Cell count

Confluent cells were trypsynized with a trypsin-EDTA solution (0.04% and 0.02%, respectively) and plated in a 24-well plate at concentration 2.10^4 cells/well in 1 mL of MEM containing 2% of FCS for 24 hr and then incubated in serum-free medium for 24 hr, permitting cell synchronisation. After that, quiescent cells were incubated in MEM containing serum from control or Mg-deficient rats at various concentrations. For cell count, cells were detached by trypsinisation with 1 mL of trypsin-EDTA. For each well the cellular suspension was diluted in 9 mL of isotonic solution (Labinter, Aix-en-Provence, France). Cell number was counted with a Coulter Counter DN (Coultronics France SA, Mergency, France).

[^{3}H] Thymidine incorporation

VSMC were plated in a 24-well plate at 5.10^4 cells/well with 500 μL/well of MEM containing 5% of FCS. After 24-hr culture, medium was removed and replaced by serum-free medium for 24 hr. Thereafter, quiescent cells were incubated with MEM containing 6% of control or Mg-deficient serum and incubated at 37° C with 0.5 μCi [^{3}H]Thymidine (specific activity 1.04 TBq/mmol; Amersham, Buks., UK). A control of incorporation was determined by incubating VSMC with serum-free medium. After 24-hr incubation, cells were washed three times with 1 mL of cold PBS, precipitated with 1 mL of 10% TCA, and twice with 1 mL of 5% TCA, then washed twice with 1 mL of ethanol prior to drying. The cells were solubilized 2 hr at 60° C in 300 μL of 0.3N NaOH-1%SDS. 200 μL of this solution was transferred to the scintillation vial and mixed with 3 mL of PICO-FLUOR 40 (Packard Instruments, Groningen, The Netherlands). The radioactivity was counted in a liquid scintillation counter (Packard Instruments).

Trypan blue assay

Quiescent cells were cultured in 24-well plates with 6% of control and Mg-deficient serum, and cytoxicity assay was performed every day for 5 days by using a trypan blue solution (0.4%, p/v), according to the manufacturer's protocol (Sigma).

Lipid and protein analyses in VSMC

VSMC were cultured as described in the cell count section. Cells were exposed to 6% of control or Mg-deficient serum for 4 days. After that, wells were rinsed with HBSS, trypsinized (100 μL of Trypsin-EDTA solution/well), and 150 μL of HBSS were added to each well. The pooled cellular suspension from six wells (1.5 mL) for each tested serum was homogenized. Two hundred fifty microliters (in duplicate) were removed for determination of protein content and the remaining 1 mL for determination of lipid content. Total cellular lipids were extracted using chloroform-methanol method according to Folch et al.[12] Triglyceride and total cholesterol content in lipid extracts were measured as previously described by us.[13] Protein content was determined with BCA protein assay kit (Pierce Chemical Co., Rockford, IL USA).

Oil Red O-staining

Lipid inclusions in cells were stained according the method described by Ganter and Jalles.[14] After incubation with tested sera, cells were washed three times with 1 mL of HBSS and fixed with Boin's liquid (RAL, Villers St Paul, France) for 24 hr. After that, cells were washed three times with desionized water and 1 mL of isopropylic alcohol 60% (vol/vol), followed by staining with 1 mL of Oil Red O (0.5 g/100 mL of isopropylic alcohol) for 2 hr and washing with 1 mL of isopropylic alcohol (60%, vol/vol) then three times with deionized water. For nucleus staining, cells were

incubated with 1 mL of hematoxylin (RAL) for 3 min and washed three times with deionized water.

Scanning electron microscopy

Quiescent cells plated in 24-well plates and incubated with 6% of control or Mg-deficient serum for 4 days were fixed in 3% glutaraldehyde, 0.2 M cacodylate buffer (pH 7.4) for 1 hr at 4° C. After that, they were washed three times in this buffer, post fixed with 1% osmic acid for 1 hr at 4° C, washed in the same buffer, and dehydrated with 70, 95, and 100% ethanol for 10 min each. Samples were transferred into a critical-point drying apparatus, coated with gold, and examined with a scanning electron microscope (SEM 505, Philips).

Statistical analysis

Results were expressed as means ± SEM. Statistical significance of differences between groups was evaluated by Student's *t* paired or unpaired test. Results were considered significant at $P < 0.05$.

