Endothelial senescence after high-cholesterol, high-fat diet challenge in baboons

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1Department of Genetics, 3Department of Physiology and Medicine, Southwest Foundation for Biomedical Research; 2Southwest National Primate Research Center, San Antonio; 4Cardiothoracic Research Laboratory, Texas Heart Institute, St. Luke’s Episcopal Hospital, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston; 5Department of Medicine/Division of Hematology, University of Texas Health Science Center, San Antonio, Texas

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Shi Q, Hubbard GB, Kushwaha RS, Rainwater D, Thomas CA 3rd, Leland MM, VandeBerg JL, Wang XL. Endothelial senescence after high-cholesterol, high-fat diet challenge in baboons. Am J Physiol Heart Circ Physiol 292: H2913–H2920, 2007. First published February 2, 2007; doi:10.1152/ajpheart.01405.2006.—Increasing evidence indicates that replicative senescence and premature endothelial senescence could contribute to endothelial dysfunction. This study aims at testing the hypothesis that a high-fat diet may lead to premature vascular endothelial senescence in a nonhuman primate model. We isolated endothelial cells from left and right femoral arteries in 10 baboons before and after a 7-wk high-fat dietary treatment. We compared the morphological alterations, replicative capacities, and senescence-associated β-galactosidase activities (SA-β-gal) at these two time points. We found that high-fat diet increased the prevalence of endothelial senescence. Replicative capacities declined dramatically, and SA-β-gal activities increased significantly in postdietary challenge. There was no change in telomeric length using quantitative flow fluorescence in situ hybridization analysis, suggesting that some stressors lead to cell senescence independent of telomere dysfunction. Our findings that high-fat diet causes endothelial damage through the premature senescence suggest a novel mechanism for the diet-induced endothelial dysfunction.

senescence-associated β-galactosidase; intercellular adhesion molecule; vascular cell adhesion molecule

ACCELERATED OR PREMATURE SENECE of cells is defined as irreversible proliferation arrest, which could occur in vivo or in vitro in response to various stressors. In the settings of in vitro cell culture, primary cells could become prematurely senescent either because of intrinsic growth restriction or exposure to certain types of stress. Chronic oxidative stress or repeated physical injury, such as endothelial denudation, can result in emergence of senescent endothelial cells (11, 18). Studies suggest that functional changes associated with senescence could be involved in atherosclerosis in humans and animals (23, 25); in addition, progressive telomere shortening in vivo was observed in the regions susceptible to atherosclerosis (12). In addition to known pathogenic mechanisms, traditional risk factors, such as hypercholesterolemia, could also create an intracellular prooxidative stress environment and induce premature cellular senescence.

It is therefore logical to speculate that high-fat diet could result in oxidative stress and lead to endothelial senescence, which may provide a novel mechanism in the causal link of the atherogenic diet and atheroma formation. In a previous study, we challenged 10 baboons with a high-cholesterol high-fat (HCHF) diet for 7 wk. Endothelial cells were isolated from femoral arteries before and after the dietary challenge. We found that endothelial cells harvested after the HCHF diet did not grow as readily as those collected from the same animals before the diet. There were also increased expressions of cellular adhesion molecules [intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)] as the result of the HCHF diet (30). These results suggest that a premature cellular senescence may have been triggered by the HCHF diet in these baboons.

In the current study, we examined the hypothesis that HCHF diet may damage arterial vessels through the induction of endothelial senescence and whether cellular senescence plays a role in the observed slowing down of cellular growth (replicative arrest). By comparing samples from the same animals before and after the HCHF diet, we found increased ratios of senescent cells, lower replicative capacities, and increased senescence-associated β-galactosidase (SA-β-gal) activities in endothelial cells after the dietary challenge. Our results suggest that the atherogenic diet induces premature endothelial senescence, reflecting a novel mechanism for diet-induced atherosclerosis.

