Swimming Prevents Vulnerable Atherosclerotic Plaque Development in Hypertensive 2-Kidney, 1-Clip Mice by Modulating Angiotensin II Type 1 Receptor Expression Independently From Hemodynamic Changes

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Abstract—Exercise is known to reduce cardiovascular risk. However, its role on atherosclerotic plaque stabilization is unknown. Apolipoprotein E \( E^{-/-} \) mice with vulnerable (2-kidney, 1-clip: angiotensin [Ang] II–dependent hypertension model) or stable atherosclerotic plaques (1-kidney, 1-clip: Ang II–independent hypertension model and normotensive shams) were used for experiments. Mice swam regularly for 5 weeks and were compared with sedentary controls. Exercised 2-kidney, 1-clip mice developed significantly more stable plaques (thinner fibrous cap, decreased media degeneration, layering, macrophage content, and increased smooth muscle cells) than sedentary controls. Exercise did not affect blood pressure. Conversely, swimming significantly reduced aortic Ang II type 1 receptor mRNA levels, whereas Ang II type 2 receptor expression remained unaffected. Sympathetic tone also significantly diminished in exercised 2-kidney, 1-clip mice compared with sedentary ones; renin and aldosterone levels tended to increase. Ang II type 1 downregulation was not accompanied by improved endothelial function, and no difference in balance among T-helper 1, T-helper 2, and T regulatory cells was observed between sedentary and exercised mice. These results show for the first time, in a mouse model of Ang II–mediated vulnerable plaques, that swimming prevents atherosclerosis progression and plaque vulnerability. This benefit is likely mediated by downregulating aortic Ang II type 1 receptor expression independent from any hemodynamic change. Ang II type 1 downregulation may protect the vessel wall from the Ang II proatherogenic effects. Moreover, data presented herein further emphasize the pivotal and blood pressure–independent role of Ang II in atherogenesis. (Hypertension. 2009;53:782-789.)

Key Words: hypertension ■ vulnerable plaque ■ angiotensin ■ atherosclerosis ■ swimming

Atherosclerosis, a complex multifactorial chronic disease, represents a major health burden in modern society. A number of risk factors are strongly associated with the initiation and growth of atherosclerotic plaques. However, the mechanisms that cause a stable plaque to become vulnerable remain largely unknown. We have shown recently, in a mouse model of atherosclerosis, that angiotensin (Ang) II induces the progression of atherosclerosis and mediates plaque vulnerability beyond its effect on blood pressure.\(^1\) Progression toward a vulnerable phenotype facilitates plaque rupture with dramatic consequences.\(^2,3\) Therefore, stabilizing atherosclerotic plaques is a major goal in cardiovascular medicine. Regular physical exercise is effective and efficient in reducing cardiovascular risk.\(^4-6\) In particular, exercise has been shown to reduce the progression of atherosclerosis in patients with coronary artery disease.\(^7\) Similarly, we and others have shown that regular exercise exerts a beneficial effect on atherosclerosis extension in a variety of animal models.\(^8-11\) It is, therefore, not surprising that many national and international guidelines now consider regular aerobic exercise for \( \geq 30 \) minutes per day, most days of the week, to be a key element in the management of cardiovascular risk reduction. Swimming is considered equally effective as jogging, biking, or walking as a recommended aerobic exercise. However, although exercise has undoubtedly beneficial effects on cardiovascular risk reduction, little is known as to whether exercise and, in particular, swimming, may have an impact on atherosclerotic plaque progression/stabilization. Therefore, the aim of this study was to assess a putative beneficial effect of regular swimming on vulnerable

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plaque development. For this purpose, the mouse model of Ang II–mediated vulnerable plaque, developed in our laboratory,1 was used.

