Partial Gene Deletion of Endothelial Nitric Oxide Synthase Predisposes to Exaggerated High-Fat Diet–Induced Insulin Resistance and Arterial Hypertension

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Nitric oxide (NO) plays a major role in the regulation of cardiovascular and metabolic homeostasis, as evidenced by insulin resistance and arterial hypertension in endothelial NO synthase (eNOS) null mice. Extrapolation of these findings to humans is difficult, however, because eNOS gene deficiency has not been reported. eNOS gene polymorphism and impaired NO synthesis, however, have been reported in several cardiovascular disease states and could predispose to insulin resistance. High-fat diet induces insulin resistance and arterial hypertension in normal mice. To test whether partial eNOS deficiency facilitates the development of insulin resistance and arterial hypertension during metabolic stress, we examined effects of an 8-week high-fat diet on insulin sensitivity (euglycemic clamp) and arterial pressure in eNOS$^{+/-}$ mice. When fed a normal diet, these mice had normal insulin sensitivity and were normotensive. When fed a high-fat diet, however, eNOS$^{+/-}$ mice developed exaggerated arterial hypertension and had fasting hyperinsulinemia and a 35% lower insulin-stimulated glucose utilization than control mice. The partial deletion of the eNOS gene does not alter insulin sensitivity or blood pressure in mice. When challenged with nutritional stress, however, partial eNOS deficiency facilitates the development of insulin resistance and arterial hypertension, providing further evidence for the importance of this gene in linking metabolic and cardiovascular disease. *Diabetes* 53:2067–2072, 2004

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eNOS, endothelial nitric oxide synthase; HFD, high-fat carbohydrate-free diet; NC, normal diet.

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Metabolic insulin resistance is a problem of utmost clinical importance and a major risk factor for cardiovascular morbidity and mortality. Epidemiological studies indicate that insulin resistance and arterial hypertension are related (1,2), suggesting the possibility of a common underlying mechanism. Endothelial nitric oxide (NO) synthase (eNOS)-dependent NO synthesis by the vascular endothelium regulates arterial pressure (3,4) and is defective in human essential hypertension (5). Endothelium-derived NO also mediates insulin-induced stimulation of the perfusion of skeletal muscle (6), its main metabolic target tissue. In insulin-resistant individuals, insulin stimulation of endothelial NO synthesis is impaired and may contribute to defective skeletal muscle glucose uptake (7). In line with this hypothesis, NOS inhibitors reduce insulin-stimulated muscle glucose uptake in rats in vivo (8). Moreover, eNOS is expressed in the skeletal muscle (9), and NO donors stimulate glucose transport in isolated rat muscle preparations in vitro (10–12). Consistent with the concept of an important role of NO in the regulation of insulin sensitivity and arterial pressure, eNOS null mice are insulin resistant (13–15) and hypertensive (3,14,15). Extrapolation of these findings in mice to humans is problematic, because eNOS gene deficiency has not been reported in humans so far. There is evidence, however, that cardiovascular disease states such as hypertension, coronary artery disease, and myocardial infarction are associated with eNOS gene polymorphism (16–21) and impaired NO synthesis (19,20). The impaired NO synthesis, which under some conditions is directly related to the polymorphism (22,23), could predispose to insulin resistance (24,25). We hypothesized that partial eNOS deficiency in mice, when challenged with a metabolic stress, may predispose to insulin resistance and hypertension. To test this hypothesis, we assessed insulin sensitivity and arterial pressure in eNOS$^{+/-}$ and wild-type mice that were fed a normal or a high-fat diet for 8 weeks.

RESEARCH DESIGN AND METHODS

Experiments were carried out under protocols approved by the Institutional Animal Care and Use Committee. eNOS$^{-/-}$ C57BL6 mice, as previously described, were used (14,15). Female heterozygous (eNOS$^{+/-}$) and control

mice (eNOS$^{+/+}$) were generated by mating heterozygous animals from our colony. Mice of generations 9–12 were used for our studies. Starting at the age of 6 weeks, the mice were fed a normal diet (NC; UAR, Epinay sur Orge, France; energy content: 12% fat, 28% protein, and 60% carbohydrate, low nitrates) or a high-fat carbohydrate-free diet (HFD; UAR; energy content: 72% fat [corn oil and lard], 28% protein, and <1% carbohydrate, low nitrates) (26). Throughout the study period, the mice were housed with light on from 7:00 A.M. to 7:00 P.M. with food and water ad libitum.

