Cell Metabolism
Short Article

Resveratrol Prevents High Fat/Sucrose Diet-Induced Central Arterial Wall Inflammation and Stiffening in Nonhuman Primates

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SUMMARY

Central arterial wall stiffening, driven by a chronic inflammatory milieu, accompanies arterial diseases, the leading cause of cardiovascular (CV) morbidity and mortality in Western society. An increase in central arterial wall stiffening, measured as an increase in aortic pulse wave velocity (PWV), is a major risk factor for clinical CV disease events. However, no specific therapies to reduce PWV are presently available. In rhesus monkeys, a 2 year diet high in fat and sucrose (HFS) increases not only body weight and cholesterol, but also induces prominent central arterial wall stiffening and increases PWV and inflammation. The observed loss of endothelial cell integrity, lipid and macrophage infiltration, and calcification of the arterial wall were driven by genomic and proteomic signatures of oxidative stress and inflammation. Resveratrol prevented the HFS-induced arterial wall inflammation and the accompanying increase in PWV. Dietary resveratrol may hold promise as a therapy to ameliorate increases in PWV.

INTRODUCTION

The incidence of cardiovascular diseases (CVD), mainly arterial diseases of hypertension and atherosclerosis, increases exponentially beyond middle age (Lakatta, 2013). Stiffening of the central arteries is a cardinal feature of advancing age in humans beyond the age of 40 years. Over the last decade numerous epidemiological and longitudinal studies have convincingly demonstrated that carotid-femoral pulse wave velocity (PWV), a direct measure of aortic stiffness, is a highly relevant clinical measure of arterial stiffness. In humans, an increase in PWV shows a strong association with CVD-associated clinical events and all-cause mortality, even after taking other known risk factors into consideration (Najjar et al., 2008). PWV has emerged as an independent predictor for CV disease, morbidity, and mortality. There is also strong evidence to indicate that PWV provides early information about the development/progression of atherosclerosis before macroscopic alterations of the vessel

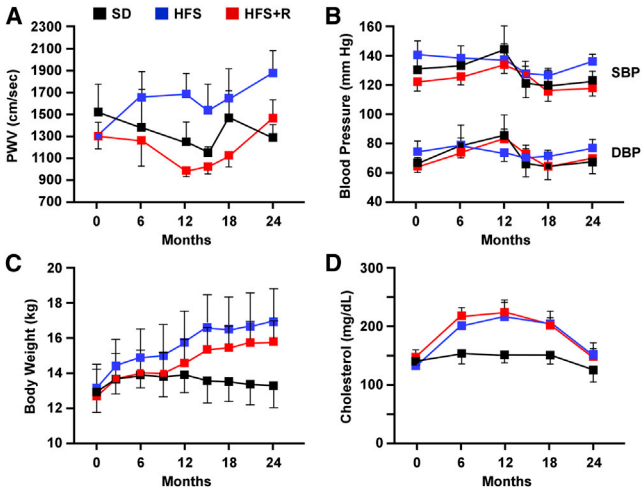


Figure 1. Physiological Measurements in Rhesus Monkeys Maintained on HFS, HFS+R, or SD Diet for up to 2 Years

(A) Pulse wave velocity.
(B) Blood pressure.
(C) Body weight.
(D) Serum cholesterol levels. Results are mean ± SEM.
See also Table S1.

wall occur (Gotschy et al., 2013) and is integral to the retardation of CV events (Reference Values for Arterial Stiffness' Collaboration, 2010). This epidemiologic perspective suggests that the reduction of PWV may carry substantial health benefits. Importantly, metabolic disease in humans accelerates the age-associated increase in PWV (Scuteri et al., 2012). Histological, genomic, and proteomic studies provide strong evidence that increased central arterial stiffness occurs in the context of an oxidative stress-driven arterial wall inflammatory profile (for review see Wang et al., 2014).

Clinical trials to assess the beneficial effects of pharmacological interventions on vascular health have shown that presently available antiinflammatory drugs, e.g., statins (Williams et al., 2009) or angiotensin receptor blockers (Hayoz et al., 2012) had only modest effects, if any, at reducing PWV. Thus, at present, there are no effective therapies available to reduce PWV, and novel strategies are required to impact on chronic arterial wall inflammation and stiffening that underlie and accelerate the progression of CV diseases, other than classic regulation of blood pressure. In this regard, vascular protective effects of the polyphenol resveratrol (Resv) have been illustrated in several different animal species (Ramprasath and Jones, 2010). Studies in mice demonstrate that the addition of Resv to a high-fat diet ameliorates arterial wall inflammation and other arterial markers associated with aging (Pearson et al., 2008). Further, in apolipoprotein E-deficient (apo $E^{-/-}$) mice, a model of atherosclerosis with very high levels of circulating cholesterol, dietary supplementation of Resv leads to improvement of lipid profile, accompanied by the prevention of intimal lesion formation and inhibition of HMG-CoA reductase to decrease cholesterol formation (Do et al., 2008). In pig models, Resv also improves myocardial perfusion, regional contractility, and decreases oxidative stress (Elmadhun et al., 2013). We have recently reported that Resv promotes metabolic and inflammatory adaptations in visceral white adipose tissue (Jimenez-Gomez et al., 2013) and prevents pancreatic β cell dedifferentiation (Fiori et al., 2013) of rhesus monkeys on a high-fat, high-sucrose (HFS) diet. In the present study, the hypothesis tested that HFS will induce arterial wall inflammation driven by oxidative stress and cause deleterious increase in central arterial wall stiffness, manifest as an increased PWV and that these effects will be ameliorated by the addition of Resv to the HFS in a clinically relevant nonhuman primate (NHP) model of metabolic disease.