Materials and Methods

Mouse Models of Stable and Vulnerable Atherosclerotic Plaques

Apolipoprotein E (ApoE)−/− mice (C57BL/6J background, IFFA CREDO, L’Arbresle Cedex, France) were fed regular rodent chow and water ad libitum throughout the study. Both male and female mice were used. All of the experiments were approved by the local institutional animal committee. Two models of renovascular hypertension were generated as described previously in 14- to 16-week-old animals: the renin-dependent Ang II–mediated 2-kidney, 1-clip (2K1C; these mice develop vulnerable plaques), and the renin-independent 1-kidney, 1-clip (1K1C; these mice develop stable plaques). Briefly, mice were anesthetized by halothane inhalation (1% to 2% in oxygen), the left kidney was exposed, and the left renal artery was clipped to reduce renal perfusion. In the 1K1C model, other than left renal artery clipping, a right nephrectomy was performed. Normotensive sham ApoE−/− animals were used as controls.

Endurance Swimming Protocol

ApoE−/− mice were randomly assigned to either the sedentary or exercise group. The exercise group underwent a 5-week swimming program, as published previously.10 Briefly, swimming was always performed in the morning between 8 and 10 AM. It consisted of a swimming period of 50 minutes per day, 5 days per week, in water constantly kept at 35°C to 36°C. Animals were progressively trained before clipping: 10 minutes of training on day 1, followed by daily 10-minute increases up to 40 minutes of training on day 4. On day 5, mouse models of stable or vulnerable plaques were generated as described above. After surgery, mice were allowed to recover for 2 days. Swimming training was then resumed at 50 minutes per day, 5 days per week for 5 weeks. After swimming sessions, wet animals were carefully dried and placed in a warm environment to avoid additional cold physiological stress and health problems.

Blood Pressure and Heart Rate

Mean blood pressure (MBP) and heart rate (HR) were measured 5 weeks after clipping at the end of the experimental protocol in the 6 groups of mice (sedentary and exercised sham, 2K1C and 1K1C ApoE−/− mice), as described previously.1 Briefly, the left carotid artery was catheterized, and the catheter was tunneled subcutaneously to exit at the back of the neck. Mice were allowed full recovery from anesthesia. The arterial line was connected to a pressure acquisition system.12 Diastolic pressures, and HR were recorded using a computerized data analysis system (DiaSys Diagnostic Systems GmbH) following the manufacturer’s instructions. Citrate synthase activity, a marker of mitochondrial content, was measured in gastrocnemius muscle homogenates using a spectrophotometric method, as described previously.10 Plasma interleukin 6 was measured using an ELISA kit following the manufacturer’s instructions (Quantikine R&D).

Evaluation of Atherosclerotic Plaque Vulnerability and Morphology

Analyses of plaque morphology and morphometry were carried out in 3-μm-thick serial histological sections of formalin-fixed and paraffin-embedded aortic sinuses, as described previously.1 Sections obtained between the appearance and disappearance of the aortic valve were analyzed by light microscopy for the identification of plaques. The 3 middle sections of the biggest atherosclerotic plaque were used for atherosclerotic evaluation after staining with Movats pentachrome. The preceding and following 3-μm-thick sections were used for immunohistochemistry. An independent investigator blinded to the study protocol evaluated each section under a light microscopy using the morphometry software Qwin (Leica Systems). After Movats staining, plaques were classified as early intermediate (essentially composed of foam cells or with a small lipid core) and advanced (large necrotic/lipid core with multiple layers). Furthermore, the following characteristics of plaque quality were evaluated: (1) fibrous cap thickness; (2) quantification of smooth muscle cell (SMC) content (α-smooth muscle actin staining); (3) presence of media degeneration (invasion of media by plaque components, media thinning, elastic laminae rupture, and media atrophy); (4) presence of layering (foam cells above or adjacent to the central lipid core); (5) inflammation of the adventitia (>20 polymorphonuclear cells); and (6) plaque macrophage content quantification (staining).3 Plaque surface at the aortic valve level was measured in stained Movats sections using the morphometry software Qwin (Leica Systems).