Results

Mean final body weights of Mg-deficient and pair-fed control rats were 86 ± 3 and 95 ± 3 g ($n = 48$, $P < 0.05$). Clinical signs of inflammation, including erythema of ears, were observed in rats fed the Mg-deficient diet. Hypomagnesemia was observed in deficient animals *(Table 1)*. Triglyceride concentration in serum was significantly greater in Mg-deficient rats than in controls, whereas serum total cholesterol was only modestly elevated *(Table 1)*. To study the effects of control and Mg-deficient sera on VSMC growth, cells were incubated 5 days with the addition of these sera, at various percentages, to the culture medium. The maximum cell growth, monitored by daily cell count, was reached on day 4, when cells were incubated with the addition of 6% of the tested sera *(Figure 1a, b)*. In these conditions (i.e., 6% of serum in the medium and 4 days of culture), the cell growth was more important in the presence of Mg-deficient serum than in the presence of control serum (32% increase) *(Table 2)*. In agreement with this result, serum from Mg-deficient rats, as compared with the control rat serum, enhances VSMC proliferation studied by [^{3}H]Thymidine incorporation *(Table 2)*. There was no cytotoxic effect of tested sera on the cultured VSMC as monitored by the trypan blue test. The staining of neutral lipids in VSMC with Oil red O revealed that VSMC cultured with addition of serum from Mg-deficient rats contained numerous lipid inclusions *(Figure 2a)*. These lipid inclusions were relatively limited in VSMC cultured in the presence of serum from control animals *(Figure 2a)*. Similar observations were made when these cells were analyzed by scanning electron microscopy,

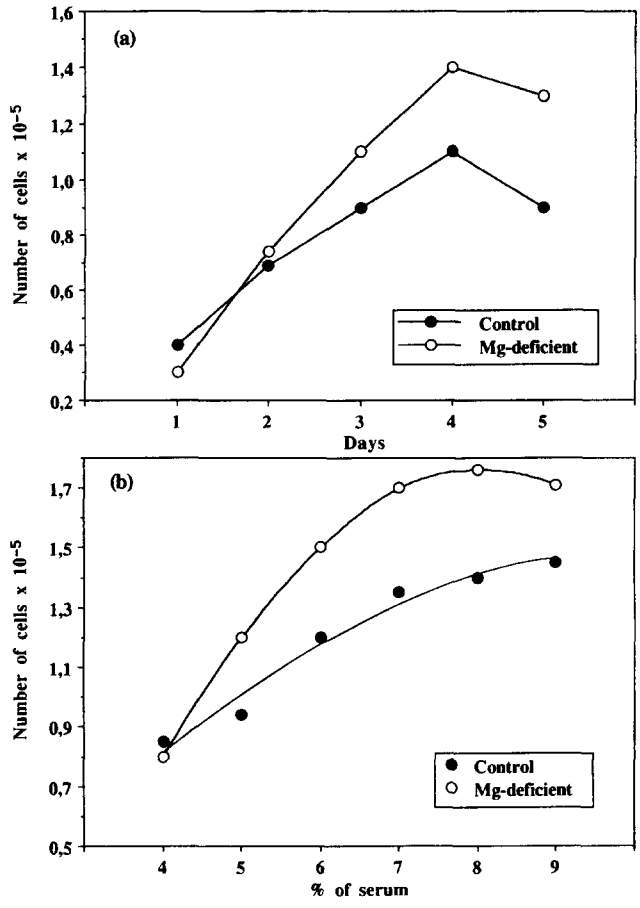

Figure 1 Effect of control and Mg-deficient serum on rat aorta smooth muscle cell growth. a) effect of the addition of 6% serum from control and Mg-deficient rats to the culture medium during five days; b) effect of serum level in the medium. Cells were plated at 2.10^4 cells/well in a 24-well plate and incubated in the medium containing various percentage of serum from control or Mg-deficient rats during five days. The cell count was performed every day. The presented curve is representative from four independent experiments.

Table 1 Magnesium and lipid concentrations in pooled sera from control and Mg-deficient rats

Serum	Magnesium	Triglycerides (mmol/L)	Total cholesterol
Control	0.82 ± 0.03	0.79 ± 0.09	1.45 ± 0.08
Mg-deficient	0.13 ± 0.01*	4.00 ± 0.40*	1.72 ± 0.12

Values are means ± SEM of six values per group; *$P < 0.01$.

i.e., more accumulation of lipids in VSMC cultured with Mg-deficient serum than with control serum *(Figure 2b)*. As shown in *Table 2*, addition of serum from Mg-deficient rats to the culture medium for 4 days brings about a marked triglyceride accumulation in VSMC as compared with the cells cultured with control serum. However, there was no significant difference in total cholesterol content in cells cultured with the addition of both sera studied *(Table 2)*.

