Reduced NO-cGMP Signaling Contributes to Vascular Inflammation and Insulin Resistance Induced by High-Fat Feeding

Norma O. Rizzo; Ezekiel Maloney; Matilda Pham; Ian Luttrell; Hunter Wessell; Sanshiro Tateya; Guenter Daum; Priya Handa; Michael W. Schwartz; Francis Kim

Objective—Diet-induced obesity (DIO) in mice causes vascular inflammation and insulin resistance that are accompanied by decreased endothelial-derived NO production. We sought to determine whether reduced NO-cGMP signaling contributes to the deleterious effects of DIO on the vasculature and, if so, whether these effects can be blocked by increased vascular NO-cGMP signaling.

Methods and Results—By using an established endothelial cell culture model of insulin resistance, exposure to palmitate, 100 μmol/L, for 3 hours induced both cellular inflammation (activation of IKKβ—nuclear factor-κB) and impaired insulin signaling via the insulin receptor substrate—phosphatidylinositol 3-kinase pathway. Sensitivity to palmitate-induced endothelial inflammation and insulin resistance was increased when NO signaling was reduced using an endothelial NO synthase inhibitor, whereas endothelial responses to palmitate were blocked by pretreatment with either an NO donor or a cGMP analogue. To investigate whether endogenous NO-cGMP signaling protects against vascular responses to nutrient excess in vivo, adult male mice lacking endothelial NO synthase were studied. As predicted, both vascular inflammation (phosphorylated IkBα and intercellular adhesion molecule levels) and insulin resistance (pAkt and peNOS levels) were increased in endothelial NO synthase−/− mice, reminiscent of the effect of DIO in wild-type controls. Next, we asked whether the vascular response to DIO in wild-type mice can be reversed by a pharmacological increase of cGMP signaling. C57BL6 mice were either fed a high-fat diet or remained on a low-fat diet for 8 weeks. During the final 2 weeks of the study, mice on each diet received either placebo or the phosphodiesterase-5 inhibitor sildenafil, 0.1 mg/kg per day orally. In high-fat diet–fed mice, vascular inflammation and insulin resistance were completely prevented by sildenafil administration at a dose that had no effect in mice fed the low-fat diet.

Conclusions—Reduced signaling via the NO–cGMP pathway is a mediator of vascular inflammation and insulin resistance during overnutrition induced by high-fat feeding. Therefore, phosphodiesterase-5, soluble guanylyl cyclase, and other molecules in the NO–cGMP pathway (eg, protein kinase G) constitute potential targets for the treatment of vascular dysfunction in the setting of obesity. (Arterioscler Thromb Vasc Biol. 2010;30:00-00.)

Key Words: nitric oxide • eNOS • vascular inflammation
much lower concentrations of NO that involve the cGMP–phosphoglycerate kinase (PKG) pathway. Inducible NO synthase is an enzyme found in activated macrophages and other immune cells that produces NO in the micromolar range. At these concentrations, NO induces oxidative DNA damage and modifications of protein structure and function that can lead to cell death. In comparison, NO produced by endothelial NO synthase (eNOS) is typically present at nanomolar concentrations and, via increased cGMP signaling and perhaps other mechanisms, has well-documented anti-inflammatory effects. For example, NO can inhibit vascular inflammatory NF-κB activity by the induction of IkBα, inhibition of NF-κB–DNA binding, or indirect inhibitory effects on NF-κB by activation of PKA. In addition, eNOS has been shown to regulate the expression of NF-κB1,2,12; and NO-mediated S-nitrosylation of the p50 subunit of NF-κB12, resulting in inhibition of NF-κB activity, is suggested to limit endothelial inflammation.