RESULTS AND DISCUSSION

Baseline characteristics of the NHPs comprised in this study while on a standard diet (SD) are detailed in Table S1 available online. A 2 year HFS diet in adult (7–13 years) male *Macaca mulatta* caused an increase in body weight, an elevation in plasma cholesterol, and an ~40% increase in aortic PWV—an index of central arterial stiffness. Daily dietary supplementation with Resv (80 mg for first year and 480 mg for the second year) prevented the increase in PWV in HFS-fed monkeys (Figure 1A). The effect on PWV was most profound at 12 months when the differences between HFS-fed and those supplemented with Resv (HFS+R) was 38% ($p < 0.05$). The gradual increase in PWV during the second year could be due to the HFS diet overwhelming the system; alternatively, the higher dose of Resv may elicit side-effects and offer lower health benefits (Mukherjee et al., 2010). Despite preventing the HFS-induced increase in PWV, Resv had no effect on blood pressure (Figure 1B), body weight (Figure 1C), serum cholesterol (Figure 1D), or total low density lipoprotein (not shown) in linear mixed model analyses of paired data. Serum levels of both intercellular adhesion molecule 1 (ICAM) and macrophage inflammatory protein 1 (MIP-1), markers of arterial wall inflammation, increased during the 2 year study in all groups and was not affected by Resv (not shown). Thus, these typical clinically measured risk factors for CV disease did not relate to events occurring within the arterial wall that lead to the reduction in PWV by the addition of Resv. Furthermore, the size and weight of the heart and gross morphometric measurements of aortic remodeling did not vary whether monkeys were fed a HFS or HFS+R diet (Table S2).

Microscopic morphometric analyses of immunolabeled aortic cross-sections were performed to determine whether Resv could prevent the signature of inflammation within the arterial wall (Figure 2; Table S2). HFS diet induced monocyte/macrophage infiltration (Figure 2A), endothelial cell loss (Figure 2B), and enhanced staining for fat deposition and calcification (Figures 2C and 2D); furthermore, ICAM-1, vascular cell adhesion molecule 1 (VCAM-1), and monocyte chemoattractant protein-1 (MCP-1) immunostaining was also increased in aortic cross-sections of HFS-fed animals, but normalized by Resv supplementation (Figures 2E and 2F) despite no effect on serum lipid profile or circulatory markers of inflammation. When compared to SD-fed controls, aortic homogenates from HFS-fed cohort exhibited significantly higher levels of the stable lipid peroxidation marker, 4-hydroxynonenal (4-HNE), and caspase 3 activity, which were reversed by Resv (Figure 2G). Thus, the antioxidant and antiapoptotic response

of Resv could be part of the mechanism used by this polyphenol to preserve vascular health (Ungvari et al., 2007; Kaneko et al., 2011).

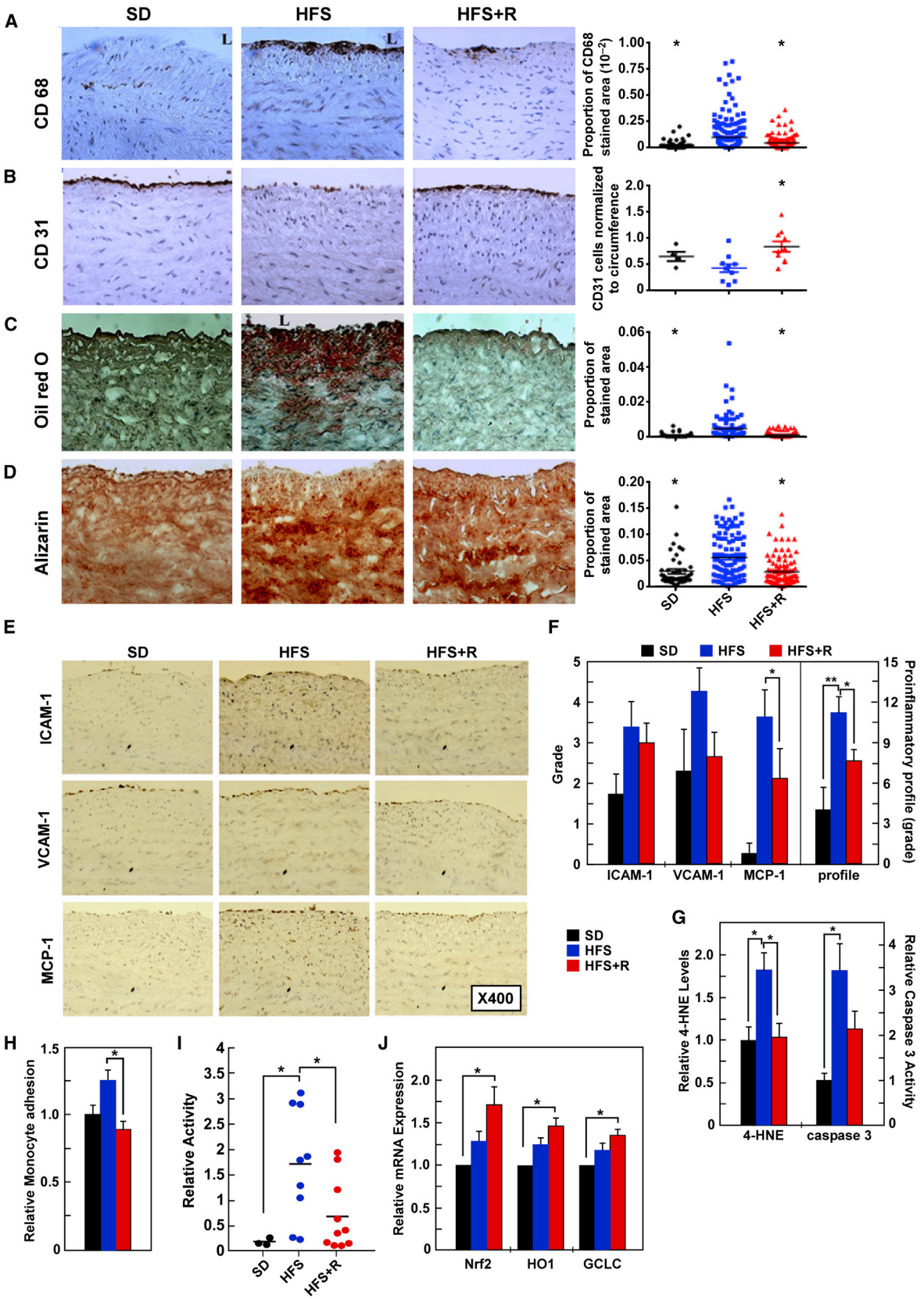
Monocyte adhesion to human coronary artery endothelial cells (CAECs) is a key initiating event of arterial wall inflammation associated with metabolic disease. To ascertain whether Resv could prevent the HFS-mediated initiation of inflammation at the monocyte-endothelial cell interface, we assessed the leukocyte adhesion properties of CAECs after a 24 hr incubation with medium containing 10% serum from monkeys on experimental diets for 2 years. Strikingly, medium supplementation with HFS+R serum significantly reduced HFS serum-mediated increase in PMA-activated human monocytic THP-1 cell adhesion onto confluent monolayer of human CAECs ($p < 0.01$, Figure 2H). As a measure of the antiinflammatory action of Resv, we determined the activation status of NF-κB in human CAECs treated either with SD, HFS, or HFS+R monkey serum (Figure 2I). The increase in NF-κB promoter activity in response to HFS monkey serum (9.28 ± 2.80-fold versus SD serum, $p = 0.017$) was reduced by ~60% in cells treated with HFS+R serum ($p = 0.025$). These data suggest that Resv suppresses NF-κB transcriptional activity to block induction of endothelial genes implicated in leukocyte recruitment and transmigration as well as tissue remodeling (Csiszar et al., 2012; Tuttolomondo et al., 2012).