Discussion

Previous experiments from our laboratory provided evidence that Mg deficiency produces a dyslipoproteinemia characterized by increased plasma levels of very low density lipoproteins (VLDL) and low density lipoproteins (LDL), and decreased plasma high density lipoprotein levels.[6] The accumulation of TGRLP in the plasma of Mg-deficient animals is a most important characteristic of hyperlipemia associated with Mg deficiency.[6,7] It has been previously demonstrated

Table 2 Effect of sera from control and Mg-deficient rats on rat aorta smooth muscle cell proliferation and lipid content

Serum	Cell growth† (cell × 10^3/well)	[^{3}H]Thymidine incorporation§ (cpm/well)	Triglycerides† (μg/mg protein)	Total cholesterol† (μg/mg protein)
Control	83 ± 10	1,102 ± 163	114 ± 4	16.7 ± 1.7
Mg-deficient	108 ± 13*	1,237 ± 140*	157 ± 16*	18.7 ± 1.2

†For cell count and lipid content measurement quiescent cells were incubated with 6% of control or Mg-deficient serum in the culture medium for four days.
§For [^{3}H]Thymidine incorporation quiescent cells were incubated with 6% of control or Mg-deficient serum and with 0.5 μCi/mL of [^{3}H]Thymidine for 24 hr.
Results are means ± SEM of four to six various pools of control and Mg-deficient sera.
*$P < 0.01$.