Endothelial-derived NO activates guanylyl cyclase in vascular smooth muscle and other cell types, thereby increasing cGMP production. Intracellular levels of cGMP are not only governed by guanylyl cyclase activity but also by phosphodiesterase conversion of cGMP back to GMP. Sildenafil (marketed as Viagra) is a phosphodiesterase type 5 (PDE-5) inhibitor that attenuates intracellular catabolism of cGMP. In addition to its use in the management of erectile dysfunction, sildenafil’s effect of enhancing signaling via cGMP is also useful in the treatment of pulmonary hypertension and congestive heart failure; it may promote ischemia-induced angiogenesis and immune regulation. A link between cGMP signaling and insulin action was identified in a recent study in which long-term treatment with sildenafil improved energy balance and insulin sensitivity in mice fed a high-fat (HF) diet; sildenafil also ameliorated diabetes-induced endothelial dysfunction in both a rat model and humans. Combined with evidence that vascular NO content decreases early in the course of DIO in mice, these observations raise the possibility that reduced NO-cGMP signaling is a trigger for the development of vascular insulin resistance and inflammation during HF feeding and, therefore, that increased signaling via this pathway may have therapeutic effects in such conditions.

In this study, we demonstrate that genetic and pharmacological interventions that reduce NO-cGMP signaling predispose to vascular inflammation and insulin resistance during nutrient excess, whereas interventions that increase signaling via this pathway exert a protective effect, both in vivo and in an endothelial cell culture model. These findings collectively suggest that NO-cGMP levels play a critical role in maintaining vascular insulin sensitivity, and that reduced signaling via this pathway is a key mediator of vascular inflammation and insulin resistance that occurs during HF feeding.

**Methods**

**Materials**

Anti–phosphorylated eNOS (serine 1177), phosphorylated Akt (serine 473), anti-Akt, anti–phosphorylated 1αB0, anti–insulin receptor substrate (IRS)-1 rabbit polyclonal antibodies and monoclonal anti–phosphorysinephrine molecule (ICAM) antibody were purchased from Cell Signaling, Beverly, Mass; anti-eNOS antibody was obtained from Transduction Labs, BD Biosciences, Lexington, Ky; and anti–intercellular adhesion molecule (ICAM) antibody was purchased from R and D Systems, Minneapolis, Minn. Total Akt and pAkt (serine 473) ELISA kits were obtained from Biosource, Camarillo, Ca. (Z)-1-[N-(2-aminoethyl)-N-(2-ammonioethyl)amino]diazan-1-IM1.2-diolate (DETANO) and 8-bromoguanosine-3',5' (8-Br)-cGMP were purchased from Alexis Biochemical; and the soluble guanylyl cyclase inhibitor 1-H-[1,2,4]oxadiazolo[4,3-a]quinolin-1-one and PKG inhibitor (KT 5823) were purchased from Cayman Chemical, Ann Arbor, Mich.

**Cell Culture**

Human microvascular endothelial cells (HMECs) were purchased from Invitrogen–Cascade Biological and were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, Utah) and 12 μg/mL of bovine brain extract (Clontech, Walkersville, Md); L-glutamine, 2 mmol/L; sodium pyruvate, 1 mmol/L; and nonessential amino acids in the presence of penicillin, 100 U/mL. The HMECs were maintained at 37°C in 5% CO₂. All Western blots were performed as previously described, using equal amounts of total protein for each condition and experiment. SDS gel electrophoresis was performed using a 4% by 20% gradient gel.

**Study Protocol**

Adult male C57BL6 wild-type (WT) mice and eNOS−/− mice were purchased from Jackson Laboratories. Age-matched groups (6 to 12 weeks old; n=10 per group) were maintained in a temperature-controlled facility with a 12-hour light-dark cycle and were fed an equicaloric diet that was either low (10% saturated fat) or high (60% saturated fat) in fat content (Research Diets; Nos. D12492 and D12450B). Body weight and food intake were measured weekly. In 1 study involving WT mice only, sildenafil tablets, 100 mg, were ground into a powder and mixed into a highly palatable “treat,” 300 mg, containing peanut butter and an HF food pellet (Research Diets) to deliver sildenafil orally at a dose of 0.1 mg/kg per day. Vehicle-treated controls received the same daily treat without sildenafil, which was reliably and rapidly consumed. Sildenafil or vehicle was given once daily for the final 2 weeks of an 8-week study protocol in which mice were fed either the HF or the low-fat (LF) diet.

