Effect of Magnesium Deficiency on Blood Pressure and Mechanical Properties of Rat Carotid Artery

Pascal Laurant, Daniel Hayoz, Hans R. Brunner, Alain Berthelot

Abstract—The purpose of this study was to determine the effect of dietary Mg deficiency (80 mg/kg versus control diet: 960 mg/kg) on blood pressure and mechanical properties of the rat common carotid artery. The internal diameter and intra-arterial pressure of carotid artery were measured continuously with an echo-tracking device. At 19 weeks, systolic, diastolic, and mean blood pressures were higher in Mg-deficient rats. Histological examination showed an increase in cross-sectional area, intima-media thickness, and media-to-lumen ratio in carotid artery of Mg-deficient rats. Mg deficiency did not modify the arterial distensibility–blood pressure curve. At mean blood pressure, arterial distensibility was significantly less in 19-week-old rats than in 5-week-old rats of both control and Mg-deficient groups. A significant interaction between age and Mg-deficient diet on arterial distensibility (P<0.04) indicates an accelerated age-dependent decreased arterial distensibility with Mg deficiency. At 19 weeks, the artery was stiffer in hypertensive Mg-deficient rats, as illustrated by a shift to higher levels of the incremental elastic modulus–stress curve. In conclusion, the increased blood pressure and the vascular morphological alterations observed in Mg-deficient rats may contribute to an accelerated alteration of the wall material, which in turn leads to a stiffening of the carotid artery. (Hypertension. 1999;33:1105-1110.)

Key Words: ultrasonography • carotid arteries • elastic modulus • calcium • magnesium deficiency

Epidemiologic and experimental evidence indicates that Mg deficiency may be considered a risk factor for cardiovascular diseases and hypertension.1,2 Several epidemiological studies have shown that Mg consumption is inversely related to blood pressure (BP).3 In humans, one study has shown that dietary Mg deprivation elevates BP.4 Experimental investigations in rats also indicate that long-term dietary Mg deficiency increases BP and induces sustained hypertension.5–7 In vitro, decreasing Mg concentration results in increased vascular tone, increased vascular reactivity in most types of large and small blood vessels. This results in increased vascular tone, increased vascular reactivity, and reduction in peripheral blood flow.1,2 Mg deficiency–induced hypertension in rats is associated with reduced arteriolar, venular, and precapillary lumen size, suggesting an increase in myogenic tone.5 Early studies have shown that Mg deficiency induces vascular lesions, including wall thickness, endothelial and smooth muscle cell hyperplasia, inflammation of the media and the intima, and fibrinoid necrosis of the blood vessels.8,9 Epidemiologically, an inverse relationship between serum Mg concentration and intima-media thickness (IMT) of the carotid artery has been recently demonstrated.10 All these findings suggest that Mg deficiency contributes to structural modifications of blood vessels, mainly arteries, that should alter their viscoelastic properties.

BP levels seem to be closely correlated to vascular structure.11 Chronic elevation of BP in both animals and humans is characterized by an increase in arterial wall thickness.11 These structural modifications should decrease the buffering function and modify the wall elastic properties of the conductance arteries. Whether Mg deficiency contributes to modify elastic properties of the conductance arteries is not known. The purpose of the present investigation was to study the effect of long-term dietary Mg deficiency intake on structural and elastic properties of the rat common carotid artery (CCA).

Methods
Thirty Wistar male rats (3 weeks old) weighing 60 g (IFFA CREDO, L’Arbresle, France) were used. Animal care, surgical preparation, and experimental procedures were approved by the government review committee. The rats were housed in plastic cages with a constant temperature of 23°C, constant humidity (50% to 60%), and a daily 12-hour light/dark cycle. They were randomly divided into a constant temperature of 23°C, constant humidity (50% to 60%), and a daily 12-hour light/dark cycle. They were randomly divided into Mg-deficient (0.008% Mg) and control (0.096% Mg) groups and pair-fed with the appropriate diets for 19 weeks. The synthetic diets contained the following (%): casein 20, starch 40, sucrose 21, cellulose 6, groundnut oil 2.5, corn oil 2.5, mineral mixture 7, vitamin mixture 1. Mg was given in the form of MgO.

Systolic BP was measured in unanesthetized restrained prewarmed rats by the indirect tail-cuff method with a sphygmomanometer (PE-3000, Narco Biosystem). The lowest and the highest values were...
discarded before the mean systolic BP of ≥ 6 clear readings was calculated.

