CALL FOR PAPERS | Cardiovascular and Cerebrovascular Aging–New Mechanisms and Insights

High-protein-low-carbohydrate diet: deleterious metabolic and cardiovascular effects depend on age

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Submitted 1 May 2014; accepted in final form 8 July 2014

Bedarida T, Baron S, Vessieres E, Vibert F, Ayer A, Marchiol-Fournigault C, Henrion D, Paul J, Noble F, Golmard J, Beaudouex J, Cottart C, Nivet-Antoine V. High-protein-low-carbohydrate diet: deleterious metabolic and cardiovascular effects depend on age. Am J Physiol Heart Circ Physiol 307: H649–H657, 2014. First published July 11, 2014; doi:10.1152/ajpheart.00291.2014.—High-protein-low-carbohydrate (HP-LC) diets have become widespread. Yet their deleterious consequences, especially on glucose metabolism and arteries, have already been underlined. Our previous study (2) has already shown glucose intolerance with major arterial dysfunction in very old mice subjected to an HP-LC diet. The hypothesis of this work was that this diet had an age-dependent deleterious metabolic and cardiovascular outcome. Two groups of mice, young and adult (3 and 6 mo old), were subjected for 12 wk to a standard or to an HP-LC diet. Glucose and lipid metabolism was studied. The cardiovascular system was explored from the functional stage with Doppler-echography to the molecular stage (arterial reactivity, mRNA, immunohistochemistry). Young mice did not exhibit any significant metabolic modification, whereas adult mice presented marked glucose intolerance associated with an increase in resistin and triglyceride levels. These metabolic disturbances were responsible for cardiovascular damages only in adult mice, with decreased aortic distensibility and left ventricle dysfunction. These seemed to be the consequence of arterial dysfunctions. Mesenteric arteries were the worst affected with a major oxidative stress, whereas aorta function seemed to be maintained with an appreciable role of cyclooxygenase-2 to preserve endothelial function. This study highlights for the first time the age-dependent deleterious effects of an HP-LC diet on metabolism, with glucose intolerance and lipid disorders and vascular (especially microvessels) and cardiac functions. This work shows that HP-LC lead to equivalent cardiovascular alterations, as observed in very old age, and underlines the danger of such diet.

Cardiovascular disease: glucose intolerance; high-protein-low-carbohydrate diet

CHANGE IN WESTERN lifestyle, with an increasingly fat-rich diet and decreased physical activity, has led to increased overweight and obesity prevalence. Type 2 diabetes and metabolic syndrome have now become endemic (40), leading to increased cardiovascular morbidity and mortality (3). This trend does not concern only mature adults, but also teenagers and young adults, in whom the prevalence of diabetes and being overweight has been increasing (29). Some nutritional strategies have become more and more prevalent, such as high-protein-low-carbohydrate (HP-LC) diets (8). However, their deleterious outcomes on glucose metabolism and arteries have already been underlined (7, 12). Aortas as well as microvessels such as mesenteric arteries are altered with aging. Oxidative stress is one of the most important factors implied in these alterations. The reactive oxygen species (ROS) producers, such as NADPH oxidase, seem to be implied, leading to the vascular inflammation (30, 35). Moreover, the deficiency of the mechanisms that should decrease the oxidative stress might also take part into this oxidative stress imbalance, like the thioredoxin (Trx) system, one of the most important in the vessels (1). These mechanisms have been widely described with aging (11,
HIGH PROTEIN-LOW CARBOHYDRATE DIET EFFECTS DEPEND ON AGE

20, 30). Nonetheless, several other conditions lead to vascular alterations, but the mechanisms responsible for them need to be explored, in particular the case of the HP-LC diet, which has already been incriminated in vascular changes (12). Our laboratory’s previous study has already shown that such a diet could become deleterious in very old mice, with major metabolic disturbances and cardiovascular alterations (2).

Our hypothesis here was that metabolic and, more importantly, cardiovascular consequences of an HP-LC diet could depend on age. To explore this hypothesis, mice 3 mo old (young) and 6 mo old (adult) were subjected to an HP-LC diet for 3 mo. The outcome of the HP-LC diet was evaluated with regard to their metabolic and cardiovascular effects from functional stage (by Doppler-echography analysis) to molecular stage of endothelial dysfunction (with ex vivo arterial experiments and molecular characterization).