Activation of the nuclear factor-E(2)-related factor-2 (Nrf2) pathway improves vascular function in patients with risk of vascular events through upregulated expression of genes that contain antioxidant response elements in their promoters (Carrizzo et al., 2013). *Nrf2* mRNA levels were significantly higher in human CAECs incubated with HFS+R serum, but not in HFS serum-treated cells (Figure 2J); moreover, expression of Nrf2 target genes, heme oxygenase-1, and gamma-glutamylcysteine synthetase, was also upregulated by HFS+R serum, consistent with previous reports in the NHP animal model (Ungvari et al., 2011). Thus, a 2 year consumption of Resv may confer atheroprotective effects to the vascular endothelium of HFS-fed monkeys via Nrf2 activation.

To identify genes that are key components of the antioxidative and antiinflammatory pathways of Resv, we performed cDNA microarray analysis of thoracic aorta. The analysis revealed the expression of 217 gene sets whose levels were significantly altered in HFS and HFS+R cohorts based on unbiased gene ontology (GO) annotations. Of these, 106 gene sets were shared between the two cohorts (Figure 3A) with all, but one gene set, exhibiting a reciprocal pattern of expression (Figure 3B). Among the top upregulated GO annotations for HFS+R versus HFS were "fatty acid metabolic process," "protein catabolic processes," "stress response." and "mitochondrion." The most highly upregulated gene in the aorta of HFS+R with respect to HFS was *DHCR24*, followed by *FBN2*, *MED1*, and *SNIP1*, while the most downregulated gene was *GAS6* (Figure 3C). DHCR24/seladin-1 encodes a multifunctional enzyme that not only scavenges H_2O_2 but also is involved in cholesterol synthesis (Lu et al., 2008). GAS6 plays a key role in vascular remodeling and calcification of vascular smooth muscle (Konishi et al., 2004; Son et al., 2007). The expression of selected genes from the microarray analyses was verified using quantitative RT-PCR analysis (Figure 3D). Significant changes in GO terms and gene expression levels shared between the HFS and HFS+R cohorts are compiled in Tables S3A and S3B. Expression of gene sets relevant to arterial inflammation that were altered by the experimental diets were: the "interleukin receptor activity," "activation of NF-κB transcription," and "nitric oxide biosynthetic process." Of significance, Resv supplementation reversed the HFS-induced reduction in *NOS3* expression, which encodes endothelial nitric oxide synthase (eNOS), consistent with recent preclinical studies showing that Resv upregulates eNOS activity to enhance the production/bioavailability of NO and subsequent endothelium-dependent vasodilation (Carrizzo et al., 2013). Moreover, HFS feeding coordinately regulated expression of genes implicated in matrix foundation structure and function and arterial remodeling, which, for the most part, was reversed by Resv supplementation (Figure S1). Thus, Resv may have beneficial effects on CV health by evoking effective protection against diet-induced activation of multiple routes that link inflammation to arterial stiffness. The raw data file and the filtered, normalized results are available online in the Gene Expression Omnibus, accession number GSE45927.

To assess molecular signatures consistent with improved vascular health following Resv supplementation, vascular smooth muscle (VSM) cells from the monkey aorta were isolated, placed in primary culture and then were subjected to quantitative proteomics by stable isotope labeling with amino acids (SILAC) combined with LC-MS/MS, as previously reported (Park et al., 2012). Of the 750 proteins identified, expression of 121 proteins significantly differed in either the HFS and/or HFS+R compared to SD (Table S3C). GO Term annotation and other Bioinformatic tools (e.g., KEGG signaling pathway, WikiPathways, and PathwayCommons analyses) revealed that the differentially altered proteins in VSM cells included several proteins involved in cytoskeletal dynamic regulation, calcium regulation, energy metabolism, and vascular functionality (Figure S2A; Table S4). A representative set of seven proteins showed a complex series of dynamic changes with experimental diets. These included proteins that were upregulated in the HFS-fed animals, but became either downregulated with Resv supplementation (e.g., Serpine1) or normalized to SD controls (e.g., Rock2, β-catenin [Ctnnb1]); proteins whose expression moved in the same direction in HFS and HFS+R groups (e.g., caveolin 1 [Cav1], microtubule-associated protein 2 [MAP2]); and proteins that responded only to Resv (e.g., neuromodulin [GAP43], synaptopodin 2 [SYNPO]) (Table S3C; Figure 4A). Serpine1, Rock2, and β-catenin are related to VSM cell inflammation (Satoh et al., 2011; Vykoukal and Davies, 2011; Xiao et al., 2014) and their expression levels were validated in aortic protein extracts by western blotting (Figure 4B).

