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Serum from magnesium-deficient rats affects vascular endothelial cells in culture: Role of hyperlipemia and inflammation

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Magnesium (Mg) deficit has been implicated as a risk for atherosclerosis. An important characteristic of experimental Mg deficiency in rats is the spontaneous inflammation with marked leukocytosis and increase in plasma concentrations of inflammatory cytokines. This deficiency is also accompanied by hyperlipemia resulting from accumulation of triglyceride-rich lipoproteins (TGRLP). The present investigation was performed to determine the effect of serum from Mg-deficient animals on cultured vascular endothelial cells. Sera were obtained from control and Mg-deficient rats fed for 8 days adequate or Mg-deficient diets. Mg-deficient animals presented an important leukocytosis and an increased interleukin (IL) 6 concentration in the plasma. Deficient rats were hypertriglyceridemic as compared with control ones, but their cholesterolemia was not modified significantly. Pooled sera from control and Mg-deficient animals were added to the culture medium of human umbilical endothelial cells (HUVEC). The results show that serum from Mg-deficient rats stimulates proliferation of cultured endothelial cells, increases adhesion of monocytes to these cells, and causes an induction of plasminogen activator inhibitor factor 1 (PAI-1) mRNA level in these cells. The present study demonstrates that the inflammatory and hyperlipemic serum from Mg-deficient animals affects various processes in endothelial cells, which are known to be implicated in atherogenesis. (J. Nutr. Biochem. 9:17–22, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Magnesium (Mg) deficit has been implicated as a risk factor for development of atherosclerosis.[1–3] Early epidemiologic studies indicate that communities with low concentrations of Mg in the drinking water tend to have higher incidence of ischemic heart disease and acute myocardial infarction.[4] Other studies demonstrated the relationships between dietary Mg and plasma lipids.[5] Several clinical disorders, such as diabetes mellitus,[6] alcoholism,[7] renal failure,[3] etc., include Mg depletion, alteration of lipid metabolism, and cardiovascular complications. On the other hand some of supplementation studies have found beneficial effect of Mg on plasma lipids[8] and platelet reactivity.[9] For example, in a study performed on patients who suffered myocardial infarction, Rasmussen et al.[8] found that oral Mg supplementation reduced plasma concentrations of triglycerides, VLDL, and apo B, and increased the apo A-I/apo B ratio. Experimental Mg deficiency enhances vascular lipid infiltration in rodents fed atherogenic diets and dietary Mg supplementation can prevent atherosclerosis.[10,11] These lesions however, are not necessarily correlated with cholesterolemia and the mechanism causing them has yet to be

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identified. Other experimental studies have shown dyslipidemia, altered platelet function, and predisposition to thrombosis during Mg deficiency.[2] Thus, epidemiologic, clinical, and experimental data indicate that Mg deficit may modulate the events of cardiovascular diseases in an unfavorable manner.

We have shown previously[12] that serum from Mg-deficient rats has atherogenic properties on cultured vascular smooth muscle cells (VSMC), i.e., it induces their growth and causes lipid accumulation in these cells. The present study was focused on the endothelial cells. The endothelial cell plays a major role in maintaining the functional integrity of the vascular wall. Beyond their role as a permeability barrier, endothelial cells are involved in the maintenance of nonthrombogenic blood-tissue interface, in the modulation of blood flow and vascular resistance, in the regulation of immune and inflammatory reactions.[13] The endothelium is constantly exposed to various potential stimuli that can initiate the atherosclerotic process, among which are hyperlipemia, shear stress, inflammatory agents, cytokines, hypertension, smoking, and glycated proteins.[14]

During experimental Mg deficiency both hyperlipemia and inflammatory process have been shown.[15,16] Several inflammatory agonists, including reactive oxygen species, cytokines, and eicosanoids are elevated in Mg-deficient animals.[15,16] Because these are known effectors of the endothelium, and given the current interest of Mg levels in cardiovascular disease, the present study was performed to determine whether the serum from Mg-deficient animals could affect cultured vascular endothelial 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 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 to 08:00 hr) and 12-hr light period. They were pair-fed with 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). Mg oxide 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). Nonfasting rats were anaesthetized with sodium pentobarbital (40 mg/kg of body weight, i.p.). Blood was collected by exsanguination via abdominal aorta into tubes without anticoagulant. Serum was obtained after clot formation and centrifugation (1,000 g, 15 min). Pools from control and Mg-deficient sera were combined, filtered through 0.2 μm filter (Millipore, Molsheim, France), decomplemented at 56°C for 30 min and then stored at −80°C. For leukocyte analysis small blood samples were collected into heparinized tubes.