Immunohistochemical Analysis

Analysis was carried out as described previously.1 Briefly, sections were stained with a biotinated mouse monoclonal IgG2a α-smooth muscle actin antibody or with a monoclonal antibody to mouse macrophage marker Mac-2 (clone M3/38, Cedarlane). Samples were observed with a photomicroscope, and pictures were acquired with a high-sensitivity color digital camera (Leica DC Camera, Leica Systems). α-Smooth muscle actin and Mac-2–positive areas were quantified by means of the Qwin morphometry software.1

Ex Vivo Endothelial Function Assessment

Aortas of mice were excised, cleaned from connective tissue, and cut into rings of ~2 mm in length. Each ring was suspended between wires attached to force transducers in a Multi Myograph System (model 610M) for the measurement of isometric tension. Rings were incubated in a chamber filled with Krebs solution (NaCl 118 mmol/L, KCl 4.65 mmol/L, CaCl2 2.5 mmol/L, KH2PO4 1.18 mmol/L, NaHCO3 24.9 mmol/L, MgSO4 1.18 mmol/L, and glucose 12 mmol/L [pH 7.4]) bubbled with 95% O2-5% CO2 at 37°C (pH 7.4) and allowed to stabilize for 60 minutes at 3 to 4 mN optimal resting tension. To study endothelial function, the vasodilator responses to the endothelium–dependent agonist ace- tylcysteine (3×10−9 to 3×10−5 mol/L) and endothelium-independent agonist sodium nitroprusside (3×10−10 to 3×10−5 mol/L) were studied. The degree of vasodilatation was expressed as the percentage reduction from the precontracted state induced by 10−4 mol/L of phenylephrine.

Real-Time Semiquantitative PCR

Total RNA from mouse spleens or thoracic-abdominal aortas was extracted with TRI Reagent (MRC, Inc) according to the manufacturer’s instructions. Reverse transcription was performed using the Quantitect kit (Qiagen). Real-time PCR (ABI Prism 7000 Sequence Detection System, Applied Biosystems) was used to determine (DiaSys Diagnostic Systems GmbH) following the manufacturer’s instructions. Citrate synthase activity, a marker of mitochondrial content, was measured in gastrocnemius muscle homogenates using a spectrophotometric method, as described previously.10 Plasma interleukin 6 was measured using an ELISA kit following the manufacturer’s instructions (Quantikine R&D).
Table 1. Hemodynamic and Hormonal Characteristics in Sedentary and 5-Week Exercised Sham, 2K1C, and 1K1C ApoE<sup>−/−</sup> Mice

<table>
<thead>
<tr>
<th>Mouse Model</th>
<th>Exercise</th>
<th>BW, g</th>
<th>MBP, mm Hg</th>
<th>HR, bpm</th>
<th>Ang-N, pmol/mL</th>
<th>PRC, ng/mL per h</th>
<th>NE, nmol/L</th>
<th>Aldosterone, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>No</td>
<td>25±1</td>
<td>118±3</td>
<td>628±11</td>
<td>309±17</td>
<td>903±84</td>
<td>7.3±0.5</td>
<td>533±78</td>
</tr>
<tr>
<td>2K1C</td>
<td>No</td>
<td>26±1</td>
<td>142±2*</td>
<td>654±13</td>
<td>193±25‡</td>
<td>1987±184‡</td>
<td>5.8±0.8</td>
<td>606±156</td>
</tr>
<tr>
<td>1K1C</td>
<td>No</td>
<td>23±1</td>
<td>150±3*</td>
<td>646±12</td>
<td>327±48</td>
<td>970±172</td>
<td>6.5±0.7</td>
<td>1049±173</td>
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<tr>
<td>Sham</td>
<td>Yes</td>
<td>25±1</td>
<td>118±2</td>
<td>570±27†</td>
<td>248±14</td>
<td>1022±169</td>
<td>4.2±1.2</td>
<td>776±138</td>
</tr>
<tr>
<td>2K1C</td>
<td>Yes</td>
<td>24±1</td>
<td>141±2*</td>
<td>607±7†</td>
<td>111±11‡</td>
<td>2059±470‡</td>
<td>4.4±0.3†</td>
<td>636±196</td>
</tr>
<tr>
<td>1K1C</td>
<td>Yes</td>
<td>23±1</td>
<td>146±3*</td>
<td>590±1†</td>
<td>321±23</td>
<td>994±456</td>
<td>5.4±0.7</td>
<td>1380±300</td>
</tr>
</tbody>
</table>

