Leptin-induced endothelial dysfunction in obesity

Mykhaylo Korda, Ruslan Kubant, Stephen Patton, and Tadeusz Malinski

Department of Chemistry and Biochemistry, Ohio University, Athens, Ohio

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Korda M, Kubant R, Patton S, Malinski T. Leptin-induced endothelial dysfunction in obesity. Am J Physiol Heart Circ Physiol 295: H1514–H1521, 2008.—Hyperleptinemia accompanying obesity affects endothelial nitric oxide (NO) and is a serious factor for vascular disorders. NO, superoxide (O_2^·), and peroxynitrite (ONOO^-) nanosensors were placed near the surface (5 ± 2 μm) of a single human umbilical vein endothelial cell (HUVEC) exposed to leptin or aortic endothelium of obese C57BL/6J mice, and concentrations of human umbilical vein endothelial cell (HUVEC) exposed to leptin or nanosensors were placed near the surface (5

The [NO][ONOO^-] balance in obese mice. In obesity, leptin

METHODS

All materials were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise noted.

Cell culture. HUVECs (ATCC No. CRL-1730, Manassas, VA) were seeded in collagen-coated flasks and incubated in MCDB-131 complete medium at 37°C under an atmosphere of 5% CO_2-95% air. After the formation of a confluent monolayer, the cells were trypsinized and then resuspended in MCDB-131 and seeded in 12-well cell culture clusters. After confluence, MCDB-131 complete medium was replaced with Eagle’s minimum essential medium without blood serum, and the confluent cells were incubated for 2 or 12 h with different concentrations of leptin or coincubated with 0.1 mmol/l of leptin and elevated concentrations of l-arginine (3 mmol/l) or l-arginine (3 mmol/l) and sepiapterin (0.1 mmol/l).

When we cross-check the response of our sensors using enzyme inhibitors, the cells were preincubated for 2 h before and during measurements with eNOS inhibitor, 0.3 mmol/l N^γ-nitro-l-arginine methyl ester (l-NAME), 2 mmol/l of membrane permeable superoxide dismutase conjugated with polyethylene glycol (PEG-SOD), or 0.01 mmol/l of peroxynitrite decomposition catalyst/scavenger, MinI(III) tetakis, (1-methyl-4-pyrindyl) porphyrin pentachloride (MinI(III)TPP), or two oxidase inhibitors 2.0 mmol/l apocynin or (0.07 mmol/l) 6,8 diallyl 5,7-dihydroxy

Address for reprint requests and other correspondence: T. Malinski, Dept. of Chemistry and Biochemistry, Ohio Univ., 350 W. State St., Athens, OH 45701-2979 (e-mail: malinski@ohio.edu).

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Fig. 1. Typical amperometric curves showing nitric oxide (NO; A), superoxide (O$_2^-$; B), and peroxynitrite (ONOO$^-$; C) release from a single human umbilical vein endothelial cell (HUVEC). Leptin (0.1 μg/ml; a) or calcium ionophore (Cal; 1 μmol/l) was used to stimulate NO, O$_2^-$, and ONOO$^-$ generation from a HUVEC incubated for 2 h without leptin (b) or with leptin (0.1 μg/ml; c).
these species was significantly higher in HUVEC incubated with leptin when compared with control cells.

To investigate the relationship between the eNOS functional state and leptin concentration, as well as the leptin exposure time, we measured the Cal-stimulated NO, O$_2^-$, and ONOO$^-$ release from HUVEC treated with different leptin concentrations during different times. The incubation of cells for both 2 and 12 h with increasing leptin concentrations resulted in a dose-dependent increase of the peak NO, O$_2^-$, and ONOO$^-$ generation (Fig. 2). The curves reflecting the production of these species reached a semiplateau at about 0.1 μg/ml leptin. It is interesting to report that the incubation of HUVEC with leptin for 2 h stimulated the NO production to a higher degree than treatment with leptin for 12 h. A 2-h exposure of cells to leptin (0.1–10 μg/ml) led to a 1.6-fold increase of NO production compared with basal conditions, whereas the same concentrations of leptin exposure for 12 h caused only a 1.3-fold increase of peak NO release. In contrast to NO, the Cal stimulated peak production of O$_2^-$ and ONOO$^-$ in the HUVECs incubated with leptin (0.1–10 μg/ml) for 12 h was about 2.0 and 1.7 times higher, respectively, than the control HUVEC. Whereas there was only a 1.3- and 1.2-fold increase, respectively, of the O$_2^-$ and ONOO$^-$ peak productions in the HUVECs incubated with 0.1–10 μg/ml leptin for 2 h.

