Resveratrol Decreases TXNIP mRNA and Protein Nuclear Expressions With an Arterial Function Improvement in Old Mice

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Abstract

Aging leads to a high prevalence of glucose intolerance and cardiovascular diseases, with oxidative stress playing a potential role. Resveratrol has shown promising effects on glucose tolerance and tends to improve endothelial function in elderly patients. Thioredoxin-interacting protein (TXNIP) was recently proposed as a potential link connecting glucose metabolism to oxidative stress. Here, we investigated the resveratrol-induced improvement of arterial aging phenotype in old mice and the expression of aortic TXNIP. Using an in vivo model of old mice with or without 3-month resveratrol treatment, we investigated the effects of resveratrol on age-related impairments from a cardiovascular Doppler analysis, to a molecular level, by studying inflammation and oxidative stress factors. We found a dual effect of resveratrol, with a decrease of age-related glucose intolerance and oxidative stress imbalance leading to reduced matrix remodeling that forestalls arterial aging phenotype in terms of intima-media thickness and arterial distensibility. These results provide the first evidence that aortic TXNIP mRNA and protein nuclear expressions are increased in the arterial aging and decreased by resveratrol treatment. In conclusion, we demonstrated that resveratrol helped to restore several aging impaired processes in old mice, with a decrease of aortic TXNIP mRNA and protein nuclear expressions.

Keywords: Resveratrol—Arterial aging—Oxidative stress—Thioredoxin-interacting protein—Glucose intolerance

Aging is associated with generalized physiological deterioration including the deregulation of glucose homeostasis and the onset of arterial dysfunction. Many countries face an aging population, especially a proportional rise in the elderly ≥80 age bracket with multiple pathologies, but there is a still lack of data on old human or animal populations due to difficulties inherent to the studies needed. Further, discrepancies have been reported in the literature concerning impaired glucose tolerance (IGT) in old mice (1,2). IGT is a characteristic defect of old people, affecting about 34% of the ≥60 age bracket (3), yet there is still no therapy available. IGT is a major risk factor for cardiovascular disease, which is still the main cause of death with an age-induced prevalence. The mechanisms underlying the age-associated increased risk for cardiovascular disease have not been fully elucidated, but the development of vascular endothelial dysfunction seems to be a major contributor (4,5). Several studies suggest that oxidative...
stress plays a key role in the occurrence of IGT and arterial aging (6–8).

Resveratrol (3,5,4’-trihydroxystilbene) is a plant-derived polyphenolic compound that has pleiotropic effects but is mainly known for its antioxidant and anti-inflammatory properties (9–11). Some teams have claimed that resveratrol increases life span or span of health and improves aortic function in monkeys (12–15) whereas others have reported zero effects or negative outcomes after resveratrol treatment (16–18). A recent human pilot study claims that resveratrol could improve arterial aging, with a potential link between TXNIP and the anti-aging effects of resveratrol (19). Authors proposed resveratrol as a new therapeutic strategy (20), but the molecular aortic modifications leading to endothelial dysfunction or the consequences in terms of functional aortic events such as matrix remodeling or aortic distensibility remain unclear.

A previous study performed in our lab demonstrated a beneficial effect of resveratrol on thio redoxin-interacting protein (TXNIP) expression in an oxidative stress context (21), and the impact of resveratrol-induced antioxidant effect on the thio redoxin (TRX)/TXNIP system was very recently confirmed (22). TXNIP was initially identified as an endogenous inhibitor of thio redoxin, known as one of the major thioredoxin systems, protecting cells by scavenging reactive oxygen species (23). TXNIP is also an α-arrestin and a physiological regulator of glucose metabolism (24). Given that TXNIP deletion preserves insulin sensitivity and that TXNIP may depress thio redoxin activities in cells (25,26), there is an emerging hypothesis that TXNIP could be a crucial link connecting glucose metabolism to oxidative stress (27). Furthermore, an inverse correlation between TXNIP expression and longevity suggests that TXNIP is implicated in the aging process (28,29). However, to our knowledge, a potential link between TXNIP and the anti-aging effects of resveratrol has never been considered. As we had previously shown a beneficial effect of resveratrol on glucose metabolism in old mice, we hypothesized that resveratrol could improve arterial aging, with decreased aortic TXNIP mRNA and protein nuclear expressions.

