Amino Acids and Mitochondrial Biogenesis

Enzo Nisoli, MD, PhD,[a,b,*] Valeria Cozzi, PhD,[c] and Michele O. Carruba, MD, PhD[a,b]

Mitochondria are sources of energy production through their role in producing adenosine triphosphate for cell metabolism. Defective mitochondrial biogenesis and function play relevant roles in the pathophysiology of relevant diseases, including obesity, diabetes mellitus, myopathies, and neurodegenerative diseases. Their function is the product of synthesis of macromolecules within the mitochondria and import of proteins and lipids synthesized outside the organelles. Both are required for mitochondrial proliferation and may also facilitate the growth of preexisting mitochondria. Recent evidence indicates that these events are regulated in a complex way by several agonists and environmental conditions, through activation of specific signaling pathways and transcription factors. Nitric oxide (NO) appears to be a novel modulator of mitochondrial biogenesis. High levels of NO acutely inhibit cell respiration by binding to cytochrome *c* oxidase. Conversely, chronic, low-grade increases of NO stimulate mitochondrial biogenesis in diverse cell types. Here, we suggest that some types of nutrients, including specific mixtures of amino acids, may improve mitochondrial biogenesis and energy production in energy-defective conditions by increasing endothelial NO synthase expression. **(Am J Cardiol 2008;101[suppl]:22E–25E)**

Mitochondria first captured the attention of cell physiologists some 50 years ago. The elucidation of their role in energy production—the passing of electrons along the series of respiratory enzyme complexes in the inner mitochondrial membrane, and the ensuing buildup of a transmembrane proton gradient that drives adenosine triphosphate (ATP) synthase—is among the most fascinating enterprises in the history of science. Recent evidence suggests that this process occurs in organelles that are not static. Mitochondria are in constant movement within cells, and numerous fusion/fission events can take place. These are accompanied by variations in mitochondrial size, number, and mass that are triggered by a variety of physiologic stimuli and differentiation states. Indeed, >1,000 genes and ~ 20% of cellular proteins are involved, and a complex regulatory network,[1,2] including factors such as the transcription factor peroxisome proliferator-activated receptor−γ coactivator-1α (PGC-1α), nuclear respiratory factors (NRF-1 and NRF-2), and mitochondrial DNA transcription factor A (Tfam), coordinates their behavior.[2] Recently, NO has been found to affect different mitochondrial functions. NO plays essential roles in the modulation of vascular tone,[3] neurotransmission,[4,5] and the immune system.[6,7] Generated by a family of NO synthases (NOS), NO binds to soluble guanylyl cyclase and to the mitochondrial cytochrome *c* oxidase and thus activates cellular signaling cascades. NO interacts with soluble guanylyl cyclase allosterically to increase cyclic guanosine monophosphate concentrations, leading to cyclic guanosine monophosphate−dependent responses recently reviewed elsewhere.[8] Binding of NO with cytochrome *c* oxidase decreases its affinity for oxygen, thus affecting mitochondrial electron flux and ATP synthesis.[9] The interaction of NO with cytochrome *c* oxidase may also have pathophysiologic implications that are only now beginning to be understood.

Nitric Oxide as a Regulator of Mitochondrial Biogenesis and Function

NO is synthesized from L-arginine and oxygen by NOS in almost all mammalian cells.[10,11] To date, 3 distinct cellular isoforms of NOS have been identified: the endothelial and neuronal isoforms are regulated by second messengers; inducible NOS is induced by cytokines and bacterial products. All 3 isoforms can be regulated by transcriptional and posttranscriptional mechanisms and are constitutively expressed in certain tissues.[10,11] Moreover, a mitochondrial NOS isoform has been described as a constitutive protein of the mitochondrial inner membrane that generates NO in a Ca^{2+}-dependent reaction,[12] even if its existence has been doubted by others.[13] It is notable that endothelial NOS (eNOS) is attached to the outer mitochondrial membrane in neurons and endothelial cells,[14,15] indicating that mitochondria may regulate NOS activity and, conversely, that endothelial NOS (eNOS) may regulate mitochondrial function.

[a]Department of Pharmacology, Chemotherapy and Medical Toxicology, University of Milan, School of Medicine, Milan, Italy; [b]Istituto Auxologico Italiano, Milan, Italy; and [c]Department of Preclinical Sciences, LITA Vialba, University of Milan, Milan, Italy.