Analysis of the time-dependent effect of constant leptin concentration on stimulated peak NO release showed the rapid rise of NO production for 1 h after the beginning of cell exposure to the hormone (Fig. 3A). After reaching the maximum (820 ± 35 mmol/l at 2 h), a significant linear decrease in NO release was observed. In contrast to NO, the O$_2^-$ and ONOO$^-$ productions sharply increased at 4 h and steadily increased thereafter. As expected, in the presence of membrane-permeable PEG-SOD (O$_2^-$ scavenger), the concentration of O$_2^-$ as well as the concentration of ONOO$^-$ sharply decreased, whereas NO concentration increased in leptin-treated cells (Fig. 4A). However, in the presence of MnTMPyP (ONOO$^-$ scavenger), only a significant decrease in ONOO$^-$ was observed.

We used the ratio of NO to ONOO$^-$ concentration ([NO]/[ONOO$^-$]) to quantify the relation between bioavailable NO and cytotoxic ONOO$^-$ in the endothelium. A high R value indicates strong eNOS coupling and high bioavailable NO and/or low nitroxidative stress (low level of ONOO$^-$).

Intriguingly, the R value was 2.8 ± 0.1 in HUVEC incubated for 2 h with 0.1 μg/ml leptin when compared with control cells (2.0 ± 0.1) (Fig. 4B). In contrast, HUVEC treated with the same leptin concentration for 12 h showed a decrease of the R value to 1.3 ± 0.1. This indicated that there was a gradual increase in eNOS uncoupling on longer exposure to leptin. The eNOS uncoupling was partially reversed in the presence of elevated l-arginine concentration (3 mmol/l) and sepiapterin (0.1 mmol/l), a precursor of tetrahydrobiopterin (a cofactor of eNOS) (Fig. 4B). In the presence of l-arginine and sepiapterin, the R value increased to 2.3 ± 0.1.

In addition, we confirmed that the release of NO and ONOO$^-$ was related to eNOS activation because eNOS inhibition of the enzyme by N$^\omega$-nitro-l-arginine methyl ester (0.3 mmol/l) significantly blocked (about 75%) Cal-stimulated release of both NO and ONOO$^-$ (Fig. 4C). We tested the potential contribution of the NAD(P)H oxidase-generated O$_2^-$ to the overall formation of ONOO$^-$ and the diminished bioavailable NO. First, we measured NO and ONOO$^-$ in the presence of apocynin, a nonspecific NAD(P)H oxidase inhibitor. We then measured NO and ONOO$^-$ in the presence of S-17834, a more selective NAD(P)H oxidase inhibitor. Treatment of endothelial cells with apocynin or S-17834 increased NO release by about 20–30% and decreased ONOO$^-$ level by 20–30%. This indicates that about a 20–30% concentration of O$_2^-$ is generated by membrane-bound NAD(P)H oxidase and about 70–80% of O$_2^-$ is generated by membrane-bound eNOS for the production of ONOO$^-$ (after Cal stimulation). Another potentially major source of NO, O$_2^-$, and ONOO$^-$ in HUVEC is mitochondria. Nevertheless, under experimental conditions used in this study (sensors located 5 ± 2 μm from the outer surface of the cell membrane), it is rather unlikely that mitochondrial O$_2^-$ or ONOO$^-$ can diffuse to, and be measured by, the sensors.

**eNOS expression and l-arginine concentration in HUVEC.** As shown in Fig. 5A, eNOS expression increased after cell incubation with leptin in a dose-dependent manner. HUVEC treated with leptin for 2 h showed a higher increase of eNOS expression compared with the cells incubated for 12 h. Figure 5B shows that during incubation, leptin-treated HUVEC had significantly lower intracellular l-arginine concentration levels than untreated cells.

**Body weight, leptin concentration, and eNOS expression.** The D-12492 mice had statistically significant increased mean body weight versus D-12450B group as early as 1 mo after diet initiation (Fig. 6A). Later the high-calorie-fed mice continued to gain body weight much faster than mice maintained on the low-calorie diet. After 105 days, the mean body weight of...
D-12492 mice was about 1.4 times higher than that of the control group. No influence of treatment with L-arginine on body weight was detected.