BW indicates body weight. N=11 to 19 in each group; for the NE measurements, N=5 to 10 in each group.

*P<0.001 vs respective sham.
†P<0.05 vs respective sedentary group.
‡P<0.05 vs respective sham and 1K1C.

mRNA levels of CD4 (T-helper cell marker), Tim3 (T-helper 1 cell marker), Gata3 (T-helper 2-cell marker), and Foxp3 (regulatory T-cell marker) in spleens, whereas mRNA levels of endothelial NO synthase (eNOS), Ang II type 1 (AT<sub>1</sub>), and AT<sub>2</sub> receptors were quantified in mouse aortas. The following primers were used: for CD4, 5'<TGGCCTTCTCCACCTGAAGTT (sense) and 5'<CCCTAAAAAGGTGTCCAGCA 3'> (antisense); for Tim3, 5'<GCCGGTGCACTGAGTTTTCCTAAG (antisense); for Gata3, 5'<CAGAACCGGCCCCTTTATCA (sense) and 5'<CATTAGGTCTCTCTCCAGA (antisense); and for Foxp3, 5'<GCCGGTGCACTGAGTTTTCCTAAG (antisense) and 5'<CAGAACCGGCCCCTTTATCA (sense) and 5'<CATTAGGTCTCTCTCCAGA (antisense) and 5'<GCCGTATGCTGACGATCA (antisense).

mRNA levels of CD4 (T-helper cell marker), Tim3 (T-helper 1 cell marker), Gata3 (T-helper 2-cell marker), and Foxp3 (regulatory T-cell marker) in spleens, whereas mRNA levels of endothelial NO synthase (eNOS), Ang II type 1 (AT<sub>1</sub>), and AT<sub>2</sub> receptors were quantified in mouse aortas. The following primers were used: for CD4, 5'<TGGCCTTCTCCACCTGAAGTT (sense) and 5'<CCCTAAAAAGGTGTCCAGCA 3'> (antisense); for Tim3, 5'<GCCGGTGCACTGAGTTTTCCTAAG (antisense); for Gata3, 5'<CAGAACCGGCCCCTTTATCA (sense) and 5'<CATTAGGTCTCTCTCCAGA (antisense); for Foxp3, 5'<GCCGGTGCACTGAGTTTTCCTAAG (antisense) and 5'<CAGAACCGGCCCCTTTATCA (sense) and 5'<CATTAGGTCTCTCTCCAGA (antisense) and 5'<GCCGTATGCTGACGATCA (antisense).

**Results**

**Sedentary Mice**

Hemodynamic and hormonal results are summarized in Table 1. Exercised ApoE<sup>−/−</sup> sham, 2K1C, and 1K1C mice were analyzed. Sedentary ApoE<sup>−/−</sup> sham, 2K1C, and 1K1C animals served as controls. As expected, in sedentary hypertensive 2K1C mice, PRC values were significantly above normal (Ang II–dependent hypertension model) as compared with control normotensive sham. In hypertensive 1K1C mice (Ang II–independent hypertension model), PRC levels were within the normal range. Plasma Ang-N in sedentary hypertensive 2K1C mice was significantly decreased (because of substrate consumption after renin activation) compared with sedentary normotensive sham and hypertensive 1K1C mice. In these 2 latter groups, plasma Ang-N levels remained normal. HR and body weight were not different among sham, 2K1C, and 1K1C sedentary animals.