Latent Semantic Indexing (LSI) heatmap analysis (Chadwick et al., 2011) of the 121-protein data set affected by diet demonstrated that nineteen proteins were implicitly involved in interrelated functional networks associated with vascular inflammation and protection, regeneration, and development (Figure S2B). Aortic VSM cells exhibited higher levels of NOV (CCN family member 3), among other proteins, when comparing HFS to either SD or HFS+R cohort (Figure 4C). NOV is a potent inhibitor of vascular repair program (Shimoyama et al., 2010). Several protein markers of endothelial dysfunction that were upregulated in response to HFS were found to be normalized to SD levels by



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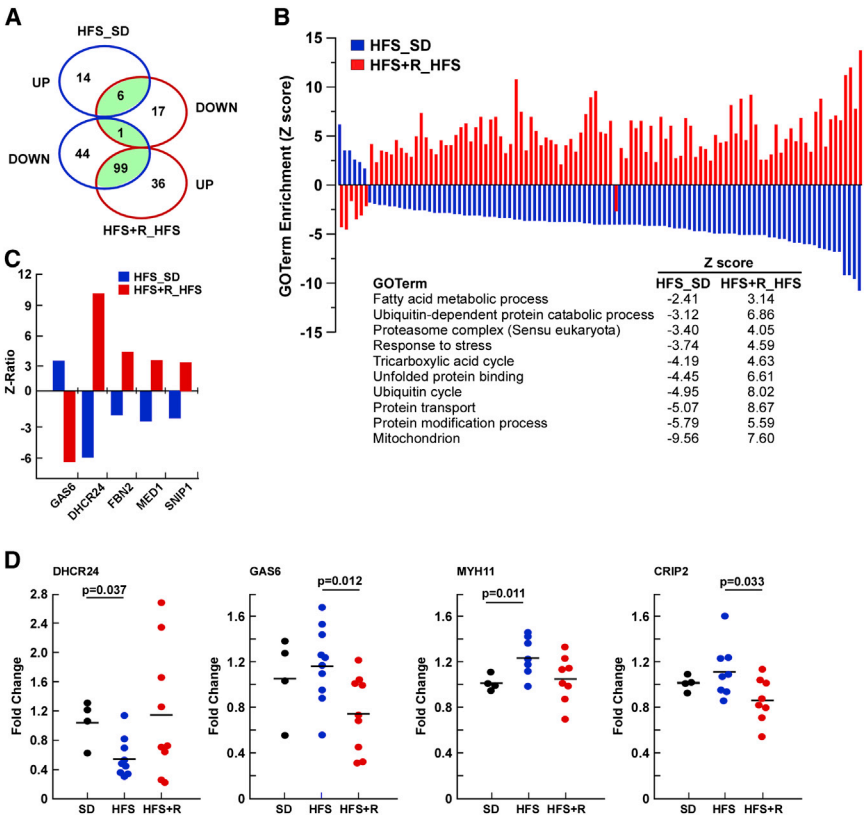

Figure 3. Resveratrol Supplementation Reverses Global Transcriptional Effects of a HFS Diet in Aorta of Rhesus Monkeys

(A) Venn diagram of GO terms significantly modified by HFS diet versus SD, and HFS+R versus HFS.

(B) Graphical representation of the significant 106 GO terms shared by HFS- (plotted in blue) and HFS+R-fed animals (plotted in red). Metabolic and catabolic processes, stress response, and mitochondrion were among the regulated GO terms in aorta. Full GO term listing is provided in the Supplemental Information.

(C) Change in expression of select genes between HFS versus SD and HFS+R versus HFS is depicted as Z ratios. List of genes whose expression in the HFS-fed group was reversed significantly by Resv supplementation is provided in the Supplemental Information.

(D) Validation of the microarray data by quantitative RT-PCR.

See also Figure S1 and Tables S3A and S3B.

Resv supplementation. These included COL3A1, cysteine-rich protein 2, and GLIPR2, the former being associated with the pathogenesis of vascular lesions (Smith et al., 2011). Moreover, the levels of cytoskeletal regulatory protein Nesprin 2 (SYNE2) were downregulated by HFS, but restored by Resv supplementation to levels that were even higher than in VSM from SD controls (Figure 4C). HFS increased expression of transglutaminase 2 (TGM2), an arterial calcification-related protein that is positively associated with hypertension and atherosclerosis (Johnson et al., 2008; Matlung et al., 2012) and crosslinking of extracellular matrix proteins (Bakker et al., 2008; Santhanam et al., 2010). This upregulation of TGM2 protein level and activity in response to HFS was restored to control levels in VSM cells from the HFS+R cohort (Figures 4C, 4D, and S3). Consistent with the established effects of NO bioavailability on TGM2 regulation (Santhanam et al., 2010), we propose that Resv supplementation offers endothelial cell protection against diet-induced metabolic stress partly though TGM2 signaling.

In summary, we provide data about functional and morphometric measurements in nonhuman primates, as well as genomic

Figure 2. Resveratrol Supplementation Retards the Adverse Molecular and Cellular Events within the Aortic Wall of Rhesus Monkeys on a HFS Diet and CAEC Incubation with Serum from HFS+R Serum Decreases Monocyte Adhesion

(A) Photomicrographs (×400) of the paraffin sections after immunostaining for CD68, a marker of monocyte/macrophage cells (left panel) (brown color determined by 3,3′-diaminobenzidine [DAB]); the average density of CD68-stained area/field/cross-section (right panel). *$p < 0.05$ versus HFS. SD, n = 4; HFS, n = 10; HFS+R, n = 10.