The shape of curves reflecting the increase of leptin concentration in serum of mice fed with high- and low-calorie diets (Fig. 6B) were very similar to the curves reflecting the gain of body weight in corresponding animal groups. Whereas the leptin concentration at the end of the experimental period (105 days) was 6.9 ± 0.8 ng/ml in the control group, the maximal leptin concentration at the same time was 2.5 times higher (16.1 ± 1.5 ng/ml) in the mice provided with the high-calorie diet and untreated water. As in the case with body weight, L-arginine did not significantly affect the leptin concentration in obese mice. A slight linear increase (about 8% maximal) in leptin concentration with the body weight was exponential for obese mice. It is interesting to note that the most remarkable increase in leptin concentration was observed in obese mice at a weight exceeding the maximal weight (about 35 g) of the low-calorie diet D-12450B mice. In addition, the 2–5 fold increase in leptin concentration correlated with the increase in eNOS in obese mice expression (about a 2-fold increase compared with control). The expression of inducible NO synthase in the endothelium did increase significantly in obese mice (data not shown). The cGMP level (aortic wall) correlated inversely with eNOS expression (Fig. 7, A and B) and directly reflected the diminished NO concentration observed in obese mice (Fig. 2A).

NO, O$_2^\cdot$, and ONOO$^-$ release in aortic endothelium. To deeper investigate the ability of leptin to affect the functional state of endothelial cells, the Cal-stimulated NO, O$_2^\cdot$, and ONOO$^-$ production in aortic endothelium of normal (control) and obese C57BL/6J mice was measured (Fig. 7C). NO concentration released from the aortic endothelium of control mice was 420 ± 12 nmol/l, about 1.7 times higher than from aortic endothelium of mice with obesity (250 ± 11 nmol/l). Obese mice treated with L-arginine showed higher NO production (297 ± 10 nmol/l, P < 0.05) compared with untreated obese mice. A further increase (~25%) in NO levels was observed after L-arginine plus sepiapterin treatment.

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**Fig. 3.** Cal (1 μmol/l)-stimulated peak concentration of NO (A), O$_2^\cdot$ (B), and ONOO$^-$ (C) as a function of incubation time. HUVECs were incubated with leptin (0.1 μg/ml) (●) or without leptin (○). *P < 0.05 vs. control (without leptin); n = 4 to 5 experiments.

**Fig. 4.** A: maximal NO, O$_2^\cdot$, and ONOO$^-$ concentration produced by HUVEC in the presence of O$_2^\cdot$ or ONOO$^-$ scavengers. NO, O$_2^\cdot$, and ONOO$^-$ releases were stimulated by Cal (1 μmol/l) from a HUVEC incubated for 12 h with leptin (0.1 μmol/l) or with polyethylene glycol (PEG)-SOD (100 U/ml) (gray bar) or MnTMPyP (0.02 mmol/l) (black bar); control (white bar). *P < 0.0001 vs. control; n = 4 to 5 experiments. B: the ratio (R) of peak NO concentration to the peak concentration of ONOO$^-$ (R = [NO]/[ONOO$^-$]). NO and ONOO$^-$ releases were stimulated by Cal (1 μmol/l) from HUVEC incubated for 2 h without leptin (control, white bar) or incubated with leptin (0.1 μg/ml) for 2 h (gray bar) or 12 h (black bar) at normal and elevated L-arginine (L-Arg: 3 mmol/l) or L-arginine (3 mmol/l) and sepiapterin (0.1 mmol/l). *P < 0.05 vs. control and **P < 0.01 vs. 12-h incubation without L-arginine, sepiapterin; n = 4 to 5 experiments. C: Cal-stimulated maximal NO and ONOO$^-$ concentration released from HUVEC in the absence (control, white bars) or in the presence (black bars) of N$^\omega$-nitro-L-arginine methyl ester (L-NAME; 0.3 mmol/l) and apocynin (3 mmol/l) or S-17834 (0.07 mmol/l). The concentration of NO and ONOO$^-$ was measured after 12 h incubation with leptin (0.1 μg/ml). *P < 0.001 vs. control, n = 4 to 5 experiments.
The CaI-stimulated peaks of O$_2$ and ONOO$^-$ release were significantly elevated in obese mice. The maximal O$_2$ production in aortic endothelium of mice maintained on a high-calorie diet and untreated water was about 3.4 times higher than in control mice. The peak ONOO$^-$ release was 1.7 times higher in obese mice than in control mice. A high-calorie diet supplementation with l-arginine or l-arginine plus sepiapterin resulted in the decrease of O$_2$ and ONOO$^-$ and an increased NO production in the aortas of obese mice by 30% and 17%, respectively. The high-calorie diet l-arginine supplementation resulted in the decrease of O$_2$ and ONOO$^-$. This effect was even more pronounced after supplementation with l-arginine plus sepiapterin (Fig. 7C).