Glucose clamp studies. The glucose turnover rate was assessed after 8 weeks of dietary treatment during a euglycemic-hyperinsulinemic clamp in freely moving mice after a 5-h fast as described previously (14,15). Insulin sensitivity was assessed at maximal (18 mU · kg^{-1} · min^{-1}; n = 8–9 animals per group) and at physiological insulin stimulation (4 mU · kg^{-1} · min^{-1}; n = 8 animals per group, HFD studies only). Briefly, 3 days before study, mice were anesthetized with halothane and an indwelling catheter was inserted into the vena cava through the femoral vein, sealed under the back skin, and exteriorized and glued at the back of the neck. On the day of the clamp, 3-[^{3}H]glucose (high-performance liquid chromatography purified; NEN Life Science, Boston, MA; 30 μCi · kg^{-1} · min^{-1}) and insulin (4 or 18 mU · kg^{-1} · min^{-1}) were infused into the femoral vein for 3 h. Euglycemia was maintained by periodically adjusting a variable infusion of 16.5% glucose. During the last hour of infusion, additional blood samples were collected at 10-min intervals for the measurement of plasma 3-[^{3}H]glucose enrichment. The glucose infusion rate was calculated as the mean of the values obtained every 10 min during the last hour of the infusion period. The glucose turnover rate was determined isotopically and calculated by dividing the rate of 3-[^{3}H]glucose infusion by the plasma 3-[^{3}H]glucose specific activity. Two mice that showed variations of these two parameters of >15% during the last hour of the 3-h infusion were not included in the calculations. Endogenous glucose production was calculated by subtracting the glucose infusion rate from the glucose turnover rate. Whole-body glucose clearance was calculated by dividing the mean whole-body glucose turnover rate by the mean steady-state plasma glucose concentration obtained during the last hour of the infusion period. At time 180 min, a blood sample was obtained for determination of the plasma insulin concentration and the mice were killed.

Blood chemical analysis. Glucose (Glucose Trinder kit 100; Sigma Diagnostics, St. Louis, MO) and insulin (ELISA; Mercodia, Uppsala, Sweden) plasma concentrations were measured in the fed state in conscious mice between 1:00 and 3:00 P.M., after a 6-h fast (n = 10–20 mice for each group). Free fatty acids were measured in at least six animals per group after an 8-h fast (NEFA-C; Wako). Nitrate (NOx) concentration was measured in plasma samples obtained by cardiac puncture, with a chemiluminescence NO analyzer (Sievers 280 NOA), after reduction of NOx to NO with VCl_3 (n = 7 mice for each group).

Measurement of arterial blood pressure and heart rate. Arterial pressure and heart rate were measured in awake, partially restricted mice (n = 5–6 mice for each group) with a fluid-filled PE-10 tubing connected to a pressure transducer. The catheter had been inserted into the carotid artery 3–5 h before the measurement under halothane anesthesia and tunneled subcutaneously to exit at the back of the neck.

Muscle blood flow. Muscle blood flow was measured in anesthetized mice (4–5% halothane inhalation for the induction, followed by 1–1.5% for the maintenance of anesthesia), using a laser Doppler probe (Periflux System 5000, Probe #403; Perimed, Järfälla, Sweden) inserted directly into the hindlimb skeletal musculature and stabilized with a micromanipulator. Fourteen mice that were fed with NC (6 eNOS$^{+/+}$, 8 eNOS$^{+/-}$) and 10 mice that were fed a HFD (5 eNOS$^{+/+}$, 5 eNOS$^{+/-}$) were studied. During the entire study, the body temperature was maintained at 37.0 ± 0.5°C with a temperature control unit (Frederick Haer and Co., Bowdoinham, ME). The blood flow signal was recorded on a personal computer using a specific data acquisition software (Powerlab 400; AD Instruments). A 90-min euglycemic glucose clamp was performed as described above.

Muscle eNOS content. Muscle eNOS content was determined by Western blotting. In brief, at the end of the clamp, the muscles from the hindlimb were dissected out of the bones and adipose tissue depots and immediately frozen in liquid nitrogen. Then they were ground, lysis buffer was added, and they were processed as previously described (27).

Equal amounts of proteins were then resolved by SDS-PAGE and transferred to nitrocellulose membranes. After incubation with a monoclonal anti-eNOS antibody (Transduction Laboratories, Lexington, KY; dilution 1:2,000) for 1 h at room temperature, immune complexes were revealed using enhanced chemiluminescence. Quantification was performed using scanning densitometry with a Fluor-S Imager from BioRad (Hercules, CA) with Quantity One software (BioRad).