On the day of the experiment (5 and 19 weeks of dietary treatment), anesthesia was induced and maintained with halothane (Halothane BP, Arovet AG) at a concentration of 1.5%. The right CCA was cannulated with a catheter (PE-30, Portex) filled with a heparinized 0.9% NaCl solution. Intra-arterial pressure was monitored with a computerized data acquisition system. The internal diameter (ID) of the left CCA was measured at the same time with an A-mode ultrasonic echo-tracking device (NIUS-02, Asulab), which has already been used and validated in humans and rats. The simultaneous arterial diameter and BP measurements were processed online to calculate a diameter-pressure relationship, which was subsequently converted into an arterial cross-sectional distensibility-pressure curve characterized over the whole range of operating BPs. The curves were compared with previously. The IMT and ID measurements were performed with 200-fold magnification in a blinded procedure. The measurements, performed on 2 carotid sections and on 6 fields per section at a 60° angle, were averaged. The intima-media cross-sectional area (CSA) of the fixed arteries was determined according to the following formula: 

$$\text{CSA} = \pi \left( \text{internal radius} + \text{IMT} \right) - \left( \text{internal radius} \right)^2$$

The media-to-lumen (M/L) ratio was calculated as 100 · IMT/ID. For estimation of incremental elastic modulus ($E_{inc}$) and mean circumferential stress ($\sigma$), arterial wall thickness was derived for each level of BP from the CSA measured at histology and from the ID measured in vivo. Wall thickness ($h$) was calculated according to the formula $h = [(\text{CSA} + \pi \text{ID}/2)/\pi]^{1/2} - \text{ID}/2$. Stress at each level of operational pressure (P) and ID was derived from the formula $\sigma = P \cdot h$. Finally, $E_{inc}$ was defined as $E_{inc} = \Delta \sigma / \Delta \text{strain} = (\text{strain}_{n+1} - \text{strain}_{n}) / (\text{ID}_{n+1} - \text{ID}_n)$ and was calculated for each increase in intra-arterial BP of 2.5 mm Hg within the operational BP range.

Before artery fixation, blood samples were drawn from the right CCA and collected in heparinized tubes. Blood was immediately centrifuged at 2000g for 15 minutes at 4°C. After appropriate dilution of the plasma, total Ca and Mg were analyzed by atomic absorption spectrophotometry. Triglycerides and total cholesterol were determined by enzymatic methods (Boehringer Mannheim).

Values are represented as mean ± SEM. Comparisons were performed with the use of 2-way ANOVA, with age and dietary Mg deficiency as main effects. A subsequent Student-Newman-Keuls test was used to examine data for specific intergroup differences. The pressure-ID, pressure-distensibility, and wall stress–$E_{inc}$ curves were established within operating BPs. The curves were compared with the use of ANOVA for repeated measures. Linear regression was analyzed with Pearson correlation coefficients. Simultaneous independent effects of different variables on mechanical parameters were assessed by stepwise multivariate linear regression. A P value < 0.05 was considered statistically significant.

**Results**

**Effect of Mg-Deficient Diet on BP**

During the first 6 weeks of magnesium deficiency, the Mg-deficient rats presented hyperemia of the ears, alopecia, and ulceration of the skin. Until week 8, BP was similar in both control and Mg-deficient rats. After 8 weeks, until the end of the experimental period, the BP of the Mg-deficient rats was significantly higher than in the control rats (P < 0.01) (Figure 1). Body weight increased with time, and Mg-deficient diet significantly altered growth of the rats (P < 0.05) (Table 1). At 19 weeks, the cardiac weight index was significantly greater in Mg-deficient rats than in control rats (0.33 ± 0.01% versus 0.26 ± 0.01%; P < 0.05). When the rats were anesthetized at 5 weeks, systolic, diastolic, and mean BPs of the control and the Mg-deficient rats were not significantly different. At 19 weeks, systolic, diastolic, and mean BPs were significantly higher in Mg-deficient rats than in control rats (P < 0.05). Two-way ANOVA, however, indicated significant interaction between age and Mg-deficient diet on BPs (P < 0.02). Pulse pressures were similar in the 2 groups (Table 1).