The R value decreased about 64% in obese mice compared with control mice (Fig. 8A). L-Arginine treatment increased the R value about 30% over the untreated obese mice, and treatments with l-arginine plus sepiapterin doubled the R value over the untreated obese mice.

**L-Arginine concentration in blood and aortic endothelium.**
The level of l-arginine in the blood of obese mice did not change significantly compared with the control mice (Fig. 8B). In contrast, obesity resulted in about a 30% decrease of l-arginine level in aortic endothelium of obese mice compared with control mice. L-Arginine supplementation caused a significant increase of l-arginine level both in blood (70%) and in aortic endothelium (30%) of the obese mice.

**DISCUSSION**
It is generally accepted that obesity is closely associated with an increased risk of hypertension and heart failure, but the mechanisms involved are still not fully understood (14, 32). It has been reported that the adipocyte-derived hormone leptin is involved in the regulation of blood vessel tonus (21). However, the effect of leptin on NO production in endothelial cells has been assessed only with the use of indirect methods (3, 12, 18, 19). In contrast, this study used highly sensitive, selective, millisecond response-time electrochemical nanosensors (22, 25, 34) to directly measure the concentration of CaI-stimulated NO, O$_2$, and ONOO$^-$ release from HUVEC treated with leptin as well as from the aortic endothelium of normal and obese mice with hyperleptinemia.

Our results clearly demonstrated that leptin does not directly stimulate NO, O$_2$, or ONOO$^-$ release from the endothelium. However, the exposure of endothelial cells to leptin resulted in the dose-dependent upregulation of eNOS expression and an enhanced NO production that lasted for about 2 h.

Leptin is known to increase cell proliferation, which profoundly influences the abundance of the eNOS transcript. Thus any factors that influence endothelial cell growth rate may confound the interpretation of the primary effects on eNOS gene expression (30). Therefore, the leptin-enhanced expres-
tion of eNOS in endothelial cells can be explained, at least in part, by a transcriptional and/or posttranscriptional mechanism. We acknowledge, however, that further work beyond the limits of the present study is required to verify the mechanism of leptin-enhanced eNOS expression in the endothelium.

A short-time exposure of HUVEC to leptin did not increase \( \text{O}_2^- \) and \( \text{ONOO}^- \) levels significantly. This indicates that an acute exposure of endothelial cells to leptin may have beneficial effects on the cardiovascular system by increasing the potential for the generation of bioavailable NO.

In contrast, long-term (12 h) exposure of endothelial cells to leptin decreased CaI-stimulated bioavailable NO despite a twofold increase in eNOS expression in the endothelium. This apparent paradox can be explained by the enhanced generation of \( \text{O}_2^- \) and \( \text{ONOO}^- \) after long-term exposure of the HUVEC to leptin. Indeed, a wide variety of proatherogenic risk factors like obesity and other cardiovascular disease states can be associated with increased oxidative stress and diminished NO bioavailability (10).

To better specify the relationship between leptin, eNOS expression, and obesity in animals, another portion of our experiments involved an inbred C57BL/6J strain of obese mice. These mice were not obese on a standard chow diet but became obese and developed hyperleptinemia when fed a high-fat diet (28). We clearly documented the exponential increase in serum leptin concentration with body weight of obese mice. The increased leptin was especially dramatic when the weight of obese mice exceeded the maximal weight of low-calorie diet mice.