(B) Photomicrographs (×400) of paraffin sections after immunostaining for CD31, a marker of endothelial cells (left panel); the number of CD31-stained endothelial cells normalized by the circumference (right panel). *$p < 0.05$ versus HFS. SD, n = 4; HFS, n = 10; HFS+R, n = 10.

(C) Photomicrographs (×400) of frozen sections after Oil O Red staining (left panel); the density of lipid deposition/field/cross-section (right panel). *$p < 0.05$ versus HFS. SD, n = 4; HFS, n = 10; HFS+R, n = 10.

(D) Photomicrographs (×400) of frozen sections after Alizarin red staining (left panel); the density of calcification/field/cross-section (right panel). *$p < 0.05$ versus HFS.

(E) Photomicrographs (×400) of frozen sections after immunostaining with antibodies for the proinflammatory molecules ICAM-1, VCAM-1, and MCP-1.

(F) Grade average for ICAM-1, VCAM-1, and MCP-1 immunostaining as well as their summation (defined as local proinflammatory profile) of cells plus matrix within the intimal and medial compartment. SD, n = 4; HFS, n = 10; HFS+R, n = 10. *$p < 0.05$, **$p < 0.01$. Data are mean ± SEM for all graphs.

(G) 4-HNE content and caspase 3 activity in aorta. SD, n = 3; HFS, n = 9; HFS+R, n = 9. *$p < 0.05$. Data are mean ± SEM for all graphs.

(H) Monocyte adhesion assay in human coronary artery endothelial cells (CAECs) cultured for 24 hr with 10% serum from SD-, HFS- or HFS+R-fed rhesus monkeys. SD, n = 3; HFS, n = 9; HFS+R, n = 10. *$p < 0.05$. Data are mean ± SEM for all graphs.

(I) NF-κB reporter assays were initiated after a 24 hr incubation of human CAECs with HFS monkey serum. SD, n = 3; HFS, n = 9; HFS+R, n = 10. *$p < 0.05$. Data are mean ± SEM for all graphs.

(J) Expression of *Nrf2*, *HO1*, and *GCLC* mRNA in human CAECs was determined by quantitative RT-PCR analysis. The effects of a 24 hr incubation with HFS and HFS+R monkey serum were normalized to SD serum. SD, n = 3; HFS, n = 9; HFS+R, n = 10. *$p < 0.05$. Data are mean ± SEM for all graphs.

See also Table S2.