At the conclusion of the protocol, each animal received an intraperitoneal injection of either vehicle (normal saline) or regular insulin (2 U in 300 μL of normal saline) after an overnight fast. Fifteen minutes later, mice were euthanized with an overdose of CO₂ followed by cervical dislocation. Thoracic aorta and surrounding connective tissue were quickly removed and snap frozen on dry ice. Protein was subsequently extracted from tissue samples; after protein levels were quantified using a kit (Micro BCA Protein Assay Kit; Pierce, Rockford Ill), equal amounts of protein were used for each condition in each assay. Total and phosphorylated Akt (serine 473) levels, a measure of PI3 kinase signal transduction, were determined using ELISA assay kits (Biosource). Total eNOS, peNOS, ICAM, and phosphorylated 1αB0 were assessed using Western blot analysis and were quantified using software (Image J; National Institutes of Health, Bethesda, Md). All procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

**Statistical Analysis**

In all experiments, densitometry measurements were normalized to controls incubated with vehicle, and fold increase above the control condition was calculated. An analysis of the results was performed using a statistical package (STATA8). Data are expressed as mean±SEM, and P<0.05 was considered statistically significant. A 2-tailed t test was used to compare mean values in 2-group comparisons. To compare responses between sildenafil- and vehicle-treated mice that also received either vehicle or insulin, data were analyzed by 2-way analysis of variance; and the Bonferroni–post hoc comparison test was used to compare mean values between groups.

**Results**

**Effect of Reduced NO Bioavailability on Vascular Inflammation and Insulin Signaling**

To determine if basal NO signaling is required to prevent endothelial inflammation and insulin resistance, we used the
Insulin Signaling in Endothelial Cells

Palmitate-Mediated Vascular Inflammation and Insulin Resistance

Figure 1. The effect of eNOS inhibitor, L-NAME, on palmitate-mediated endothelial inflammation and insulin signaling. HMECs were treated with vehicle or the eNOS inhibitor (L-NAME), 50 μmol/L, for 3 hours and then treated with BSA (C) or BSA-palmitate (F), 10 μmol/L, for 3 hours, followed by insulin stimulation (I), 100 nmol/L, for 15 minutes. Cell lysates were analyzed by Western blot, and the fold increase over vehicle, BSA control group was calculated for each experiment (n=3). A, Fold increase of phosphorylated IkBα protein normalized to GAPDH levels. B, Fold increase of ICAM protein levels normalized to GAPDH levels. C, Fold increase in IRS-1 tyrosine phosphorylation (PY) normalized to total IRS-1. D, Fold increase in pAkt normalized to total Akt. E, Fold increase in peNOS normalized to total eNOS levels. *P<0.05.

Because eNOS inhibition predisposes to the deleterious effects of palmitate, and increased NO bioavailability decreases expression of NF-κB–dependent adhesion molecule expression in endothelial cells,10,11 we determined whether interventions that increase NO bioavailability would attenuate palmitate-mediated activation of endothelial NF-κB signaling and endothelial insulin resistance. As previously shown,1 palmitate, 100 μmol/L, for 3 hours, inhibited insulin-mediated increases of IRS-1 tyrosine phosphorylation, pAkt, and peNOS protein levels in HMECs; pretreatment with DETANO, 50 μmol/L, attenuated this effect (Figure 2D and E). In the presence of DETANO, 50 μmol/L; soluble guanylyl cyclase inhibitor 1-H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, 1 μmol/L; or PKG inhibitor KT 5823, 1 μmol/L, from HMEC supernatant, as determined by ELISA. *P<0.05.