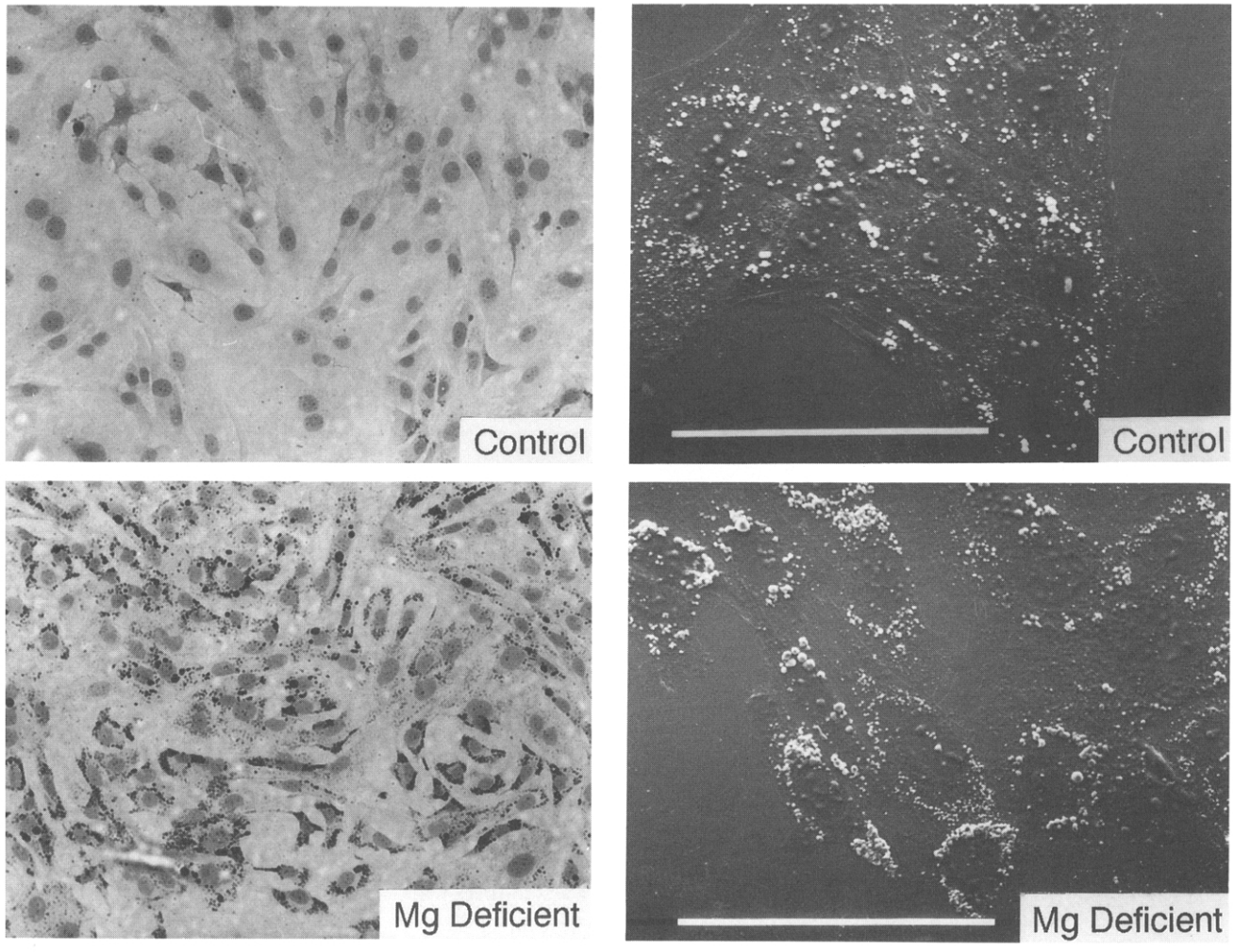

Figure 2 Accumulation of lipids in cultured rat aorta smooth muscle cells cultured for 4 days with 6% of control or Mg-deficient serum in the medium. a) Phase contrast microscopy after staining with Oil red O (× 200); lipids visible as black inclusions. b) Scan electron microscopy (× 600); lipids visible as white inclusions.

by our group that this hypertriglyceridemia mainly results from decreased lipoprotein lipase activity.[7]

The present results demonstrate for the first time that the hyperlipidemic serum from Mg-deficient rats induces VSMC proliferation and causes lipid accumulation in these cells. Previous works have shown that hyperlipidemic sera from cholesterol-fed rabbits and monkeys also induce cell proliferation and lipid accumulation in these cells.[15–19] However, in contrast to these earlier studies carried out on hypercholesterolemic sera, the present study used hyperlipidemic serum rich in TGRLP. Our observation is of particular interest because we used hyperlipidemic serum obtained from

animals that develop dyslipidemia related to a metabolic disorder and not to an increase in dietary lipid intake. There is growing evidence that hypertriglyceridemia may be associated with increased risk for cardiovascular heart disease (CHD).[20] It has been recently discussed[21] that TGRLP could be directly atherogenic and/or that the metabolic consequences of hypertriglyceridemia could account for the increased risk for CHD.

It has been shown in studies carried out on vascular endothelial cells and on macrophages that VLDL from hypertriglyceridemic human plasma are more likely to promote cellular lipid uptake than those from normolipidemic plasma, and that the predominant lipid that accumulates intracellularly after uptake of hypertriglyceridemic VLDL is triglyceride.[22,23] In agreement with these previous observations, the accumulation of triglycerides in VSMC in the present work reflects the predominant lipid in the TGRLP that accumulates in the serum of Mg-deficient rats.

The effect of the hyperlipidemic serum on the increased VSMC proliferation may be due to both lipoprotein and nonlipoprotein components.[10,24] The observed effect of serum from Mg-deficient animals on VSMC proliferation is undoubtedly not related to the low Mg concentration in this serum because the studied serum was added to the medium at a low percentage, and the medium used provides the Mg concentration (about 1 mmol/L) close to this observed in the control serum. However, other authors who have studied the effect of low Mg concentration on cultured cells[25–27] have found that Mg-deficient medium could affect the vascular cell integrity and functions. Thus, in addition to the observed effect of the serum from Mg-deficient animals on VSMC in the present study, low Mg concentration in the serum may play an important role in the vascular cell physiology and may contribute to the development of the atherosclerotic lesion.

Distinct mitogenic and growth-stimulating effects of various lipoprotein fractions isolated from human plasma have recently been demonstrated.[28,29] Additionally, LDL isolated from diabetic patients have been shown to stimulate VSMC proliferation as compared with normal LDL.[30] Earlier works carried out on rhesus monkeys have also shown that LDL isolated from these primates fed a high-fat cholesterol-supplemented diet induce VSMC proliferation to a higher extent than from controls.[16,18] Thus, a significant part of the serum effect on VSMC proliferation may be due to the lipoprotein component of the serum, in particular, in the hyperlipidemia.

Recently, we have shown increased susceptibility to peroxidation of TGRLP isolated from Mg-deficient rats compared with controls.[8,9] There is increasing evidence that oxidatively modified lipoproteins are important in atherogenesis.[31] Oxidized lipoproteins can modulate many biological processes, and when they are more extensively oxidized, they become cytotoxic. Recently Chatterjee[32] has shown that oxidized human LDL at low concentrations exerts a concentration-dependent stimulation of VSMC proliferation. Because it is well known that cells are able to catalyze the oxidation of lipoproteins, it may be hypothesized that TGRLP present in hyperlipidemic serum from Mg-deficient rats undergo oxidative modification to a greater extent than that of control rats, and that it exerts a more potent effect on cultured cells.