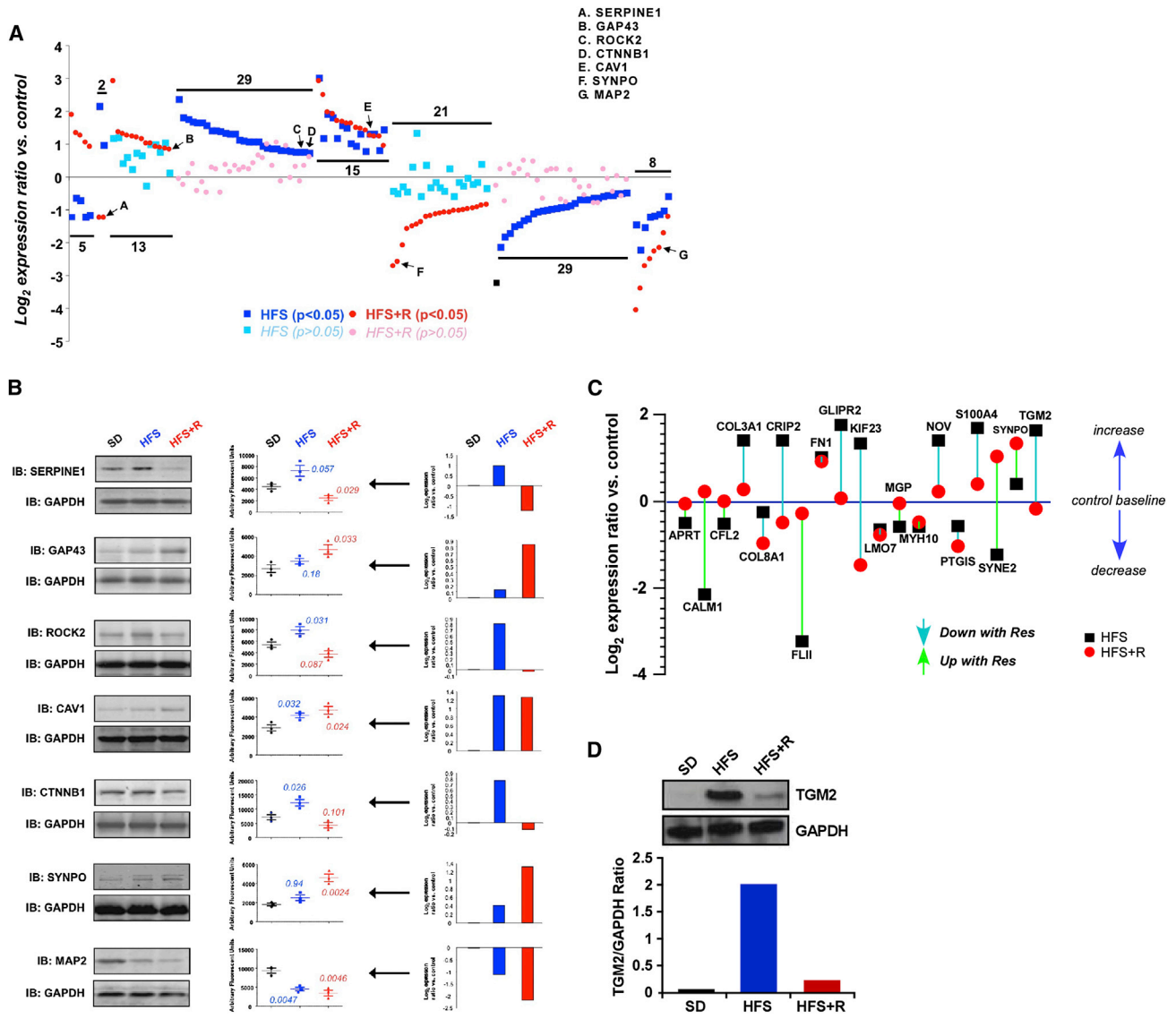

Figure 4. Resveratrol Supplementation Restores the Conversion of "Multidimensional" Protein Network of the Cytoskeletal Dynamics and Vascular Functionality

(A) Log_2-transformed SILAC expression ratio data for proteins extracted and identified in primary cultures of vascular smooth muscle (VSM) from rhesus monkey on SD, HFS, and HFS+R diet. These proteins were selected due to their implicit textual association with vascular functionality (see Supplemental Information for additional information). The proteins identified demonstrated a significantly altered degree of expression regulation in either the HFS (blue squares) or HFS+R (red circle) cohort ($p < 0.05$). Nonsignificant expression changes are identified for the HFS cohort with light blue squares and for the HFS+R cohort with pink circles.

(B) Western blot validation of several proteins identified with SILAC. Representative western blot panels display the expression profile from a set of SD, HFS, and HFS+R-fed monkeys. The associated scatterplot histograms indicate the degree of individual animal variation in expression, as well as the significant p value (t test) for HFS or HFS+R compared to SD.

(C) The effects of Resv and HFS diet treatments on 19 "multidimensional" proteins identified from multiple bioinformatic interpretation (GO-*bp*, KEGG, WikiPathways, PathwayCommons). These proteins isolated from VSM extracts were associated with all the interrogator terms.

(D) TGM2 expression in primary culture of rhesus monkey VSM cells was measured using western blot and expression levels were normalized to GAPDH.

See also Figures S2 and S3 and Tables S3C and S4.

and quantitative proteomic analyses of arterial wall tissues and cells, which establish that a 2 year dietary supplementation of Resv has a marked effect to reduce central arterial wall inflammation that underlie arterial wall stiffening, which accompanies chronic metabolic stress, advancing age, and age-associated diseases (i.e., atherosclerosis, hypertension and diabetes) that have become rampant within Western society. Thus, dietary resveratrol at doses achievable in humans can safely reduce many of the negative consequences of excess caloric intake, and it may hold promise as a therapy to ameliorate increases in PWV and metabolic stress-induced factors that accelerate CV disease.

EXPERIMENTAL PROCEDURES

Animals and Diets

During baseline assessments, all monkeys were maintained on a commercially available closed formula monkey chow (TestDiet 5038, Purina Mills). After baseline assessment, the 24 male rhesus monkeys were quasi-randomized into one of three groups: an HFS diet (n = 10), HFS+R (n = 10), or remaining on the healthy SD (n = 4). The SD was a purified biscuit consisting of 13% of kcal in fat and <5% sucrose by weight. The HFS diet was a specially formulated purified ingredient diet with 42% of kcal in fat and ~27% sucrose by weight (Harlan, Teklad); additional details are provided in the Supplemental Information. The monkeys were gradually switched to the HFS diet over a 3 week period. All groups received two meals per day of the specified diet in allotments that represent ad libitum feeding, yet consumption was isocaloric across groups. Resveratrol was supplied by DSM Nutritional Products. HFS+R monkeys received 40 mg Resv twice a day for the first year that was increased to 240 mg twice a day during the second year. The HFS and SD monkeys received a placebo treat. All procedures were approved by the Animal Care and Use Committee of the NIA Intramural Research Program.

Gene Expression

Microarray and quantitative RT-PCR techniques were carried out according to standard procedures to determine the effects of Resv on gene expression in rhesus monkey aorta. Full methodological details are described in Supplemental Experimental Procedures. The primer sequences used for quantitative RT-PCR are summarized in Table S5.

Determination of 4-HNE Content and Caspase-3 Activity in Aorta Samples

Frozen aorta samples were pulverized and aliquots were homogenized in assay-specific lysis buffers. Tissue content in 4-HNE was assessed using the OxiSelect HNE-His Adduct ELISA kits (Cell BioLabs).

Caspase-3 activity, a useful marker of apoptosis, was measured using the Caspase-Glo 3/7 assay system (Promega), as previously described (Ungvari et al., 2007). Luminescence intensity was measured using an Infinite M200 plate reader (Tecan US) and was normalized to the sample protein concentration.

Effect of Monkey Serum on Human CAECs

Primary human CAECs (Cell Applications) were cultured in MesoEndo Endothelial Cell Growth Medium (Cell Applications) supplemented with 10% fetal calf serum and 1% antibiotics (GIBCO) until the time of treatment. For treatment, cells (passage 4 and up) were incubated for 24 hr in serum-free medium supplemented with serum collected from SD, HFS, and HFS+R monkeys (10% final concentration). Monocyte adhesion assay and quantitative RT-PCR analysis were carried out (see Supplemental Information for additional details).

Transient Transfection and NF-κB Reporter Gene Assay

Transcriptional activity of NF-κB was tested in monkey serum-treated CAECs by a reporter gene assay as described (Ungvari et al., 2011). A NF-κB reporter comprised of an NF-κB response element upstream of firefly luciferase (NF-κB-Luc, Stratagene) together with a Renilla luciferase plasmid under the control of CMV promoter was used in this assay.

