# Mitochondrial biogenesis: which part of "NO" do we understand?

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**Summary**

**A recent paper by Nisoli et al.[1] provides the first evidence that elevated levels of nitric oxide (NO) stimulate mitochondrial biogenesis in a number of cell lines via a soluble guanylate-cyclase-dependent signaling pathway that activates PGC1α (peroxisome proliferator-activated receptor γ coactivator-1α), a master regulator of mitochondrial content. These results raise intriguing possibilities for a role of NO in modulating mitochondrial content in response to physiological stimuli such as exercise or cold exposure. However, whether this signaling cascade represents a widespread mechanism by which mammalian tissues regulate mitochondrial content, and how it might integrate with other pathways that control PGC1α expression, remain unclear.** ***BioEssays*** **25:538–541, 2003.**


## Introduction

Mitochondria produce the bulk of the ATP required for the normal function of most tissues. As such, the mitochondrial content of a given tissue largely reflects its specific demands for respiratory energy. In specialized tissues such as brown fat, which has a thermogenic role in neonates and hibernating animals, mitochondria express an uncoupling protein (UCP1) that functionally dissociates electron transport from oxidative phosphorylation, allowing the energy released in this process to be dissipated as heat.[2] Increased physiological demand for aerobic ATP production or the generation of heat results in mitochondrial proliferation. Several families of unrelated transcription factors, most notably the nuclear respiratory factors (NRFs), modulate the increased expression of nuclear-encoded mitochondrial proteins necessary for increased biogenesis of the organelle.[3] The activity of many of these transcription factors is in turn controlled by the co-activator PGC1α, and its related family members.[4] PGC1α also potentiates the expression of a large number of additional genes via its interactions with members of the nuclear hormone receptor superfamily.[5]

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Despite significant advances in our understanding of the role of transcription factors and co-activators in mediating the transcriptional control of respiratory gene expression, the mechanisms by which the expression of these proteins is in turn regulated remain largely unknown. Investigations of the control of mitochondrial biogenesis in the skeletal muscle of transgenic mice have demonstrated that increases in PGC1α levels and mitochondrial content are dependent on the activity of at least two proteins, CaMKIV[6] and AMP kinase,[7] which are regulated by changes in intracellular calcium concentrations and the ATP:AMP ratio, respectively. Thus, an attractive hypothesis is that the expression and activity of PGC1α and other determinants of mitochondrial content are controlled by effector proteins that are capable of monitoring changes in intracellular energy metabolism by virtue of their sensitivity to metabolic activity. If the ability to sense metabolic disturbances is in fact central to the regulation of PGC1α expression and activity, data from several studies suggest that increased mitochondrial content results from an integrated response to multiple as opposed to individual intracellular signals.[8] It is important to point out, however, that the relative roles of different intracellular signals in the regulation of mitochondrial content may be tissue-specific, a reflection of differences in metabolic organization and reliance upon aerobic ATP production in different cell types.

## A"NO"ther modulator of PGC1α?

NO is synthesized by a family of three nitric oxide synthases (NOSs), whose expression is cell-type specific. Two of these are constitutively expressed, calcium-sensitive isoforms (neuronal NOS and endothelial NOS [eNOS]), while the third is an inducible isoform (inducible NOS).[9] Originally described as an endothelial-derived relaxation factor, NO is now recognized as a molecule that exerts a myriad of regulatory as well as cytotoxic effects in different cell types.[10] The recent study by Nisoli et al.[1] describes yet another role for NO in stimulating mitochondrial biogenesis by upregulating the expression of PGC1α and mitochondrial content through a soluble guanylate cyclase (sGC)-sensitive, cGMP-dependent signaling pathway. Pharmacological manipulation of either NO or cGMP levels yielded comparable results in a number of unrelated cell lines, suggesting that this signaling pathway may represent a conserved mechanism by which many tissue types regulate mitochondrial content. However, a number of

important questions remain about the relative importance of altered intracellular NO production and its physiological role in modulating mitochondrial content.

One concern is whether the levels of NO that were achieved as a result of pharmacological manipulation are physiologically meaningful. Certainly the present and previous work[11] of the authors excluded the possibility that any of the observed effects were due to cytotoxic exposure to the agents in question. If, however, these cell lines cannot either generate or sustain the production of comparable amounts of NO in response to physiological stimuli, the in vivo significance of the signaling pathway with respect to the activation of PGC1α would be greatly diminished. Based on the length of time that the cells were treated (4–6 days) with relatively high concentrations of the NO donor S-nitrosoacetyl penicillamine (SNAP) (100 μM), and the fact that only nanomolar concentrations of NO have been detected in tissues (10–450 nM),[10] this appears to be a legitimate possibility.

