0013-7227/07/$15.00/0
Printed in U.S.A.

Endocrinology 148(10):4545–4547

doi: 10.1210/en.2007-0866

NO to Obesity: Does Nitric Oxide Regulate Fat Oxidation and Insulin Sensitivity?

Nitric oxide (NO) is a free radical gas that acts as a pleiotropic transmitter in many diverse functions (1). Most importantly, its production by endothelial NO synthase (eNOS) mediates vasodilation and inhibits thrombocyte aggregation. In other cells such as immune cells or neurons, NO is produced by the inducible NO synthase (iNOS) and neuronal NO synthase (nNOS), and mediates an inflammatory response or acts as an atypical neurotransmitter, respectively. Furthermore, it was suggested that NO plays an important role in the regulation of energy balance because administration of the nonspecific NO synthase inhibitor *N*-nitro-L-arginine-methyl ester (L-NAME) reduced weight gain and food intake in mice (2). In nNOS-knockout mice, the appetite-suppressant activity of leptin was markedly reduced, suggesting that NO is a downstream signal of leptin (3). Because deletion of the iNOS protected mice against high-fat diet-associated insulin resistance (4), it was also suggested that NO ("nitrosative stress") is a player in the pathogenesis of insulin resistance (5). However, such a link between NO and insulin sensitivity is not consistent with studies in eNOS knockout mice, in which deletion of one allele led to reduced insulin sensitivity in response to a high-fat diet (6), and with the concept that endothelial dysfunction is reciprocal to insulin sensitivity (7). Such unresolved inconsistencies may be the reason why NO has not yet made its way into the current textbooks as a major player in the regulation of adiposity and insulin sensitivity or a pathogenetic factor for obesity and diabetes.

The report by Tsuchiya *et al*. (8) in this issue of *Endocrinology* readdresses the role of NO in the regulation of energy balance, supports the view that NO synthase is involved in this process, and offers potential mechanistic links with obesity and diabetes. The authors show that a chronic application of the established NO synthase inhibitor L-NAME significantly reduced body weight gain in mice. Intriguingly, this effect was larger in mice on a high-fat diet; here, L-NAME markedly reduced the volume of fat cells as well as triglyceride accumulation. Consistent with this phenomenon, L-NAME reversed the detrimental effects of the high-fat diet on hepatic triglyceride content, glucose tolerance, and *in vivo* insulin sensitivity. The authors conclude that these effects are due to increased energy dissipation because L-NAME elevated the mRNA levels of uncoupling proteins 1 and 3 in muscle and brown adipose tissue, respectively. Most interestingly, L-NAME also increased the expression of peroxisome proliferator-activated receptor δ in muscle. This transcription factor has recently been shown to be of central importance for fat oxidation in muscle (9). In addition, generation of NO in mitochondria by the mitochondrial NO synthase reduces respiration and oxygen consumption (10). Thus, it appears reasonable to assume that the primary effect of a chronic inhibition of NO production by L-NAME is an enhanced oxidation of fatty acids (Fig. 1).

The evidence presented by Tsuchiya *et al*. (8) demonstrates that inhibition of NO synthesis partially protects mice from the adipogenic effects of a high-fat diet. However, the study leaves a few questions unanswered. Most importantly, the here presented data do not provide any direct evidence for an increase in energy expenditure or an enhanced oxidation of fatty acids in muscle in response to L-NAME. Furthermore, there is a striking difference between the present results and those by Morley and Flood (2). In the previous study, administration of L-NAME for 9 d produced a significant inhibition of food intake in obese mice. In contrast, reduced adiposity observed in the present study could not be explained by reduced caloric intake. However, it should be noted that small changes of daily food intake or energy expenditure may lead to large effects on adiposity over time. Morbidly obese mice such as the New Zealand obese mouse consume approximately 10% more calories and have a slightly lower body temperature (by 0.7 C) than lean controls (11); quantification of even smaller differences is technically challenging. Finally, it has to be assumed that the effect of L-NAME represents the net result of a nonspecific inhibition of eNOS, iNOS, and other isotypes. According to the previous data, these tissue-specific isotypes appear to exert diverging effects on insulin sensitivity (4, 6). To establish convincingly the role of NO in the regulation of energy balance, these points require clarification by additional experimentation involving tissue-specific deletion of NO synthesis, and the direct demonstration of altered fatty acid metabolism and energy expenditure. In addition, opposite effects of an exposure to increased NO levels would strengthen the concept considerably.