METHODS

Cell isolation, culture, and treatment. Animals were immobilized under 10 mg/kg ketamine hydrochloride, and a 4-cm segment of femoral artery in the upper part of the thigh was obtained under sterile surgical procedure. Before the diet challenge, the femoral artery was biopsied from one leg, e.g., right leg; at the end of the diet challenge, the femoral artery biopsy was carried out in the other leg, e.g., left leg. The constituents of the HCHF diet are shown in Table 1. Surgery was performed by experienced veterinarians, and procedures were approved by the International Animal Care and Use Committee of Southwest Foundation for Biomedical Research. The artery was gently cannulated and then injected with 0.1% collagenase (Invitrogen, Carlsbad, CA) with incubation at 37°C for 20 min for digestion. The released cells were seeded immediately on 1.0% gelatin-coated

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Table 1. Measured composition of the basal and HCHF diets

<table>
<thead>
<tr>
<th></th>
<th>Basal Diet</th>
<th>HCHF Diet</th>
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<tbody>
<tr>
<td>Energy, kcal/g</td>
<td>3.1</td>
<td>3.8</td>
</tr>
<tr>
<td>Carbohydrates, %</td>
<td>75</td>
<td>39</td>
</tr>
<tr>
<td>Protein, %</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Fat, %</td>
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<td>41</td>
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<tr>
<td>Cholesterol content, mg/g</td>
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<td>6.37</td>
</tr>
<tr>
<td>Fat composition</td>
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</tr>
<tr>
<td>Monounsaturated/polyunsaturated</td>
<td>0.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Saturated fat, %</td>
<td>23.9%</td>
<td>44.4%</td>
</tr>
<tr>
<td>Monounsaturated fat, %</td>
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<td>40.7%</td>
</tr>
<tr>
<td>Polyunsaturated fat, %</td>
<td>50.9%</td>
<td>14.9%</td>
</tr>
<tr>
<td>Fatty acid composition, %</td>
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<td></td>
</tr>
<tr>
<td>Myristic acid (14:0)</td>
<td>1.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Palmitic acid (16:0)</td>
<td>17.0</td>
<td>24.8</td>
</tr>
<tr>
<td>Palmitoleic acid (16:1)</td>
<td>1.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Stearic acid (18:0)</td>
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<td>17.8</td>
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<td>Oleic acid (18:1)</td>
<td>25.6</td>
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<tr>
<td>Linoleic acid (18:2)</td>
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</tr>
<tr>
<td>Linolenic acid (18:3)</td>
<td>3.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

HCHF, high cholesterol high fat.

culture plates. The endothelial growth medium was F-12K supplemented with 20% FCS, 75 µg/ml endothelial-derived growth factor (Sigma, St. Louis, MO), 50 µg/ml heparin, 10 mM HEPES (Invitrogen), 2 mM glutamine, and antibiotics (Invitrogen). Baboon femoral artery endothelial cells (BFAECs) were successfully isolated from all 10 experimental baboons. Cells were allowed to reach 70–90% confluence before the in vitro analyses. Senescent cells usually have distinctive morphological modifications. We therefore documented the microscopic images of all BFAECs in the study.

Immunocytochemical staining for SA-β-gal activity. Immunological detection of SA-β-gal was conducted using FITC-conjugated rabbit polyclonal anti-β-galactosidase antibody (Abcam) at a dilution of 1:10,000. BFAECs were grown on cover slip slides, fixed in 4% paraformaldehyde in PBS for 20 min at room temperature (RT). The cells were permeabilized by 0.1% Triton X-100. After three washes with PBS, the slides were blocked by 5% BSA for 1 h at RT and then incubated with primary antibodies overnight at 4°C. The nuclei were stained with 100 ng/ml 4',6-diamidino-2-phenylindole (DAPI; Sigma) or 5 U/ml Texas red-conjugated phalloidin (Molecular Probes) to stain DNA and actin. Cells were counterstained with hematoxylin and eosin.