MATERIALS AND METHODS

The Paris Descartes University ethics committee approved all of the animal procedures and protocols used here (Protocol No. CEEA34.SB.008.12).

Animals and diets. C57BL/6J male mice (3 and 6 mo old) were obtained from Janvier (Le Genest-St-Isle, France) and treated in compliance with the European Parliament and Council Directive 2010/63/EU. They were housed in a temperature- and humidity-controlled room with a 12:12-h light-dark cycle and had food and water ad libitum. After 10 days of adaptation, the mice were randomized according to the two diets for the next 12 wk: a standard diet M20 (SDS), delivering an 18% protein supply as given to adult mice in the animal care unit, or an HP-LC diet (Certificate U8954, Safe) delivering a 31% protein supply as energy content, corresponding to a doubling of the protein supply. Young mice 3 mo old were fed for 12 wk with a standard diet (YS) \((n = 10)\), or an HP-LC diet (YD) \((n = 10)\). Adult mice 6 mo old were fed for 3 mo with a standard diet (AS) \((n = 20)\), or an HP-LC diet (AD) \((n = 20)\). Mice were weighed every 2 wk throughout the 12 wk of the study.

Glucose tolerance test. At the end of the trial, after a 6-h fasting period (6–12 AM), blood samples were taken from tail sections, and glucose levels were measured using a glucometer (One Touch Easy, Life Scan). Glucose tolerance test (GTT) was then assessed by injecting intraperitoneally 2 mg/g body wt glucose (G30 Aguettant), as previously described (2). Blood glucose was measured every 30 min during the 2 h after glucose injection. In AD mice only, 30 min after glucose injection, glycemia were all above the limit of quantification of the glucometer (33 mmol/l). Thus the value of 33 mmol/l was considered as individual time points and as an integrated area under the full GTT curve.

Metabolic measurements. Blood samples were clotted for 30 min and centrifuged for 10 min at 2,000 g. Serum triglycerides, cholesterol, and HDL-cholesterol were measured with DxC800 (Beckman). HDL fractions were measured with a Lipoprint HDL kit (Eurobio). A liquid-phase multiplexed technique (Luminex Bioplex, BioRad) was used to determine systemic insulin and resistin levels (Mouse Serum Adipokine-2plex kit). The insulin resistance index HOMA-IR (homeostasis model of assessment-insulin resistance) was calculated.

Locomotor activity. Twelve weeks after the beginning of the trial, locomotor activity was evaluated for 30 min in an actimeter (Immertime, Pessac, France) composed of eight cages \((34 \times 21 \times 19 \text{ cm})\) under low illumination \((<5 \text{ lux})\), as described previously (22). Measurements were started 5 min after introducing the animals into the actimeter, and horizontal and vertical activities were automatically recorded for 25 min.

Doppler echography. Doppler echography was carried out on anesthetized mice (isoflurane inhalation, induced at 3.5% then maintained with 1.5%) with an ultrasound biomicroscope (Vevo 770 Visual Sonic). Aortic diameters were measured continuously to calculate intima-media thickness in diastole: (adventitia-adventitia distance \(-\) internal diameter)/2. Aortic distensibility was calculated as \(\frac{\text{[systole diameter \(-\) diastole diameter]/diastole diameter}}{\text{Heart dimensions were measured in systole and diastole to calculate several parameters: left ventricular posterior wall thickening, shortening fraction, ejection fraction, and cardiac flow. Heart rates were obtained from electrocardiograms.}

Blood pressure measurements. Mice were anesthetized (isoflurane inhalation, induced at 5%, and then maintained with 2%). Arterial blood pressures were measured with a catheter in the carotid artery. Systolic, diastolic, mean, and pulse blood pressures were recorded with AcqKnowledge software.