Statement of author disclosure: Please see the Author Disclosures section at the end of this article.

*Address for reprints: Enzo Nisoli, MD, PhD, Department of Pharmacology, Chemotherapy and Medical Toxicology, University of Milan School of Medicine, via Vanvitelli 32, 20129 Milan, Italy.

E-mail address: enzo.nisoli@unimi.it.

0002-9149/08/$ – see front matter
doi:10.1016/j.amjcard.2008.02.077

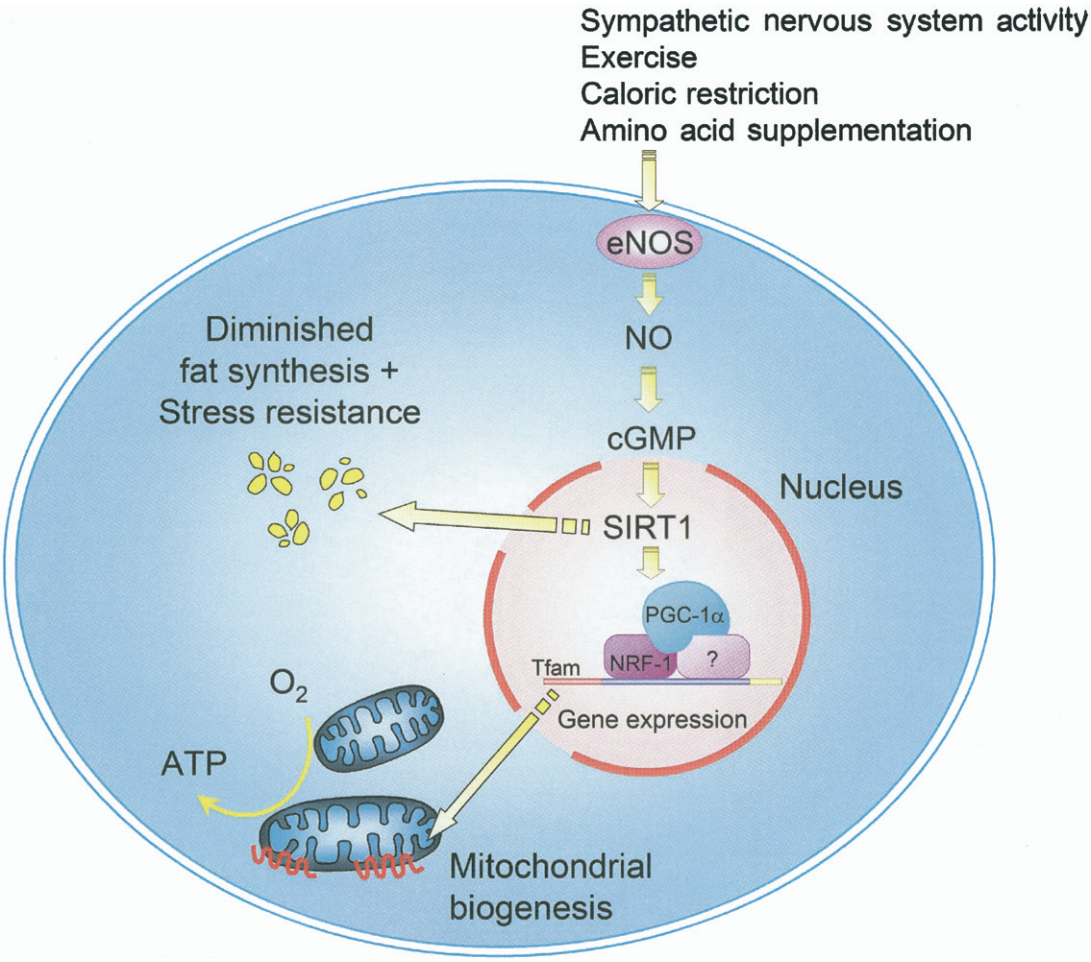

Figure 1. Different stimuli, including activation of the sympathetic nervous system, physical activity, caloric restriction, and amino acid supplementation may induce cyclic guanosine monophosphate (cGMP) production through an increase in endothelial nitric oxide synthase (eNOS) levels in skeletal and cardiac muscle. Mitochondrial genes involved in mitochondrial biogenesis are upregulated as a consequence, leading to increased mitochondrial biogenesis and function with adenosine triphosphate (ATP) production. NO = nitric oxide; NRF-1 = nuclear respiratory factor-1; PGC-1α = peroxisome proliferator-activated receptor–γ coactivator-1α; SIRT1 = sirtuin-1; Tfam = mitochondrial DNA transcription factor A.