Methods

Experimental Animals

C57BL/6J male mice (6 and 22 months old) were obtained from Janvier (Le Genest-Saint-Ise, France) and maintained according to the recommendations prescribed under European Parliament and Council Directive 2010/63/EU. They were housed in a temperature- and humidity-controlled room with a 12h/12h light/dark cycle and free access to water and food. After a 10-day adaptation period, they were randomized into four groups according to age and resveratrol treatment for the 12-week treatment [i.e. a control group (control; 9-month-old mice), a control resveratrol group (CR; 9-month-old mice fed a standard diet, n = 20), a control resveratrol group (OR; 25-month-old mice fed a standard diet added with resveratrol (0.04% w/w), n = 20) and an old resveratrol group (OR; 25-month-old mice fed a standard diet added with resveratrol (0.04% w/w), n = 20)]. This protocol was approved by the local ethics committee (registration numbers: P2.VNA.060.08 and CEEA34.SB.008.12).

Six-hour Fasting Glycemia and Intraperitoneal Glucose Tolerance Test

At the end of the trial, after a 6-hour 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 glucometer; One Touch Ultra test strips). A glucose tolerance test was carried out by intraperitoneally injecting 2mg/g body weight of glucose (G30 Aguettant) as described previously (30,31). Blood glucose was measured every 30 minutes during the 2 hours after glucose injection. Blood glucose concentrations were assessed as an integrated area under the curve of the intraperitoneal glucose tolerance test calculated using the trapezoid rule.

Doppler Ultrasound Imaging

Doppler ultrasound imaging was carried out on anesthetized mice (isoflurorn inhalation, induced at 3.5% then maintained at 1.5%) with an Ultrasound Biomicroscope (Vevo 770, Visual Sonics), using RMV-704 and RMV-707B probes at central frequency 40 and 30 MHz, respectively. Internal and external diameters of aortas were measured during systole and diastole. Thicknesses were assessed during diastole and calculated as thickness = (adventitia–adventitia distance – internal diameter)/2. Distensibility was calculated as distensibility = (systole diameter – diastole diameter)/diastole diameter.

Heart dimensions measured in both systole (s) and diastole (d) were left ventricular posterior wall thickness (LVPW), left ventricular internal diameter (LVID), and interventricular septum thickness (IVS). Heart rates were obtained with an electrocardiogram.

Several parameters were calculated:

- Left ventricular posterior wall thickening: PWTh = (LVPW.s − LVPW.d)/LVPW.d
- Left ventricular telesystolic and telediastolic volume (Dodge method): 
  \[ \text{LV Vol} = \left( \frac{7.0}{(2.4 + \text{LVID})} \right) \times \text{LVID}^3 \]
- Ejection fraction: \[ \text{EF} = \frac{\text{LV Vol.d} − \text{LV Vol.s}}{\text{LV Vol.d}} \]
- Cardiac flow: \[ \text{CF} = \text{HR} \times \frac{\text{LV Vol.d} − \text{LV Vol.s}}{\text{LV Vol.s}} \]
- Left ventricular mass: \[ \text{LV mass} = 0.8 \times 1.053 \times (\text{LVID.d} + \text{LVPW.d} + \text{IVS.d})^3 \text{LVID.d}^3 \]

Blood Pressure Measurements

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

Systemic Parameters

The vena cava was sectioned from anesthetized (5% isoflurorn inhalation) mice. Blood samples were collected to measure serum calcium and phosphate parameters (Architect Ci8200, Abbott) as per the suppliers’ instructions.