Arterial reactivity experiments. Aorta and mesenteric artery vasoreactivity experiments were carried out as previously described (25). Briefly, 2-mm-long segments of thoracic aorta and second-order mesenteric artery were dissected out and mounted on a wire myograph. Two wires were inserted into the lumen of the arteries and fixed to a force transducer and a micrometer, respectively. The arterial segments were bathed in a 5-ml organ bath containing a physiological salt solution maintained at a pH of 7.4, a P\(_{\text{O}_2}\) of 160 Torr and a P\(_{\text{CO}_2}\) of 37 Torr. Optimal wall tension was then applied, and artery viability was tested using a potassium-rich solution (80 mmol/l). A cumulative concentration-response curve for the endothelium-dependent vasoreactivity experiments was carried out as previously described (25). Briefly, 2-mm-long segments of thoracic aorta and second-order mesenteric artery were crushed with an Ultra-Turrax J25 mill (Fisher-Bioblock) for 1 min in Trizol (Invitrogen). RNA was extracted as previously described (2). An aliquot of 1 \(\mu\)l of total RNA was treated with DNase I (Invitrogen) and converted into complementary DNA (cDNA) using Superscript II reverse transcriptase, oligo(dT)\(_{2-18}\) primers, and RNase OUT recombinant ribonuclease inhibitor (Invitrogen). cDNA products were subjected to real-time polymerase chain reaction (ABI 7900HT Fast Real-Time PCR). Quantitect SYBR Green PCR and Quantitect primer assay kits (Qiagen) were used to quantify interleukin (IL)-1, TNF-\(\alpha\), COX-2, p47\(\text{phox}\) (NADPH oxidase subunit), NOS III, and Trx1 gene expressions. All of the reactions were carried out in triplicate in a final volume of 20 \(\mu\)l, following the manufacturer’s instructions. Ribosomal protein L4 was used as a housekeeping gene after a validation step to verify equal loading of RNA and cDNA for the reverse transcription and PCR reactions. Data were analyzed by the 2\(^{-\Delta\Delta\text{ct}}\) method (24).

IL-1. NADPH oxidase, and Trx1 aorta and mesenteric artery labeling. Segments of aorta and mesenteric arteries were embedded in Tissue Tek OCT Compound, frozen at \(-40^\circ\text{C}\) and stored at \(-80^\circ\text{C}\). Sections 20 \(\mu\)m thick were incubated with a primary antibody raised against IL-1 (rabbit polyclonal Abcam), p47\(\text{phox}\) (NADPH oxidase subunit) (rabbit polyclonal Santa Cruz), or Trx1 (mouse monoclonal...
Abcam). Labeling was visualized with Alexa Fluor 488 goat anti-rabbit IgG or anti-mouse IgG (Invitrogen). The endothelium was labeled with rat monoclonal anti-CD31 antibody (BD Pharmingen) and visualized with Alexa Fluor 550 goat anti-rat IgG. Nuclei were counterstained with To-Pro3 (Invitrogen). Negative controls (primary antibodies substituted by nonimmune IgG isotype) did not yield any detectable labeling. Images were recorded on a Leica TCS SP2 confocal microscope. Each endothelial protein expression was quantified using National Institutes of Health ImageJ software. Three sections of each aorta were recorded, and four independent sectors of each section were analyzed. Protein staining intensities were measured within the endothelium and divided by surface area to give fluorescence intensity values (in fluorescence intensity units/m^2).

Statistical analyses. Mann-Whitney tests were used to compare YD with YS mice, and AD with AS mice, respectively (Graph Pad Prism). Results were expressed as means ± SE; statistical significance was set at P ≤ 0.05.

RESULTS

Longitudinal study. For overall survival, young mice fed standard diet (YS) and HP-LC diet (YD), and adult mice fed standard diet (AS) exhibited 100% survival at day 84, while adult mice fed HP-LC diet (AD) exhibited 84.6% survival. The two deaths occurred at around 3 wk after the HP-LC diet introduction. YS and YD mice exhibited a similar pattern with 17.7 and 16.3% weight increase, respectively (Fig. 1A). In adult mice, AD mice exhibited a significant weight decrease at day 14 compared with their initial weight (−4.0%, P ≤ 10^-4), while AS mice exhibited weight increase (+2.7%, P ≤ 0.05).