Measurement of glucose utilization in isolated muscle. After cervical dislocation, the soleus muscles were rapidly isolated, tied separately to silk threads by the tendons, and immersed for 15 min in an incubation medium (Krebs-Ringer bicarbonate [pH 7.3] supplemented with 1% BSA [Fraction V, pH 7.0] and 2 mmol/l sodium pyruvate). Under an atmosphere that contained 5% CO_2 and 95% O_2, the muscles were then incubated in the medium with or without 10 nmol/l insulin for 60 min at 37°C. Thereafter, the muscles were immersed for 20 min in the incubation medium supplemented with 2-[^{3}H]deoxyglucose (0.1 mmol/l, 0.5 μCi/ml). During this immersion, the 2-[^{3}H]deoxyglucose is metabolized and accumulates as 2-[^{3}H]deoxyglucose-6-phosphate. To stop the reaction, the muscles were immersed in ice-cold saline buffer, washed for 30 min, and then dissolved in 1 mol/l NaOH at 55°C for 60 min. An aliquot of the extract was neutralized with 1 mol/l HCl and spun down, and the ^{3}H-labeled radioactivity was counted in the presence of a scintillation buffer. Sample aliquots were used for protein determination.

Statistical analysis. Data were analyzed using the JMP software package (SAS Institute, Cary, NC). Statistical analysis was done with ANOVA for between-group comparisons and with the two-tailed t test for single comparisons. Relations between variables were analyzed by calculating Pearson product-moment correlation coefficient. All data are presented as mean ± SE. $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Body weight was comparable in eNOS$^{+/-}$ and wild-type mice throughout the study period (data not shown). eNOS expression in skeletal muscle tissue was roughly 50% lower in heterozygous than in control mice and remained unchanged during HFD.

Effects of HFD on insulin sensitivity. In mice that were fed NC, the glucose infusion (96.0 ± 6.4 vs. 109.9 ± 5.9 mg · kg^{-1} · min^{-1}) and glucose clearance rates (0.97 ± 0.10 vs. 1.08 ± 0.06 dl · min^{-1} · kg^{-1}) during the hyperinsulinemic clamp studies with the maximal insulin infusion rate were comparable in eNOS$^{+/-}$ and wild-type mice (Fig. 1*A* and *B*, Table 1). In contrast, in mice that were fed HFD, the glucose infusion (54.4 ± 2.6 vs. 82.2 ± 3.1 mg · kg^{-1} · min^{-1}) and glucose clearance rates (0.55 ± 0.03 vs. 0.82 ± 0.03 dl · min^{-1} · kg^{-1}) were roughly 35% lower in eNOS$^{+/-}$ than in wild-type mice ($P < 0.001$; Fig. 1*A* and *B*). In eNOS$^{+/-}$ mice that were fed HFD, insulin resistance was also more marked during clamps using a physiological insulin infusion rate (glucose infusion rate, 29.1 ± 1.3 vs. 43.6 ± 6.8 mg · kg^{-1} · min^{-1} in eNOS$^{+/-}$ and control mice, respectively). During the high-dose insulin clamps, hepatic glucose production was completely suppressed in all groups. During the low-dose insulin clamps, hepatic glucose production in HFD-fed mice was incompletely but equally suppressed in both groups (5.8 ± 1.1 and 4.7 ± 2.5 mg · kg^{-1} · min^{-1} in eNOS$^{+/-}$ and control mice, respectively). During the clamp studies, the plasma glucose concentration was comparable in all conditions. In NC-fed mice, the fasting plasma insulin concentration was comparable in both strains (12.9 ± 1.6 and 13.0 ± 1.2 μU/ml), whereas during HFD, it was almost 2.5-fold higher in eNOS$^{+/-}$ than in wild-type mice ($P < 0.05$; Fig. 1*C*). The fasting blood glucose concentration was not altered by the HFD (Fig. 1*C*).

Effects of HFD on vascular NO synthesis, arterial pressure, and heart rate. In humans, fat administration may induce endothelial dysfunction (28). To determine the impact of an HFD on vascular NO production in mice, we measured the plasma concentration of NOx. It was ~60% lower in HFD- than in NC-fed mice ($P < 0.05$; Fig. 1*D*). This defect of diet-induced vascular NO production was associated with the development of arterial hypertension (Fig. 1*E*). After 8 weeks of HFD, mean arterial pressure had increased from 97 ± 6 to 130 ± 4 mmHg ($P < 0.01$) in eNOS$^{+/-}$ and from 99 ± 2 to 117 ± 1 mmHg ($P < 0.001$) in