Enzymes whose inhibition reduces high-fat induced adiposity and insulin resistance are potential targets for a pharmacological intervention in obesity. Unfortunately, global inhibition of NO synthesis will lead to undesired cardiovascular and other side effects. In the present study, L-NAME produced a substantial increase of blood pressure, from 112–144 mm Hg, and a compensatory reduction in heart rate. Such side effects would certainly represent a difficult obstacle for a chronic drug therapy of obesity. Thus, without dissociation of the vascular effects from those on energy balance, possibly by a selective and tissue-

Abbreviations: eNOS, Endothelial NO synthase; iNOS, inducible NO synthase; L-NAME, *N*-nitro-L-arginine-methyl ester; nNOS, neuronal NO synthase; NO, nitric oxide.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

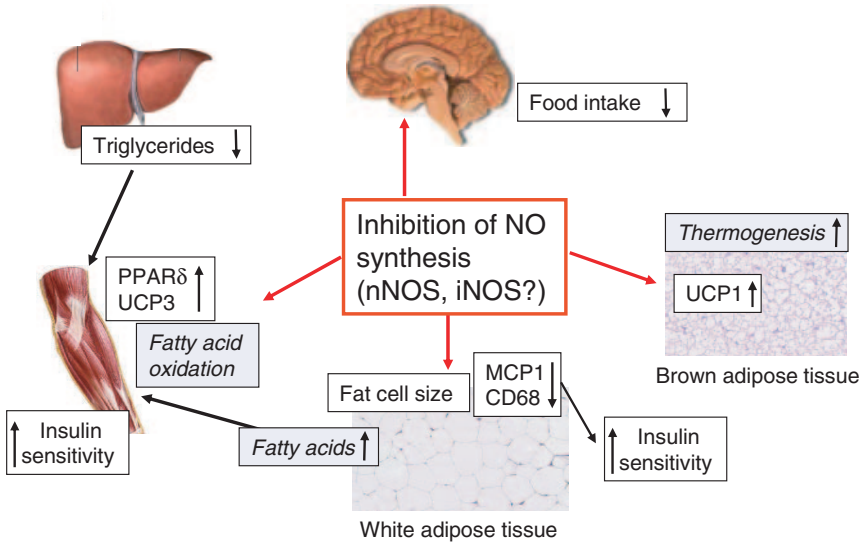

FIG. 1. Effects of a chronic inhibition of NO synthesis by L-NAME on parameters of adiposity and energy balance in mice. The synopsis depicts parameters assayed in Refs. 2 and 8. *Light blue boxes and italics* indicate conclusions. MCP, Monocyte chemoattracting protein; PPAR, peroxisome proliferator-activated receptor; UCP, uncoupling protein.

specific inhibition of iNOS in muscle and/or adipose tissue, it is hard to envision a therapeutic benefit of this approach.

After the discovery of leptin more than 10 yr ago, obesity research has been one of the fastest moving fields, and has provided a large body of knowledge as to the neuroendocrine and hormonal control of hunger, satiety, and energy expenditure (12). Furthermore, a substantial number of genes involved in the regulation of fat storage has been identified through their knockout phenotypes. In contrast, there is much less progress in the search for new therapeutics of obesity, and very few "druggable" targets have been identified so far. The few candidates that made it through large clinical trials, such as cannabinoid receptor antagonists, struggle due to their limited efficacy, potential side effects, and small therapeutic window. However, the report by Tsuchiya *et al*. (8) reminds us that a large number of unknown or untested antiobesity targets exists, awaiting detailed examination. From genome-wide mutagenesis or knockout screens in *Drosophila melanogaster* or *Caenorhabditis elegans*, respectively, we can estimate that the total number of genes involved in the regulation of energy balance is in the hundreds (13, 14). Data from mice are consistent with this estimate. A recent metaanalysis of genome-wide searches for susceptibility loci (quantitative trait loci) indicated that at least 50 chromosomal segments have already been identified that harbor alleles associated with adiposity or leanness (15). Identification of these by positional cloning is difficult and time consuming but appears feasible based on rapidly evolving technology (16). Furthermore, genome-wide association studies in humans carry the potential to lead to new insights into the mechanisms of triglyceride storage; proof of principle has been provided by the recent discovery of the fat mass and obesity associated (FTO) gene (17). Thus, identification and examination of druggable targets of antiobesity therapy remain to be high-hanging fruits. However, in the face of the threat posed by the rapidly increasing incidence of obesity and diabetes, we certainly cannot afford to wait for them to fall into our lap.