Analyses in blood and serum

The differential leukocyte counts for neutrophils, monocytes, lymphocytes were made from a blood smear stained with the May-Grunwald and Giemsa stain. Mg in serum was determined by atomic absorption flame spectrometry (Perkin Elmer 400) after dilution in lanthanum chloride solution containing 1 g La/L. Triglyceride (Biotrol, Paris, France) and total cholesterol (BioMérieux, Charbonnières-les-Bains, France) concentrations were determined by enzymatic procedures. Tumor necrosis factor (TNF)-α and interleukin (IL)-6 concentrations were determined by biological assays.[17] Standards used were serially diluted human recombinant TNF-α and IL-6 (NIBSC, Hertfodshire, England).

Cell culture

Human umbilical vein endothelial cells (HUVEC) were obtained by collagenase digestion as described.[18] Cells were serially passaged at a 1/5 split ratio in medium 199 (Life Technologies, San Giuliano Milanese, Italy) with 10% fetal calf serum (FCS), 10 U/mL heparin, 0.2 × glutamine, antibiotics, antimycotics, and bovine Endothelial Cell Growth Factor (50 ng/mL) (Boehringer, Mannheim, Germany) in tissue culture dishes coated with gelatin (Sigma, St Louis, MO, USA). For the adhesion assay, monoblastoid U937 cell line was grown in RPMI (Sigma) containing 10% FCS.

Cell proliferation assay

HUVEC were seeded at the density of 10,000 cells/sq cm and were exposed to either 5 or 10% of the serum from control or Mg-deficient rats. After 2 or 5 days, cells were trypsinized and counted using a Burker chamber. Cultures with 10% FCS were used as controls. Cytoxicity was tested by using a trypan blue solution (0.4%), according to the manufacturer's protocol (Sigma).

Adhesion assay

HUVEC were cultured in 24-wells dishes in the presence of 10% of tested sera or with IL-1 (10 ng/mL) as a positive control for 4 hr at 37°C with 5% CO_2. After three washes with serum-free medium, 1×10^5 U937 cells in M-199 medium were added to each well. After 1 hr, the nonadherent U937 cells were rinsed off and the wells were fixed with 11% glutaraldehyde in phosphate-buffered saline (PBS). The number of attached U937 was counted in 20 microscopic fields defined by eyepiece. The experiments were performed in triplicate.[19]

Purification of RNA and Northern blot analysis of plasminogen activator inhibitor factor (PAI)-1

Total RNA were obtained from confluent cultures of HUVEC treated with tested sera. The cells were rinsed with PBS, lysed in RNAzolB, and total RNA was purified. RNA was electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde, capillary blotted on a Hybond-N+ membrane (Amersham, Buckinghamshire, UK) and UV cross-linked. The cDNA fragment for human PAI-1 was labeled with a random-primed DNA labeling kit (Boehringer) and filter was hybridized in 0.5 M sodium phosphate (pH, 7.2) containing 7% sodium dodecyl sulfate, 1% bovine serum albumin, 1 mM EDTA, and 20% formamide at 65°C for 20 hr and extensively washed at high stringency (0.1 × SSC, 65°C).[20] Hybridization signal was visualized by autoradiography.

Statistical analysis

Results were expressed as means ± SEM. Statistical significance of differences between groups was evaluated by Student's *t* unpaired test (Instat, GraphPad, San Diego, CA, USA). Results were considered significant at $P < 0.05$.

Table 1 Blood parameters in control and Mg-deficient rats after 8 days on the experimental diets

Rats	Control	Mg-deficient
Mg (mmol/L)	0.83 ± 0.02	0.14 ± 0.01**
Leukocytes (10^9/L)	4.4 ± 0.9	8.0 ± 0.01*
Interleukin 6 (pg/mL)	40 ± 2	100 ± 12**
Triglycerides (mmol/L)	0.83 ± 0.07	4.21 ± 0.35**
Total cholesterol (mmol/L)	1.48 ± 0.07	1.70 ± 0.10

Values are means ± SEM of 12 values per group.
*$P < 0.05$.
**$P < 0.001$.