Effect of Increased NO Bioavailability on Palmitate-Mediated Vascular Inflammation and Insulin Signaling in Endothelial Cells

Because eNOS inhibition predisposes to the deleterious effects of palmitate, and increased NO bioavailability decreases expres-
Effect of Increased cGMP Levels on Inflammatory Signaling in Endothelial Cells
The inhibitory effects of NO on NF-κB–dependent adhesion protein expression reportedly involve mechanisms that are both dependent on and independent of guanylyl cyclase/cGMP activity.6,20 To investigate the role of the guanylyl cyclase pathway in palmitate-induced endothelial inflammation, we used the cell-permeable cGMP analogue, 8-Br–cGMP. Pretreatment of HMECs with 8-Br–cGMP, 50 μmol/L, for 12 hours attenuated increases of phosphorylated IkBα induced by either palmitate or lipopolysaccharide (LPS); similar to palmitate, LPS induces cellular inflammatory responses in endothelial cells exposed to palmitate. Similarly, treatment with vehicle or 8-Br–cGMP, HMECs were stimulated with 100-nmol/L insulin for 15 minutes, and pAkt levels were assessed by Western blot. *P<0.05.

Vascular Inflammation and Insulin Resistance in eNOS−/− Mice
To test the hypothesis that the absence of vascular eNOS predisposes the vasculature to the development of inflammation and insulin resistance in vivo, we studied both eNOS−/− and WT mice fed either an LF or an HF diet for 4 weeks. As expected, HF feeding in WT mice was associated with rapid weight gain compared with LF-fed controls, and this effect did not differ by genotype, suggesting that eNOS is not required for weight gain in this setting (Figure 4A). However, compared with WT controls, eNOS−/− mice exhibited significant reductions in insulin-mediated activation of pAkt and pεNOS were reduced in HF compared with LF-fed mice, consistent with our previous findings.7 In comparison, these defective vascular responses to HF feeding were not detected in mice treated with sildenafil (Figure 5B and C). Similar results were obtained when vascular inflammation was assessed. Thus, protein levels of phosphorylated IkBα and ICAM were increased in the thoracic aorta of vehicle-treated mice in response to HF feeding (but were not observed in LF-fed controls); in sildenafil-treated mice, these adverse responses to HF feeding were attenuated by daily sildenafil treatment at this dose.
that in eNOS
results were obtained using a mouse model of obesity and
hypothesis that vascular NO-cGMP signaling plays a physi-
together, these results provide direct evidence in support of the
deleterious vascular effects of HF feeding were prevented
availability of either NO or cGMP reverse palmitate-
eNOS is inhibited, whereas interventions that increase bio-
to the inflammatory effects of palmitate increases when
an endothelial cell culture model, we found that susceptibility
properties, and because vascular NO levels decline early in
that NF-

tissue in vivo, and growing evidence implicates these
lar tissue in vivo, and vascular tissue21,28; interventions that block in-
mediation underlying vascular inflammation associated with
vascular NO-cGMP signaling as a potential target in the
treatment of vascular inflammation associated with obesity and related metabolic
disorders.

Because endothelial NO signaling has anti-inflammatory
properties, and because vascular NO levels decline early in
the course of DIO, we hypothesized an etiologic role for
reduced NO-cGMP signaling in the pathogenesis of vascular
inflammation and insulin resistance in this setting. By using
an endothelial cell culture model, we found that susceptibility
to the inflammatory effects of palmitate increases when
eNOS is inhibited, whereas interventions that increase bio-
availability of either NO or cGMP reverse palmitate-
mediated NF-κB activation and insulin resistance. Similar
results were obtained using a mouse model of obesity and
insulin resistance induced by HF feeding. Herein, we found
that in eNOS−/− mice, genetic deficiency of vascular NO
is associated with vascular inflammation and insulin resistance,
even for mice on an LF diet, suggesting that endothelial NO
signaling is required to restrain these responses. Conversely,
the deleterious vascular effects of HF feeding were prevented
in healthy mice by daily oral dosing of sildenafil, a PDE-5
inhibitor that increases signaling downstream of NO. To-
gether, these results provide direct evidence in support of the
hypothesis that vascular NO-cGMP signaling plays a physi-
ological role in protecting against vascular inflammation and
insulin resistance and suggest that DIO (when inhibiting
eNOS) is a mediator of these deleterious vascular responses.

