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Anti-obesogenic role of endothelial nitric oxide synthase

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Abstract

The prevalence of obesity has increased remarkably in the past four decades. Because obesity can promote the development of type 2 diabetes and cardiovascular disease, understanding the mechanisms that engender weight gain and discovering safe anti-obesity therapies are of critical importance. In particular, the gaseous signaling molecule, nitric oxide (NO), appears to be a central factor regulating adiposity and systemic metabolism. Obese and diabetic states are characterized by a deficit in bioavailable NO, with such decreases commonly attributed to downregulation of endothelial NO synthase (eNOS), loss of eNOS activity, or quenching of NO by its reaction with oxygen radicals. Gain-of-function studies, in which vascular-derived NO has been increased pharmacologically or genetically, reveal remarkable actions of NO on body composition and systemic metabolism. This review addresses the metabolic actions of eNOS and the potential therapeutic utility of harnessing its anti-obesogenic effects.

Keywords

nitric oxide; obesity; diabetes; adipose tissue; mitochondria; metabolism

Introduction

The rising prevalence of obesity is a principal health challenge in the United States and abroad. As of 2008, 10% of adults were obese, and approximately 1.5 billion were overweight (Ahima). In the United States, recent estimates indicate that greater than one-third of adults and 17% of children are obese (Ogden, Carroll, Kit, & Flegal). This is associated with an increase in pre-diabetic states, with >30% of the US population meeting the criteria for pre-diabetes (Ervin; Roger et al.). Furthermore, obesity is associated with multiple other co-morbidities including cardiovascular disease and cancer (Calle, Thun, Petrelli, Rodriguez, & Heath, 1999). The current high prevalence of obesity has also equated

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to a substantial economic burden of nearly $150 billion per year in health care costs (Zamosky).

While lifestyle changes and lack of exercise are undeniable risk factors for weight gain (Hu, Li, Colditz, Willett, & Manson; Robinson; Roger et al.; Smith et al.; Williamson et al.), an excess consumption of foods appears to be one of the key factors in the epidemic of obesity. In the US, the average human consumption of calories has increased by at least 200 kcal/d per person in the past three decades, and this is partly attributable to an increase in the intake of high-energy-density foods (Briefel & Johnson; Duffey & Popkin; Kant & Graubard; Nielsen & Popkin; Popkin et al.; Y. C. Wang, Bleich, & Gortmaker). Such dietary habits negatively affect a broad range of cardiovascular functions and promote the onset of T2D (Roger et al.).

Insulin resistance is a cardinal feature of T2D and has been identified in multiple prospective studies as the initial defect promoting development of disease (Reaven & Chen). It is typically defined as a decrease in sensitivity to the metabolic actions of insulin. Insulin maintains glucose homeostasis by promoting glucose uptake in skeletal muscle and by suppressing glucose production from the liver (Muniyappa, Montagnani, Koh, & Quon). Loss of insulin signaling therefore leads to hyperinsulinemia, hyperglycemia, and T2D. Hence, any treatment strategy to prevent diabetes must necessarily target insulin resistance.

Nitric oxide (NO) has emerged as a critical regulator of both adiposity and insulin sensitivity. In obese and diabetic states, the bioavailability of NO is decreased in both animal models (Bender, Herrick, Lott, & Klabunde, 2007; Kim et al., 2008) and adult and adolescent humans (Gruber et al., 2008; Higashi et al., 2001). Because the availability of NO is dependent upon its generation and degradation, lower levels observed in obese states may be due to downregulation of NOS, diminished NOS activity, or by reaction of NO with reactive oxygen species such as superoxide. In particular, eNOS abundance and activity is reported to decrease remarkably in obese and diabetic states and, as discussed below, is likely a central feature regulating body composition.