Results

Mean final body weights of Mg-deficient and pair-fed control rats were 87 ± 3 and 94 ± 3 g (n = 12, $P > 0.05$). Clinical signs of inflammation including erythema of ears were observed in rats fed the Mg-deficient diet. Severe hypomagnesemia was observed in deficient animals (*Table 1*). Deficient rats presented markedly increased leukocytosis (*Table 1*), because of the increase in polymorphonuclear cell number (data not shown). IL-6 concentrations were higher in the serum from deficient rats as compared to controls (*Table 1*), however TNF-α was undetectable (<2 pg/mL) in the serum from both groups. Triglyceride concentration in serum was significantly greater in Mg-deficient rats than in controls, whereas serum total cholesterol was not significantly different between both groups (*Table 1*).

To study the effects of tested sera on endothelial cell growth, HUVEC were exposed to various concentrations of serum from control or Mg-deficient rats and counted after different times. In cells exposed to the serum obtained from Mg-deficient rats, a 2 fold increase in cell number was observed after 2 and 5 days of treatment (*Figure 1*).

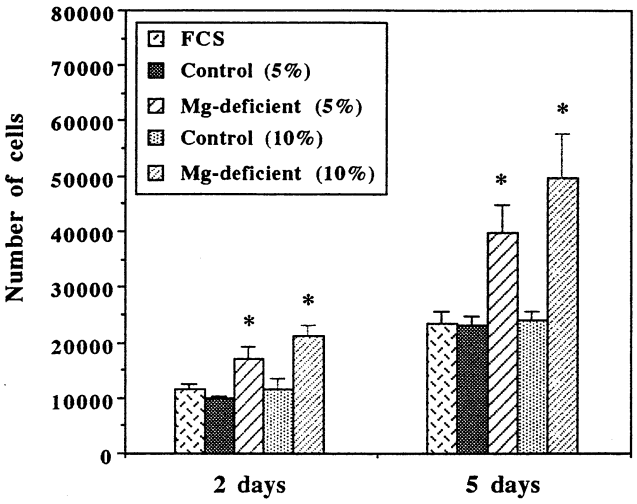

Figure 1 Effect of the serum from control and Mg-deficient rats on HUVEC growth HUVEC were seeded at the density of 10,000 cells/sq cm and were exposed to either 5 or 10% of the serum from control or magnesium-deficient rats for 2 or 5 days. The medium with 10% of fetal calf serum (FCS) was used as control. Results are means ± SEM from three serum pools, each pool was prepared from four sera; *$P < 0.05$, significantly different between sera from control and Mg-deficient rats.

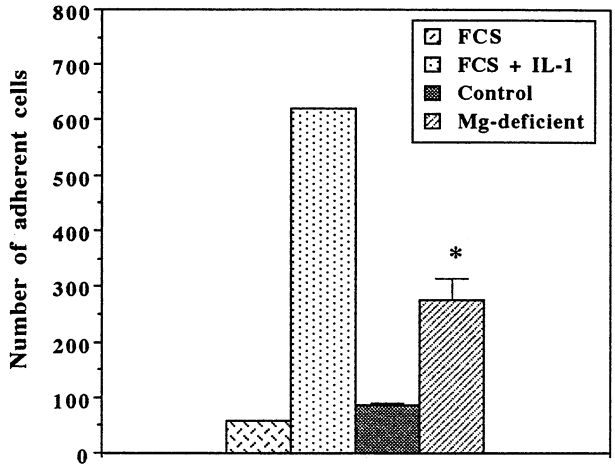

Figure 2 Effect of the serum from control and Mg-deficient rats on the monocyte adhesion to HUVEC. HUVEC were treated for 4 hr with 10% of the serum from control or Mg-deficient rats followed by addition of U937 cells. The media containing fetal calf serum (FCS), with or without IL-1, were used as controls. Results are means ± SEM from three serum pools, each pool was prepared from four sera; *$P < 0.05$, significantly different between sera from control and Mg-deficient rats.