Glucose regulation. Fasting glycemia was not different between young (YS and YD groups) and adult mice (AS and AD groups) (Table 1). No significant difference was observed between the responses of YS and YD mice to the GTT (Fig. 1B). After glucose load, compared with the AS group, AD mice exhibited increased glycemia at each time (Fig. 1B), leading to an upper area under the curve (2,024 ± 87 vs. 3,172 ± 149 mmol·l^-1·min^-1) for AS and AD mice, respectively, +57%, (P < 0.01). To explore the difference between AS and AD mice, glucose regulation parameters were analyzed. Compared with AS mice, the AD group showed no modification in insulin level or HOMA-IR, but exhibited a 47% increased resistin level (P ≤ 0.01) (Table 2).

Lipid profile. Compared with the YS group, YD mice did not exhibit any modification in triglyceride serum level, but had increased HDL-cholesterol and total cholesterol levels (+75 and +61%, respectively) with no modification in non-HDL cholesterol level (Fig. 1, C and D). Compared with AS mice, the AD group exhibited increased triglyceride, total cholesterol, HDL-cholesterol, and non-HDL cholesterol levels (respectively, +48, +50, +26, and +74%). For the HDL subfraction, only the large HDL cholesterol part increased (+7.8 points, P ≤ 0.05), while the nonlarge HDL part decreased (−8.4 points, P ≤ 0.05).

Table 1. Fasting glycemia in young and adult mice fed standard or high-protein-low-carbohydrate diet

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<th>YS</th>
<th>YD</th>
<th>AS</th>
<th>AD</th>
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<tr>
<td>Fasting glycemia</td>
<td>9.0 ± 0.6</td>
<td>9.9 ± 0.6</td>
<td>10.7 ± 0.5</td>
<td>12.0 ± 0.5</td>
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Values are means ± SE in mmol/l; n = 9–11 per group. YS and AS (respectively, young and adult mice fed standard diet) and YD and AD (respectively, young and adult mice fed high-protein-low-carbohydrate diet) mice were compared. No significant difference was observed between the different groups.
DISCUSSION

The HP-LC diet use is increasingly widespread (13, 31). However, such a diet may become deleterious, depending on the context in which it is used, as underlined by our laboratory’s previous study on very old mice (2). To explore the age-related effects of a chronic HP-LC diet, its metabolic and cardiovascular consequences were studied in young and adult mice.

In young mice, no change in glucose tolerance or in triglyceride level induced by HP-LC was observed. An increased cholesterol level was reported, but this dyslipidemia seemed to have no deleterious outcome in terms of cardiovascular aging, as shown by vascular and cardiac Doppler-echography results. Although the consequences of this dyslipidemia in young mice have not been evaluated with arterial reactivity experiments, Aorta molecular phenotype. Compared with the AS group, AD mice exhibited an increased inflammation gene expression for IL-1, TNF-α (12.8-fold, \( P < 0.001 \)), and COX-2 (2.8-fold, \( P < 0.05 \)) (Fig. 3A). For oxidative stress mediators, no modification was observed for \( p^{47}_{\text{phox}} \), Trx1, or NOS III gene expression (Fig. 3, B and C).

For endothelial protein expression in aorta in adult mice, compared with AS mice, the AD group exhibited increased IL-1 and \( p^{47}_{\text{phox}} \) subunit expression by 102 and 30%, respectively, while Trx1 protein expression decreased by 20%.

Mesenteric artery molecular phenotype. In mesenteric artery, inflammation gene expression (IL-1, TNF-α) was not changed in AD mice compared with the AS group, while COX-2 gene expression decreased 2.6-fold (\( P < 0.05 \)) (Fig. 3A). AD mice exhibited increased \( p^{47}_{\text{phox}} \) gene expression with no change in NOS III gene expression compared with AS mice (Fig. 3B). Trx1 expression was decreased (Fig. 3C).

For endothelial protein expression in mesenteric arteries, compared with AS mice, AD mice exhibited increased IL-1 and \( p^{47}_{\text{phox}} \) subunit by 55 and 118%, respectively; Trx1 endothelial protein decreased by 53% (Fig. 3).