NO may act on mitochondria at several levels. Because it regulates blood flow to tissues, it supplies respiratory substrates to mitochondria and redistributes heat generated by respiring mitochondria indirectly; it regulates the binding to and release of oxygen from hemoglobin,[16] and thus the supply of oxygen to mitochondria directly. NO also regulates mitochondrial function by binding to cytochrome *c* oxidase, the terminal enzyme in the electron-transport chain. It competes with oxygen, inhibiting the activity of the enzyme,[17–19] and thus negatively regulates mitochondrial oxidative phosphorylation, particularly at the low oxygen concentrations usually found in tissues.[20]

Moreover, treatment of various cells with NO donors increases their mitochondrial DNA content, and this is sensitive to removal of NO by the NO scavenger oxyhemoglobin.[21] This effect occurs through increased expression of PGC-1α (the principal regulator of mitochondrial biogenesis), NRF-1, NRF-2, and Tfam[2] (Figure 1). It depends on the secondary messenger cyclic guanosine monophosphate through which NO frequently acts. Such NO-dependent mitochondrial biogenesis occurs in numerous cell types and is not restricted to a specific cell lineage or species.[21,22] An important aspect of this effect is that it generates functionally active mitochondria capable of generating ATP through oxidative phosphorylation.[22] The significance of this profound change in energy metabolism remains to be investigated. However, mitochondrial activity is known to play critical roles in various processes, such as the switch of skeletal muscle fibers from glycolytic to oxidative metabolism,[23] and the regeneration of cardiac and skeletal muscles.[24,25]

Defective Nitric Oxide System and Mitochondrial Biogenesis Lead to Metabolic Disorders

Studies of eNOS$^{-/-}$ mice have demonstrated an obligatory role of endothelial NOS in mitochondrial biogenesis. Brown fat from these mice is functionally inactive, and exposure of the animals to cold blunts mitochondrial biogenesis and,

unlike in wild-type animals, results in a steep decline in core temperature.[21] In addition, deletion of endothelial NOS is sufficient to reduce mitochondrial mass even in tissues that have basal expression levels of neuronal NOS, and possibly inducible NOS, such as the brain, liver, muscle, and heart.

The importance of NO as a mitochondrial biogenetic stimulus has broad implications for pathology. Impairment of mitochondrial function is associated with neurodegenerative diseases, neuromuscular disorders, liver and heart failure, and type 2 diabetes.[24,26–29] The potential role of NO in diabetes and obesity is particularly relevant. In eNOS$^{-/-}$ mice, oxygen consumption (an indicator of metabolic rate) is decreased, indicating impaired brown fat–dependent thermogenesis. In genetic models of obesity, defective energy expenditure is linked to increased food intake and body weight gain. Compared with wild-type mice, eNOS$^{-/-}$ mice exhibit similar levels of food consumption, but have greater body weight. Their increased body weight could be owing to higher feed efficiency (ie, weight gain/food intake) caused by defective energy expenditure.[21] Therefore, generating new metabolically active mitochondria might be an approach to treatment of disorders in which impaired energy expenditure is evident.

Interestingly, a large number of studies during the last 70 years have demonstrated that caloric restriction, without malnutrition, is the only approach that extends life span in numerous organisms, from yeast to rodents and possibly primates. In mammals, caloric restriction delays the onset of age-associated diseases, including cancer, atherosclerosis, and diabetes. We have recently provided evidence that caloric restriction induces eNOS and cyclic guanosine monophosphate, and that the resulting surge of NO activates expression of a broad array of mitochondrial proteins, increases production of mitochondrial DNA, with higher respiration and ATP levels, in different tissues and organs, including white and brown fat, brain, liver, and heart.[30]