Clinical studies have demonstrated a profound effect of
FFA on NO production. In healthy patient volunteers, the
ingestion of a single HF meal transiently impairs endothelial
function, as measured by flow-mediated brachial artery
vasodilation.25 The infusion of high doses of intralipid plus
heparin into healthy volunteers increases circulating FFA
concentrations from a starting concentration of 350 μmol/L
to a peak of 3800 μmol/L; methacholine-induced vasodila-
tion was reduced by as much as 20%, indicating that elevated
FFA levels induce endothelial dysfunction.26 In a separate
study,27 increasing FFA levels resulted in impairment of basal and
insulin-mediated NO production. These human studies
suggest that the production of NO is impaired in the presence
of high circulating levels of FFA. Previously, we demon-
strated that the cellular mechanism of FFA-mediated impair-
ment of NO is dependent on NF-

Cellular inflammation has emerged as an important mech-
anism underlying insulin resistance in muscle, liver, adipose
tissue, and vascular tissue31,28; interventions that block in-
flammatory responses have been shown to improve insulin
sensitivity in vivo.28,29 Consistent with these observations, we
report that whereas reduced NO signaling (induced by phar-
macological inhibition of eNOS) enhances endothelial
inflammation induced by palmitate, increased NO-cGMP sig-
alling has the opposite effect in both cultured endothelial
cells and a mouse model of DIO. Specifically, we found that
in mice, daily oral administration of sildenafil for 2 weeks
fully reversed the vascular inflammation and insulin resis-
tance induced by HF feeding at a dose that had no effect on
body weight gain or fat mass. This observation identifies
vascular NO-cGMP signaling as a potential target in the
treatment of vascular inflammation associated with human
obesity and related metabolic disorders.

That IKKβ–NF-κB activation mediates palmitate-induced
insulin resistance in endothelial cells was established in a
previous study1 showing that IKKβ inhibition blocks this
effect, whereas IKKβ activation recapitulates it. Combined
with evidence that signaling via the innate immune receptor
TLR4 is required for IKKβ–NF-κB activation by palmitate in
endothelial cells,21 these findings suggest that NO antago-
izes the ability of palmitate to increase endothelial signaling
via TLR4 and to subsequently activate IKKβ–NF-κB.
Whether the mechanism underlying this NO effect involves
an interaction directly with NF-κB or with upstream signaling
molecules is unknown. Several possibilities exist. First, NO is
reported to scavenge superoxide, thereby reducing the gen-
eration of hydrogen peroxide and impeding the activation of
NF-κB and the subsequent expression of inflammatory medi-
ators that promote leukocyte adhesion30,31 and macrophage
recruitment.32 Second, NO upregulates and stabilizes IκBα, the
inhibitor of NF-κB, thereby suppressing NF-κB activity33; it
may also inhibit DNA binding by NF-κB, thereby decreasing
the transcription of genes involved in cellular inflammation.9
Additional studies are warranted to identify mechanisms
underlying NO-mediated inhibition of the endothelial re-
sponse to palmitate, and to determine whether NO exerts
similar protective effects in other cell types, as suggested by evidence that reduced eNOS activity exacerbates liver injury in response to the bacterial endotoxin, LPS (similar to palmitate, LPS induces inflammation through TLR4 activation). 34 Conversely, increased NO signaling by either NO donor administration or eNOS overexpression can prevent liver injury in animal models of hepatotoxicity. 35,36

