Background—Arterial remodeling in response to atherosclerosis may be outward (positive) or inward (negative) and is an important mechanism in the clinical manifestations of atherosclerosis and restenosis after percutaneous coronary interventions. Postmortem and intravascular ultrasound studies of arterial remodeling do not allow serial and noninvasive data to be obtained. In a rabbit model of atherosclerosis, we sought to validate MRI as a new tool for documentation of arterial remodeling.

Methods and Results—Watanabe heritable hyperlipidemic rabbits underwent serial MRI at baseline and 6 months after aortic balloon denudation. The lumen area had a small but significant ($P = 0.006$) increase, from $4.36 \pm 0.16$ to $4.89 \pm 0.12$ mm$^2$. There was a large, significant ($P < 0.0001$) increase in the outer wall area, from $7.96 \pm 0.19$ to $10.46 \pm 0.19$ mm$^2$. The vessel wall area (a marker of atherosclerotic burden) increased significantly ($P < 0.0001$), from $3.61 \pm 0.07$ to $5.57 \pm 0.09$ mm$^2$. Thus, the increase in atherosclerotic burden over time was completely accounted for by positive arterial remodeling. The subgroup used for histopathological validation confirmed a significant ($P < 0.0001$) agreement between histopathology and MRI for assessment of all 3 parameters.

Conclusions—MRI can provide serial and noninvasive data about the arterial wall, allowing assessment of arterial remodeling in this rabbit model. Thus, MRI appears to be a useful tool for the investigation of arterial remodeling both in native atherosclerosis and after percutaneous coronary intervention. (Circulation. 2000;101:586-589.)

Key Words: remodeling ■ atherosclerosis ■ magnetic resonance imaging

Atherosclerotic plaques may develop and even progress without compromising the arterial lumen in the early phases because of outward growth of the vessel wall, a concept known as “positive” or “outward” arterial remodeling. Remodeling of the arterial wall is an important mechanism in determining luminal narrowing of native atherosclerotic lesions and restenosis after percutaneous coronary interventions. However, because of the difficulty in studying atherosclerotic lesions longitudinally over time, little is known about the mechanisms involved in the remodeling process. Moreover, recent data have suggested that arterial remodeling may be “negative” or “inward” even in the early stages of plaque development. To date, apart from postmortem studies, intravascular ultrasound has been used to provide information on the remodeling phenomenon. However, this is an invasive imaging methodology, which limits its usefulness for serial analysis of atherosclerotic lesions. Thus, an imaging technique that could noninvasively and serially monitor the vessel wall could greatly assist our understanding of this process.

MRI is a noninvasive imaging modality that has been used to document atherosclerotic burden both in humans and in animal models of atherosclerosis. It has been validated in a rabbit model of abdominal aortic atherosclerosis with cholesterol feeding and balloon injury, and it permits serial imaging of the same atherosclerotic lesions.

The Watanabe heritable hyperlipidemic (WHHL) rabbit has an intrinsic deficiency in LDL receptors, thus more closely approximating humans with atherosclerosis than the cholesterol-fed rabbit models.

In this study, we investigated arterial remodeling with serial MRI in WHHL rabbits that have undergone balloon injury to the abdominal aorta to accelerate and localize the atherosclerosis.

Methods

Animal Model

WHHL rabbits (n=11; age, 3 months; weight, 3.0 kg) underwent aortic denudation of the aorta from the renal arteries to the iliac bifurcation as previously described 1 week after arrival at the institution. Full lipid profiles were analyzed from serum specimens obtained at the time of the MRI studies. All experiments were approved by the Mount Sinai School of Medicine animal management program.
MR Imaging
All rabbits had serial MRI performed on 2 occasions, immediately before aortic balloon denudation and 6 months later, with a 1.5-T MRI system (Signa GEMS) and a conventional phased-array volume coil. The MRI protocol used was based on previously validated work. Sequential axial images (3 mm thick) of the aorta from the arch to the iliac bifurcation were obtained by use of a fast spin-echo sequence with an in-plane resolution of 350 μm (proton density weighted: TR/TE, 2300/17 ms; T2w: TR/TE, 2300/60 ms; field of view, 9 × 9 cm; matrix, 256 × 256; echo train length, 8; signal averages, 4). Fat suppression and flow saturation pulses were used as previously described.

MRI Analysis
The MR images were transferred to a Macintosh computer for further analysis. The MR images from the same animals at the 2 time points were matched by use of distance from the renal arteries and the iliac bifurcation for registration. Thus, each segment of the abdominal aorta could be compared at baseline and 6 months by MRI, allowing true serial data to be obtained. The MR images from the validation subgroup were matched with corresponding histopathological sections for the aortic specimens as described above. Cross-sectional areas of the lumen and outer boundary of each aortic section were determined by manual tracing with Image Pro-Plus (Media Cybernetics) by an observer blinded to the identity of the rabbits. From these measurements, lumen area, outer vessel area, and vessel wall area (outer vessel area minus lumen area) were calculated. The outer wall was defined as the vessel wall–epicardial fat interface.