eNOS is important for regulating vascular and metabolic function

The nitric oxide synthase (NOS) family of enzymes catalyze NADPH- and O_2-dependent oxidation of L-arginine to L-citrulline, producing NO in the process (Alderton, Cooper, & Knowles, 2001; Hill, Dranka, Bailey, Lancaster, & Darley-Usmar). NO synthesis depends also on the availability of several cofactors, including flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), tetrahydrobiopterin (BH_4), as well as the prosthetic group, heme (H. Li & Poulos, 2005). Endothelial NOS (eNOS), the primary subject of this review, is expressed in the vascular endothelium, but has also been identified in neurons, epithelial cells, and cardiomyocytes (Dudzinski & Michel, 2007). Its activity is controlled by Ca^{2+} and calmodulin, post-translational modifications (Oess, Icking, Fulton, Govers, & Muller-Esterl, 2006; Sessa, 2004), and shear stress (Balligand, Feron, & Dessy, 2009; Kone, Kuncewicz, Zhang, & Yu, 2003). Other isoforms of NOS, i.e., neuronal NOS (nNOS) and inducible NOS (iNOS), are commonly expressed in different tissues and cells, and, in general produce higher quantities of NO (Hill et al.).

While NO is well known to have diverse biological actions (including regulation of learning and memory, platelet aggregation, leukocyte-endothelial interactions, immune function, and angiogenesis/arteriogenesis (Forstermann & Sessa)), it is most renowned for its vascular actions. The discovery that endothelial cells control acetylcholine-induced relaxation of smooth muscle (Furchgott & Zawadzki) was one of several impetuses responsible for the designation of NO as endothelial-derived relaxing factor (EDRF). Following Furchgott's findings, a series of studies showed that NO synthesized by eNOS in endothelial cells diffuses into the tunica media where it activates soluble guanylate cyclase (sGC), generating cyclic GMP (cGMP) and eliciting vessel relaxation (Forstermann, Mulsch, Bohme, & Busse; Gryglewski, Moncada, & Palmer; Moncada, Palmer, & Gryglewski; Palmer, Ferrige, & Moncada; Rajfer, Aronson, Bush, Dorey, & Ignarro; Rapoport, Draznin, & Murad; Rapoport & Murad).

Although NO's most-celebrated role is a result of its reaction with the metalloprotein sGC, NO and its oxidation species have the ability to react with other biomolecules as well. NO primarily reacts with ferrous iron and other radical species, with the highest affinity interactions being with the iron-containing proteins sGC, cytochrome *c* oxidase, and hemoglobin. The presence of other radical species such as superoxide (O_2^-) (Beckman, 2009; Pacher, Beckman, & Liaudet, 2007; Szabo, Ischiropoulos, & Radi, 2007; Trujillo, Ferrer-Sueta, & Radi, 2008) can result in the formation of products such as peroxynitrite (Beckman, 2009; Pacher et al., 2007; Szabo et al., 2007), which has different biomolecular targets and causes nitration of tyrosine residues (Hill et al.). NO can react also with O_2 to form oxidized species such as N_2O_3, which can S-nitrosate or promote the S-oxidization of protein side chains (Hill & Bhatnagar; West, Hill, Xuan, & Bhatnagar). Also, NO reacts with thiyl radicals to form S-nitrosated proteins. Cysteinyl thiols of glutathione and proteins are commonly targets of NO and its oxidized species and become not only S-nitrosated or S-oxidized ($SO_{2/3}$), but S-glutathiolated as well (Hill & Bhatnagar; West et al.). These modifications frequently modulate enzyme activity (Hill & Bhatnagar).

In addition to its vasodilatory actions, NO modulates oxygen delivery to cells and tissues by regulating oxygen binding and release from hemoglobin. It regulates oxygen consumption as well by binding and inhibiting cytochrome *c* oxidase, with such binding dependent on both mitochondrial activity and the O_2 level (Cooper & Giulivi, 2007; Shiva et al., 2005). Hence, it can extend O_2 gradients in tissues by regulating hemoglobin action and by inhibiting O_2 consumption in mitochondria (Thomas, Liu, Kantrow, & Lancaster, 2001). Exposure of cells to relatively high concentrations of NO promotes mitochondrial biogenesis (Kelly & Scarpulla, 2004; Nisoli et al., 2003; Nisoli et al., 2004), thereby increasing overall respiratory capacity.

NO bioavailability is decreased in obese and diabetic states

Several studies link a decrease in eNOS-derived NO to diabetes. A T(-786)C variant of the eNOS gene is associated with insulin resistance (Ohtoshi et al.; Vecoli et al.; Yoshimura et al.), along with several other genetic variants in the eNOS locus, which are associated with T2D (Monti et al.). eNOS variants also appear to increase susceptibility for insulin

resistance, hypertriglyceridemia, and low HDL (Gonzalez-Sanchez et al.), and worsen endothelial function in individuals prone to T2D (Rittig et al.).