![Figure 1. Systolic blood pressure measured in conscious, restrained, and prewarmed Wistar rats fed control (□——□) or Mg-deficient (○——○) diets. Data are mean ± SEM; n = 16 rats per group. *P < 0.05, **P < 0.01, significant difference in blood pressure.](http://hyper.ahajournals.org/)}
TABLE 1. Body Weight, Hemodynamic Measurements, and Mechanical Parameters of Carotid Arteries in Anesthetized Control and Mg-Deficient Rats at 5 and 19 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>Mg-Deficient (n=8)</th>
<th>Control (n=7)</th>
<th>Mg-Deficient (n=7)</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>274±4</td>
<td>255±4*</td>
<td>461±14†</td>
<td>369±10†</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>128±2</td>
<td>122±2</td>
<td>129±4</td>
<td>141±3†</td>
<td>&lt;0.0006 NS</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>90±3</td>
<td>85±3†</td>
<td>95±4</td>
<td>106±3†</td>
<td>&lt;0.0002 NS</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>103±3</td>
<td>98±2</td>
<td>106±4</td>
<td>118±3†</td>
<td>&lt;0.0001 NS</td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>38±2</td>
<td>37±2</td>
<td>34±2</td>
<td>35±2</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>ID, μm</td>
<td>994±38</td>
<td>924±35</td>
<td>1252±42†</td>
<td>1041±63*</td>
<td>&lt;0.0004 NS</td>
</tr>
<tr>
<td>Distensibility, 10⁻³/mm Hg</td>
<td>8.05±0.41</td>
<td>8.62±0.43</td>
<td>5.94±0.20†</td>
<td>5.16±0.23†</td>
<td>&lt;0.0001 NS</td>
</tr>
<tr>
<td>Stress, 10⁶ dyne/cm²</td>
<td>4.12±0.36</td>
<td>2.73±0.16*</td>
<td>5.58±0.41†</td>
<td>3.46±0.39*</td>
<td>&lt;0.004 &lt;0.001 NS</td>
</tr>
<tr>
<td>Eₑₑₑ, 10⁶ dyne/cm²</td>
<td>8.10±0.95</td>
<td>5.34±0.46</td>
<td>19.27±1.70†</td>
<td>15.95±1.12†</td>
<td>&lt;0.0001 NS</td>
</tr>
</tbody>
</table>

BW indicates body weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; and PP, pulse pressure. The mechanical parameters (ID, distensibility, stress, Eₑₑₑ) were measured at mean blood pressure. Wall stress and Eₑₑₑ were extrapolated and calculated on the basis of histomorphometric measurements; see text. n = number of rats. Values are mean±SEM.

*P<0.05 vs age-matched control rats.
†P<0.05 vs 5-week treated Mg-deficient rats.
‡P<0.05 vs 5-week control rats.

Effect of Mg-Deficient Diet on Histomorphometric Characteristics of CCA
The IMT, CSA, and M/L ratio of the fixed CCA artery were significantly greater (P<0.05) in Mg-deficient rats at 5 and

Figure 2. Relationship between intra-arterial pressure and ID, between intra-arterial pressure and arterial distensibility, and between circumferential wall stress and Eₑₑₑ of the CCA in Wistar rats fed control (□) or Mg-deficient (○) diets for 5 and 19 weeks. Data are mean±SEM; n=7 to 8 rats per group.

Discussion
The data of the present study provide the first in vivo evidence that dietary Mg deficiency induces changes in the mechanical properties of the rat CCA. The increased thickness of the carotid artery wall, concomitant with the chronic elevation of BP observed in Wistar rats fed with the Mg-deficient diet for 19 weeks, was associated with an increase in
arterial stiffness and an accelerated age-dependent decrease in arterial distensibility. No measurable mechanical alterations appeared at the early phase of dietary Mg deficiency (when BP did not change), despite an increase in IMT. These findings first suggest that the mechanical properties of large arteries depend on BP level and arterial wall structure.13

Increased BP and sustained chronic wall hypertrophy have been previously reported in rats fed a Mg-deficient diet.5–7 The fact that Mg deficiency elevated both systolic and diastolic BP, as previously described,6 suggests that Mg deficiency increases ventricular ejection and peripheral resistance. The greater cardiac weight index, indicating the existence of ventricular hypertrophy, would support an increase in isotropic activity in hypertensive Mg-deficient rats. In addition, chronic Mg deficiency, or hypomagnesemia, elevates vascular tone, potentiates vasoconstrictor activity to various agonists, and attenuates responses to various dilator agents,5,6 leading to increased peripheral resistance and thus to increased BP.