The findings of the present study are also consistent with recent work on AMP-activated kinase (AMPK), inflammation, and NO. Although the AMPK pathway is traditionally thought of as an intracellular fuel gauge and regulator of metabolism, it is also important in the regulation and maintenance of endothelial function. 37 AMPK and NO are integrally related because AMPK has been shown to directly activate eNOS activity 38,39; recently, NO has been shown to be an endogenous activator of AMPK, 40 suggesting a reciprocal relationship between AMPK and eNOS activity. Furthermore, palmitate-mediated activation of NF-κB and insulin resistance can be inhibited by AMPK. 41 Therefore, a reduction in NO bioavailability could result in reduced activation of AMPK, leading in turn to increased palmitate-mediated activation of vascular inflammation or increased susceptibility to the inflammatory effects of palmitate. In addition, increased NO levels could activate AMPK activity; through its “anti-inflammatory” effects, AMPK could attenuate palmitate-mediated activation of NF-κB. Thus, it is possible that the protective effects of NO-cGMP could be mediated by AMPK; further studies to address this question are warranted.

Although our findings suggest a key role for NO to prevent or limit vascular inflammation induced by overnutrition, it is important to recognize that at the same time NO biosynthesis is inhibited by activation of the IKKβ-NF-κB pathway in endothelial cells. 7 The mechanism underlying this observation involves the effect of NF-κB activation of inhibiting signal transduction via the IRS-phosphatidylinositol 3-kinase pathway, a key positive regulator of eNOS activity. Because the course of reduced NO levels during HF feeding coincides with the onset of vascular inflammation and insulin resistance, 7 reduced NO signaling may both mediate and be a consequence of vascular inflammation in the setting of DIO. These considerations support a bidirectional model in which the effect of nutrient excess on inhibiting endothelial NO production predisposes to inflammation that, in turn, inhibits eNOS and further reduces NO synthesis and release. Thus, we propose that reduced vascular NO levels are an integral component of a vicious cycle that operates in states of nutrient excess. Consistent with this hypothesis, studies in mice have shown that although insulin-stimulated induction of vascular phosphorylated eNOS is measurably reduced (by approximately 40%) within the first week of HF feeding, this response declines progressively over time such that it is entirely absent after 8 weeks on this diet. 7 This hypothesis is also in agreement with the negative feedback loop proposed Grumbach et al, 12 which explains the observation that in endothelial cells, shear stress activates eNOS and increases NO signaling on the one hand, while also activating NF-κB on the other hand. To explain these seemingly paradoxical findings, the researchers hypothesized that eNOS activation by shear stress serves to prevent sustained activation of NF-κB by shear stress. Accordingly, we propose that the early decline of NO signaling in DIO 7 predisposes endothelial cells to inflammation; this, in turn, causes insulin resistance, leading to eNOS inhibition that further reduces NO bioavailability and thereby exacerbates endothelial inflammation.

Direct evidence in support of this hypothesis stems from our observation that aortic samples from eNos−/− mice fed an LF diet display the same pattern of inflammation and insulin resistance that occurs in the vasculature of WT mice rendered obese by HF feeding. This vascular response is characterized by activation of the IKKβ–NF-κB pathway and induction of the adhesion molecule ICAM, and by resistance to the ability of insulin to activate either phosphatidylinositol 3-kinase or eNOS in aortic tissue. This change in vascular inflammation does not appear to be a result of changes in blood pressure between eNos−/− and WT mice because no difference in ICAM expression was detected when both mouse strains were fed a chow diet (Supplemental Figure III) (supplemental material is available online at http://atvb.ahajournals.org). Complementary evidence is provided by our observation that a 2-week course of the PDE-5 inhibitor sildenafil fully reversed the deleterious vascular consequences of DIO in WT mice and thereby recapitulates the protective effect exerted by deficiency of TLR4. Similarly, both TLR4 deficiency 21 and sildenafil, 15 administration can ameliorate systemic insulin resistance in mice with DIO.