Beyond eNOS polymorphisms, a primary mechanism by which NO is decreased in obesity is through diminished expression of eNOS. A decrease in eNOS abundance occurs in both adipose tissue and skeletal muscle of obese humans and rodents (Georgescu et al.; Kraus et al.; Perez-Matute, Neville, Tan, Frayn, & Karpe; Brian E. Sansbury et al.; Valerio et al.). In particular, it appears that the cytokine tumor necrosis factor-α (TNFα), which is implicated in the initiation of insulin resistance (Hotamisligil, Shargill, & Spiegelman), downregulates eNOS abundance (Anderson, Rahmutula, & Gardner; Lai, Mohamed, Monge, & Stewart; T. Michel & Lamas; Neumann, Gertzberg, & Johnson; Valerio et al.) by decreasing the stability of eNOS mRNA (Alonso, Sanchez de Miguel, Monton, Casado, & Lopez-Farre; Sanchez de Miguel et al.), thereby shortening its half-life (Yoshizumi, Perrella, Burnett, & Lee, 1993). Destabilization of *Enos* may be due, at least in part, to upregulation of elongation factor 1-α1 (Yan, You, Chen, Liao, & Sun).

The NO-producing activity of eNOS is also diminished in metabolic disease. Conditions related with nutrient excess were shown to upregulate caveolin-1, a negative regulator of eNOS (Ju, Zou, Venema, & Venema; J. B. Michel, Feron, Sacks, & Michel), in the aorta of obese rats (Yang et al.). Furthermore, ceramide,(which increases in abundance in obese states (Bikman & Summers)) decreases eNOS activity by disrupting the eNOS-Akt complex from HSP90 (Q. J. Zhang et al.).

Critical changes in eNOS phosphorylation occur in obesity as well. The eNOS enzyme can be phosphorylated at several sites, including: tyrosine (Y) resides—Y81 and Y567; serine (S) residues—S114, S615, S633, and S1177; and threonine (T) residues—T495 [for review, see (Kolluru, Siamwala, & Chatterjee; Rafikov et al.)]. In particular, the eNOS phosphorylation site—serine 1177 (Ser1176 in mice), the phosphorylation of which increases NO output from the enzyme (McCabe, Fulton, Roman, & Sessa)—is diminished by nutrient excess (Elrod et al.; Q. Li et al.; Taguchi, Kobayashi, Matsumoto, & Kamata; Zhong et al.) or high fat feeding (Kim et al.; Kim et al.; Brian E. Sansbury et al.; Symons et al.) in mice, and is similarly decreased in obese rats (Naruse et al.; Park et al.; Zecchin et al.) and pigs (Low Wang et al.). This eNOS phosphorylation site is regulated by Akt (Dimmeler et al.), which is stimulated by insulin (Hermann, Assmus, Urbich, Zeiher, & Dimmeler). Insulin receptor signaling stimulates the Akt-eNOS pathway, which is known to regulate post-prandial blood flow and nutrient disposition to peripheral tissues. Consequently, endothelial insulin resistance is sufficient to decrease NO bioavailability and promote endothelial dysfunction (Duncan et al.), and diminished eNOS phosphorylation due to insulin resistance appears to be responsible for diminished glucose uptake in skeletal muscle of high fat-fed mice (Kubota et al.).

Loss of eNOS phosphorylation under conditions of nutrient excess may be due to several factors, one of which is fatty acids, which can promote insulin resistance (Kim et al., 2008). Elevated free fatty acids (FFAs; e.g., palmitic acid) decrease NO production or availability in humans (Steinberg et al., 2000; Steinberg et al., 1997), animal models (X. Du et al., 2006), isolated arteries, and cultured cells (Kim et al., 2005) (Edirisinghe, McCormick