Table 3. Cardiovascular Doppler-echography results in young and adult mice fed standard or high-protein-low-carbohydrate diet

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<tr>
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<th>YD</th>
<th>AS</th>
<th>AD</th>
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<tr>
<td><strong>Cardiac Doppler echography</strong></td>
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<tr>
<td>Heart rate, beats/min</td>
<td>493 ± 17</td>
<td>493 ± 17</td>
<td>461 ± 9</td>
<td>441 ± 10</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>18.5 ± 1.6</td>
<td>20.0 ± 1.5</td>
<td>17.8 ± 0.5</td>
<td>17.5 ± 0.6</td>
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<tr>
<td>Posterior wall thickening, %</td>
<td>22.8 ± 3.2</td>
<td>17.2 ± 2.4</td>
<td>35.57 ± 1.83</td>
<td>35.70 ± 3.71</td>
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<tr>
<td>Ejection fraction, %</td>
<td>49.12 ± 1.6</td>
<td>53.43 ± 1.23</td>
<td>50.19 ± 1.28*</td>
<td>50.19 ± 1.28*</td>
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<tr>
<td>Shortening fraction, %</td>
<td>23.27 ± 1.6</td>
<td>24.70 ± 1.0</td>
<td>27.66 ± 0.7</td>
<td>25.39 ± 0.8*</td>
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<tr>
<td><strong>Aorta Doppler</strong></td>
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<tr>
<td>Intima-media thickness, μm</td>
<td>110.5 ± 7.7</td>
<td>110.0 ± 13.5</td>
<td>85.8 ± 10.2</td>
<td>101.7 ± 9.8</td>
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<td>Distensibility, %</td>
<td>9.0 ± 1.3</td>
<td>5.9 ± 1.1</td>
<td>9.8 ± 0.9</td>
<td>6.7 ± 0.9*</td>
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Values are means ± SE; \( n = 10–20 \) per group. YD and AD mice were compared, respectively, with YS and AS groups. *\( P < 0.05 \).

Table 4. Locomotor activity in adult mice fed standard or high-protein-low-carbohydrate diet

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<th>AD</th>
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<tr>
<td>Locomotion</td>
<td>285.5 ± 21.6</td>
<td>205.6 ± 24.5**</td>
</tr>
<tr>
<td>Rearing</td>
<td>109.4 ± 13.7</td>
<td>76.1 ± 14.2*</td>
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Values are means ± SE of nos. of actions; \( n = 17–20 \) per group. AD mice were compared with AS group. *\( P < 0.05 \). **\( P < 0.01 \).

Doppler echography. Compared with the YS group, YD did not exhibit any modification in cardiac parameters or in vascular parameters (Table 3). Compared with AS mice, AD mice exhibited decreased ejection fraction and shortening fraction by 3.2 and 2.6 points, respectively, with no modification in posterior wall thickening. AD mice also exhibited decreased aortic distensibility (−32%), with a trend toward increase intima-media thickness compared with AS mice.

Blood pressure measurements. AS and AD mice exhibited comparable blood pressure values: systolic (86.9 ± 1.7 vs. 89.6 ± 0.8 mmHg), diastolic (72.3 ± 2.1 vs. 72.8 ± 1.8 mmHg), and mean (80.4 ± 1.8 vs. 83.2 ± 1.2 mmHg). However, pulse pressure tended to increase in AD mice (14.8 ± 0.8 vs. 17.0 ± 1.6 mmHg, AS vs. AD, \( P = 0.07 \)).

Locomotor activity. During the 25 min of the test, both horizontal and vertical activities were decreased in AD mice compared with the AS group by 28.0% for horizontal locomotion (\( P < 0.01 \)) and 30.0% for rearing (\( P < 0.05 \)) (Table 4).

Arterial reactivity experiments. Compared with the AS group, AD mice exhibited decreased acetylcholine-induced relaxation in mesenteric resistance arteries, while no modification was observed in the aorta (conduction artery) (Fig. 2, A and B). After incubation with catalase and Tempol, acetylcholine-mediated relaxation was improved in AD mice in mesenteric arteries only, suggesting that oxidative stress reduced acetylcholine-induced relaxation in microvessels (Fig. 2, C and D).