Aorta Calcium Content

As described Henrion and colleagues (32), frozen segments of aortas were weighed and dried. Dry tissue samples were dissolved in nitric acid (14 N) for 3 days then centrifuged for 10 minutes at 2,000g. Calcium was measured by atomic absorption spectrometry using an air/acetylene flame at the calcium resonance wavelength of 422.7 nm (Perkin Elmer Analyst 100 Atomic Absorption Spectrometer with a multielement hollow cathode lamp). Results were expressed in mmol of calcium per gram dry tissue.
Quantitative Real-Time Polymerase Chain Reaction Analysis

Frozen segments of aortas were crushed with an Ultra-Turrax J25 instrument (Fisher-Bioblock) for 40 seconds in Trizol (Invitrogen). RNA was extracted by Chomczynski's method (33). One micrograms of total aortic RNA was treated with DNase I (Invitrogen) and converted into complementary DNA (cDNA) using Superscript II reverse transcriptase, oligo (dT)12-18 primers, and RNase OUT Recombinant Ribonuclease Inhibitor (Invitrogen). cDNA products were subjected to real-time polymerase chain reaction (qPCR) using an ABI 7900HT Fast Real-Time PCR. Quantitect SYBR Green PCR kits and a Quantitect primer assay (Qiagen) were used to quantify matrix metalloproteinase 2 and 9 (MMP2 and MMP9), tissue inhibitor of metalloproteinases 1 (TIMP1), osteocalcin (OC), bone morphogenetic protein 2 (BMP2), matrix Gla protein (MGP), osteopergerin (OPG), receptor activator of NFκB (RANK), interleukin 1β (IL1β), tumor necrosis factor α (TNFα), NO synthase III (NOS III), p47phox (NADPH oxidase subunit), thioredoxin-1 (TRX1), and TXNIP gene expression. All reactions were carried out in triplicate in a final volume of 20 μL according to the manufacturer’s instructions. Ribosomal protein L4 (RPL4) was used as housekeeping gene after a validation step to verify equal loading of RNA and cDNA in the reverse transcription and PCR reactions. Data were analyzed with the 2−ΔΔCt method as described by Livak and Schmittgen (34).

Aorta Histology and Immunocytochemistry Labeling

Serial sections of 5 μm were cut along the paraffin-embedded aorta and stained with Sirius red to enable collagen visualization.

In parallel, segments of aorta were embedded in Tissue Tek OCT Compound frozen at −40 °C and stored at −80 °C. After fixing (paraformaldehyde 4%) and blocking, 20-μm thick sections were incubated with a primary antibody raised against TXNip (rabbit polyclonal anti-TNXip, Abcam) (1/100), p47phox (rabbit polyclonal anti-p47phox, Santa Cruz) (1/200), or TXNIP (mouse monoclonal anti-TNXIP, MBL) (1/200). Labeling was revealed with Alexa Fluor 488 goat antirabbit IgG (Invitrogen) or Alexa Fluor 488 goat anti-mouse IgG (1/200). Endothelium was labeled with rat monoclonal anti-CD31 antibody (BD Pharmingen) (1/200) and visualized with Alexa Fluor 555 goat antirat IgG (1/200). Nuclei were counterstained with To-Pro3 (Invitrogen) (1/500). Negative controls (primary antibodies substituted by nonimmune IgG isotype) showed no detectable labeling. Images were recorded on a Leica TCS SP2 confocal microscope. Three sections of each aorta were recorded and four independent sectors of each section were analyzed. Protein expressions (TXNip, p47phox and TXNIP) were quantified using NIH ImageJ software. Briefly, protein staining intensities were measured 10 times within the aorta and divided by surface area to give fluorescence-density values (expressed in arbitrary fluorescence intensity units/μm²). TXNIP staining intensities were measured inside nuclei masks of each section, delimited by the To-Pro3 labeling, then expressed as fluorescence intensity/nuclei area (in arbitrary fluorescence intensity units/μm²).