Quantitative measurement of SA-β-gal. Positive SA-β-gal staining has been reported to reflect replicative senescence of human endothelial cells, and the areas of staining represent the enzymatic activities (11, 17). SA-β-gal activity at pH 6.0 was found specifically in senescent endothelial cells but not in quiescent or terminally differentiated cells. We determined SA-β-gal activities using cytochemical staining. The quantitative SA-β-gal analysis was done using computerized image analysis software Image-Pro 4.5.1 (MediaCybernetics). Under a ×40 objective lens, we selected ~150 cells/slide at random. We captured the specific color that was identical to the products of SA-β-gal and used the area measurement option provided by the software program to count area sizes and objective intensities. Based on previous investigations that area size was closely correlated to enzymatic activities (11, 17), we chose area sizes and averaged them (mean ± SD). Results of pre- and postdiet SA-β-gal were compared statistically with the paired Student’s t-test.

Apoptosis assay. Cellular apoptosis rates were evaluated by Annexin V-PE Apoptosis Detection Reagent (Abcam). Briefly, 1 × 10³–10⁶ cells were suspended in 1 ml phycoerythrin (PE)-conjugated Annexin V for 5 min at RT in the dark. Before analysis, propidium iodide (PI) was added at a final concentration of 1.0 µg/ml. Cells were then analyzed by FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA) using 488-nm excitation. Gates were set based on light scatter, collected PE Annexin V fluorescence at 530 ± 20 nm, and PI fluorescence > 600 nm.

Fluorescence in situ hybridization for telomere. To determine the telomere length in BFAECs, we used a kit from Applied Biosystems, which uses a peptide nucleic acid (PNA) telomere probe (Flu-OO-CCC-TAA-CCC-CCTTAA-CC-CC-TEE) to hybridize the in situ tandem repeat sequence in BFAECs in interphase/metaphase preparations. The PNA telomere probe is labeled with FITC fluorophore. The cells were resuspended by adding 8 ml of 75 mmol/l KCl, and incubated for 30 min at RT. We then added 2 ml freshly prepared fixative in 3:1

Fig. 1. A: morphological alternations of baboon femoral artery endothelial cells (BFAECs) as a result of dietary challenge. Photomicrographs show the morphological characteristics of representative senescent cells from BFAECs before (B) and after (C) the high-cholesterol high-fat (HCHF) diet. Arrows in B and C indicate individual senescent BFAECs seen at high prevalence in the postdiet group (magnifications ×400).
(vol/vol) methanol-acetic acid on top of the hypotonic suspension and mixed carefully by inverting the tube. This fixation step was repeated three times. The cells were washed two times in PBS containing 0.1% BSA in a 1.5-ml Eppendorf tube. Typically, 6 \times 10^5 cells/sample were used per tube. The cell suspension was then dropped on slides. An aliquot of 10 \mu l PNA telomere probe was applied to the target area of the slide. The slide was placed in a 74°C preheated block and maintained at 74°C for 90 s before ramped down to RT. The slides were immersed in 1\times PBS and 0.1% Tween 20 [preheated to 57 ± 1°C for 20 min followed by rinsing the slide in 2\times saline-sodium citrate (SSC) and 0.1% Tween 20]. The cells were counterstained with 60 ng/ml DAPI in 2\times SSC and 0.1% Tween 20 solution. Images of interphase and metaphase spreads were viewed by fluorescence microscopy and captured using a cooled charge-coupled device camera and MetaPhore software.