In the present study the CCA of Mg-deficient rats had a greater CSA, IMT, and M/L ratio, whereas external diameter did not change. These findings indicate both vascular remodeling and wall hypertrophy. The smaller ID of CCA observed in vivo in Mg-deficient rats may reflect an enhanced arterial tone, as will be discussed below. However, a confounding factor is that the body weight gain in Mg-deficient rats was lower than in control rats. The difference in ID therefore might also be accounted for by the difference in growth.

Increasing BP elevates stress of the arterial wall. To counteract the rise in wall tension, chronic hypertension induces outward hypertrophic remodeling of the large blood vessels. As a consequence of chronic increased BP, wall thickening normalizes circumferential wall stress.12 Our findings, however, report that the Mg-deficient diet induced thickening in the arterial wall before elevation of BP. In addition, wall stress was lower in Mg-deficient than in control rats before and after elevation in BP. These findings suggest that the adaptive process that tends to maintain optimal wall tensile stress in response to BP levels was ineffective in Mg-deficient rats and that Mg deficiency may stimulate growth and/or proliferation of arterial wall constituents independently of BP elevation. Early studies have shown that Mg deficiency induces morphological changes in arteries, including hyperplasia and proliferation of endothelial and smooth muscle cells, calcification, fibrinoid necrosis, and edema with inflammatory infiltration.8,9 Furthermore, Mg deficiency increases the production of inflammatory and mitogenic factors by vascular smooth muscle and endothelial cells.2

The present study demonstrates, at mean BP, an intergroup positive linear relationship between IMT of CCA and plasma total Ca or triglyceride concentrations and a negative linear relationship between wall stress and the same plasma parameters. These findings strongly support the hypothesis that the elevation of circulating Ca and triglyceride levels induced by Mg deficiency1,2,9,14,15 may contribute to increased IMT of CCA of the rat. Ca stimulates growth of various cells and mediates migration, proliferation, matrix production, and necrosis of vascular smooth muscle cells.16–18 Extracellular Mg concentration influences Ca entry, binding, translocation, and intracellular mobilization in vascular smooth muscle cells.1,19,20 Hence, the decrease in extracellular Mg concentration, or hypomagnesemia, will lead to an enhanced intracellular Ca level. Ca overloading in heart and blood vessels occurs as a general consequence of Mg deficiency.6,9

| Table 2: Histomorphometric Characteristics of Carotid Arteries From Control and Mg-Deficient Rats at 5 and 19 Weeks |

<table>
<thead>
<tr>
<th></th>
<th>5 Weeks Control (n=8)</th>
<th></th>
<th>5 Weeks Mg-Deficient (n=8)</th>
<th></th>
<th>19 Weeks Control (n=7)</th>
<th></th>
<th>19 Weeks Mg-Deficient (n=7)</th>
<th></th>
<th>P (ANOVA) Age Effect</th>
<th>Mg Effect</th>
<th>Age×Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMT, μm</td>
<td>22.2±0.8</td>
<td></td>
<td>27.2±0.7*</td>
<td></td>
<td>25.7±0.4‡</td>
<td></td>
<td>33.2±0.7*†</td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>ED, μm</td>
<td>601±11</td>
<td></td>
<td>615±19</td>
<td></td>
<td>632±15</td>
<td></td>
<td>610±8</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CSA, 10³ · μm²</td>
<td>40.5±1.9</td>
<td></td>
<td>50.3±2.3*</td>
<td></td>
<td>48.9±1.3‡</td>
<td></td>
<td>60.2±1.4†</td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>M/L, %</td>
<td>8.0±0.3</td>
<td></td>
<td>9.8±0.3*</td>
<td></td>
<td>8.9±0.3</td>
<td></td>
<td>12.2±0.4†</td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.03</td>
</tr>
</tbody>
</table>

ED indicates external diameter. n=number of rats. Values are mean±SEM.
*P<0.05 vs age-matched control rats.
‡P<0.05 vs 5-week control rats.

| Table 3: Biochemical Parameters Obtained in Plasma From Control and Mg-Deficient Rats at 5 and 19 Weeks |