Because it is well established that monocyte adhesion to the endothelium is an early event in the pathogenesis of atherosclerosis,[13] we ascertained whether the serum from Mg-deficient rats modulated U937-endothelial cell interactions. HUVEC were exposed to the aforementioned serum as well as to the corresponding control for 4 hr. *Figure 2* shows the induction of U937 adhesion to endothelial cells by serum from Mg-deficient rats. The increased adhesion of monocyte-like cells to endothelial cells required protein synthesis since exposure of HUVEC to cycloheximide (5 μg/mL) for 30 min before the addition of the serum blocked the effect (not shown).

Because positive correlations have been established between plasma PAI-1 levels and known risk factors for the development of atherosclerosis, including hypertriglyceridemia,[21] we studied the expression of PAI-1 in HUVEC exposed to the serum from control or Mg-deficient rats for 4 hr. Northern blot analysis of total RNA revealed increased levels of PAI-1 mRNA in HUVEC exposed to serum from Mg-deficient rats (*Figure 3*). Both species of PAI-1 mRNA (3.2 and 2.3 kb) were elevated under the aforementioned experimental conditions.

Discussion

We have previously demonstrated that the serum from Mg-deficient rats induces VSMC proliferation and causes lipid accumulation in these cells.[12] The present results demonstrate for the first time that this serum also affects various processes in endothelial cells, which may be implicated in atherogenesis. Mg-deficiency in the rat induces an inflammatory state and severe hyperlipemia. It was proposed by Weglicki et al.[15] from rodent dietary studies that Mg deficiency initiates a neurogenic inflammatory se-

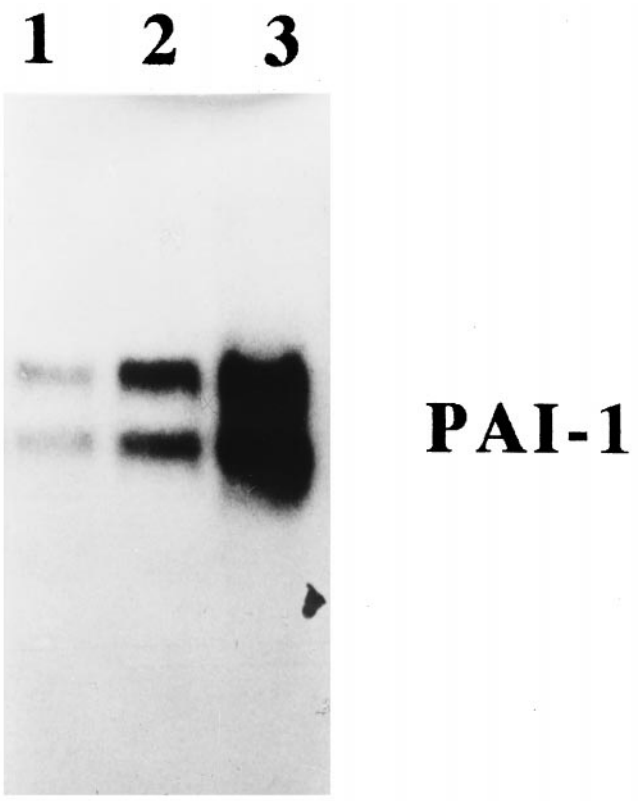

Figure 3 Northern blot analysis of the plasminogen activator inhibitor factor (PAI)-1 mRNA in HUVEC exposed to the serum from control and Mg-deficient rats. *Lane 1*: Fetal calf serum; 2: serum from control rats; 3: serum from Mg-deficient rats.

quence and that this could account for much of the vascular dysfunction during dietary Mg deficiency. In agreement with previous works[15,16] we found the clinical signs of inflammation, a marked leukocytosis as well as increased plasma levels of IL-6 in Mg-deficient rats, whereas TNF-α was undetectable. The inflammation observed in Mg-deficient animals may play an important role in the development of hypertriglyceridemia in these animals since it is well known that several cytokines cause a rapid increase in serum triglycerides by stimulating liver lipogenesis and by decreasing the activity of lipoprotein lipase.[22] The accumulation of triglyceride-rich lipoproteins (TGRLP) in the plasma of Mg-deficient animals is a most important characteristic of hyperlipemia associated with Mg deficiency.[23] It has been previously demonstrated by our group that this hypertriglyceridemia mainly results from decreased lipoprotein lipase activity.[23,24]