Statistical Analyses

PWV, body weight, and serum cholesterol repeated-measures data were analyzed using linear mixed-effects models. A group or group × time interaction were considered statistically significant at $p \leq 0.05$. For histochemical analysis, a one-way ANOVA was done to compare mean values for the SD, HFS, and HFS+R groups. Pairwise comparisons were made using the Bonferroni multiple comparison test. Data are presented as mean ± SEM. In each western blot histogram, data represent the means ± SE. Statistical analyses (Student's t test) were performed using GraphPad Prism (GraphPad Software). $p \leq 0.05$ was considered statistically significant.

ACCESSION NUMBERS

The Gene Expression Omnibus accession number for the data reported in this paper is GSE45927.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and five tables and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2014.04.018.

AUTHOR CONTRIBUTIONS

J.A.M. was involved in protocol design and implementation of the nonhuman primates (NHP) study, data interpretation and analysis, writing and editing the manuscript. M.W. was involved in all immunohistochemical analyses, study design, and final editing of the manuscript. M.B. was involved in data interpretation and analysis, writing and editing the manuscript, and creation of the figures.

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REFERENCES

Bakker, E.N., Pistea, A., and VanBavel, E. (2008). Transglutaminases in vascular biology: relevance for vascular remodeling and atherosclerosis. J. Vasc. Res. *45*, 271–278.

Carrizzo, A., Puca, A., Damato, A., Marino, M., Franco, E., Pompeo, F., Traficante, A., Civitillo, F., Santini, L., Trimarco, V., and Vecchione, C. (2013). Resveratrol improves vascular function in patients with hypertension and dyslipidemia by modulating NO metabolism. Hypertension *62*, 359–366.

Chadwick, W., Keselman, A., Park, S.S., Zhou, Y., Wang, L., Brenneman, R., Martin, B., and Maudsley, S. (2011). Repetitive peroxide exposure reveals pleiotropic mitogen-activated protein kinase signaling mechanisms. J. Signal Transduct. *2011*, 636951.

Csiszar, A., Sosnowska, D., Wang, M., Lakatta, E.G., Sonntag, W.E., and Ungvari, Z. (2012). Age-associated proinflammatory secretory phenotype in vascular smooth muscle cells from the non-human primate Macaca mulatta: reversal by resveratrol treatment. J. Gerontol. A Biol. Sci. Med. Sci. *67*, 811–820.

Do, G.M., Kwon, E.Y., Kim, H.J., Jeon, S.M., Ha, T.Y., Park, T., and Choi, M.S. (2008). Long-term effects of resveratrol supplementation on suppression of atherogenic lesion formation and cholesterol synthesis in apo E-deficient mice. Biochem. Biophys. Res. Commun. *374*, 55–59.

Elmadhun, N.Y., Sabe, A.A., Robich, M.P., Chu, L.M., Lassaletta, A.D., and Sellke, F.W. (2013). The pig as a valuable model for testing the effect of resveratrol to prevent cardiovascular disease. Ann. N Y Acad. Sci. *1290*, 130–135.

Fiori, J.L., Shin, Y.K., Kim, W., Krzysik-Walker, S.M., González-Mariscal, I., Carlson, O.D., Sanghvi, M., Moaddel, R., Farhang, K., Gadkaree, S.K., et al. (2013). Resveratrol prevents β-cell dedifferentiation in nonhuman primates given a high-fat/high-sugar diet. Diabetes *62*, 3500–3513.

Gotschy, A., Bauer, E., Schrodt, C., Lykowsky, G., Ye, Y.X., Rommel, E., Jakob, P.M., Bauer, W.R., and Herold, V. (2013). Local arterial stiffening assessed by MRI precedes atherosclerotic plaque formation. Circ Cardiovasc Imaging *6*, 916–923.

Hayoz, D., Zappe, D.H., Meyer, M.A., Baek, I., Kandra, A., Joly, M.P., Mazzolai, L., Haesler, E., and Periard, D. (2012). Changes in aortic pulse wave velocity in hypertensive postmenopausal women: comparison between a calcium channel blocker vs angiotensin receptor blocker regimen. J. Clin. Hypertens. (Greenwich) *14*, 773–778.

Jimenez-Gomez, Y., Mattison, J.A., Pearson, K.J., Martin-Montalvo, A., Palacios, H.H., Sossong, A.M., Ward, T.M., Younts, C.M., Lewis, K., Allard, J.S., et al. (2013). Resveratrol improves adipose insulin signaling and reduces the inflammatory response in adipose tissue of rhesus monkeys on high-fat, high-sugar diet. Cell Metab. *18*, 533–545.

Johnson, K.A., Polewski, M., and Terkeltaub, R.A. (2008). Transglutaminase 2 is central to induction of the arterial calcification program by smooth muscle cells. Circ. Res. *102*, 529–537.

Kaneko, H., Anzai, T., Morisawa, M., Kohno, T., Nagai, T., Anzai, A., Takahashi, T., Shimoda, M., Sasaki, A., Maekawa, Y., et al. (2011). Resveratrol prevents the development of abdominal aortic aneurysm through attenuation of inflammation, oxidative stress, and neovascularization. Atherosclerosis *217*, 350–357.

Konishi, A., Aizawa, T., Mohan, A., Korshunov, V.A., and Berk, B.C. (2004). Hydrogen peroxide activates the Gas6-Axl pathway in vascular smooth muscle cells. J. Biol. Chem. *279*, 28766–28770.

Lakatta, E.G. (2013). The reality of aging viewed from the arterial wall. Artery Res. *7*, 73–80.

Lu, X., Kambe, F., Cao, X., Kozaki, Y., Kaji, T., Ishii, T., and Seo, H. (2008). 3beta-Hydroxysteroid-delta24 reductase is a hydrogen peroxide scavenger, protecting cells from oxidative stress-induced apoptosis. Endocrinology *149*, 3267–3273.