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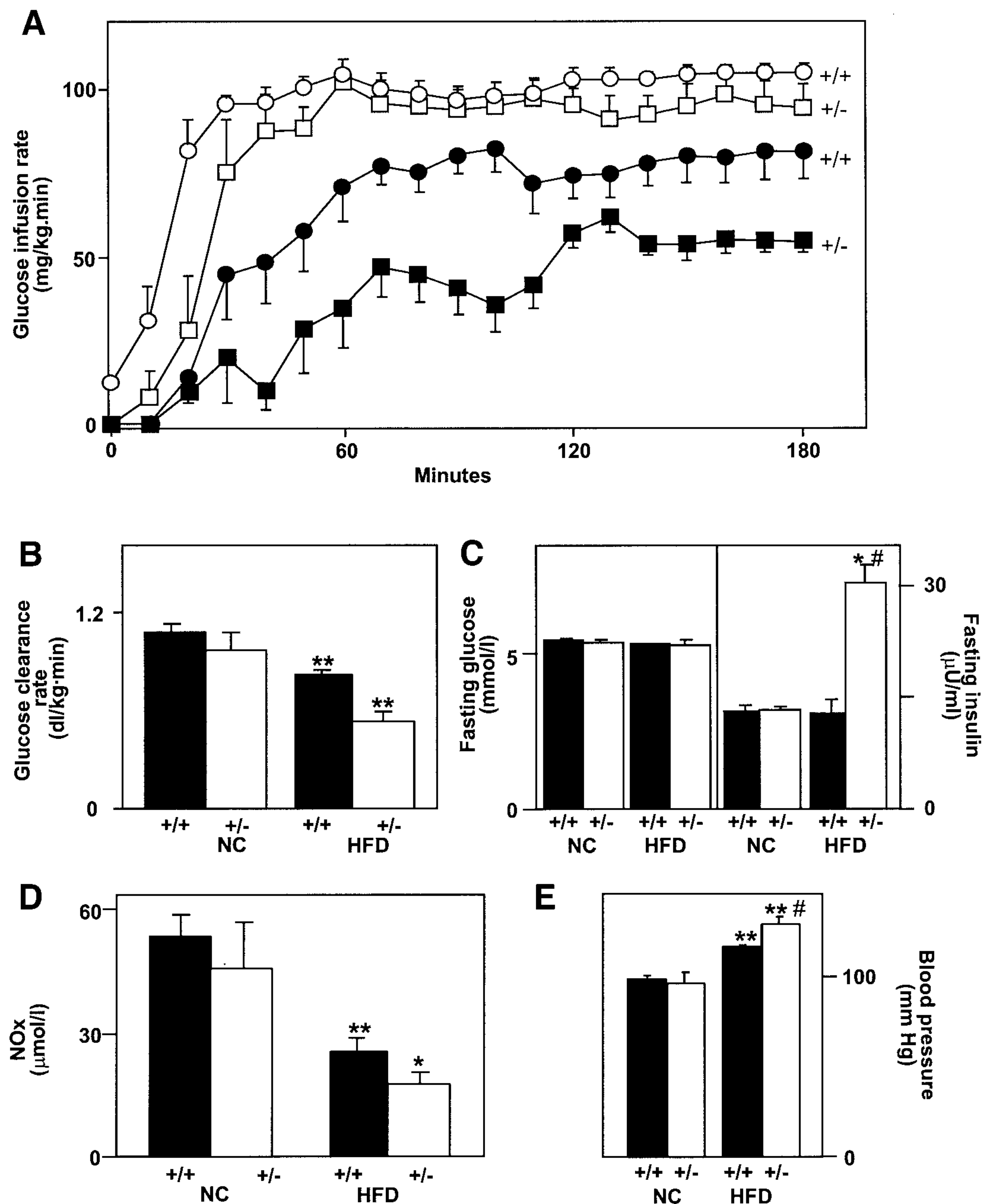

FIG. 1. ***A*: Glucose infusion rates during hyperinsulinemic-euglycemic clamp studies in eNOS$^{+/-}$ (*n* = 9) and wild-type mice (*n* = 8) fed NC (open symbols) or HFD (filled symbols; eNOS$^{+/-}$, *n* = 9; wild type, *n* = 8). *P* < 0.001, eNOS$^{+/-}$ vs. wild type during HFD; *P* < 0.001, NC vs. HFD, for both strains. Data are mean ± SE. *B*: Glucose clearance rate during the steady-state phase of the euglycemic clamp studies. *P* < 0.001, eNOS$^{+/-}$ vs. wild type during HFD, *P < 0.05, **P < 0.01, vs. NC. Data are mean ± SE. *C*: Fasting plasma concentrations of glucose and insulin (NC: eNOS$^{+/-}$ and wild type, *n* = 10; HFD: eNOS$^{+/-}$, *n* = 15, wild type, *n* = 20). *D*: Plasma concentration of nitrite and nitrates (*n* = 7 in each group). *E*: Mean arterial pressure in eNOS$^{+/-}$ and wild-type mice fed NC or HFD (*n* = 6–7 in each group). *C–E*: *P < 0.05, **P < 0.01 vs. corresponding NC; #P < 0.05 eNOS$^{+/-}$ vs. wild type.**

wild-type mice. The diet-induced increase in arterial pressure was significantly larger in eNOS$^{+/-}$ than in wild-type mice ($P < 0.05$). Similarly, during HFD, heart rate was significantly faster in eNOS$^{+/-}$ mice than in control mice (516 ± 31 vs. 395 ± 29 bpm; $P = 0.008$), whereas during NC, it was comparable in both groups (469 ± 25 vs. 439 ± 41 bpm; $P > 0.1$). Free fatty acid plasma concentration did not differ during HFD between wild-type and heterozygous mice (1.37 ± 0.21 vs. 1.08 ± 0.34 μmol/l; $P > 0.1$).