Flow cytometry to determine telomere shortening. We adapted the flow fluorescence in situ hybridization protocol in the assay as described previously with modifications (1, 14, 21, 27). Briefly, cells were fixed in 4% formaldehyde in PBS and then washed three times. Each sample was divided equally among two Eppendorf tubes. Following centrifugation for 15 s at 13,000 rpm, cell pellets were resuspended in 300 \mu l hybridization mixture (70% formamide-30% buffer containing 10 mM NaCl, 1\times Denhardt’s solution, 0.1 mg/ml each of RNA and salmon sperm DNA, and 20 mM Tris, pH 7.5) and 200 ng PNA/sample. Samples were subjected to heat denaturation of DNA at 80°C in a heating block for 10 min before being ramped down to RT. The samples were incubated in the dark at RT for 2 h. The cells were washed and incubated two times at RT for 10 min with 5 ml wash solution I (10 mM Tris•Cl, pH 7.4, 70% (vol/vol) final deionized formamide, 0.1% (wt/vol) BSA) and then washed with 5 ml wash solution II [0.1 M Tris•Cl, pH 7.4, 0.15 M NaCl, 0.1% (vol/vol) Tween 20] prewarmed at 57 ± 1°C for 20 min. Finally, the cells were washed in 1 ml of 2\times SSC and 0.1% Tween 20 before being resuspended in 200–500 \mu l DNA staining buffer (1\times PBS, 0.1% BSA, 10 \mu g/ml RNase, and 0.06 \mu g/ml PI). The calibration and linearity of the flow cytometry were checked by running the fluorescent Molecular of Equivalent Soluble Fluorochrome (MESF) beads before cell samples to establish the standard fluorescence curve. The samples were analyzed, leaving the FITC detector at the same voltage setting as that used for the MESF beads. We then drew a gate at the G1 cell cycle phase and displayed a histogram for FITC fluorescence gated on this population. We used the MESF beads that have known numbers of fluorochrome molecules on their surfaces to determine the exact changes in telomere length, which can be calculated based on fluorescence (FL) intensity by flow cytometry using an internal MESF standard curve with the equation as follows: telomere length (kb) = 0.019 \times [FL channel counts − FL channel counts (blank)] (14, 27). This approach allows us to compare the telomere shortening in multiple samples at the same time with relatively low cell counts (1–5 \times 10^5).

Immunochemistry and confocal microscopy. Immunological detection of SA-β-gal was conducted using FITC-conjugated rabbit polyclonal anti-β-galactosidase antibody (Abcam). BFAECs were grown on cover slip slides, fixed in 4% paraformaldehyde in PBS, and permeabilized by 0.1% Triton X-100. After three washes with PBS, the slides were blocked by 5% BSA for 1 h at RT and then incubated with anti-β-gal antibody overnight at 4°C at 1:1,000 dilution. Cells were counterstained with DAPI at 100 ng/ml and actin with Texas red phalloidin at 5 U/ml.

 Colony-forming assay. Two hundred cells were plated in six-well dishes in endothelial culture medium and incubated for 14 days. At the end of experiment, the medium was removed, and the dishes were stained with 0.5% crystal violet (Sigma) in methanol for 5 min. The cells were washed two times with distilled water, and the number of colonies was counted under microscope. Only the colonies containing >100 cells were counted. Colony-forming units (CFUs) refer to the averaged total colony numbers in duplicate wells.

RESULTS

Characteristics of senescent endothelial cells induced by the HCHF diet. Under routine culture conditions, normal endothelial cells showed a typical cobblestone shape with tight connection between cells during confluence under the phase-contrast microscope (Fig. 1A). BFAECs harvested from the same baboon after the dietary challenge at the same passage (passage 3) had an atypical appearance, including diverse morphotypes such as elongation (Fig. 1B), flattened cytoplasm...
(Fig. 1C) and multinucleation. The cell size was much bigger than those collected before the dietary challenge. Although cytoskeleton structures were highly prominent, intercellular contacts were less than that of normal cells. Senescent cells were scattered randomly over the culture flasks. They exhibited a higher prevalence in BFAECs collected after the dietary challenge.