In addition, incubation with BH4 and L-arginine did not change acetylcholine-induced relaxation in mesenteric arteries of AD mice (Fig. 2E). Incubation with the COX-2 inhibitor CAY 4004 decreased aorta acetylcholine-induced relaxation (Fig. 2F).

Arterial superoxide production. Compared with AS mice, the AD group exhibited a 100% increase superoxide production in mesenteric arteries (\( P < 0.05 \)), but not in aorta (Table 5).
Fig. 2. Arterial reactivity. Concentration-response curves for acetylcholine in AS and AD mice in mesenteric resistance artery (A) and in aorta (B) are shown. Experiments were repeated after incubation of the arterial segments with catalase-Tempol in AD mice in mesenteric artery (C) and aorta (D), and with tetrahydrobiopterin (BH$_4$)-L-arginine in mesenteric arteries (E) and with cyclooxygenase (COX)-2 inhibitor (CAY 4004) in aorta (F). Values are means ± SE; n = 4–8 per group. *P ≤ 0.05. **P ≤ 0.01.
our results are in line with those of a previous study showing that dyslipidemia had no effect on the endothelial function in 3-mo-old mice. The pathways involved in the dilation of mouse arteries evolve during maturation and aging, demonstrating that the endothelial biology is dynamic with time and most likely results from time-dependent injuries and repairs (15). Adult mice exhibited an aging arterial phenotype with an increased pulse pressure and aorta distensibility, a decreased aorta intima-media thickness leading to cardiac consequences, such as left ventricle hypertrophy with a trend to increase left ventricle mass (data not shown), and posterior wall thickening (19).

**HP-LC diet-induced insulin resistance in adult mice.** The HP-LC diet introduction was responsible for an early weight loss during the first 2 wk, probably explaining the use of such diets for body weight control (38). However, after 3 mo, this diet did not induce weight loss. Caloric intakes were not statistically different in HP-LC and standard fed mice (data not shown). After 12 wk of HP-LC diet, glucose homeostasis was explored with a GTT that exhibited a glucose intolerance state in adult mice. The glucose regulation alteration observed in adult mice was confirmed by the increased resistin level, a marker previously associated with insulin resistance, even though insulin level and HOMA-IR were unchanged (28). The glucose intolerance highlighted in this context reminded that commonly observed during aging (32). The combination of glucose intolerance and hypertriglyceridemia might be the outcome of an increase in free fatty acids level (33, 34). Regarding cholesterol profile, HP-LC-fed mice exhibited increased cholesterol levels compared with the control group. With regard to cholesterol fraction distribution, we observed a shift from nonlarge to large HDL fractions (7). Large HDL fractions are enriched with apolipoprotein E and bind to LDL receptors with high affinity; large HDL fractions may increase atheromatous risk under this type of diet, as already suggested (9). Furthermore, HDL cholesterol-to-total cholesterol ratio was reduced, which may be predictive of increased susceptibility to atherosclerosis (23). Interestingly, 8 wk after HP-LC diet stopping, a preservation of glucose intolerance was observed in this study in a female additional cohort (data not shown).