The altered parameters that we detected in the serum from Mg-deficient rats can partially explain the modulation of endothelial behavior we described, because cytokines and lipoproteins are known to affect endothelial cell proliferation, adhesion properties, and fibrinolytic activity.[25] Indeed, cytokines activate endothelial cells; for instance the pro-inflammatory cytokines IL-1 and TNF induce the expression of adhesion molecules, which are responsible for the enhanced interactions of endothelial with circulating cells, and upregulate PAI-1, which promotes thrombosis. IL-6 also affects endothelial function by modulating the expression of adhesion molecules as well as cell proliferation.[25] Similarly, lipoproteins have been shown to alter endothelial cell behavior. There is growing evidence that hypertriglyceridemia may be associated with increased risk for cardiovascular heart disease (CHD).[26] It has been suggested[27,28] that TGRLP could be directly involved in atherogenesis. It has been shown that very low density lipoproteins (VLDL) from hypertriglyceridemic human plasma are more likely to promote lipid uptake in vascular endothelial cells and on macrophages than those from normolipidemic plasma.[27,28] Moreover, it was shown that hyperlipidemic sera from cholesterol-fed rabbits and monkeys induced VSMC proliferation.[29–32] Distinct mitogenic and growth-stimulating effects of various lipoprotein fractions isolated from human plasma have also been demonstrated.[33,34] Recently we have shown increased susceptibility to peroxidation of TGRLP isolated from Mg-deficient rats as compared to the controls.[35] There is increasing evidence that oxidatively modified lipoproteins are important in atherogenesis.[36] Mildly oxidized lipoproteins modulate many biologic processes, whereas extensively oxidized LDL are cytotoxic. Low concentrations of oxidized LDL induce the binding of monocytes to the endothelium,[19] stimulate endothelial growth,[37] enhance the expression of several cytokines,[14] and activate a prothrombotic program. Because it is well known that cells—among which are endothelial cells—are able to catalyze the oxidation of lipoproteins it may be hypothesized that TGRLP present in hyperlipemic serum from Mg-deficient rats undergo oxidative modification to a greater extent than that of control rats and, therefore, exert a more potent effect on cultured cells. This hypothesis is supported by our recent study[38] on TGRLP isolated from Mg-deficient rats that are more oxidized in the presence of cultured VSMC and induce more cell growth than control ones.

The effects on endothelial cells of serum from Mg-deficient animals cannot be attributed solely to the low Mg concentration because high Mg concentration was used in the culture medium (about 1 mmol/L). On the other hand, it is enough to expose endothelial cells to Mg-deficient medium to affect their functions[39–42] (our preliminary unpublished results).

The three events we studied in endothelial cells exposed to the serum of Mg-deficient animals are of pivotal importance in atherogenesis. It is well known that adherence of circulating monocytes to the endothelium is an early step in atherogenesis, rapidly followed by monocyte transmigration in the subendothelial space where differentiation into macrophages takes place. Similarly, endothelial cells chronically exposed to the serum from Mg-deficient animals proliferate faster and, since they have a limited lifespan, they reach cellular senescence earlier than unstimulated cells; cell senescence is accompanied by the failure to proliferate, thus impairing the capacity of repairing any injury to the endothelial monolayer. Our finding that the serum from Mg-deficient animals induces PAI-1 is also worthy of note, because PAI-1 seems to have a major clinical relevance; indeed, increased PAI-1 levels have been observed in various clinical conditions such as coronary artery disease, venous thrombosis and in survivors of myocardial infarction.[43–45] Moreover, positive correlations

have also been established between plasma PAI-1 levels and known risk factors for the development of atherosclerosis,[21,46] and PAI-1 expression was found to be increased in atherosclerotic vs normal-appearing arterial tissues.[47]

In conclusion, we suggest that some of inflammatory factors and lipoproteins present in the serum from Mg-deficient animals act and cooperate in altering endothelial functions. Because during experimental Mg deficiency both inflammatory processes and hyperlipemia occur, and given the increasing interest of Mg levels in cardiovascular disease, the present study offers an explanation to the role of Mg in atherogenesis. In the future, the present work should be extended by using the microscopy and ultra microscopy to study the lesion development in arteries of the Mg-deficient animals. Studies performed on animals with induced vascular injury (for example fed with oxidized cholesterol or after balloon angioplasty) will be of particular interest.