Histopathology
The rabbits (n = 4) were euthanized within 24 hours of MRI by intravenous injection of Sleepaway 5 mL IV (Fort Dodge Animal Health) after receiving heparin (100 U/kg) to prevent postmortem blood clotting. The aortas were excised and perfusion-fixed as described. Serial sections of the aorta were cut at 3-mm intervals matching the corresponding MR images. The aortic specimens were embedded in paraffin, and sections 5 μm thick were cut and stained with combined Masson’s trichrome elastin stain.

Histopathology Analysis
The histopathological sections were digitized to the same computer and the same parameters were analyzed with the method described above for MR image analysis. The outer wall of the vessel was defined as the dense adventitia–epicardial fat interface on histopathology.

Statistical Analysis
Paired Student’s t tests were used to compare the MR image–derived parameters from the same sites in the abdominal aorta at the 2 time points. Comparisons between MRI and histopathological assessment of vessel parameters in the validation subgroup were performed by simple linear regression analysis. All probabilities are 2-sided, with
Results

Serum Lipid Profiles
The mean baseline serum cholesterol (at age 3 months) was 990±91 mg/dL. Six months later (age 9 months), it was 449±57 mg/dL. This age-related drop in serum cholesterol is a well-described characteristic of WHHL rabbits.14

Validation Subgroup Data
To confirm the ability of MRI to assess changes in the arterial wall parameters, a correlation between the techniques was performed. There was a significant ($P<0.0001$) correlation between MRI and histopathological analysis for assessment of lumen area ($r=0.80$), outer vessel area ($r=0.84$), and vessel wall area ($r=0.83$), consistent with previous studies (Figure 1).12,13

Serial MRI Data
To monitor changes in the lumen and outer vessel wall areas over time (indicating degree of stenosis and arterial remodeling, respectively), we compared matched MR images of the abdominal aorta in the same rabbits at the 2 time points (Figure 2). At baseline, the mean areas for the predetermined parameters were lumen area, 4.36±0.16 mm$^2$; outer vessel area, 7.96±0.19 mm$^2$; and vessel wall area, 3.61±0.07 mm$^2$. Six months after the balloon injury, the same parameters were lumen area, 4.89±0.12 mm$^2$; outer vessel area, 10.46±0.19 mm$^2$; and vessel wall area, 5.57±0.09 mm$^2$ (Figure 3). Over this 6-month period, there was a significant ($P<0.0001$) increase in the vessel wall area, a surrogate of atherosclerotic thickening. Rather than a concomitant decrease in the lumen area, there was a small but significant ($P=0.006$) increase in the lumen area. An outward or positive remodeling accounted for this increase in atherosclerotic plaque burden, as evidenced by the significant ($P<0.0001$) increase in the outer vessel area.

Discussion
Atherosclerosis has classically been assessed by the degree of luminal narrowing. However, it is now known that significant atherosclerosis can exist without any compromise to the
lumen. This concept is clearly demonstrated in this model of aortic atherosclerosis in WHHL rabbits. Noninvasive serial MRI accurately documents the increase in atherosclerotic burden despite there being no decrease, or even an increase, in the arterial lumen, as indicated by the increase in vessel wall area over the 6-month period (positive arterial remodeling). Angiographic analysis would have indicated not only no increase but even a decrease in atherosclerotic burden over time, as noted by the small but significant increase in lumen area over the 6-month period. Conversely, MRI allows the serial and noninvasive assessment of vessel wall parameters in this model and thus the potential for providing insights into the mechanisms and the natural history of arterial remodeling.

Arterial remodeling has been described for >10 years. However, the mechanisms involved remain unclear because of the difficulty in obtaining longitudinal studies over the lengthy time interval during which remodeling most likely occurs and because of limited data from relevant animal models. Although we are not able to draw conclusions about the early remodeling process in humans or even other animal models from our findings, the feasibility of using MRI to document arterial remodeling in vivo permits future studies at multiple time points. Indeed, it is clear that early arterial remodeling is not always positive. This further reinforces the need for an imaging modality that can serially and noninvasively provide information about the arterial remodeling process in humans. Intravascular ultrasound has been the only imaging technique used to study the effects of remodeling and atherosclerosis. However, it is an intrinsically invasive modality, which limits its usefulness for longitudinal studies.

MRI has been shown to accurately quantify the vessel wall area in the abdominal aorta of rabbits fed cholesterol. Furthermore, in rabbit models of atherosclerosis, both intravascular ultrasound and surface MRI accurately estimate vessel wall area and thus atherosclerotic burden when compared with histology. The ability of MRI to provide serial and noninvasive information about the arterial wall in this model provides us with a useful imaging tool to assist the investigation of arterial remodeling in future studies, both in primary atherosclerosis and in restenosis after percutaneous intervention.

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