Hallam, & Kappagoda, 2006). Insulin resistance due to FFAs is likely caused, at least in part, by activation of Toll-like receptor 2 (TLR2) (Jang, Kim, Hwang, Quon, & Kim) or Toll-like receptor 4 (TLR4) and NF-κB (Kim et al.; Kim et al.). In addition, hyperglycemia was suggested increase O-linked N-acetylglucosamine (OGlcNAc) modification of eNOS, which diminishes its activity (X. L. Du et al.). A PKCβII-mediated diminishment in Akt and eNOS responsiveness to insulin has also been reported (Naruse et al.; Park et al.), and an apparently Akt-independent impairment of eNOS phosphorylation may occur (Symons et al.). It is unclear whether or how each of these signaling pathways integrates to modulate NO production in obese and diabetic states.

eNOS activity and NO generation are dependent on proper enzyme coupling, which is regulated by cofactors, dimerization (Rodriguez-Crespo, Gerber, & Ortiz de Montellano; Rodriguez-Crespo & Ortiz de Montellano), and post-translational modifications (Alp & Channon; Forstermann & Sessa; H. Li & Forstermann; Zweier, Chen, & Druhan). The cofactor BH_4 is critical for optimal eNOS activity, and it is depleted by excessive levels of reactive oxygen or nitrogen species (Channon). Obese and diabetic states are associated with decreased BH_4 and increased levels of its oxidized form, BH_2 (Cai, Khoo, & Channon; Chander et al.; Ding & Triggle; Pannirselvam, Verma, Anderson, & Triggle; Shinozaki et al.). This is important because deficiency in BH_4 or elevations in BH_2 uncouple NOS, resulting in superoxide production and peroxynitrite generation (Alp & Channon). Indeed, decreases in the BH_4 to BH_2 ratio are responsible for glucose-induced eNOS uncoupling (Crabtree, Smith, Lam, Goligorsky, & Gross) and replenishment of BH_4 pools is an effective treatment in multiple pathologies (e.g., (Alp & Channon; Crabtree & Channon; Forstermann & Li; Kietadisorn, Juni, & Moens; H. Li & Forstermann)).

Commonly, 3-nitrotyrosine (3-NT) modifications are found at sites of eNOS uncoupling, and 3-NT-modified proteins are observed in abundance in tissues from obese and diabetic animals (Brodsky et al.; Chander et al.; Molnar et al.; Brian E. Sansbury et al.). In line with a potential role of peroxynitrite in regulating eNOS function, diabetic patients showed elevated levels 3-NT protein adducts, which localized with caveolae; these patients demonstrated diminished flow-mediated dilation of coronary arterioles (Cassuto et al.), which was rescued by the BH_4 supplement, sepiapterin (Cassuto et al.). Numerous additional studies also demonstrate a role for reactive species such as peroxynitrite and superoxide to promote eNOS uncoupling (Bitar et al.; Dikalova et al.; Landmesser et al.; Satoh et al.; Xu, Xie, Reece, Pimental, & Zou). Nonetheless, the contribution of damaging reactive species to endothelial function remains unclear, as other studies suggest that, rather than uncoupling eNOS, superoxide activates the enzyme (Q. Zhang et al.), leaving open the possibility that loss of NO bioavailability could be due to quenching of NO and not to uncoupling of the enzyme. However, multiple other factors, such as asymmetric dimethyl arginine (ADMA), insufficient L-arginine levels or glutathio(ny)lation of the eNOS enzyme, can promote eNOS uncoupling and endothelial dysfunction also (Chen et al.; Forstermann & Sessa; Lei, Luo, Qin, & Xia; Risbano & Gladwin; Toutouzas, Riga, Stefanadi, & Stefanadis), suggesting the uncoupling of the enzyme is a contributor to decreases in NO production.