SNAP-mediated increases in NO production have been shown to signal through cGMP-independent pathways.[12] In addition, several signal transduction pathways that regulate PGC1α expression independent of changes in intracellular cGMP levels have also been described.[2,6,7,13] Do the data in the present experiments support the contention that the observed NO effects on PGC1α expression are transduced exclusively through a sGC-sensitive, cGMP-dependent signaling pathway? Some insight into this question comes from their studies on the human monocytic cell line U937. The authors point out that U937 cells do not express any of the known NOS isoforms[1] and a previous study concluded that U937 cells do not have any detectable NO-sensitive sGC activity.[12] Despite this, the response of PGC1α mRNA levels to treatment with the NO donor SNAP, the sGC inhibitor ODQ and the cGMP analog 8-Br-cGMP was similar to the other cell lines tested. In addition, the data show that ODQ and 8-Br-cGMP are only partially capable of abrogating or mimicking the observed effects of SNAP on PGC1α in all cell types. This suggests that a significant component of the observed NO-dependent activation of PGC1α involves cGMP-independent signaling pathways.

Whether or not the observed effects of NO are mediated in part by cGMP-independent signaling, the ability of elevated cGMP levels alone to potentiate increases in PGC1α expression is clear. Sustaining sufficient intracellular production of cGMP in order to trigger a PGC1α-dependent increase in mitochondrial content is contingent upon the activity of at least three distinct groups of proteins; NOSs, sGC and phosphodiesterases. Significant differences in the tissue distribution and/or the activity of any or all of these proteins could therefore have a marked impact on the overall importance of this pathway in modulating either PGC1α expression or activity. For example, expression of the α ($\alpha_1$, $\alpha_2$) and β ($\beta_1$, $\beta_2$) subunits of sGC is very low in liver relative to other human tissues.[14] Whether additional inter-tissue differences exist in the subunit composition of sGC, which has been shown to have a pronounced effect on NO-stimulated rates of cGMP production,[15] is unknown. Nonetheless, either or both of these factors may help to explain why, despite a significant increase in eNOS expression and NO production in stably transfected HeLa cells,[16] Nisoli et al.[1] only observed modest effects on PGC1α expression and mitochondrial biogenesis relative to those obtained in other cell types in the presence of either the NO donor SNAP or the cGMP analog 8-Br-cGMP.

### "NO" effects on mitochondrial properties

In contrast to the rather modest effects of eNOS overexpression on mitochondrial content in HeLa cells, targeted disruption of the eNOS gene in vivo resulted in a significant reduction in the levels of several indices of mitochondrial content. This implies that eNOS-dependent NO signaling is important in maintaining basal mitochondrial content in a number of mammalian tissues. Whether the reduced mitochondrial content is caused by the lack of autocrine or paracrine actions of eNOS-derived NO remains unknown. Intriguingly, the magnitude of the adaptive increase in mitochondrial content of brown adipose tissue (BAT) in response to cold exposure was comparable in eNOS$^{-/-}$ mice and wild-type littermates (~threefold), perhaps reflecting the contribution(s) of NO-independent signaling pathways on PGC1α expression.[2] Alternatively, increased expression and activity of iNOS, which Nisoli and colleagues have previously demonstrated in BAT upon sympathetic stimulation,[17,18] may functionally compensate for the lack of eNOS protein, thereby preserving NO-dependent signaling events.

Regardless of the mechanisms by which NO is generated under basal conditions or in response to physiological stimuli, an equally important element of the findings of Nisoli et al.[1] that remains unresolved concerns the exact nature of the NO effects on mitochondrial properties. Despite their thorough molecular analyses, the extent to which the effects of manipulating in vitro and in vivo NO production produce changes in mitochondrial content and function remain unclear. Increases of twofold to fourfold in the levels of mitochondrial DNA and nuclear-encoded mitochondrial proteins in cultured cells treated with either SNAP or 8-Br-cGMP were accompanied by much more modest changes in total mitochondrial number and volume, 45 and 61%, respectively. Such non-stoichiometric changes suggest that the most important changes induced by altering NO levels involved the reorganization of existing mitochondria and their properties. Also, NO is known to bind reversibly to the binuclear center of cytochrome c oxidase, inhibiting cellular respiration.[10] It is therefore conceivable that this effect of NO could at least in part abrogate any increases in cellular respiration that might have arisen from increased mitochondrial content. It is also unclear from their in vivo studies whether the reduced rate of