On the other hand, malnutrition and cachexia may induce mitochondrial deficits. In fact, common micronutrient deficiencies accelerate mitochondrial decay. For example, heme biosynthesis occurs predominantly in the mitochondria. Interference with heme synthesis causes specific loss of complex IV of the mitochondrial respiratory chain, with consequent release of oxidants.[31] Moreover, iron deficiency (note that 25% of menstruating women in the United States ingest <50% of the recommended daily allowance of iron) also causes release of oxidants and mitochondrial decay.[32] Vitamin B_6 deficiency (10% of Americans ingest <50% of the recommended daily allowance of this nutrient) also can cause heme deficiency. The consequences appear to be accelerated aging and neural decay. Finally, a deficiency of vitamins B_{12}, B_6, B_9 (folic acid), B_3 (niacin), C, or E, or inadequate intake of iron or zinc, appears to mimic radiation in damaging DNA by causing single- and double-strand breaks, oxidative lesions, or both, with mitochondrial deficit.[33]

Amino Acids and Nitric Oxide Production

With regard to stimulation of synthesis of endogenous NO, by use of a substrate for NOS, L-arginine seems important. Intriguingly, whether exogenous L-arginine promotes endothelial synthesis of NO, reverses endothelial dysfunction, and therefore improves the clinical status of all patients is still uncertain. Dietary supplementation with L-arginine, which increases serum NO concentration and PGC-1α expression in white adipose tissue, has been shown to reduce fat mass in Zucker diabetic Fatty rats 4–10 weeks after the treatment initiation compared with control rats.[34] Preliminary results seem to suggest that a specific mixture of amino acid (AA) supplements may increase endothelial NOS expression and NO production, leading to improvement of the mitochondrial biogenesis and function that are defective in several tissues, including fat and skeletal and cardiac muscle, of aged (12-month-old) male mice (E. Nisoli and M. O. Carruba, unpublished results, 2007) (Figure 1).

Conclusion

Defective mitochondrial biogenesis and function play relevant roles in the pathophysiology of relevant diseases, including obesity, diabetes, myopathies, and neurodegenerative diseases. Our results imply that oral supplementation with a specific mixture of AAs may be an alternative approach to be investigated for treatment of metabolic syndrome and prevention of the cardiovascular risk in aging.

Author Disclosures

The authors who contributed to this article have disclosed the following industry relationships:

Enzo Nisoli, MD, PhD, has no financial arrangement or affiliation with a corporate organization or a manufacturer of a product discussed in this supplement.

Valeria Cozzi, PhD, has no financial arrangement or affiliation with a corporate organization or a manufacturer of a product discussed in this supplement.

Michele O. Carruba, MD, PhD, has no financial arrangement or affiliation with a corporate organization or a manufacturer of a product discussed in this supplement.

1. Attardi G, Schatz G. Biogenesis of mitochondria. *Annu Rev Cell Biol* 1988;4:289–333.
2. Kelly DP, Scarpulla RC. Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. *Genes Dev* 2004;15:357–368.
3. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987;327:524–526.
4. Garthwaite J, Charles SL, Chess-Williams R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests a role as intercellular messenger in the brain. *Nature* 1988;336:385–387.
5. Knowles RG, Palacios M, Palmer RMJ, Moncada S. Formation of nitric oxide from L-arginine in the central nervous system: a transduc-