So, does obesity itself decrease NO availability? Obesity in humans is associated with decreased blood flow in response to shear stress (Arcaro et al.), bradykinin (Laine et al.; Van Guilder, Stauffer, Greiner, & Desouza), methacholine (Steinberg et al.), substance P and acetylcholine (Van Guilder et al.), and insulin (Tack, Ong, Lutterman, & Smits; Westerbacka et al.), which would appear to suggest that obesity is causally linked with decreased vascular NO bioavailability. Other studies support this hypothesis as well (Andersson et al.; Bhattacharjee, Alotaibi, Kheirandish-Gozal, Capdevila, & Gozal; Bhattacharjee et al.; Georgescu et al.; Grassi et al.; Gupta et al.; Han, Patel, Lteif, Chisholm, & Mather; Lambert et al.; Mahmud, Hill, Cuerden, & Clarson; Miadi-Messaoud et al.; Parikh et al.; Sturm et al.; Weil et al.). Nevertheless, the question remains: Does increased adiposity somehow decrease eNOS-derived NO and availability, or are losses in vascular NO due only to conditions associated with obesity? Several studies suggest that the state of being corpulent is not causative in decreasing vascular NO bioavailability. For example, morbidly obese humans appear to have endothelial dysfunction only when insulin resistance is present (El Assar et al.), and, severely obese humans, in the absence of insulin resistance, have better flow-mediated dilation compared with both normal and obese insulin-sensitive subjects (Biasucci et al.). Moreover, in overweight (insulin-sensitive) individuals, capillary recruitment may actually be higher compared with lean controls (Czernichow et al.). Hence, it appears that either insulin resistance or conditions directly linked with the insulin resistant phenotype are to blame for loss of NO bioavailability in obesity.

Regulation of obesity and insulin resistance by eNOS

Does eNOS-derived NO affect insulin resistance and obesity? This question has been addressed by multiple pharmacological studies and genetic studies, which, collectively, have helped clarify critical roles for eNOS-derived NO in regulating obesity and insulin resistance. Human studies show that L-arginine supplementation has favorable effects on adiposity and insulin sensitivity (Alizadeh et al.; Bogdanski et al.; Bogdanski et al.; Lucotti et al.; Monti et al.; Suliburska, Bogdanski, Szulinska, Pupek-Musialik, & Jablecka; Wascher et al.). Results from animal studies also show that L-arginine decreases fat mass, increases muscle mass, and improves insulin sensitivity (Clemmensen, Madsen, Smajilovic, Holst, & Brauner-Osborne). Dietary L-arginine supplementation in rats increases brown fat and skeletal muscle mass and reduces serum concentrations of triglycerides, glucose, homocysteine, free fatty acids, dimethylarginines, and leptin (Fu et al.; Jobgen et al.). L-arginine has a similar effect on pigs (Tan et al.).

Interestingly, sildenafil—which prevents the degradation of cGMP and is used to treat erectile dysfunction in humans—increases insulin sensitivity and prevents obesity in high fat-fed mice (Ayala et al.), potentially by promoting “browning” of white adipose tissue (Mitschke et al.). Sildenafil increases mitochondrial biogenesis in human adipose tissue *ex vivo* as well (De Toni et al.). Other compounds that activate the NO pathway also support a role for NO in improving insulin sensitivity. Beraprost (a stable prostaglandin analog), when given to endothelial-specific insulin receptor substrate 2 (Irs2) knockout mice, restored eNOS phosphorylation, capillary recruitment, and insulin and glucose delivery to skeletal muscle (Kubota et al.). Additionally, S-nitrosation in response to L-arginine, insulin, or sodium nitroprusside was shown to be important for regulating vascular endothelial insulin

uptake and transendothelial transport (H. Wang, Wang, Aylor, & Barrett). Thus, it appears that NO may regulate obesity and insulin resistance by both cGMP-dependent and –independent pathways.

Although chronic treatment with NOS inhibitors causes weight loss and promotes insulin sensitivity in animals (Morley & Flood; Stricker-Krongrad, Beck, & Burlet; Tsuchiya et al.), their acute application causes systemic insulin resistance (Baron et al.), in part by promoting metabolic changes in the liver (Meshkani & Adeli). Furthermore, BH_4, which is oxidized to BH_2 in the diabetic state (Meininger et al.; Meininger et al.; Xu et al.), administered to STZ-treated mice lowered blood glucose levels in an eNOS-dependent manner. Increasing BH_4 was shown to also improve glucose tolerance and insulin sensitivity in *ob/ob* mice (Abudukadier et al.). This was suggested to be due to eNOS-mediated activation of AMPK in the liver (Abudukadier et al.), which suppresses hepatic glucose production (Viollet et al.). Therefore, eNOS uncoupling in liver appears to negatively regulate systemic glucose metabolism in obese, diabetic states.