Matlung, H.L., Neele, A.E., Groen, H.C., van Gaalen, K., Tuna, B.G., van Weert, A., de Vos, J., Wentzel, J.J., Hoogenboezem, M., van Buul, J.D., et al. (2012). Transglutaminase activity regulates atherosclerotic plaque composition at locations exposed to oscillatory shear stress. Atherosclerosis *224*, 355–362.

Mukherjee, S., Dudley, J.I., and Das, D.K. (2010). Dose-dependency of resveratrol in providing health benefits. Dose Response *8*, 478–500.

Najjar, S.S., Scuteri, A., Shetty, V., Wright, J.G., Muller, D.C., Fleg, J.L., Spurgeon, H.P., Ferrucci, L., and Lakatta, E.G. (2008). Pulse wave velocity is an independent predictor of the longitudinal increase in systolic blood pressure and of incident hypertension in the Baltimore Longitudinal Study of Aging. J. Am. Coll. Cardiol. *51*, 1377–1383.

Park, S.S., Wu, W.W., Zhou, Y., Shen, R.F., Martin, B., and Maudsley, S. (2012). Effective correction of experimental errors in quantitative proteomics using stable isotope labeling by amino acids in cell culture (SILAC). J. Proteomics *75*, 3720–3732.

Pearson, K.J., Baur, J.A., Lewis, K.N., Peshkin, L., Price, N.L., Labinskyy, N., Swindell, W.R., Kamara, D., Minor, R.K., Perez, E., et al. (2008). Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab. *8*, 157–168.

Ramprasath, V.R., and Jones, P.J. (2010). Anti-atherogenic effects of resveratrol. Eur. J. Clin. Nutr. *64*, 660–668.

Reference Values for Arterial Stiffness' Collaboration (2010). Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. Eur. Heart J. *31*, 2338–2350.

Santhanam, L., Tuday, E.C., Webb, A.K., Dowzicky, P., Kim, J.H., Oh, Y.J., Sikka, G., Kuo, M., Halushka, M.K., Macgregor, A.M., et al. (2010). Decreased S-nitrosylation of tissue transglutaminase contributes to age-related increases in vascular stiffness. Circ. Res. *107*, 117–125.

Satoh, K., Fukumoto, Y., and Shimokawa, H. (2011). Rho-kinase: important new therapeutic target in cardiovascular diseases. Am. J. Physiol. Heart Circ. Physiol. *301*, H287–H296.

Scuteri, A., Orru', M., Morrell, C.H., Tarasov, K., Schlessinger, D., Uda, M., and Lakatta, E.G. (2012). Associations of large artery structure and function with adiposity: effects of age, gender, and hypertension. The SardiNIA Study. Atherosclerosis *221*, 189–197.

Shimoyama, T., Hiraoka, S., Takemoto, M., Koshizaka, M., Tokuyama, H., Tokuyama, T., Watanabe, A., Fujimoto, M., Kawamura, H., Sato, S., et al. (2010). CCN3 inhibits neointimal hyperplasia through modulation of smooth muscle cell growth and migration. Arterioscler. Thromb. Vasc. Biol. *30*, 675–682.

Smith, L.B., Hadoke, P.W., Dyer, E., Denvir, M.A., Brownstein, D., Miller, E., Nelson, N., Wells, S., Cheeseman, M., and Greenfield, A. (2011). Haploinsufficiency of the murine Col3a1 locus causes aortic dissection: a novel model of the vascular type of Ehlers-Danlos syndrome. Cardiovasc. Res. *90*, 182–190.

Son, B.K., Kozaki, K., Iijima, K., Eto, M., Nakano, T., Akishita, M., and Ouchi, Y. (2007). Gas6/Axl-PI3K/Akt pathway plays a central role in the effect of statins on inorganic phosphate-induced calcification of vascular smooth muscle cells. Eur. J. Pharmacol. *556*, 1–8.

Tuttolomondo, A., Di Raimondo, D., Pecoraro, R., Arnao, V., Pinto, A., and Licata, G. (2012). Atherosclerosis as an inflammatory disease. Curr. Pharm. Des. *18*, 4266–4288.

Ungvari, Z., Orosz, Z., Rivera, A., Labinskyy, N., Xiangmin, Z., Olson, S., Podlutsky, A., and Csiszar, A. (2007). Resveratrol increases vascular oxidative stress resistance. Am. J. Physiol. Heart Circ. Physiol. *292*, H2417–H2424.

Ungvari, Z., Bailey-Downs, L., Gautam, T., Sosnowska, D., Wang, M., Monticone, R.E., Telljohann, R., Pinto, J.T., de Cabo, R., Sonntag, W.E., et al. (2011). Age-associated vascular oxidative stress, Nrf2 dysfunction, and NF-kappaB activation in the nonhuman primate Macaca mulatta. J. Gerontol. A Biol. Sci. Med. Sci. *66*, 866–875.

Vykoukal, D., and Davies, M.G. (2011). Vascular biology of metabolic syndrome. J. Vasc. Surg. *54*, 819–831.

Wang, M., Jiang, L., Monticone, R.E., and Lakatta, E.G. (2014). Proinflammation: the key to arterial aging. Trends Endocrinol. Metab. *25*, 72–79.

Williams, B., Lacy, P.S., Cruickshank, J.K., Collier, D., Hughes, A.D., Stanton, A., Thom, S., and Thurston, H.; CAFE and ASCOT Investigators (2009). Impact of statin therapy on central aortic pressures and hemodynamics: principal results of the Conduit Artery Function Evaluation-Lipid-Lowering Arm (CAFE-LLA) Study. Circulation *119*, 53–61.

Xiao, Q., Zhang, F., Grassia, G., Hu, Y., Zhang, Z., Xing, Q., Yin, X., Maddaluno, M., Drung, B., Schmidt, B., et al. (2014). Matrix metalloproteinase-8 promotes vascular smooth muscle cell proliferation and neointima formation. Arterioscler. Thromb. Vasc. Biol. *34*, 90–98.