**Effect of Exercise on MBP, HR, Body Weight, PRC, Total Cholesterol, and Citrate Synthase Levels**

Five weeks of swimming training had no significant effect on MBP in any group of mice (Table 1). Analysis of systolic and diastolic blood pressure values showed similar unchanged results between sedentary and exercised mice (data not shown). Along the same line, as observed in sedentary animals, after exercise, PRC remained significantly higher in 2K1C mice as compared with similarly trained sham and 1K1C animals (Table 1). Correspondingly, the plasma Ang-N substrate significantly decreased in exercised 2K1C mice (Table 1). As expected, HR was significantly lower in animals after swimming as compared with sedentary controls (P<0.05; Table 1). Body weight was not affected by swimming (Table 1). Likewise, plasma total cholesterol levels were not different among exercised and sedentary mice (data not shown). To verify that, indeed, swimming mice significantly exercised, muscle citrate synthase was measured and was found to be significantly increased after 5 weeks of swimming training in all 3 groups of mice (data not shown).

**Swimming Prevents Atherosclerosis Progression in Hypertensive 2K1C Mice**

Plaque analysis in sedentary mice showed in 2K1C animals a more vulnerable phenotype than plaques from similarly hypertensive 1K1C and normotensive sham mice, as described previously (decreased fibrous cap thickness, increased media degeneration, layering, and adventitia inflammation; P<0.05; Table 2). Moreover, SMC content was significantly decreased in plaques from 2K1C mice, as evidenced by the decrease in α-smooth muscle actin (P<0.05; Figure 1A). Along the same line, in plaques from 2K1C mice, macrophage content was significantly increased (P<0.05; Figure 1B). Contrasting with sedentary 2K1C mice, plaques from sedentary hypertensive 1K1C mice showed a significantly more stable phenotype similar to that found in sedentary normotensive sham animals (Table 2 and Figure 1A and 1B).

Analysis of plaques from exercised mice revealed prevention of atherosclerosis progression in 2K1C animals. In fact, qualitative plaque evaluation from hypertensive 2K1C mice, after 5 weeks of swimming, showed a more stable phenotype as compared with equally hypertensive sedentary 2K1C
animals (significantly thicker fibrous cap, decreased media degeneration, layering, and adventitia inflammation; Table 2 and Figure 1C through 1E). Along the same line, α-smooth muscle actin content was significantly increased by 143% (P < 0.05) in exercised as compared with sedentary 2K1C mice (Figure 1A). In addition, macrophage plaque content was significantly reduced by 35% (P < 0.05) in exercised as compared with sedentary 2K1C mice (Figure 1B). The plaque phenotype of these exercised 2K1C mice was similar to that found in sedentary normotensive sham and sedentary hypertensive 1K1C animals.

Plaque staging also differed among exercised and sedentary 2K1C mice. Sedentary hypertensive 2K1C animals mainly developed advanced lesions (89% of mice), whereas only 25% of mice developed advanced plaques in the exercised hypertensive 2K1C group (P < 0.05; Table 2). Swimming exercise did not seem to significantly affect the plaque phenotype of sham and hypertensive 1K1C mice as compared with sedentary ones (Table 2 and Figure 1A and 1B). No difference in plaque staging was observed among exercised sham or 1K1C and sedentary sham or 1K1C mice (Table 2).