Genetic models in which eNOS has been deleted or overexpressed have helped to further elucidate the mechanisms by which NO regulates obesity and insulin resistance. Deletion of eNOS causes insulin resistance, hyperlipidemia, and hypertension (Duplain et al., 2001), and partial deletion of the gene can exaggerate insulin resistance, glucose intolerance, and hypertension under conditions of nutrient excess (Cook et al.; Cook et al.). Mice in which both eNOS and nNOS are absent show similar results, with deletion of eNOS appearing responsible for insulin resistance in both skeletal muscle and liver (Shankar, Wu, Shen, Zhu, & Baron). Similarly, mice lacking eNOS, nNOS, and iNOS (i.e., triple knockout mice), show increased visceral obesity, hypertension, hypertriglyceridemia, and impaired glucose tolerance (Nakata et al.).

The metabolic phenotype caused by eNOS deletion or otherwise low endothelial derived NO appears to relate directly to changes in substrate metabolism in liver, skeletal muscle and adipose tissue. In skeletal muscle, eNOS KO mice have lower mitochondrial content and fatty acid oxidation than WT mice, and they demonstrate markedly lower energy expenditure (Le Gouill et al., 2007). Supplementation of eNOS KO mice with nitrate, which can be reduced to nitrite and NO in the body, decreased not only blood pressure, but visceral adipose tissue and triglycerides as well (Carlstrom et al.).

Our studies in mice overexpressing eNOS suggest a remarkable ability of eNOS to regulate metabolism and body composition. Mice overexpressing eNOS in the vasculature show an anti-obesogenic phenotype characterized by resistance to accumulation of white adipose tissue in response to a high fat diet, a higher metabolic rate, resistance to diet-induced hyperinsulinemia, and remarkably lower plasma levels of free fatty acids and triglycerides (B. E. Sansbury et al., 2012). As shown in **Figure 1**, overexpression of eNOS resulted in decreased weight gain on a high fat diet, which was due to diminished expansion of the adipose tissue.

An eNOS phosphomimetic point mutant mouse model (Atochin & Huang; Kashiwagi et al.) showed a very similar phenotype: mutation of eNOS ser1176 to an aspartic acid increased

endothelial NO production as well as promoted resistance to diet-induced weight gain and hyperinsulinemia, whereas mutation of this residue to an alanine promoted insulin resistance and permitted the development of an obese state (Huang; Kashiwagi et al.).

That eNOS KO mice have elevated plasma levels of triglycerides and free fatty acids compared with WT mice (Cook et al.; Duplain et al.), while eNOS transgenic mice show diminished abundance of the lipids (Brian E. Sansbury et al.) suggests that eNOS regulates lipid oxidation or synthesis. Indeed, eNOS KO mice show diminished fat oxidation capacity in skeletal muscle (Le Gouill et al.). Furthermore, administration of a NOS inhibitor to rats increases serum triglycerides and diminishes fatty acid oxidation in the liver (Khedara, Kawai, Kayashita, & Kato), potentially by decreasing carnitine palmitoyl transferase activity (Khedara, Goto, Morishima, Kayashita, & Kato). NOS inhibitor-dependent decreases in fatty acid oxidation occur in heart as well (Recchia et al.). In hepatocytes, NO donors increase β-oxidation in a cGMP-dependent manner by inhibiting acetyl CoA carboxylase, thereby stimulating the activity of carnitine palmitoyl transferase (Garcia-Villafranca, Guillen, & Castro). NO also diminishes fatty acid synthesis in hepatocytes (Garcia-Villafranca et al.), which is consistent with studies showing that inhibitors of NOS (Goto et al.) or deletion of eNOS increases lipid synthesis in liver (Schild et al.). Similarly, in skeletal muscle, loss of eNOS increases neolipogenic gene expression while decreasing those genes that promote fatty acid oxidation (Le Gouill et al.). These data suggest that eNOS may regulate peroxisome proliferator activated receptor (PPAR)-α, which is well known to regulate lipid metabolism (Lefebvre, Chinetti, Fruchart, & Staels). Indeed, our studies show that overexpression of eNOS increases PPARα expression in adipose tissue (Brian E. Sansbury et al.), suggesting that endothelial-derived NO increases the molecular machinery required to program cells to burn fat. However, it is possible that NO primes fat oxidation in other ways as well. For example, recent studies demonstrate S-nitrosation of multiple enzymes involved in metabolism. In particular, very long chain acyl-coA dehydrogenase (VLCAD), a liver enzyme important in β-oxidation, was shown to be nitrosated at Cys238, which increases the catalytic efficiency of the enzyme. This modification was dependent on eNOS activity, as nitrosation of the enzyme was absent in eNOS KO mice (Gould, Doulias, Tenopoulou, Raju, & Ischiropoulos). Lastly, it is possible that NO-induced increases in mitochondrial mass (Nisoli et al.; Nisoli et al.; Piantadosi & Suliman) could be sufficient to increase metabolic rate and prevent obesity. This would be consistent with studies showing that cGMP-dependent increases in mitochondrial biogenesis prevent obesity (Miyashita et al.) as well as several other studies demonstrating a link between augmented mitochondrial mass and resistance to diet-induced weight gain [e.g., (Fang et al.; Hwang et al.; Yadav et al.; Yamamoto et al.)].