Effects of HFD on insulin stimulation of skeletal muscle blood flow in vivo and muscle glucose utilization in vitro. In eNOS knockout mice, insulin resistance is associated with impaired insulin stimulation of skeletal muscle perfusion (14). To study the effect of HFD on insulin-stimulated muscle perfusion, we measured hindlimb muscle blood flow during clamp studies. During NC, muscle blood flow had increased by ~45% by the end of the clamp in both strains of mice. HFD almost completely abolished the insulin-induced stimulation of muscle blood flow in eNOS$^{+/-}$ and wild-type mice (Fig. 2*A*). In skeletal muscle, insulin resistance could be constitutive or induced by external factors. To address this point, we studied

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TABLE 1
Fasted parameters in control and mutant mice

Genotype	Diet	Fasting plasma glucose (mmol/l)	Fasting plasma insulin (μU/ml)	Glucose infusion rate ($mg/kg \cdot min^{-1}$)	Glucose clearance rate ($dl/min \cdot kg^{-1}$)
$eNOS^{+/+}$	NC	5.4 ± 0.1	12.9 ± 1.6	109.9 ± 5.9	1.08 ± 0.06
$eNOS^{+/+}$	HFD	5.3 ± 0.1	12.8 ± 3.1	82.2 ± 3.1	0.82 ± 0.03
$eNOS^{+/-}$	NC	5.3 ± 0.1	13.0 ± 1.2	96.0 ± 6.4	0.97 ± 0.10
$eNOS^{+/-}$	HFD	5.3 ± 0.2	30.6 ± 3.4*	54.4 ± 2.6†	0.55 ± 0.03†

Data are mean ± SE. *$P < 0.05$, $eNOS^{+/-}$ HFD vs. $eNOS^{+/+}$ HFD; †$P < 0.01$, $eNOS^{+/-}$ HFD vs. $eNOS^{+/+}$ HFD.

isolated muscle glucose utilization. We found that basal and insulin-stimulated glucose transport were comparable in soleus muscles from heterozygous and control mice that were fed NC or HFD (Fig. 2*B*).

DISCUSSION

Recently, we found that eNOS null mice are insulin resistant and hypertensive (13–15). As complete gene deficiencies are seldom found in human disease, we examined whether a partial deletion of eNOS also affects insulin sensitivity and blood pressure homeostasis. We found that when fed an NC, $eNOS^{+/-}$ mice had normal insulin sensitivity and were normotensive. When fed HFD for 8 weeks, however, $eNOS^{+/-}$ mice developed exaggerated insulin resistance at both physiological and maximal insulin-stimulated rates, as evidenced by fasting hyperinsulinemia and glucose infusion rates during euglycemic clamp studies that were ~40% lower than in wild-type mice. Moreover, HFD caused an exaggerated increase of the arterial pressure in $eNOS^{+/-}$ mice. These findings indicate that one eNOS gene provides sufficient eNOS protein expression and activity to maintain normal insulin sensitivity and arterial blood pressure under usual conditions. During a metabolic stress (HFD), however, eNOS deficiency amplified a pathological mechanism observed under normal conditions and led to exaggerated insulin resistance and arterial hypertension.

In mice that were fed HFD, hepatic glucose production was equally suppressed in both groups, indicating that the lower glucose infusion rate in $eNOS^{+/-}$ mice was mostly accounted for by decreased glucose uptake in peripheral tissues. In eNOS null mice, insulin resistance is associated with impaired stimulation of muscle blood flow (14). In $eNOS^{+/-}$ mice that were fed NC, insulin stimulation of muscle blood flow was comparable to that of wild-type mice, indicating that one allele is sufficient to maintain a normal response to this stimulus. During the HFD, insulin stimulation of muscle blood flow was markedly impaired in both groups, possibly related to impaired vascular NO synthesis (as reflected by the ~60% decrease of NOx plasma concentration in both groups). These findings suggest that impaired insulin stimulation of muscle blood flow and, in turn, substrate delivery contributed to HFD-induced insulin resistance in wild-type and $eNOS^{+/-}$ mice.