### Table 2. Plaque Quality Assessment and Staging in Sedentary and 5-Week Exercised Sham, 2K1C, and 1K1C ApoE−/− Mice

<table>
<thead>
<tr>
<th>Mouse Model</th>
<th>Exercise</th>
<th>Media Degeneration</th>
<th>Layering</th>
<th>Thinned Fibrous Cap</th>
<th>Adventitia Inflammation</th>
<th>Advanced Plaque Staging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>No</td>
<td>2/7</td>
<td>2/7</td>
<td>2/7</td>
<td>0/7</td>
<td>2/7</td>
</tr>
<tr>
<td>2K1C</td>
<td>No</td>
<td>9/9‡</td>
<td>9/9†‡</td>
<td>9/9†‡</td>
<td>5/9†‡</td>
<td>8/9‡</td>
</tr>
<tr>
<td>1K1C</td>
<td>No</td>
<td>7/8</td>
<td>2/8</td>
<td>4/8</td>
<td>0/8</td>
<td>4/8</td>
</tr>
<tr>
<td>Sham</td>
<td>Yes</td>
<td>5/8</td>
<td>2/8</td>
<td>4/8</td>
<td>1/8</td>
<td>4/8</td>
</tr>
<tr>
<td>2K1C</td>
<td>Yes</td>
<td>3/8*</td>
<td>3/8*</td>
<td>3/8*</td>
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</tr>
<tr>
<td>1K1C</td>
<td>Yes</td>
<td>6/7</td>
<td>4/8</td>
<td>4/8</td>
<td>0/8</td>
<td>4/8</td>
</tr>
</tbody>
</table>

Number of mice presenting the characteristic over total number of mice in the group. N = 7 to 9 in each group.

*P < 0.05 vs sedentary 2K1C mice.

†P < 0.05 vs sedentary 1K1C mice.

‡P < 0.05 vs sedentary sham mice.

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**Figure 1.** SMC and macrophage plaque content. SMC (A) and macrophage (B) content were immunohistochemically quantitated. Representative plaques from sedentary sham, sedentary 2K1C, and exercised 2K1C mice are shown (C through E). Sedentary hypertensive 2K1C mice show significantly decreased SMC content (A) and significantly increased macrophage content (B) as compared with sedentary normotensive sham and similarly hypertensive 1K1C mice. Sedentary normotensive sham and hypertensive 1K1C animals had similar SMC and macrophage content. After 5 weeks of swimming, hypertensive 2K1C mice presented significantly increased SMC and significantly decreased macrophage content, not different from that found in sedentary and trained normotensive sham and hypertensive 1K1C mice. N = 7 to 12 in each group. Phenotype of atherosclerotic plaques from aortic sinus in mice was assessed by Movat's staining. Compared with sedentary sham (C) advanced staging and signs of plaque vulnerability are visible in sedentary hypertensive 2K1C mice (D). After 5 weeks of swimming, plaques show a more stable phenotype similar to that found in sedentary normotensive mice (E).
As we have reported previously, the plaque surface in sedentary animals was significantly (P<0.05) and similarly increased in hypertensive 2K1C (168 704±21 897µm²) and 1K1C (172 142±31 194 µm²) mice as compared with sham normotensive animals (63 931±13 145µm²). Swimming significantly decreased the plaque surface of hypertensive mice to values similar to those found in normotensive sham sedentary mice (57% decrease in 2K1C mice and 56% decrease in 1K1C mice; P<0.05).

**Effect of Swimming on AT1 and AT2 Receptor Expression, Endothelial Function, eNOS Expression, T-Cell Phenotype, and Interleukin 6 Level**

In the ApoE<sup>−/−</sup> 2K1C mouse model, plaque vulnerability is Ang II mediated, as we have shown previously. Five weeks of regular swimming prevented the development of vulnerable plaques in ApoE<sup>−/−</sup> 2K1C mice. Interestingly, in these animals, MBP and PRC were unaffected by exercise. Therefore, we investigated whether the beneficial effect of swimming may be induced by modulating vascular Ang II AT<sub>1</sub> receptor expression. Quantitative mRNA analysis showed similar aortic AT<sub>1</sub> expression levels among sedentary sham, 2K1C, and 1K1C mice (Figure 2A). After 5 weeks of swimming, a downregulation in aortic AT<sub>1</sub> receptor expression was observed in all of the groups of mice (Figure 2A). This reduction was significant in exercised normotensive sham and hypertensive 2K1C mice compared with sedentary sham and 2K1C mice, respectively (P<0.05). In exercised 1K1C animals, AT<sub>1</sub> expression was reduced by 23%, but the reduction did not reach significance (Figure 2A). Notably, the decrease in aortic AT<sub>1</sub> mRNA levels in 2K1C animals was significantly (P<0.05) and similarly correlated with the reduction in plaque macrophage content (r=0.80) and with the increase in SMC content (r=0.58). Downregulation of AT<sub>1</sub> expression may result in an upregulation of the AT<sub>2</sub> Ang II receptor. Therefore, mRNA analysis of AT<sub>2</sub> was performed in the same aortic samples. Results showed no change in AT<sub>2</sub> expression among sedentary and exercised mice. Moreover, no difference in AT<sub>2</sub> expression was observed among normotensive and hypertensive mice (Figure 2B).