Endothelial-TXNIP KO Model

Animals experiments were approved by the Ethics Committee on Animal Resources of Paris Descartes University (Registration numbers: CEA34.VNA.129.12). To generate endothelial-specific TXNIP knockout mice (KO), TXNIPlox/lox mice (B6;129S-Txniplox/lox/J, purchased from Jackson Laboratory, #16847) were crossed with Cre recombinase transgenic mice under control of endothelial cell-specific cadherin 5 promoter (B6.Cg-Tg(Cdh5-crf)36Mlia/J, purchased from Jackson Laboratory, #6137) (35,36). Endothelial-specific TXNIP knockout mice (Cdh5-Cre-TXNIPlox/lox) were obtained. Wild-type littermates (WT) were used as controls. Genotyping was performed as recommended by Jackson Laboratory. Animals were divided into three groups (n = 4–7) according to genotype and diet for 12 weeks, that is, a WT control group (WT; 9-month-old mice fed with a standard diet), a diet-induced aging wild-type group (DA WT; 9-month-old mice fed with a high-protein low-carbohydrate diet) and a diet-induced aging endothelial-specific TXNIP KO group (DA KO; 9-month-old mice fed with a high-protein low-carbohydrate diet). From ages 6 to 9 months old, DA WT and DA KO mice received a high-protein low-carbohydrate diet (Certificate U8954, Safe), containing 37% casein, 10% cellulose, 35% lard, 14.5% corn oil, 1% mineral 205 B Safe, 2.5% vitamin 200 Safe, and delivering a 31% protein supply as energy content, corresponding to a doubling of the standard protein supply. A previous study had found that at the end of the trial, mice under this 3 months diet presented a diet-induced aging phenotype compared to controls (31). This diet-induced aging was characterized by metabolic and vascular disorders such as IGT and age-related aortic phenotype (i.e. increase of aortic NADPH oxidase and IL1β protein expression) leading to decreased aortic distensibility (31).

Statistical Analysis

One-way analysis of variance and Dunn’s multiple comparison tests were used (GraphPad Prism). Results were expressed as means ± SEM; statistical significance was set at p < .05.

Results

Age-Related and Resveratrol-Induced Effects on Glucose Tolerance

The kinetics of serum glucose clearance were analyzed in 6-hour fasted mice after an intraperitoneal glucose bolus. Old mice (O) presented a reduced fasting glycemia and an increased area under the curve during the 2 hours of the glucose tolerance test compared to controls (−15.1%, p < .01 and 167.9%, p < .05, respectively) (Table 1 and Supplementary Figure 1).

Resveratrol treatment had no effect on the control group. Nonetheless, glycemia area under the curve and glucose level 30 minutes after glucose loading of old resveratrol mice (OR) was significantly decreased compared to the O group (−40.5%, p < .01 and −24.5%, p < .05, respectively) (Table 1).

Age-Related and Resveratrol-Induced Effects on Cardiovascular Doppler Measurements

Cardiovascular Doppler ultrasound found that O mice showed increased aortic thickness (131.8%, p < .05) with decreased aortic distensibility (−39.2%, p < .01) (Figures 1A and B) compared to controls. Having established the age-related deleterious effects in the aortic phenotype, we next investigated the impact on cardiac function and blood pressure. For cardiac function and blood pressure assessments, O mice showed no change in ejection fraction, posterior wall thickening or pressures (Table 2) compared to controls.

Control resveratrol mice (CR) showed no significant differences compared to control mice in terms of aortic thickness, aortic distensibility (data not shown), and cardiac measurements. In contrast, our results showed that a 12-week resveratrol treatment
improved aortic distensibility (133.7%, *p < .05) and tended to decrease aortic thickness (−15.9%) in the OR mice. Furthermore, comparison of the data between OR mice and the matched controls did not reveal any differences in cardiac function or blood pressures (Table 2).

In a model of DA WT with decreased aortic distensibility (−33.8%, *p < .05), DA KO showed improved aortic distensibility (155.9%, *p < .01) (Figure 1B).

### Age-Related and Resveratrol-Induced Effects on Aortic Remodeling and Calcification
To clarify whether aortic calcification or remodeling explained the above aortic phenotype modifications, we measured gene expression of matrix and calcification mediators (Figure 2A), phosphatemia (Figure 2B), calcemia (Figure 2B), aortic calcium content (Figure 2C), and collagen content (Figure 2D).

O mice showed increased aortic calcium content (141.8%, *p < .05) (Figure 2C), with no significant change in systemic calcemia and phosphatemia compared to controls (Figure 2B). In parallel, for potential calcification mediators, O mice did not differ from controls in osteocalcin (OC), bone morphogenic protein (BMP2), matrix Gla protein (MGP), osteoprotegerin (OPG), or receptor activator of the NFκB (RANK) gene expressions (Figure 2A). However, for matrix remodeling mediators, O mice showed 2.38-fold higher matrix metalloproteinase 2 (MMP2) and 2.68-fold higher tissue inhibitor of metalloproteinases 1 (TIMP1) gene expression compared to matched controls (*p < .05), with no significant change in systemic calcemia.