The observation that during HFD, for a comparable impairment of insulin stimulation of muscle blood flow, insulin resistance was more marked in $eNOS^{+/-}$ than in wild-type mice, suggests that additional mechanisms contributed to metabolic insulin resistance in $eNOS^{+/-}$ mice. eNOS is expressed in skeletal muscle tissue (9), where NO regulates metabolic and contractile processes (11). In isolated skeletal muscle preparations of eNOS null mice, the basal and insulin-stimulated glucose transport is impaired (14). Here we found that, consistent with normal glucose uptake in vivo, glucose uptake in vitro in response to insulin was also normal in $eNOS^{+/-}$ mice, although soleus muscle eNOS expression was ~50% lower than in control mice. Moreover and consistent with previous results in normal mice, the low-carbohydrate HFD did not alter basal and insulin-mediated glucose uptake in vitro in

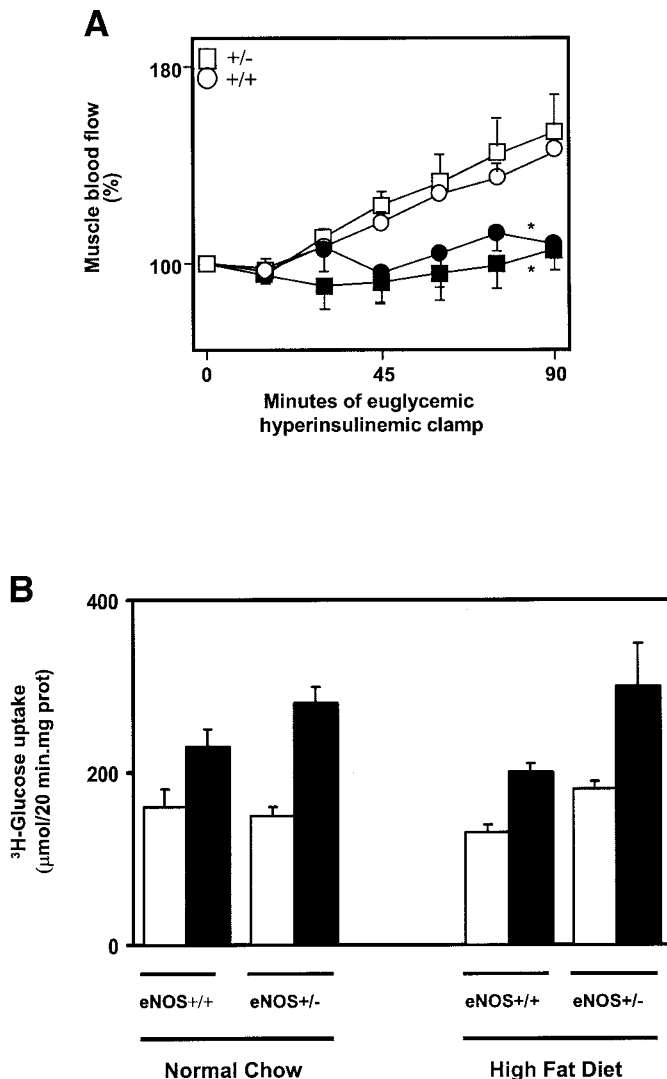

FIG. 2. ***A*: Hindlimb muscle blood flow during 90-min euglycemic-hyperinsulinemic clamp studies in $eNOS^{+/-}$ ($n = 6$) and wild-type mice ($n = 8$) fed NC (open symbols, $n = 6–8$ for each group) or HFD (filled symbols, $n = 5$ for each group). Data are mean ± SE. $P < 0.05$, NC vs. HFD, for both strains. *B*: Basal and insulin-stimulated 2-deoxyglucose uptake in soleus muscle of $eNOS^{+/-}$ (NC, $n = 7$; HFD, $n = 8$) and control mice (NC, $n = 7$; HFD, $n = 8$). Data are mean ± SE.**

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control and eNOS$^{+/-}$ mice (29). These findings suggest that differences in eNOS expression in skeletal muscle tissue did not contribute importantly to exaggerated metabolic insulin resistance in HFD-fed eNOS$^{+/-}$ mice in vivo.

Exaggerated insulin resistance in HFD-fed eNOS$^{+/-}$ mice does also not seem to be related to differences in free fatty acid levels, which were found to be comparable in the two groups. Finally, despite marked insulin resistance at the end of the 8-week HFD, the fasting plasma glucose concentration remained normal in eNOS$^{+/-}$ mice, suggesting that the compensatory hyperinsulinemia was sufficient to maintain glucose concentration within normal limits.