Mg-deficiency in the rat induces an inflammatory state with increased concentrations of cytokines (IL-1, IL-6, and tumor necrosis factor).[33] Because cytokines are able to induce VSMC proliferation and lipid accumulation,[10,34] the increased level of inflammatory cytokines may constitute an additional factor by which serum from Mg-deficient animals affects VSMC. On the other hand, inflammatory response observed in Mg-deficient animals may play an important role in the development of hypertriglyceridemia in these animals because it is well known that several cytokines cause a rapid increase in serum triglycerides by stimulating liver lipogenesis and by decreasing the activity of LPL.[35]

In conclusion, the present study indicates that serum from Mg-deficient animals contains a factor(s) that stimulates the proliferation of arterial medial cells, and that hyperlipemia associated with Mg deficiency may cause lipid accumulation in vascular cells. Further study is required to elucidate the factor(s) by which this serum modulates VSMC proliferation.

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References

1 Seelig, M. S. (1980). *Magnesium Deficiency in Pathogenesis of Disease.* Plenum Press, New York, NY USA
2 Rayssiguier, Y. and Gueux, E. (1986). Magnesium and lipids in cardiovascular disease. *J. Am. Coll. Nutr.* **5,** 507–519
3 Durlach, J. (1988). *Magnesium in Clinical Practice.* John Libbey, London, UK
4 Altura, B. T., Brust, M., Bloom, S., Barbour, R. L., Stempak, G., and Altura, B. M. (1990). Magnesium dietary intake modulates blood lipid levels and atherogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **87,** 1840–1844
5 Rayssiguier, Y. (1990). Magnesium and lipid metabolism. In *Metal Ions in Biological System. Magnesium and its role in biology, nutrition and physiology,* Vol 26, (H. A. Siegel, ed.) p. 341–358, Marcel Dekker, New York, NY USA
6 Gueux, E., Mazur, A., Cardot, P., and Rayssiguier, Y. (1991). Magnesium deficiency affects plasma lipoprotein composition in rats. *J. Nutr.* **121,** 1222–1227
7 Rayssiguier, Y., Noé, L., Etienne, J., Gueux, E., Cardot, P., and Mazur, A. (1991). Effect of magnesium deficiency on post-heparin lipase activity and tissue lipoprotein lipase in the rat. *Lipids* **26,** 182–186
8 Gueux, E., Cubizolles, C., Bussière, L., Mazur, A., and Rayssiguier, Y. (1993). Oxidative modification of triglyceride-rich lipoproteins in hypertriglyceridemic rats following magnesium deficiency. *Lipids* **28,** 573–575
9 Rayssiguier, Y., Gueux, E., Bussière, L., Durlach, J., and Mazur, A. (1993). Dietary magnesium affects susceptibility of lipoproteins and tissues to peroxidation in rats. *J. Am. Coll. Nutr.* **12,** 133–137
10 Raines, E. W. and Ross, R. (1993). Smooth muscle cells and the pathogenesis of the lesions of atherosclerosis. *Br. Heart J.* **69,** S30–S37 (Suppl)
11 Travo, P., Barett, G., and Burnstock, G. (1980). Differences in proliferation of primary cultures of vascular smooth muscle cells taken from male and female rats. *Blood Vessels* **17,** 110–116
12 Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–506
13 Mazur, A., Rémésy, C., Gueux, E., Levrat, C., and Demigné, C. (1990). Effects of diets rich in fermentable carbohydrates on plasma lipoprotein levels and on lipoprotein catabolism in rats. *J. Nutr.* **120,** 1037–1045
14 Ganter, P. and Jalles, G. (1970). *Histochimie Normale et Pathologique,* vol. 2, Gauthier-Villars Edition, Paris

15 Fisher-Dzoga, K., Fraser, R., and Wissler, R.W. (1976). Stimulation of proliferation in stationary primary cultures of monkey and rabbit aortic smooth muscle cells. *Exp. Mol. Pathol.* **24,** 346–359

16 Fischer-Dzoga, K. and Wissler, R. W. (1976). Stimulation of proliferation in stationary primary cultures of monkey aortic smooth muscle cells. *Atherosclerosis* **24,** 515–525