The present study provides direct evidence that obesity causes an increase in leptin level that inversely correlates with NO bioavailability in the aortic endothelium and with cGMP in the aortic wall. Simultaneously, with the suppression of NO production in obese mice, we registered the considerable in-

![Fig. 7](image_url1)

**A** eNOS expression in aortic endothelial cells of C57BL/6J mice maintained on a low-calorie diet (control, white bars) and high-calorie diet (60 kcal fat; black bars) for 105 Days. **B** cGMP content in aortic wall of C57BL/6J mice maintained on a low-calorie diet (control, black bars) and a high-calorie diet (60 kcal fat; white bars) \( *P < 0.005, n = 4 \) to 5 mice. **C** Cal (1 μmol/l)-stimulated NO, \( \text{O}_2^- \), and \( \text{ONOO}^- \) release from aortic endothelial cells of C57BL/6J mice maintained for 105 days on low-calorie diet (black bars) or high-calorie (60 kcal fat, white bars) with or without supplementation with l-arginine (100 mg·kg\(^{-1}\)·day\(^{-1}\)) or l-arginine (100 mg·kg\(^{-1}\)·day\(^{-1}\)) and sepiapterin (10 mg·kg\(^{-1}\)·day\(^{-1}\)). \( *P < 0.01 \) vs. control (low-calorie diet); \( #P < 0.05 \) vs. obese mice without l-arginine supplementation; \( n = 4 \) to 5 mice.

![Fig. 8](image_url2)

**A** the ratio of NO concentration to the concentration of ONOO\(^-\) \( (R = \frac{[\text{NO}]}{[\text{ONOO}^-]}) \). NO and ONOO\(^-\) releases were stimulated by Cal (1 μmol/l) from endothelial cells of the aortas of C57BL/6J mice maintained for 105 days on low-calorie diet (1) or on high-calorie (60 kcal fat) diet (2) with supplementation of l-arginine (100 mg·kg\(^{-1}\)·day\(^{-1}\)) (3) or with supplementation with l-arginine (100 mg·kg\(^{-1}\)·day\(^{-1}\)) and sepiapterin (10 mg/kg/day) (4). \( *P < 0.01 \) vs. control (low-calorie diet); \( #P < 0.05 \) vs. obese mice without supplementation with l-arginine or l-arginine and sepiapterin; \( n = 5 \) mice.

**B** l-arginine concentration in serum and aortic endothelium of C57BL/6J mice maintained for 105 days on low-calorie diet (1) or on high-calorie (60 kcal fat) without (2) or with supplementation with l-arginine (100 mg·kg\(^{-1}\)·day\(^{-1}\)) (3). \( *P < 0.01 \) vs. control (low-calorie diet); \( #P < 0.05 \) vs. obese mice without supplementation with l-arginine; \( n = 5 \) mice.
crease in $O_2^-$ and ONOO$^-$ generation. The most probable mechanism for the NO release diminishment in this case, like in the experiments with cell culture, is the overproduction of $O_2^-$, resulting in the fast reaction with NO to form ONOO$^-$. This direct quantitative data are concordant with previous indirect studies that indicate the role of oxidative stress in obesity (11, 27). Dobrian et al. (9) recently reported that there was a $\sim$1.8-fold decrease of the plasma and urine nitrate and nitrite levels and the same increase in $O_2^-$ production from the aortas from obese Sprague-Dawley rats compared with lean ones. At the same time, the eNOS expression in aorta of obese animals was increased $\sim$8-fold compared with control. This apparent discrepancy can be clearly explained with the data presented in this study.

To explain the differences in the effect of leptin exposure on NO production versus $O_2^-$ and ONOO$^-$ production, it may help to realize that the NO sensor used in this study detects only the net concentration of NO (i.e., NO that is not consumed in fast chemical reactions and can freely diffuse to a target cell and trigger cGMP production). This net concentration depends not only on the activity of eNOS but also on the production of $O_2^-$. The rapid Ca$^2+$ stimulation of eNOS produces a large NO release accompanied by $O_2^-$ production, suggesting that some of the production of $O_2^-$ is calcium dependent, similar to the production of NO by eNOS.

Also, it is helpful to realize that both membrane-bound eNOS and NAD(P)H oxidase may contribute to the overall generation of ONOO$^-$ (23, 33). At low to moderate activity of eNOS, the main generator of $O_2^-$ in the vascular wall is NAD(P)H oxidase (23). In this study, after rapid Ca$^2+$-stimulation of eNOS, the contribution of eNOS generated $O_2^-$ to the ONOO$^-$ production was about three times higher than the contribution of NAD(P)H oxidase generated $O_2^-$. Furthermore, endothelial mitochondria can be a generator of $O_2^-$ and subsequently ONOO$^-$. But, the nanotechnological set-up used in this study is not suitable for measuring $O_2^-$ or ONOO$^-$ directly in mitochondria without disturbing cell membranes and stimulating NO and $O_2^-$ release. These studies elucidate mainly NO, $O_2^-$ generation by membrane-bound eNOS.