In addition to metabolic insulin resistance, HFD induced arterial hypertension. Our findings suggest that this was related, at least in part, to impaired vascular NO synthesis. The observation that for comparable values of NOx plasma concentration the arterial hypertension was more pronounced in the eNOS$^{+/-}$ mice suggests that additional mechanisms may play a role. Insulin stimulates sympathetic nervous activity in rodents and humans (24,25). Thus, in eNOS$^{+/-}$ mice, basal hyperinsulinemia-induced sympathetic overactivity could contribute to exaggerated arterial hypertension during HFD. Consistent with this hypothesis, heart rate was faster in eNOS$^{+/-}$ than in control mice during HFD. Parenthetically, sympathetic overactivity could also represent one of the factors facilitating insulin resistance in eNOS$^{+/-}$ mice (30).

Recently, vascular endothelial insulin receptor knockout mice have been generated and were found to have normal insulin sensitivity blood pressure under normal conditions but showed insulin resistance and altered blood pressure control when challenged with changes in dietary salt intake (31). Taken together with the present data, these findings suggest that under normal conditions, either a specific loss of insulin stimulation of vascular endothelial NO release (VENIRKO mice) or a generalized partial defect of eNOS-driven NO synthesis (eNOS$^{+/-}$ mice) is largely inconsequential with regard to blood pressure homeostasis and insulin sensitivity, although in the VENIRKO mice, the potential role of insulin-stimulated endothelial NO production in the regulation of whole-body insulin sensitivity might have been overlooked, because the clamp studies were of very short duration and a high insulin infusion rate was used (i.e., it remains possible that a shift in insulin sensitivity may have been detected if lower insulin infusion rates had been used). When hit by an additional challenge, however, normal eNOS function seems to represent a line of defense to maintain normal insulin sensitivity and vascular function.

Our data in mice indicate that there exists an important interaction between genetic and environmental factors in the regulation of vascular NO synthesis and glucose and blood pressure homeostasis. In human populations, the prevalence of eNOS polymorphism ranges from 5 to 35% (16–21). We speculate that whereas under normal, unstressed conditions a partial defect of NO synthesis may not alter the phenotype, under a metabolic stress, such as the one represented by a Western-type diet, it may facilitate the development of insulin resistance and arterial hypertension.

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REFERENCES

1. Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, Shitrit A, Fuchs Z: Hyperinsulinemia: a link between hypertension obesity and glucose intolerance. *J Clin Invest* 75:809–817, 1985
2. Lucas CP, Estigarribia JA, Darga LL, Reaven GM: Insulin and blood pressure in obesity. *Hypertension* 7:702–706, 1985
3. Shesely EG, Maeda N, Kim HS, Desai KM, Krege JH, Laubach VE, Sherman PA, Sessa WC, Smithies O: Elevated blood pressures in mice lacking endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A* 93:13176–13181, 1996
4. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC: Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature* 377:239–242, 1995
5. Forte P, Copland M, Smith LM, Milne E, Sutherland J, Benjamin N: Basal nitric oxide synthesis in essential hypertension. *Lancet* 349:837–842, 1997
6. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P: Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 94:2511–2515, 1994
7. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD: Obesity/insulin resistance is associated with endothelial dysfunction: implications for the syndrome of insulin resistance. *J Clin Invest* 97:2601–2610, 1996
8. Roy D, Perreault M, Marette A: Insulin stimulation of glucose uptake in skeletal muscles and adipose tissues in vivo is NO dependent. *Am J Physiol* 274:E692–E699, 1998
9. Kapur S, Bedard S, Marcotte B, Cote CH, Marette A: Expression of nitric oxide synthase in skeletal muscle: a novel role for nitric oxide as a modulator of insulin action. *Diabetes* 46:1691–1700, 1997
10. Higaki Y, Hirshman MF, Fujii N, Goodyear LJ: Nitric oxide increases glucose uptake through a mechanism that is distinct from the insulin and contraction pathways in rat skeletal muscle. *Diabetes* 50:241–247, 2001
11. Balon TW, Nadler JL: Evidence that nitric oxide increases glucose transport in skeletal muscle. *J Appl Physiol* 82:359–363, 1997
12. Young ME, Radda GK, Leighton B: Nitric oxide stimulates glucose transport and metabolism in rat skeletal muscle in vitro. *Biochem J* 322 (Suppl.):223–228, 1997
13. Shankar RR, Wu Y, Shen HQ, Zhu JS, Baron AD: Mice with gene disruption of both endothelial and neuronal nitric oxide synthase exhibit insulin resistance. *Diabetes* 49:684–687, 2000
14. Duplain H, Burcelin R, Sartori C, Cook S, Egli M, Lepori M, Vollenweider P, Pedrazzini T, Nicod P, Thorens B, Scherrer U: Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. *Circulation* 104:342–345, 2001
15. Cook S, Hugli O, Egli M, Vollenweider P, Burcelin R, Nicod P, Thorens B, Scherrer U: Clustering of cardiovascular risk factors mimicking the human metabolic syndrome X in eNOS null mice. *Swiss Med Wkly* 133:360–363, 2003
16. Miyamoto Y, Saito Y, Kajiyama N, Yoshimura M, Shimasaki Y, Nakayama M, Kamitani S, Harada M, Ishikawa M, Kuwahara K, Ogawa E, Hamanaka I, Takahashi N, Kaneshige T, Teraoka H, Akamizu T, Azuma N, Yoshimasa Y, Yoshimasa T, Itoh H, Masuda I, Yasue H, Nakao K: Endothelial nitric oxide synthase gene is positively associated with essential hypertension. *Hypertension* 32:3–8, 1998
17. Hingorani AD, Liang CF, Fatibene J, Lyon A, Monteith S, Parsons A, Haydock S, Hopper RV, Stephens NG, O'Shaughnessy KM, Brown MJ: A common variant of the endothelial nitric oxide synthase (Glu298—>Asp) is a major risk factor for coronary artery disease in the UK. *Circulation* 100:1515–1520, 1999
18. Shimasaki Y, Yasue H, Yoshimura M, Nakayama M, Kugiyama K, Ogawa H, Harada E, Masuda T, Koyama W, Saito Y, Miyamoto Y, Ogawa Y, Nakao K: Association of the missense Glu298Asp variant of the endothelial nitric