**HP-LC diet disrupts cardiovascular functions in adult mice.** Many studies reported a lack of adverse effects of HP-LC diet on cardiovascular risk factors (6, 13), but a few have already pointed out deleterious outcome (12). Such a diet used for a long period in adult mice induced cardiac function deterioration with decreases in fraction shortening and ejection fraction without changes in cardiac output and rate. The same diet used for 3 mo in older mice (25 mo old) led to an even more serious cardiac phenotype, with decreases in fraction shortening and ejection fraction associated with a lower posterior wall thickening compared with matched-aged mice fed a standard diet (data not shown). In adult mice fed HP-LC diet, these impairments might be the consequences of an increase in left ventricle internal diameter (3.0 vs. 3.2 mm for AS and AD mice, respectively, \( P = 0.07 \)) and telesystolic volume (34.0 vs. 42.2 \( \mu l, P < 0.05 \) for AS and AD group, respectively). These cardiac impairments might reflect the beginning of the cardiac insufficiency that could lead to a decrease of locomotor activity, as described by the New-York Heart Association (18). The arterial damages induced by the HP-LC diet with a decreased aortic distensibility and a strong trend toward increased intima-media thickness could be responsible for these cardiac impairments. Blood pressure was unchanged in AD mice, but pulse pressure tended to increase. In aging, a long-term increase in pulse pressure is reported to have impact on the microvasculature (27). Medial calcification (aorta calcium contents measured by atomic absorption spectrometry) and collagen content (Sirius red staining) were not modified (data not shown). These elements, hence, cannot be responsible for impairments of relaxant responses as previously reported in aged microvessels (30). Endothelial dysfunction may be implicated in arterial damage. Local arterial inflammation could be responsible for this endothelial dysfunction. In adult mice fed an HP-LC diet, inflammation was evidenced by the marked induction of pro-inflammatory cytokines in arteries (11, 30). Also, the increased oxidative stress could be involved in the endothelial dysfunction and arise from the decrease in Trx1 expression and/or the increase in NADPH oxidase expression, as previously suggested (20). However, the increase in aortic pulse pressure by arterial stiffening would increase the transmission of the pulsatility to the microvessels, responsible for the degradation of structures and the impaired functions of the microvasculature (26). Vascular reactivity was assessed both in the aorta and in mesenteric resistance arteries of adult mice. In contrast to mesenteric arteries, acetylcholine-mediated dilation was not affected by HP-LC in the aorta of AD mice. Mesenteric artery acetylcholine-induced relaxation was reduced as previously reported in old subjects (30). Oxidative stress was involved in the endothelium-dependent relaxation of diabetic microvessels (14). In our study, the combination of Tempol, a SOD mimetic, and catalase reduced ROS concentration and improved acetylcholine-mediated relaxation (5). The BH4-L-arginine preincubation confirmed the preservation of the NO pathway in this microvessel’s aging (30, 39). This higher ROS level might lead to peroxynitrite production and underline the major role of oxidative stress in this microvascular aging, as observed in old subjects (11). The enhanced peroxynitrite formation in vessels may regulate COX-2 activity, thereby producing synergistic

Table 5. **Arterial superoxide production in adult mice fed standard or high-protein-low-carbohydrate diet**

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<tr>
<td>Aorta</td>
<td>116.7 ± 7.8</td>
<td>113.5 ± 14.4</td>
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<tr>
<td>Mesenteric artery</td>
<td>41.0 ± 3.9</td>
<td>82.5 ± 15.6*</td>
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Values are means ± SE in fluorescence intensity units; \( n = 5 \) per group. AD mice were compared with AS group. \(*P \leq 0.05. \)**

Fig. 3. Gene and endothelial protein expressions. Gene and endothelial protein expressions of interleukin (IL)-β (A), NADPH oxidase (B), and thioredoxin-1 (Trx1) (C) in aorta and mesenteric arteries of AD and AS mice are shown. AD is compared with AS group. Values are means ± SE; \( n = 4–8 \) per group. Values are fold change for gene expression and fluorescence intensity, and arbitrary units for endothelial protein expression. \(*P \leq 0.05. \)**

AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00291.2014 • www.ajpheart.org
Aorta and mesenteric artery expression

**A** IL1 gene expression

**Endothelial IL1 protein expression**

**B** p47-phox gene expression

**Endothelial p47-phox protein expression**

**C** Trx1 gene expression

**Endothelial Trx1 protein expression**
inflammatory response in the vascular wall. Indeed, the enhanced aortic IL-1β observed in this work may stimulate COX-2 expression by smooth muscle cells contributing to the inflammatory process and to the development of neointima lesions (4, 10). Thus COX-2 implication has been studied in inflammatory process and to the development of neointima COX-2 expression by smooth muscle cells contributing to the remodeling in resistance arteries from obese Zucker rats is associated with endothelial dysfunction. Hypertension 50: 248–254, 2007.


Le Marec T, Marie-Claire C, Noble F, Marie N. Chronic and intermittent morphine treatment differently regulates opioid and dopamine sys-