CR mice showed no significant differences to Control group in terms of aortic calcification or remodeling measurements. OR mice showed unchanged systemic parameters, aortic calcium content, or potential calcification mediators (Figure 2A–C) but MMP2 gene

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**Table 1. Effects of Resveratrol on Glucose Tolerance.**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CR</th>
<th>O</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glycemia—G0 (mmol/L)</td>
<td>9.26±0.22</td>
<td>10.11±0.28</td>
<td>7.86±0.16 **</td>
<td>8.76±0.27</td>
</tr>
<tr>
<td>G30 (mmol/L)</td>
<td>23.75±1.69</td>
<td>24.23±1.08</td>
<td>23.31±1.33</td>
<td>17.59±1.13 *</td>
</tr>
<tr>
<td>G120 (mmol/L)</td>
<td>12.0±0.75</td>
<td>12.53±0.40</td>
<td>10.71±0.44</td>
<td>9.73±0.88</td>
</tr>
<tr>
<td>Area under the curve (AUC)</td>
<td>532.7±73.7</td>
<td>678.3±67.4</td>
<td>894.6±84.4 *</td>
<td>532.2±69.3 **</td>
</tr>
</tbody>
</table>

Notes: Values represent mean ± SEM *n = 9–13 mice in each group. CR = control resveratrol group; O = old group; OR = old resveratrol group.

*p < .05, **p < .01 versus Control; #p < .05, ##p < .01 OR versus O.

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**Table 2. Effects of Resveratrol on Cardiac and Blood Pressure Parameters.**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CR</th>
<th>O</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beat/min)</td>
<td>478±8</td>
<td>471±9</td>
<td>490±10</td>
<td>472±10</td>
</tr>
<tr>
<td>Cardiac flow (mL/min)</td>
<td>18.7±0.6</td>
<td>17.9±0.3</td>
<td>18.4±0.3</td>
<td>17.5±0.5</td>
</tr>
<tr>
<td>Left ventricular mass</td>
<td>98.0±3.0</td>
<td>103.3±2.9</td>
<td>105.0±1.9</td>
<td>105.6±4.3</td>
</tr>
<tr>
<td>Posterior wall thickening (%)</td>
<td>41.7±3.5</td>
<td>34.7±1.8</td>
<td>35.3±3.0</td>
<td>25.4±2.0</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>55.5±1.5</td>
<td>50.7±1.8</td>
<td>57.6±1.6</td>
<td>53.0±1.4</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>86.9±1.7</td>
<td>87.5±1.0</td>
<td>84.0±4.2</td>
<td>85.9±6.4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73.2±2.1</td>
<td>75.4±1.9</td>
<td>62.7±6.8</td>
<td>50.5±11.7</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>80.4±1.8</td>
<td>80.9±1.8</td>
<td>78.6±5.1</td>
<td>72.5±6.5</td>
</tr>
</tbody>
</table>

Notes: Values represent mean ± SEM *n = 16–24 mice for echocardiography and *n = 12 mice for blood pressure measurement in each group. CR = control resveratrol group; O = old group; OR = old resveratrol group.
expression was downregulated (2.59-fold, \( p < .05 \)) whereas MMP9 and TIMP1 gene expression remained unchanged (Figure 2A).

Close examination of collagen contents in old mice showed a 19.3% decrease after resveratrol treatment (\( p < .01 \)) compared with matched untreated controls (Figure 2D).

### Age-Related and Resveratrol-Induced Effects on Aortic Inflammation

As proinflammatory stimuli are known to lead to arterial aging, we next focused on a potential effect on aortic inflammation.

Aorta of O mice showed upregulated IL1\( \beta \) and TNF\( \alpha \) gene expression (2.9-fold and 3.8-fold, respectively; \( p < .05 \)) while TNF\( \alpha \) protein expression rose by 172\% (\( p < .05 \)) (Figure 3A and B).

However, resveratrol mice showed no change in IL1\( \beta \) or TNF\( \alpha \) expression versus matched controls (Figure 3A and B).