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References

1 Seelig, M.S. (1980). *Magnesium Deficiency in Pathogenesis of Disease*. Plenum Medical Book, New York, NY, USA, 488 pp.
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, 380 pp.
4 Durlach, J., Bara, M., and Guiet-Bara, A. (1989). Magnesium level in drinking water: Its importance in cardiovascular risk. In *Magnesium in Health and Disease* (Y. Itokawa and J. Durlach, eds.), pp. 173–182, John Libbey, London, UK
5 Fehily, A.M., Yarnell, J.W., Bolton, C.N., and Butland, B.K. (1988). Dietary determinants of plasma lipids and lipoproteins: The Caerphilly Study. *Eur. J. Clin. Nutr.* **42,** 405–413
6 Garland, H.O. (1992). New experimental data on the relationship between diabetes mellitus and magnesium. *Magnesium Res.* **5,** 193–202
7 Hristova, E.N., Rehak, N.N., Cecco, S., Ruddel, M., Herion, D., Eckardt, M., Linnoila, M., and Elin, R.J. (1997). Serum ionized magnesium in chronic alcoholism: Is it really decreased? *Clin. Chem.* **43,** 394–399
8 Rasmussen, H.S., Aurup, P., Goldstein, K., McNair, P., Mortensen, P.B., Larsen, O.G., and Lawaetz, H. (1989). Influence of magnesium substitution therapy on blood lipid composition in patients with ischemic heart disease. A double-blind, placebo controlled study. *Arch. Intern. Med.* **149,** 1050–1053
9 Nadler, J.L., Malayan, S., Luong, H., Shaw, S., Natarajan, R.D., and Rude, R.K. (1992). Intracellular free magnesium deficiency plays a key role in increased platelet reactivity in type II diabetes mellitus. *Diabetes Care* **15,** 835–841
10 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. USA* **87,** 1840–1844
11 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.), pp. 341–358, Marcel Dekker, New York, NY, USA
12 Bussière, L., Mazur, A., Gueux, E., and Rayssiguier, Y. (1994). Hypertriglyceridemic serum from magnesium-deficient rats induces proliferation and lipid accumulation in cultured vascular smooth muscle cells. *J. Nutr. Biochem.* **5,** 585–590
13 Ross, R. (1993). The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature* **362,** 801–809
14 Saxena, U. and Goldberg, I.J. (1994). Endothelial cells and atherosclerosis: Lipoprotein metabolism, matrix interactions and monocyte recruitment. *Curr. Opin. Lipidol.* **5,** 316–322
15 Weglicki, W.B., Phillips, T.M., Mak, I.T., Cassidy, M.M., Dickens, B.F., Stafford, R., and Kramer, J.J. (1994). Cytokines, neuropeptides and reperfusion injury during magnesium deficiency. *Ann. N.Y. Acad. Sci.* **723,** 246–257
16 Rayssiguier, Y., Malpuech, C., Nowacki, W., Rock, E., Gueux, E., and Mazur, A. (1996). Inflammatory response in magnesium deficiency. In *Advances in Magnesium Research. Magnesium in Cardiology*. Vol. 1 (R. Smetana, ed.), pp. 415–421, John Libbey, London, UK
17 Givalois, L., Dornand, J., Mekaouche, M., Solier, M.D., Bristow, A.F., Ixart, G., Siaud, P., Assenmacher, I., and Barbanel, G. (1994). Temporal cascade of plasma level surges in ACTH, cortisone, and cytokines in endotoxin-challenged rats. *Am. J. Physiol.* **267,** R164–R170
18 Maciag, T., Hoover, G.A., Stemerman, M.B., and Weinstein, R. (1981). Serial propagation of human endothelial cells in vitro. *J. Cell Biol.* **91,** 420–426
19 Maier, J.A.M., Barenghi, L., Pagani, F., Bradamante, S., Comi, P., and Ragnotti, G. (1994). The protective role of high-density lipoprotein or oxidized-low-density-lipoprotein-induced U937/endothelial cell interactions. *Eur. J. Biochem.* **221,** 35–41
20 Maier, J.A.M., Statuto, M., and Ragnotti, G. (1994). Endogenous interleukin 1-alpha must be transported to the nucleus to exert its activity in human endothelial cells. *Mol. Cell. Biol.* **14,** 1845–1851
21 Juhan-Vague, I., Vague, P., Alessi, M.C., Badier, C., Valadier, J., Aillaud, M.F., and Atlan, D. (1987). Relationships between plasma insulin triglyceride, body mass index, and plasminogen activator inhibitor-1. *Diabetes Metab.* **13,** 331–336
22 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
23 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
24 Nassir, F., Mazur, A., Giannoni, F., Gueux, E., Davidson, N.O., and Rayssiguier, Y. (1995). Magnesium deficiency modulates hepatic lipogeneis and polipoprotein gene expression in the rat. *Biochim. Biophys. Acta* **1257,** 125–132
25 Mantovani, A., Bussolino, F., and Dejana, E. (1992). Cytokine regulation of endothelial cell function. *FASEB J.* **6,** 2591–2599
26 Austin, M.A. (1991). Plasma triglyceride and coronary heart disease. *Arterioscler. Thromb.* **11,** 2–14
27 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. *Arterioscler. Thromb.* **13,** 472–481
28 Gianturco, S.H. and Bradley, W.A. (1991). A cellular basis for the atherogenicity of triglyceride-rich lipoproteins. *Atherosclerosis Rev.* **22,** 9–14
29 Fischer-Dzoga, K., Fraser, R., and Wissler, R.W. (1976). Stimulation of proliferation in stationary primary cultures of monkey and rabbit aortic smooth muscle cells. 1. Effects of lipoprotein fractions of hyperlipemic serum and lymph. *Exp. Mol. Pathol.* **24,** 346–359
30 Fischer-Dzoga, K. and Wissler, R.W. (1976). Stimulation of proliferation in stationary primary cultures of monkey and rabbit aortic smooth muscle cells. 2. Effect of varying concentrations of hyperlipemic serum and low density lipoproteins of varying dietary fat origins. *Atherosclerosis* **24,** 515–525
31 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. Molec. Pathol.* **26,** 359–374
32 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
33 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