Synopsis

Collectively, these studies suggest that eNOS-derived NO has powerful anti-obesity and insulin-sensitizing effects. It is likely that the enzyme increases fat oxidation and lipid synthesis in tissues such as liver, skeletal muscle, and fat. The relatively low levels of adiposity and plasma free fatty acids and triglycerides in models in which eNOS is overexpressed or permanently activated is consistent with this mechanism (Kashiwagi et al.; Brian E. Sansbury et al.). The favorable effects of eNOS on glucose metabolism and insulin

sensitivity appear to be due to its ability to stimulate the transport of insulin and glucose to key peripheral tissues such as skeletal muscle and to regulate gluconeogenesis. In addition, eNOS overexpression or activation prevents diet-induced hyperinsulinemia (Kashiwagi et al.; Brian E. Sansbury et al.) suggesting that it could impact glucose metabolism by regulating insulin secretion. Exploiting the beneficial metabolic actions of eNOS is a promising prospect for anti-obesity therapies.

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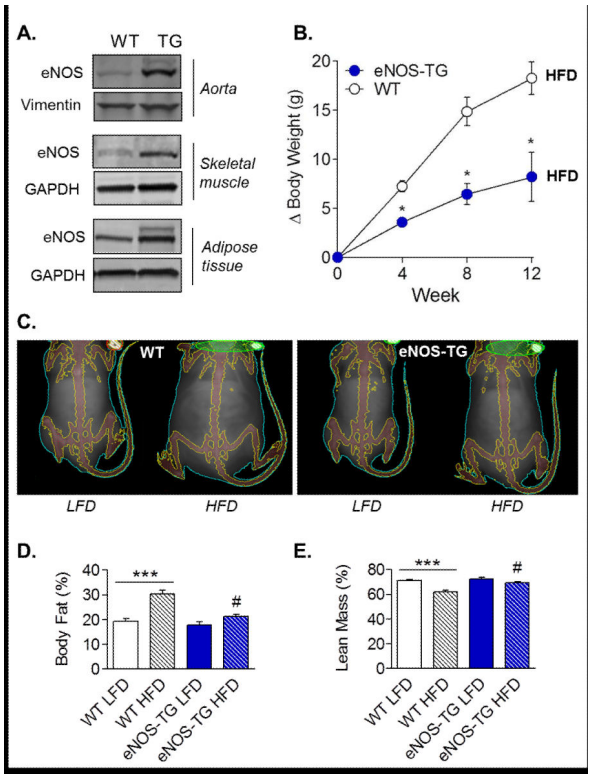

Figure 1. Overexpression of eNOS prevents obesity

(**A**) Immunoblot analysis: eNOS expression in aorta, skeletal muscle, and adipose tissue of wild-type (WT) and eNOS transgenic (eNOS-TG) mice. (**B**) Change in body weight during high fat feeding: mice were fed a high fat diet (HFD) for up to 12 weeks, and the change in body weight was measured. (**C**) Representative Dexascan images of WT and eNOS-TG mice fed a low fat diet (LFD) or HFD for 6 weeks. (**D** and **E**) Dexascan analysis of body fat and lean mass percentage. Figure adapted with permission from (Brian E. Sansbury et al.).