**Effects of dietary challenge on replicative arrest.** Primary endothelial cells normally have a population doubling (PD) time ranging from 1.2 to 0.8 PD/day and cease to replicate after 46 cumulative PD in culture (13, 18). Among the 10 baboons studied in our experiments, all BFAECs grew well with more than 10 passages (~30 PD with 1:3 split in the culture) in biopsied samples from arteries taken before diet. The cells collected from the same animals after the HCHF diet did not proliferate as well as the cells collected before the challenge. They showed slower growth rates and greater durations of time to reach confluence. To determine replicative capacities, we measured the CFUs by seeding a single BFAEC suspension to reach confluence. To determine replicative capacities, we measured the CFUs by seeding a single BFAEC suspension containing 200 cells/inoculation in six-well culture dishes. After incubation for 2 wk under the routine culture conditions, CFUs were counted under the microscope.

As shown in Fig. 2, the number of CFUs was significantly reduced in BFAECs collected from baboons after the challenge. The average size of colonies of BFAECs before diet consisted of as many as 100–350 cells. In contrast, the postdiet cells did not form typical colonies, which were much smaller and contained ~10–50 cells/colony as shown in Fig. 2A. In addition to being smaller, the colonies formed were fewer in number by comparison with the colonies formed from prediet BFAECs (Fig. 2, B and C, P < 0.05, 2-way ANOVA). Our data suggest that the HCHF diet directly causes endothelial growth arrest. Additionally, the quantities of CFU differ significantly among individual baboons, suggesting individual differences in susceptibility to diet-induced endothelial dysfunction and consequent vascular lesions.

*Endothelial apoptosis challenged by the HCHF diet.* We also examined apoptotic markers by staining the cells with PE-labeled Annexin V, which is generally considered to be one of the early markers of apoptotic changes. FACS analysis showed a very low rate of apoptosis (0.25–3.5%) in cells cultured at passage 3 (Fig. 3A). Although some showed increased postdiet apoptosis, others decreased; and the mean percentages of apoptosis between prediet and postdiet BFAECs were not statistically different. One example of apoptosis in endothelial cells before and after the diet challenge along with a positive control (Jurkat cells treated by 5 μM camptothecin for 24 h) is presented in Fig. 3, B and C. Our findings suggest that damaged BFAECs seen after the HCHF diet may not be the result of increased apoptosis. However, because the sample size in the current study was small, the role of apoptosis in diet-induced endothelial injury needs to be confirmed in more animals.

*SA-β-gal activity increased by the HCHF diet.* To further evaluate the cellular changes, we determined accumulation of SA-β-gal activities. We found that both staining areas and prevalence of SA-β-gal positive cells significantly increased in postdiet BFAECs (Fig. 4, A and B), indicating that higher enzymatic activities occurred as a result of the diet challenge. To determine more rigorously the nature of SA-β-gal in senescent endothelial cells, a polyclonal antibody was used to stain the cells (17). As shown in Fig. 4, C–G, binding of fluorescent dyes was stronger in senescent cells, as defined by their characteristic cellular morphologies, than in the normally shaped endothelial cells. The image of the multinucleated, elongated senescent cell shows stronger FITC fluorescence staining than the normal cells. It is the cytoplasm that is primarily stained, indicating that SA-β-gal expression is elevated only in senescent cells.

We used the same approach and quantitatively measured the staining objects on culture slides. With the Image-Pro computer-assisted program, we estimated enzymatic activities in eight

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**Fig. 3.** Apoptosis rates in BFAECs as the result of the diet challenge. A: apoptotic cells in individual endothelial cultures before and after the diet challenge. Data were the means of 3 independent experiments with duplicates in each assay. B: representative baboon subjected to dietary challenge. C: positive control of Jurkat cells treated with 5 μM camptothecin for 24 h.

**Fig. 4.** Multinucleated, elongated senescent cell shows stronger FITC fluorescence staining than the normal cells. It is the cytoplasm that is primarily stained, indicating that SA-β-gal expression is elevated only in senescent cells.
baboons (Table 2). SA-β-gal staining areas increased after HCHF diet from 1.3- to 23.1-fold. The enzymatic data were further validated immunohistochemically in which specific anti-SA-β-gal was used to detect the distribution and the abundance of the SA-β-gal protein (data not shown). Our results also revealed substantial interindividual variation among the tested baboons both at baseline and in response to the diet. We then compared the changes in pre- and postdiet BFAECs using the paired t-test and found the change was significantly different (P = 0.0048). These data suggest that the HCHF diet induces accelerated endothelial senescence in baboons.