17 Chen, R. M., Getz, G. S., Fischer-Dzoga, K., and Wissler, R. W. (1977). The role of hyperlipidemic serum on the proliferation and necrosis of aortic medial cells in vitro. *Exp. Mol. Pathol.* **26,** 359–374

18 Fless, G. M., Kirchhausen, T., Fischer-Dzoga, K., Wissler, R. W., and Scanu, A. M. (1982). Serum low density lipoproteins with mitogenic effect on cultured aortic smooth muscle cells. *Atherosclerosis* **41,** 171–183

19 Campbell, J. H., Reardon, M. F., Campbell, R. G., and Nestel, P. J. (1985). Metabolism of atherogenic lipoproteins by smooth muscle cells of different phenotype in culture. *Arteriosclerosis* **5,** 318–328

20 Austin, M. (1991). Plasma triglyceride and coronary heart disease. *Arter. Thromb.* **11,** 2–14

21 Gianturco, S. H., Bradley, W. A., Nozaki, S., Vega, G. L., and Grundy, S. M. (1993). Effect of Lovastatin on the levels, structure and atherogenicity of VLDL in patients with moderate hypertriglyceridemia. *Arter. Thromb.* **13,** 472–481

22 Gianturco, S. H., Brown, S. A., Via, D. P., and Bradley, W. A. (1986). The β-VLDL receptor pathway of murine p 388D1 macrophage. *J. Lipid Res.* **27,** 412–420

23 Gianturco, S. and Bradley, W. (1991). A cellular basis for the atherogenicity of triglyceride-rich lipoproteins. *Ather. Rev.* **22,** 9–14

24 Libby, P., Miao, P., Ordovas, J. M., and Schaefer, E. J. (1985). Lipoproteins increase growth of mitogen-stimulated arterial smooth muscle cells. *J. Cell. Physiol.* **124,** 1–8

25 Dikens, B. F., Weglicki, W. B., Li, Y.-S., and Mak, I. T. (1992). Magnesium deficiency in vitro enhances free radical-induced intracellular oxidation and cytotoxicity in endothelial cells. *FEBS Lett.* **3,** 187–191

26 Kawamura, K. and Kummerow, F.A. (1992). Effect of magnesium deficiency on 15-hydroxyeicosatetraenoic acid in cultured human umbilical arterial endothelial cells. *Mag. Res.* **5,** 109–113

27 Zhou, Q. and Kummerow, F. A. (1993). Modifying effect of 26-hydroxycholesterol on low-magnesium-induced atherosclerotic changes in the cultured human arterial smooth muscle cell. *Mag. Res.* **6,** 121–126

28 Harris-Hooker, S., Sanford, G.L., Montgomery, V., Rivers, R., and Emmett, N. (1992). Influence of low density lipoproteins on vascular smooth muscle cell growth and motility: modulation by extracellular matrix. *Cell. Biol. Int. Rep.* **16,** 433–450

29 Björkerud, S. and Björkerud, B. (1994). Lipoproteins are major and primary mitogens and growth promoters for human arterial smooth muscle cells and lung fibroblasts in vitro. *Arter. Thromb.* **14,** 288–298

30 Oikawa, S.-I., Hori, S., Sano, R., Suzuki, N., Fujii, Y., Abe, R., and Goto, Y. (1987). Effect of low density lipoprotein on DNA synthesis of cultured human arterial smooth muscle cells. *Atherosclerosis* **64,** 7–12

31 Berliner, J. A. and Haberland, M. E. (1993). The role of oxidized low density lipoprotein in atherogenesis. *Curr. Opin. Lipid* **4,** 373–381

32 Chatterjee, S. (1992). Role of oxidized human plasma low density lipoproteins in atherosclerosis: effects on smooth muscle cell proliferation. *Mol. Cell. Biochem.* **111,** 143–147

33 Weglicki, W. B., Stafford, R. E., Freedman, A. M., Cassidy, M. M., and Phillips, T. M. (1993). Modulation of cytokines and myocardial lesions by vitamin E and chloroquine in a Mg-deficient rat model. *Am. J. Physiol.* **264,** C723–C726

34 Stein, O., Shiloni, E., and Stein, Y. (1991). Effect of TNF on triacylglycerol in cultured vascular smooth muscle cells. *Biochim. Biophys. Acta* **1082,** 33–36

35 Grunfeld, C. and Feingold, K. R. (1992). Tumor necrosis factor, interleukin, and interferon induced changes in lipid metabolism as a part of host defence. *Proc. Soc. Exp. Biol. Med.* **200,** 224–227