tion mechanism for stimulation of the soluble guanylate cyclase. *Proc Natl Acad Sci U S A* 1989;86:5159–5162.
6. Hibbs JB Jr, Taintor RR, Vavrin Z, Rachlin EM. Nitric oxide: a cytotoxic activated macrophage effector molecule. *Biochem Biophys Res Commun* 1988;157:87–94.
7. Stuehr DJ, Nathan CF. Nitric oxide: a macrophage product responsible for cytostasis and respiratory inhibition in tumour target cells. *J Exp Med* 1989;169:1543–1555.
8. Schlossmann J, Hofmann F. cGMP-dependent protein kinases in drug discovery. *Drug Discov Today* 2005;10:627–634.
9. Moncada S, Bolanos JP. Nitric oxide, cell bioenergetics and neurodegeneration. *J Neurochem* 2006;97:1676–1689.
10. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991;43:109–142.
11. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. *Biochem J* 2001;357:593–615.
12. Ghafourifar P, Cadenas E. Mitochondrial nitric oxide synthase. *Trends Pharmacol Sci* 2005;26:190–195.
13. Lacza Z, Pankotai E, Csordas A, Gero D, Kiss L, Horvath EM, Kollai M, Busija DW, Szabo C. Mitochondrial NO and reactive nitrogen species production: does mtNOS exist? *Nitric Oxide* 2006;14:162–168.
14. Henrich M, Hoffmann K, Konig P, Gruss M, Fischbach T, Godecke A, Hempelmann G, Kummer W. Sensory neurons respond to hypoxia with NO production associated with mitochondria. *Mol Cell Neurosci* 2002;20:307–322.
15. Gao S, Chen J, Brodsky SV, Huang H, Adler S, Lee JH, Dhadwal N, Cohen-Gould L, Gross SS, Goligorsky MS. Docking of endothelial nitric oxide synthase (eNOS) to the mitochondrial outer membrane: a pentabasic amino acid sequence in the autoinhibitory domain of eNOS targets a proteinase K-cleavable peptide on the cytoplasmic face of mitochondria. *J Biol Chem* 2004;279:15968–15974.
16. Wolzt M, MacAllister RJ, Davis D, Feelisch M, Moncada S, Vallance P, Hobbs AJ. Biochemical characterization of *S*-nitrosohemoglobin: mechanisms underlying synthesis, NO release, and biological activity. *J Biol Chem* 1999;274:28983–28990.
17. Cleeter MW, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AH. Reversible inhibition of cytochrome *c* oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide: implications for neurodegenerative diseases. *FEBS Lett* 1994;345:50–54.
18. Brown GC, Cooper CE. Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Lett* 1994;356:295–298.
19. Clementi E, Brown GC, Feelisch M, Moncada S. Persistent inhibition of cell respiration by nitric oxide: crucial role of *S*-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc Natl Acad Sci U S A* 1998;95:7631–7636.
20. Clementi E, Brown GC, Foxwell N, Moncada S. On the mechanism by which vascular endothelial cells regulate their oxygen consumption. *Proc Natl Acad Sci U S A* 1999;96:1559–1562.
21. Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, Bracale R, Valerio A, Francolini M, Moncada S, Carruba MO. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science* 2003;299:896–899.
22. Nisoli E, Falcone S, Tonello C, Cozzi V, Palomba L, Fiorani M, Pisconti A, Brunelli S, Cardile A, Francolini M, et al. Mitochondrial biogenesis by NO yields functionally active mitochondria in mammals. *Proc Natl Acad Sci U S A* 2004;101:16507–16512.
23. Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, et al. Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. *Nature* 2002;418:797–801.
24. Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP. Peroxisome proliferator-activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest* 2000;106:847–856.
25. Stamler JS, Meissner G. Physiology of nitric oxide in skeletal muscle. *Physiol Rev* 2001;81:209–237.
26. Kopecky J, Clarke G, Enerback S, Spiegelman BM, Kozak LP. Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. *J Clin Invest* 1995;96:2914–2923.
27. Hansford RG, Tsuchiya N, Pepe S. Mitochondria in heart ischaemia and aging. *Biochem Soc Symp* 1999;66:141–147.
28. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc Natl Acad Sci U S A* 2003;100:8466–8471.
29. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstråle M, Laurila E, et al. PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 2003;34:267–273.
30. Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L, Falcone S, Valerio A, Cantoni O, Clementi E, Moncada S, Carruba MO. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* 2005;310:314–317.
31. Atamna H, Liu J, Ames BN. Heme deficiency selectively interrupts assembly of mitochondrial complex IV in human fibroblasts: relevance to aging. *J Biol Chem* 2001;276:48410–48416.
32. Walter PB, Knutson MD, Paler-Martinez A, Lee S, Xu Y, Viteri FE, Ames BN. Iron deficiency and iron excess damage mitochondria and mitochondrial DNA in rats. *Proc Natl Acad Sci U S A* 2002;99:2264–2269.
33. Ames BN, Wakimoto P. Are vitamin and mineral deficiencies a major cancer risk? *Nat Rev Cancer* 2002;2:694–704.
34. Fu WJ, Haynes TE, Kohli R, Hu J, Shi W, Spencer TE, Carroll RJ, Meininger CJ, Wu G. Dietary L-arginine supplementation reduces fat mass in Zucker diabetic fatty rats. *J Nutr* 2005;135:714–721.