### Age-Related and Resveratrol-Induced Effects on Aortic Oxidative Stress

As it is well-known that aging increases oxidative stress, and as resveratrol has antioxidant effects (8–10), we further investigated whether the resveratrol-induced improvement in age-related aortic phenotype resulted from a regulation of the oxidative stress.

Compared to controls, O mice showed no significant difference in NOS-III gene expression, but, p47phox (NADPH oxidase subunit) gene expression tended to increase and p47phox protein expression significantly increased with aging (132.9\%, \( p < .05 \)) (Figure 3).

The CR group did not differ from controls on oxidative stress measurements. In contrast to NOS III, p47phox gene expression was strongly downregulated (3.5-fold, \( p < .01 \)) in OR mice compared with matched untreated controls (Figure 3A). To confirm this result, aortic protein expression measurement was performed. As shown in...
Figure 3B, p47phox protein expression decreased (−30.6%, \( p < .05 \)) after resveratrol treatment.

In a model of DA WT, characterized by increased p47phox protein expression (128.0%, \( p < .05 \)), DA KO mice were preserved from this increase (−33.4%, \( p < .05 \)) (Figure 3B).

**Age-Related and Resveratrol-Induced Effects on Aortic TRX1/TXNIP Expression**

Given that age and resveratrol affected the regulation of oxidative stress, we hypothesized that the system TRX/TXNIP could be involved.
As shown in Figure 4A, in contrast to TRX1, TXNIP gene expression increased 2.6-fold \( (p < .05) \) in O mice compared to controls. O mice showed a 163\% increase \( (p < .01) \) in total TXNIP protein expression (Figure 4B). Given that a recent study showed that TXNIP was maintained in the nucleus in response to inflammatory or oxidative stress stimuli \( (37) \), we tested nuclear TXNIP expression and found that it had increased \( (252\%, p < .05) \) in O mice aorta (Figure 4B).

Resveratrol treatment had no effect on TRX and TXNIP regulation in the control group. However, our results demonstrated that resveratrol treatment induced a 4.6-fold decrease in TXNIP gene expression in OR mice \( (p < .01) \) without change in TRX1 gene expression (Figure 4A). Resveratrol treatment significantly reduced nuclear TXNIP aortic contents \( (-34\%, p < .05) \) in contrast to total TXNIP protein expression compared with O mice (Figure 4B).

**Discussion**

The benefits of resveratrol as a way to delay age-related deterioration have been investigated in several studies \( (15, 38, 39) \). Old mice showed a decreased body weight associated with decreased albuminemia, cholesterolemia, and leptinemia, leading us to...
conclude that they showed a malnutrition state, characteristic of elderly (2). Our previous study found that resveratrol improved insulin resistance in old mice with a decrease in insulin level and HOMA-IR (2). Furthermore, old mice presented IGT that was reduced after a 3-month resveratrol treatment by speeding up glucose clearance after a glucose bolus. These data support a recent pilot study in humans where resveratrol improved meal tolerance in older adults with IGT (19). IGT increases risks for cardiovascular disease (40). Here, we analyzed the effects of resveratrol on the cardiovascular system in old mice, looking at phenotypical to molecular events.

Focusing on age-related aortic phenotype, the association between arterial remodeling, increased intima-media thickness and decreased distensibility revealed aortic aging. Three months of resveratrol supplementation curbed this arterial aging without any effect on heart functions. Several hypotheses might explain these resveratrol-induced improvements in age-related aortic damages.

First, blood pressure might be the target of resveratrol, as reported by Rivera, whereas our results showed that resveratrol did not remodulate blood pressure in old mice (41). Furthermore, we also studied aortic remodeling and calcification, as arterial aging is well known to be associated with medial calcifications (42,43). On one hand, the data confirmed that our old mice presented this age-related aortic calcification and remodeling. On the other hand, in agreement with a reduction in MMP2 gene expression and phenotypical fibrosis, resveratrol treatment seemed to have an impact on aortic remodeling, regardless of any effect on calcification.