Hans-Georg Joost and Matthias H. Tschöp
Department of Pharmacology (H.-G.J.)
German Institute of Human Nutrition Potsdam-Rehbruecke
Arthur-Scheunert-Allee 114-116
D-14558 Nuthetal, Germany
and Department of Psychiatry (M.H.T.)
Obesity Research Centre and Genome Research Institute
University of Cincinnati College of Medicine
Cincinnati, Ohio 45237

Acknowledgments

Received June 27, 2007. Accepted July 9, 2007.

Address all correspondence and requests for reprints to: Dr. Hans-Georg Joost, German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114-116, D-14558 Nuthetal, Germany. E-mail: joost@mail.dife.de.

Disclosure Summary: The authors have nothing to declare.

References

1. **Bredt DS** 1999 Endogenous nitric oxide synthesis: biological functions and pathophysiology. Free Radic Res 31:577–596
2. **Morley JE, Flood JF** 1994 Effect of competitive antagonism of NO synthetase on weight and food intake in obese and diabetic mice. Am J Physiol 266: R164–R168
3. **Calapai G, Corica F, Corsonello A, Sautebin L, Di Rosa M, Campo GM, Buemi M, Mauro VN, Caputi AP** 1999 Leptin increases serotonin turnover by inhibition of brain nitric oxide synthesis. J Clin Invest 104:975–982
4. **Perreault M, Marette A** 2001 Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. Nat Med 7:1138–1143
5. **Kaneki M, Shimizu N, Yamada D, Chang K** 2007 Nitrosative stress and pathogenesis of insulin resistance. Antioxid Redox Signal 9:319–329
6. **Cook S, Hugli O, Egli M, Menard B, Thalmann S, Sartori C, Perrin C, Nicod P, Thorens B, Vollenweider P, Scherrer U, Burcelin R** 2004 Partial gene deletion of endothelial nitric oxide synthase predisposes to exaggerated high-fat diet-induced insulin resistance and arterial hypertension. Diabetes 53:2067–2072
7. **Kim JA, Montagnani M, Koh KK, Quon MJ** 2006 Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 113:1888–1904
8. **Tsuchiya K, Sakai H, Suzuki N, Iwashima F, Yoshimoto T, Shichiri M, Hiratay Y** 2007 Chronic blockade of nitric oxide synthesis reduces adiposity and improves insulin resistance in high fat-induced obese mice. Endocrinology 148:4548–4556

9. **Barish GD, Narkar VA, Evans RM** 2006 PPAR δ: a dagger in the heart of the metabolic syndrome. J Clin Invest 116:590–597
10. **Giulivi C, Kato K, Cooper CE** 2006 Nitric oxide regulation of mitochondrial oxygen consumption I: cellular physiology. Am J Physiol Cell Physiol 291: C1225–C1231
11. **Jürgens HS, Schürmann A, Kluge R, Ortmann S, Klaus S, Joost HG, Tschöp MH** 2006 Hyperphagia, lower body temperature, and reduced running wheel activity precede development of morbid obesity in New Zealand obese mice. Physiol Genomics 25:234–241
12. **Coll AP, Farooqi IS, O'Rahilly S** 2007 The hormonal control of food intake. Cell 129:251–262
13. **Dohrmann CE** 2004 Target discovery in metabolic disease. Drug Discov Today 9:785–794
14. **Ashrafi K, Chang FY, Watts JL, Fraser AG, Kamath RS, Ahringer J, Ruvkun G** 2003 Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. Nature 421:268–272
15. **Wuschke S, Dahm S, Schmidt C, Joost HG, Al-Hasani H** 2007 A meta-analysis of quantitative trait loci associated with body weight and adiposity in mice. Int J Obes (Lond) 31:829–841
16. **Buchmann J, Meyer C, Neschen S, Augustin R, Schmolz K, Kluge R, Al-Hasani H, Jurgens H, Eulenberg K, Wehr R, Dohrmann C, Joost HG, Schurmann A** 2007 Ablation of the cholesterol transporter adenosine triphosphate-binding cassette transporter G1 reduces adipose cell size and protects against diet-induced obesity. Endocrinology 148:1561–1573
17. **Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI** 2007 A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316:889–894

Endocrinology **is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**