<table>
<thead>
<tr>
<th></th>
<th>5 Weeks Control (n=8)</th>
<th></th>
<th>5 Weeks Mg-Deficient (n=8)</th>
<th></th>
<th>19 Weeks Control (n=7)</th>
<th></th>
<th>19 Weeks Mg-Deficient (n=7)</th>
<th></th>
<th>P (ANOVA) Age Effect</th>
<th>Mg Effect</th>
<th>Age×Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg, mmol/L</td>
<td>0.87±0.05</td>
<td></td>
<td>0.36±0.06*</td>
<td></td>
<td>0.75±0.02</td>
<td></td>
<td>0.21±0.01†</td>
<td></td>
<td>&lt;0.003</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>Ca, mmol/L</td>
<td>2.69±0.04</td>
<td></td>
<td>2.89±0.03*</td>
<td></td>
<td>2.85±0.02‡</td>
<td></td>
<td>2.96±0.03*</td>
<td></td>
<td>&lt;0.006</td>
<td>&lt;0.0003</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>1.65±0.05</td>
<td></td>
<td>1.87±0.06*</td>
<td></td>
<td>1.93±0.10</td>
<td></td>
<td>2.41±0.12†</td>
<td></td>
<td>&lt;0.0002</td>
<td>&lt;0.002</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.13±0.11</td>
<td></td>
<td>1.14±0.06</td>
<td></td>
<td>1.32±0.09</td>
<td></td>
<td>1.79±0.17†</td>
<td></td>
<td>&lt;0.002</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

n=number of rats. Values are mean±SEM.
*P<0.05 vs age-matched control rats.
†P<0.05 vs 5-week matched Mg-deficient rats.
‡P<0.05 vs 5-week control rats.
vascular diseases. Recently, it has been shown that Mg intake plays an important modulatory role in controlling lipid metabolism induced by Mg deficiency are unclear, Ca might play a crucial role in the development of vascular atherosclerotic lesions. In our study distensibility significantly decreased with age. In both groups of rats, when assessed under isobaric conditions and at mean BP. Two-way ANOVA indicated that the age-dependent decrease in arterial distensibility was more pronounced in the carotid artery of Mg-deficient rats. Furthermore, it was demonstrated that an intergroup negative linear relationship exists between arterial distensibility (assessed at mean BP) and BP, whereas under isobaric conditions, arterial distensibility was similar in both hypertensive Mg-deficient and control rats. These findings suggest that distensibility is highly related to operating BP levels and that, at mean BP, hypertensive Mg-deficient rats would exhibit an accelerated age-dependent decrease in arterial distensibility, which would contribute to an increase in arterial stiffness. Recent clinical reports have shown a positive linear relationship between aortic distensibility and intracellular free Mg concentration in hypertensive subjects, suggesting that intracellular Mg deficiency may contribute to arterial stiffness in hypertension. Furthermore, the same authors demonstrate that intracellular free Mg depletion observed with age may be one possible cellular mechanism mediating the age-related decrease in arterial distensibility.

Distensibility is dependent on the geometry of blood vessels and the stiffness of vascular wall components defined by E_{inc}. In hypertensive Mg-deficient rats, E_{inc} plotted against wall stress, which is recognized as the best determinant of wall stiffness, showed a significant increased stiffness of the wall constituent of the CCA. In contrast, at the early phase of dietary treatment, before the elevation of BP in Mg-deficient rats, E_{inc} was similar for a given level of stress for the 2 groups. It appears that with a longer duration of dietary Mg deficiency, the wall constituents of the arteries become significantly more rigid than those of the arteries of control rats for equivalent wall stress. At mean BP, the E_{inc} values, however, did not differ between hypertensive Mg-deficient and control rats, suggesting that the vascular wall keeps an additional elasticity to maintain a “normal” distensibility. Although the mechanisms involved in the elevation of E_{inc} in relation to wall stress in carotid artery from Mg-deficient rats are unclear, they could be related in part to differences in the content, characteristics, and/or organization of the structural components of the vascular wall. Differences in any one of these factors could contribute to the difference in the stiffness of the arterial wall between hypertensive Mg-deficient and control rats. Mg deficiency causes changes in the composition of blood vessels, including calcification, increased collagen, and decreased elastin content. Thinning, fragmentation of the elastic membranes, and modification in synthesis, turnover,
and composition of elastin have also been reported. All these findings indicate that Mg deficiency may alter wall elasticity. In conclusion, chronic Mg deficiency induced elevation in BP and growth of the rat CCA. Chronic BP elevation and arterial growth contribute to an alteration of elastic properties of vascular wall, including an accelerated age-dependent decrease in distensibility and an increased wall stiffness with Mg deficiency.

Acknowledgment
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References
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