Telomere length in endothelial cells before and after HCHF Diet. To investigate if HCHF diet-induced senescence is dependent on the telomere shortening, we used a FITC-labeled telomere-specific PNA probe to hybridize to the telomere repeats in individual cells. Figure 5 shows the images of FISH seen under a fluorescence microscope. The telomere probes were located inside the nuclei at interphase stages (Fig. 5, A and B). HeLa cells have been used as a positive control to show the binding of telomere probes to the ends of chromosomes at metaphase (Fig. 5, C and D at ×400 and ×600 magnifications).

In baboons examined in our current investigations, the telomere length before diet was 25.19 – 35.47 and 19.15 – 30.17 kb after the diet challenge, as shown in Fig. 5 E. The overall reduction in telomere lengths was not statistically significant, which suggests that telomere shortening may not contribute to diet-induced senescent changes in BFAECs. However, the findings need to be confirmed in a larger number of animals that are exposed for a longer period of dietary challenge.

Table 2. Morphometric measurement of SA-β-gal activities in histochemistry slides

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Before Diet</th>
<th>After Diet</th>
<th>Degree of Increase</th>
<th>Significance (P values)</th>
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<tbody>
<tr>
<td>10041</td>
<td>11.01±13.68</td>
<td>56.81±226.22</td>
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<td>0.0048*</td>
</tr>
<tr>
<td>13676</td>
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</table>

Values are means ± SE. SA-β-gal, senescence-associated β-galactosidase.

*P values, one-way ANOVA, at 0.95 confidence, comparison between before and after diet treatments.

In baboons examined in our current investigations, the telomere length before diet was 25.19 – 35.47 and 19.15 – 30.17 kb after the diet challenge, as shown in Fig. 5 E. The overall reduction in telomere lengths was not statistically significant, which suggests that telomere shortening may not contribute to diet-induced senescent changes in BFAECs. However, the findings need to be confirmed in a larger number of animals that are exposed for a longer period of dietary challenge.
DISCUSSION

The results of the present study revealed that a HCHF diet challenge for 7 wk in baboons accelerates the onset of replicative senescence in a longitudinal experimental observation. Although previous studies described senescent cells in the atherosclerotic arteries (23), endothelial senescence has mainly shown to be induced by repeated arterial injury or after chronic oxidative stress (11, 18). Our present findings that the HCHF diet leads to the emergence of vascular senescence are unique; no such description has been reported previously. In addition, we have demonstrated that increased telomere shortening may not be responsible for this premature endothelial senescence.

It has been increasingly recognized that adequate animal models are critical for the investigation of vascular senescence (6). Atherosclerosis is a complex disease that involves an inherent aging mechanism and requires certain time for the atheromatic injury to evolve and to fully develop. Commonly used small animals such as mice or rabbits have significant differences with respect to life span, age-associated changes, and time required for atherosclerosis development. On the other hand, baboon is a relatively long-lived nonhuman pri-

mate. The nature of slowly progressing atherosclerotic lesions in baboons when challenged by the HCHF diet closely resembles human pathogenesis. Baboon endothelial cells have shown similar dysfunctional changes as humans when challenged by HCHF diet (29). We suggest that baboons may be a more suitable model for investigations on diet-induced vascular senescence or endothelial dysfunction than mice or rabbits.