One of the most probable mechanisms underlying the leptin-stimulated endothelial dysfunction may be the imbalance between eNOS expression and intracellular l-arginine and/or tetrahydrobiopterin level. According to our results, leptin significantly increases the amount of eNOS protein in HUVEC and endothelium of obese mice. This enhanced eNOS expression in sustained hyperleptinemia was also recently observed by other investigators (9, 26, 36). Coincident with the increase in eNOS expression, we observed a tendency toward diminishment of the l-arginine concentration in leptin-incubated HUVEC and a significantly diminished l-arginine level in obese animals with hyperleptinemia. l-Arginine is required not only as a substrate for NO synthesis but also as a stabilizer of eNOS, preventing it from uncoupling. The relative l-arginine and/or eNOS cofactor tetrahydrobiopterin insufficiencies result in the uncoupling of the oxidative and reductive domains of eNOS, which results in $O_2^-$ generation (6, 17). The net effect of the reaction between NO and increased $O_2^-$ caused the diminishment of NO bioavailability. The [NO]/[ONOO$^-$] ratios were significantly decreased in both HUVEC treated with leptin for 12 h and aortic endothelium of obese mice. Furthermore, in our experiments, the obese mice treated with l-arginine or l-arginine and sepiapterin (a precursor of tetrahydrobiopterin) showed significantly higher NO production and lower $O_2^-$ and ONOO$^-$ release from the aortic endothelium compared with the untreated obese mice.

The most potentially damaging property of the eNOS dimer (called anticooperative bonding) occurs because the two eNOS monomers can act independently (24). During moderate l-arginine or tetrahydrobiopterin deficiencies, after activation, it is very probable that one monomer of an eNOS dimer could be coupled and rapidly making NO, while the adjacent monomer of the same eNOS dimer is uncoupled and rapidly making $O_2^-$. The near diffusion-limited reaction of $O_2^-$ with NO to form ONOO$^-$ is even faster than the dismutation of $O_2^-$ by superoxide dismutase. Unlike NO and $O_2^-$, which are not strong oxidants, ONOO$^-$ is a potent oxidant. ONOO$^-$ is relatively unstable in its anionic form. However, when protonated, the peroxynitrous acid (HONO$^+$) at high concentrations may freely diffuse several cell diameters before it rearranges to form nitric acid (HNO$^+$) or undergoes homolytic or heterolytic cleavage to generate highly toxic oxidative species, including 'NO$_2^-$, NO$_2^+$, and 'OH. These products are among the most reactive and damaging species in biological systems. They may initiate a cascade of events leading to the oxidation of proteins, DNA, and lipids and finally resulting in cytotoxicity and cellular dysfunction (2). The sum of unfavorable events developed in cells due to ONOO$^-$ hyperproduction is collectively called “nitrooxidative stress,” which is a major component of oxidative stress.

There is another contributing mechanism that may partially help explain our results. It has been suggested that increased free fatty acid concentration in obesity may be related to increased levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase (2). l-Arginine competes with ADMA for the eNOS enzyme (29). Therefore, an increase in NO production after supplementation with l-arginine to obese mice may be due in part to an increase of the arginine-to-ADMA ratio in endothelial cells and the reduction of the ADMA inhibitory effect.

In summary, we have provided direct evidence that, despite leptin-induced increased eNOS expression, bioavailable NO is reduced, whereas $O_2^-$ and ONOO$^-$ levels are increased in obesity. Increased $O_2^-$ and ONOO$^-$ production that contributes to high oxidative/nitrooxidative stress in endothelial cells is most likely due to a discrepancy between enhanced eNOS expression and diminished intracellular l-arginine concentration in obesity. Thus long-term exposure of endothelium to leptin may lead to enzymatic uncoupling of the oxidative and reductive domains of eNOS. l-Arginine and sepiapterin treatment during the long term hyperleptinemia accompanying obesity increases l-arginine (and most likely tetrahydrobiopterin) concentration in aortic endothelium and significantly reduces eNOS uncoupling (decreasing $O_2^-$ and ONOO$^-$ production and preserving bioavailable NO). Leptin-induced [NO]/[ONOO$^-$] imbalance in the vascular endothelium observed in obesity is similar to the redox state that has been reported in other endothelium-impaired function disorders, including hypertension, atherosclerosis, diabetes, and others (9, 32, 33).
LEPTIN-INDUCED ENDOTHelial DYSFUNCTION IN OBESITY

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