One potential advantage of a therapeutic approach based on PDE-5 inhibition is that, unlike approaches that increase NO levels, this strategy averts the risks associated with excessive NO signaling. At high concentrations, NO is cytotoxic and its release from activated macrophages and other immune cells (via activation of inducible NO synthase) plays a key role in the host defense against pathogens. Yet, excessive NO can have detrimental effects even when derived from eNOS. For example, transgenic eNOS expression paradoxically increased vascular lesion formation in atherosclerosis-prone apolipoprotein E−/− mice. 42 One mechanism forwarded to explain this finding is that excessive NO production cannot act via a mechanism independent of soluble guanylyl cyclase to increase oxidative stress and favor the development of atherosclerosis. 43 This sequence of events would not be expected to arise from PDE-5 inhibition or, alternatively, from direct activators of soluble guanylyl cyclase or the cGMP target, protein kinase G. Therefore, each of these molecules constitutes a potential target for the treatment of vascular dysfunction in the setting of obesity.

Thoracic aortic tissue lysates (Figures 4 and 5) contain endothelial cells, vascular smooth muscle cells, and adventitial tissues; changes in Akt and inflammatory signaling could reflect changes in nonendothelial tissues. To verify the inflammatory effects of palmitate on smooth muscle cells, we used a mouse smooth muscle culture model (Supplemental Figure I). As expected, palmitate increased inflammatory markers (interleukin 6 and tumor necrosis factor α) and attenuated insulin-mediated Akt phosphorylation, suggesting that changes observed in the in vivo experiments are consistent with changes in both the endothelial and smooth muscle cell population. We are unable to determine the relative
contribution of the different tissue types; however, data on the changes in eNOS phosphorylation are relatively specific to endothelial tissues. The overall significance and contribution of SMC inflammation during HF feeding deserve further study.

In conclusion, our findings suggest that NO-CGMP signaling plays a physiological role in attenuating the vascular inflammation induced by nutrient excess both in vivo and in an endothelial cell culture model. Consequently, the effect of DIO in reducing vascular NO-CGMP signaling is implicated in the mechanism underlying vascular inflammation and insulin resistance in this setting. Therefore, therapeutic strategies designed to increase vascular NO-CGMP signaling may be valuable in the prevention and treatment of obesity-associated cardiovascular disease.

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Disclosures
None.

References


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Supplement Material

Supplementary Figure I. Effect of palmitate-mediated inflammation and insulin signaling on smooth muscle cells. Mouse carotid artery smooth muscle cells were treated with palmitate-BSA (100 µM) for either 6 or 12 h or BSA control (con) and fold increase was calculated for each experiment (n=3). A. Fold increase in IL-6 concentration in response to palmitate as determined by ELISA. B-C. Fold increase in TNF-α and ICAM. D. SMC were treated with palmitate-BSA (F) or BSA control (C) followed by insulin stimulation (I) (100 nM, 15 min). Fold increase in pAkt normalized to total Akt is shown. * p<0.05

Supplementary Figure II. Effect of daily sildenafil during HF feeding and effect of HF in eNos-/- mice on thoracic iNOS expression. A. WT mice were fed a HF or LF diet for 8 wk and during the last 2 wk of the diet received daily doses of sildenafil. B. WT or eNos -/- mice were fed both a LF or HF diet for 4 wk. Fold increase over low-fat control mice was calculated. *p<0.05, n=5 in each group.

Supplementary Figure III. Effect of chow and LF-feeding in WT and eNos-/- mice. Both WT and eNos-/- mice were fed standard chow (low saturated fat) or LF (10% saturated fat) for 4 wk. ICAM expression was measured from thoracic aortic tissues.
Supplementary figure I
A

Fold Increase (iNOS)

LF HF LF HF Sildenafil

B

Fold Increase (iNOS)

LF HF LF HF WT eNOS−/−

Supplementary Figure II
Supplementary Figure III