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oxide synthase gene with myocardial infarction. *J Am Coll Cardiol* 31:1506–1510, 1998
19. Cai H, Wilcken DE, Wang XL: The Glu-298—>Asp (894G—>T) mutation at exon 7 of the endothelial nitric oxide synthase gene and coronary artery disease. *J Mol Med* 77:511–514, 1999
20. Wang XL, Sim AS, Badenhop RF, McCredie RM, Wilcken DE: A smoking-dependent risk of coronary artery disease associated with a polymorphism of the endothelial nitric oxide synthase gene. *Nat Med* 2:41–45, 1996
21. Shoji M, Tsutaya S, Saito R, Takamatu H, Yasujima M: Positive association of endothelial nitric oxide synthase gene polymorphism with hypertension in northern Japan. *Life Sci* 66:2557–2562, 2000
22. Ohtoshi K, Yamasaki Y, Gorogawa S, Hayaishi-Okano R, Node K, Matsuhisa M, Kajimoto Y, Hori M: Association of (−)786T-C mutation of endothelial nitric oxide synthase gene with insulin resistance. *Diabetologia* 45:1594–1601, 2002
23. Philip I, Plantefeve G, Vuillaumier-Barrot S, Vicaut E, LeMarie C, Henrion D, Poirier O, Levy BI, Desmonts JM, Durand G, Benessiano J: G894T polymorphism in the endothelial nitric oxide synthase gene is associated with an enhanced vascular responsiveness to phenylephrine. *Circulation* 99:3096–3098, 1999
24. Sartori C, Scherrer U: Insulin, nitric oxide and the sympathetic nervous system: at the crossroads of metabolic and cardiovascular regulation. *J Hypertens* 17:1517–1525, 1999
25. Scherrer U, Sartori C: Defective nitric oxide synthesis: a link between metabolic insulin resistance, sympathetic overactivity and cardiovascular morbidity. *Eur J Endocrinol* 142:315–323, 2000
26. Burcelin R, Dolci W, Thorens B: Long-lasting antidiabetic effect of a dipeptidyl peptidase IV-resistant analog of glucagon-like peptide-1. *Metabolism* 48:252–258, 1999
27. Vollenweider P, Menard B, Nicod P: Insulin resistance, defective insulin receptor substrate 2-associated phosphatidylinositol-3′ kinase activation, and impaired atypical protein kinase C (ζ/λ) activation in myotubes from obese patients with impaired glucose tolerance. *Diabetes* 51:1052–1059, 2002
28. Steinberg HO, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A, Bayazeed B, Baron AD: Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. *J Clin Invest* 100:1230–1239, 1997
29. Burcelin R, Crivelli V, Dacosta A, Roy-Tirelli A, Thorens B: Heterogeneous metabolic adaptation of C57BL/6J mice to high-fat diet. *Am J Physiol Endocrinol Metab* 282:E834–E842, 2002
30. Scherrer U, Sartori C: Insulin as a vascular and sympathoexcitatory hormone: implications for blood pressure regulation, insulin sensitivity, and cardiovascular morbidity. *Circulation* 96:4104–4113, 1997
31. Vicent D, Ilany J, Kondo T, Naruse K, Fisher SJ, Kisanuki YY, Bursell S, Yanagisawa M, King GL, Kahn CR: The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. *J Clin Invest* 111:1373–1380, 2003

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