Ang II, via the AT<sub>1</sub> receptor, has been linked to endothelial dysfunction. Therefore, we assessed whether aortic AT<sub>1</sub> mRNA downregulation might be accompanied by improved endothelial function in our 2K1C exercised mice. For this purpose, endothelium-dependent and -independent vasorelaxations in isolated aortic rings were studied. Five weeks of swimming did not improve endothelial function in 2K1C mice (Figure 3A). Similarly, endothelium-independent sodium nitroprusside–induced vasorelaxation and phenylephrine-induced vasoconstriction were unchanged between exercised and sedentary mice (data not shown). Likewise, expression of eNOS, a key mediator of endothelial function and atherogenesis, was not altered in exercised compared with sedentary 2K1C mice (Figure 3B).

Ang II, via its AT<sub>1</sub> receptor, is also known to modulate the T-cell phenotype, thus playing a role in atherogenesis. For this purpose, cell-type-specific transcript expression was studied in splenocytes of exercised and sedentary 2K1C mice. Results showed unchanged CD4 expression after swimming.
rabbits with chronic heart failure undergoing treadmill exercise by downregulating aortic AT1 receptor expression.1 The beneficial effect of swimming was likely mediated by reduced AT1 levels. Nevertheless, the universality of the observed exercise effect on AT1 receptor expression will have to be assessed in various organs and vascular beds and after various types of exercise to fully evaluate the potential role of this adaptation. We reported recently13 that AT1 blockade in 2K1C sedentary ApoE−/− mice prevents vulnerable plaque development. This supports the actual finding that decreased vascular AT1 expression may indeed play a direct role in plaque development. Moreover, a recent report shows that double AT1−/−/ApoE−/− mice have significantly reduced atherosclerosis size in nonexercised conditions.20 This observation further supports the role of AT1 receptors in plaque development.

Decreased aortic AT1 expression in exercised 2K1C mice was not accompanied by a compensatory upregulation of AT2 receptor expression nor by a decrease in blood pressure. Conversely, the observed modest increase in renin and aldosterone levels accompanied by a sizable reduction in Ang-N (−42% in 2K1C mice) suggested a stimulated RAAS and enhanced substrate consumption. Thus, in our mice, the benefit of exercise cannot be explained by reduced Ang II production or a hemodynamic effect.

After 5 weeks of swimming, 2K1C mice also showed a diminished sympathetic tone (significant decrease in plasma NE levels). This suppressive effect of exercise on the sympathetic system has also been observed by others.18 These trends for swimming-induced hormonal changes were seen in all 3 groups of exercised mice. An exercise-mediated reduction in sympathetic drive (reduced HR and NE levels) may necessitate a compensatory increase in RAAS activity to maintain adequate renal perfusion. Reduced AT1 receptor expression would, thus, reflect increased Ang II concentrations.

On the other hand, it is known that the RAAS and the sympathetic system work together in a synergistic way to assure blood pressure homeostasis.21 A cross-talk between the RAAS and the sympathetic system is, therefore, conceivable also at the receptor level. Reduced NE levels may have indirectly or directly reduced aortic AT1 receptor expression in our exercised mice.