34 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. *Arterioscler. Thromb.* **14,** 288–298

35 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

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

37 Maier, J.A.M., Barenghi, L., Bradamante, S., and Pagani, F. (1996). Induction of human endothelial cell growth by mildly oxidized low density lipoprotein. *Atherosclerosis* **123,** 115–121

38 Bussière, L., Mazur, A., Gueux, E., Nowacki, W., and Rayssiguier, Y. (1995). Triglyceride-rich lipoproteins from magnesium-deficient rats are more susceptible to oxidation by cells and promote proliferation of cultured vascular smooth muscle cells. *Magnesium Res.* **8,** 151–158

39 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

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

41 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. *Magnesium Res.* **6,** 121–126

42 Wiles, M.E., Wagner, T.L., and Weglicki, W.B. (1997). Effect of acute magnesium deficiency (MgD) on aortic endothelial cell (EC) oxidant production. *Life Sci.* **60,** 221–236

43 Hamsten, A., Wiman, B., De Faire, U., and Blomback, M. (1985). Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. *New Engl. J. Med.* **313,** 1557–1563

44 Nilsson, I.M., Ljungner, H., and Tengborn, L. (1985). Two different mechanisms in patients with venous thrombosis and defective fibrinolysis: Low concentration of plasminogen activator or increased concentration of plasminogen activator inhibitor. *Br. J. Med.* **290,** 1453–1456

45 Schneiderman, J., Adar, R., and Savion, N. (1991). Changes in the plasmatic tissue-type plasminogen activator and PAI activity during acute arterial occlusion associated with severe ischemia. *Thromb. Res.* **62,** 401–408

46 Auwerx, J., Bouillon, R., Collen, D., and Geboers, J. (1988). Tissue-type plasminogen activator antigen and plasminogen activator inhibitor in diabetes mellitus. *Arteriosclerosis* **8,** 68–72

47 Schneiderman, J., Sawdey, M.S., Keeton, M.R., Bordin, G.M., Bernstein, E.F., Dilley, R.B., and Loskutoff, D.J. (1992). Increased type 1 plasminogen activator inhibitor gene expression in atherosclerotic human arteries. *Proc. Natl. Acad. Sci. USA* **89,** 6998–7002