Oxidative stress is known as the first step in endothelial dysfunction in arterial aging, and could induce inflammation and matrix remodeling, leading to an increase in intima-media thickness and a decrease in aortic distensibility (44,45). Proinflammatory cytokine and pro-oxidative genes and proteins have been studied in the aorta. Our study is nevertheless limited by the fact that we only used one reference gene. Although expression levels of calcification mediators, TRX1 and NOS-III genes did not change in this aging model, some matrix remodeling, inflammatory, oxidative stress, and TXNIP genes were upregulated. The protein study confirmed the increases in TNFα, IL1β, and NADPH oxidase expression in old aortas revealing enhanced oxidative stress and inflammatory state. Resveratrol, with its antioxidant and anti-inflammatory properties, has been reported to ameliorate the arterial age-related oxidative and pro-inflammatory secretory phenotype (8–10,46). However, there was no resveratrol-induced protective effect on pro-inflammatory cytokines, suggesting that resveratrol is unable to improve these arterial aging phenotypes via inflammatory regulation in our old mice. In contrast, resveratrol treatment prevented the aging-induced expression of NADPH oxidase gene and protein. In parallel, we found that resveratrol reduced TXNIP gene expression, supporting previous evidence that TXNIP inhibition by genetic or pharmacological (with resveratrol) approaches improves redox imbalance in several models of acute oxidative stress (21,22). Conversely, given the involvement of TXNIP in NADPH oxidase induction and ROS production, a TXNIP deficiency could protect mesangial cells from a rise in ROS increase and collagen accumulation (47). Indeed, the resveratrol-induced decrease in aortic TXNIP expression found here might be responsive to the downregulation of NADPH oxidase. To support this idea, our results showed that TXNIP inhibition in endothelial cells prevents the increase in NADPH oxidase expression induced by diet-induced aging. In accordance with Shah, we observed a decrease in collagen accumulation associated with the reduced TXNIP gene and NADPH oxidase expression, suggesting that resveratrol treatment could prevent NADPH oxidase expression and collagen accumulation via TXNIP in the aorta (47).

However, this decrease in TXNIP gene expression after resveratrol treatment is not associated with decreased protein content in the aorta. This discrepancy might be explained by a slow turnover of existing TXNIP protein, leading to greater protein stability (48) which could be enhanced by TRX (49). In fact, TXNIP is a known scaffold protein with cytosolic and nuclear localization. In the cytosol, TXNIP could bind TRX or ITCH (leading to proteosomal degradation). In a context of diminished oxidative stress, TXNIP seems to bind TRX, acting as a scaffold protein, escaping proteosomal degradation, promoting TXNIP movement from the nucleus and preventing nuclear regulation of transcription factors like NF-κB and AP1 (37,50). To test this hypothesis, we studied nuclear TXNIP levels in a context of reduced oxidative stress induced by resveratrol. Interestingly, we confirmed decreased nuclear TXNIP levels in this context of diminished oxidative stress that might lead to decreased NF-κB activation (51). This resveratrol-induced decrease in NF-κB activation has often been reported in vasculature (52,53). TXNIP-TRX complexes may mediate the activation of plasma membrane signaling to promote cell survival and migration (50). The consequences would be improved matrix remodeling leading to a marked reduction in intima-media thickness and an increase in aortic distensibility independently of blood pressure variation (41). Finally, the preserved aortic distensibility induced by TXNIP inhibition in endothelial cells found here confirms the implication and key role of TXNIP in arterial aging.

This study found effects of resveratrol that decrease oxidative stress imbalance, thereby forestalling the arterial aging phenotype, with decreased aortic TXNIP mRNA and protein nuclear expressions. Even if it would be interesting to test resveratrol in the DA TXNIP KO model to better understand the potential link between resveratrol and TXNIP, these results suggest for the first time that TXNIP protein might play a key role in arterial damages in old mice through a dual implication in oxidative stress and metabolic regulation. Resveratrol may possess clinically relevant effects, but further research is needed, ideally in a large-scale clinical trial, to evaluate its therapeutic antiaging effects.

Supplementary Material
Please visit the article online at http://gerontologist.oxfordjournals.org/ to view supplementary material.

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Conflict of Interest
There are no financial or contractual agreements that might cause conflicts of interest or be perceived as causing conflicts of interest. There is no existing financial arrangement between any of the authors and a company whose product takes a prominent place in the submitted manuscript.

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