Furthermore, baboons may also be an appropriate animal model for investigating gene-diet interactive effects on endothelial functional profiles. In this study, colony-forming ability varied among individuals by more than twofold before the dietary challenge and by more than sixfold after dietary challenge (Fig. 2C). Apoptosis varied by more than fourfold both before and after the challenge (Fig. 3), and SA-β-gal activities varied by 66-fold before the challenge and 4.3-fold after the challenge (Table 1). These results suggest that genes may interact with dietary factors to produce major differences in endothelial biological characteristics and that those differences may have an important role in determining individual susceptibility to atherosclerosis.
The present observation of diet-induced vascular senescence has significant implications for endothelial dysfunction and subsequently atherogenesis. Senescent endothelial cells are more morphologically and functionally prone to atherosclerotic development than normal cells (17). The endothelial lining of the arterial wall plays a crucial role in defending against adverse “environmental” factors in the circulation, e.g., elevated low density lipoprotein. In culture, we have observed that vascular endothelial cells become senescent after the HCHF dietary treatment; cells are enlarged, flattened, and disassociated from each other. It is likely that this type of cell is unable to provide an adequate protective barrier against low density lipoprotein and monocyte infiltration. The increased SA-β-gal activities in our study are consistent with the reports of a high SA-β-gal activity in atherosclerotic lesions in autopsied coronary arteries (15, 23, 24). Moreover, senescent cells are not completely inactive. They release regulatory factors that can disrupt the architecture of neighboring cells and/or can stimulate neighboring cells to proliferate, triggering local inflammation and tissue remodeling (5, 8, 19). Although more studies are required, together with our previous findings of increased levels of cellular adhesion molecules (VCAM-1 and ICAM-1) in plasma of postdietary baboons (30), it appears that oxidative stress and inflammatory changes could be responsible for the endothelial dysfunctional changes induced by the HCHF diet and create conditions that favor vascular diseases. Furthermore, senescent cells in the vascular wall could be potential sites for the development of atherosclerotic lesions (16). What we have observed in BFAECs could be an indicator of what would happen systemically when an individual is exposed to HCHF diet for years. It is known that accelerated senescence is a common pathway for organismal aging when cells encounter detrimental factors. Accumulated organismal aging becomes systemic aging, which is the most significant risk factor for atherosclerosis in humans (10, 19, 20). It should be noted, however, that experimental evidence is still needed to establish a direct link between endothelial senescence and atherogenesis.

Traditionally, a gradual shortening of the telomeres occurs during the proliferation of human cells. Recent studies, however, suggest that the dynamics of telomere length regulation are a more complex process and that not all cellular senescence processes are accompanied by this shortening process (2, 3, 26). Two pathways currently known can cause somatic cell senescence, i.e., telomeric and nontelomeric pathways (2, 4, 28). Some stimuli, such as oxidation-induced DNA strand breaks, cause senescence via accelerated telomeric shortening or attrition. However, a variety of environmental and intracellular stimuli may induce a senescence-like state without telomeric shortening (26, 31). Examples of such nontelomeric-dependent stimuli include γ-irradiation, oxidative stress, expression of Ha-ras oncogene, accumulation of DNA cross links, and demethylating agents. Cell senescence in vivo can also be found without telomere shortening (22). We used PNA telomere probes and did not detect significant telomere shortening in BFAECs. This finding suggests that HCHF-induced cellular senescence is independent of telomere shortening, which is similar to oxidative stress-induced senescence (7, 9). It should be noted, however, it is possible that the exposure to the HCHF diet was not long enough to induce accelerated telomere erosion, and the sample size may be too small to detect significant changes. The same is true for the role of 
apoptosis in endothelial senescence. New studies, such as prospective investigations, will be required to definitely determine whether HCHF diet accelerates telomere erosion (10, 28) and whether apoptosis is increased.

In summary, our study has demonstrated accelerated endothelial senescence in baboons challenged by a HCHF diet. This novel feature of endothelial dysfunction together with proinflammatory changes may be responsible for accelerated atherogenesis induced by this diet. It can be further implicated that long-term use of HCHF diet can accelerate the organismal aging process and result in premature aging and associated diseases, including atherosclerosis or hypertension. Although more studies are needed to investigate the mechanisms responsible for this accelerated endothelial cell aging, our study presents a novel pathogenic aspect contributing to diet-induced vascular diseases.

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REFERENCES