The beneficial effect on plaque progression was clearly manifest only in exercised hypertensive 2K1C animals. This

**Figure 4.** Spleen mRNA expression for T-cell markers. The expression of transcripts for markers of T-helper cells (CD4; A), T-helper 1 cells (Tim 3; B), T-helper 2 cells (Gata3; C), and regulatory T cells (Foxp3; D) was analyzed by RT-PCR in splenocytes from 2K1C mice. N=4 to 6.

**Effect of Swimming on Plasma NE and Aldosterone Levels**

Swimming is known to affect sympathetic tone. Therefore, plasma NE was measured in sedentary and exercised mice. NE plasma levels decreased after 5 weeks of swimming in all of the groups of mice, and this reduction reached statistical significance in the exercised 2K1C group (Table 1).

Aldosterone plays a pivotal role in blood pressure homeostasis and is a key element of the renin-angiotensin-aldosterone system (RAAS). Therefore, plasma levels were determined after exercise and compared with those found in sedentary animals. Mean plasma aldosterone was consistently increased mean plasma renin, the increase was not significant.

**Discussion**

Our results show, for the first time, in a mouse model of Ang II–mediated vulnerable atherosclerotic plaques, that swimming can prevent atherosclerosis progression and plaque vulnerability. Moreover, the pivotal and blood pressure–independent role of Ang II in atherogenesis is newly emphasized.1 The beneficial effect of swimming was likely mediated by downregulating aortic AT1 receptor expression independent of any hemodynamic change.

In sedentary ApoE−/− mice, aortic AT1 receptor expression was not significantly different among sham, 2K1C, and 1K1C animals. After 5 weeks of swimming, all of the groups of mice showed a similar decrease in Ang II receptor mRNA levels. These findings of reduced AT1 mRNA levels in aortas of exercising mice, to our knowledge, have not been shown before. Alternatively, other authors showed reduced AT1 mRNA and protein levels in the central nervous system of rabbits with chronic heart failure undergoing treadmill exercise.
is not surprising. In young sham mice, lesions are at an early stage and spontaneously progress rather slowly in the absence of stimuli (high-fat diet, hypertension, RAAS stimulation, etc.). Similarly, plaques from volume-overload hypertensive 1K1C mice, although at a more advanced stage than seen in sham normotensive animals, are of a stable phenotype. On the contrary, 2K1C mice rapidly develop advanced and vulnerable lesions during the 5 weeks after clipping because of increased endogenous Ang II production and, therefore, in these mice, the beneficial effect of exercise on atherosclerosis progression was readily demonstrated.

The question arises as to the possible mechanisms by which diminished vascular Ang II receptor expression could affect plaque progression. Certainly, a lower local Ang II signaling may protect the vessel wall from the proatherogenic effects of Ang II. One of the most frequently claimed negative effects of Ang II is impaired endothelial function. Therefore, we investigated whether exercise-induced AT1 downregulation could result in improved aortic endothelial function. This was not the case, and along the same line, aortic ENOS expression was not affected by swimming in our 2K1C mice.

Several lines of evidence suggest that regular aerobic exercise reduces established markers and mediators of inflammation, which are known to play a key role in the pathophysiology of atherosclerosis. Exercise reduces proinflammatory cytokines while increasing anti-inflammatory ones in patients with coronary syndromes. Correspondingly, Ang II is known to negatively affect these same mediators. Therefore, we assessed whether, in our exercised 2K1C mice, modulation of the T-cell phenotype may explain the beneficial effect on plaque progression. However, no difference in balance among T-helper 1, T-helper 2, and T-regulatory cells was observed between sedentary and exercised mice.

**Perspectives**

Results reported here, if confirmed in clinical studies, should have clinical implications in terms of cardiovascular event protection. In fact, for hypertensive patients with an activated RAAS and presenting with clinical or subclinical atherosclerosis, swimming exercise may be an attractive adjuvant therapy for the prevention of atherosclerosis progression.

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**Disclosures**

None.

**References**


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