oxygen consumption in eNOS$^{-/-}$ mice relative to wild-type littermates is directly attributable to a functionally compromised population of organelles or simply a reduced mitochondrial content across tissues. In the only in vivo study to date on mitochondrial function in tissues from eNOS$^{-/-}$ mice, Momken et al.[9] found that the metabolic organization and rates of mitochondrial substrate utilization were preserved in cardiac and fast-twitch glycolytic skeletal muscle fibers, while being significantly reduced in slow-twitch oxidative skeletal muscle fibers. Taken together, these data raise the possibility that a lack of eNOS expression only affects the mitochondrial properties of a subset of tissues within the organism that collectively results in lower basal rates of oxygen consumption.

## Perspectives

The findings of Nisoli et al.[1] clearly demonstrate that elevated levels of NO stimulate mitochondrial biogenesis by upregulating the expression of PGC1α. How important physiologically meaningful fluctuations in NO production are relative to other regulatory inputs that also act to modulate PGC1α expression remains unknown (Fig. 1). Certainly, the comparable fold increase in mitochondrial content upon cold exposure in BAT of eNOS$^{-/-}$ mice and wild-type littermates argues against a role for eNOS-dependent NO production in controlling adaptive changes in mitochondrial content, at least in this tissue. The tissue-specific effect of abrogating eNOS-derived NO production[1,9] further suggests that altered NO production may only be critical to the regulation of basal mitochondrial content in certain tissues. However, a more general effect of NO on the overall regulation of mitochondrial content would be masked in eNOS$^{-/-}$ mice if the mechanisms by which NO is produced and metabolized under basal and adaptive conditions differ considerably both within and among tissues. Some of the tissue-specific nature of the eNOS$^{-/-}$ mitochondrial phenotype may in fact be related to the differential distribution of factors that are important in mitigating the magnitude of NO effects on mitochondrial content and function. Taking skeletal muscle as an example, there are several fiber-type-specific properties that could conceivably impinge upon NO metabolism. These include fiber-type-specific differences in calcium transients,[19] in the levels of the bioactive NO scavenger myoglobin,[20] and in the relative expression of neuronal NOS versus eNOS.[21] Clarifying the exact nature of the signaling pathway, and potential tissue-specific differences in its various elements, is therefore critical to delineating its mode(s) of action, and assessing how widespread an impact NO has on regulating mitochondrial properties. Of significant interest will be whether NO can also activate another PGC-1 family member important to mitochondrial biogenesis, PGC1-related co-activator (PRC), which has a markedly different tissue distribution to that of PGC1α.[22] Future insight into the interplay and relative importance of individual NOS isoforms in modulating the expression of PGC-1 family members will

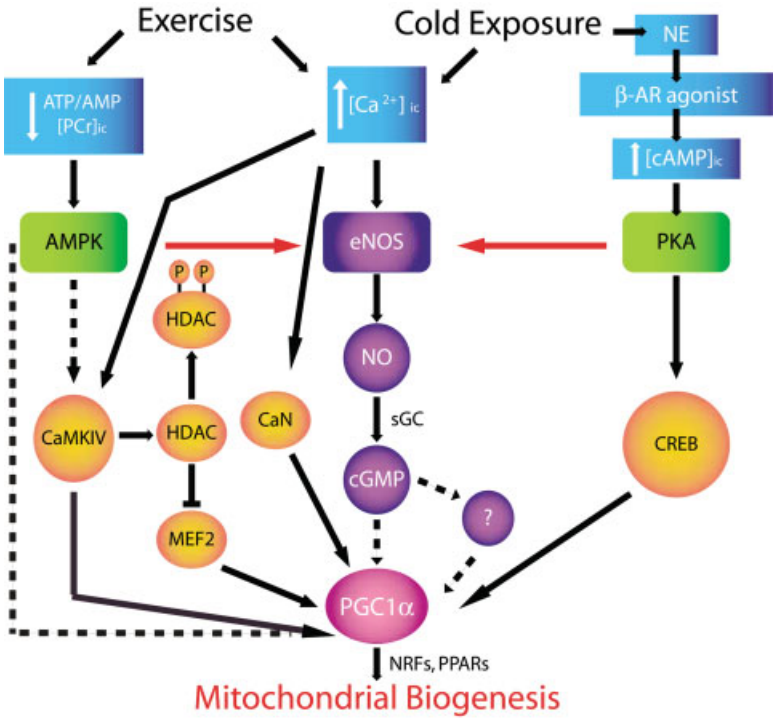


**Figure 1.** Signal transduction pathways that modulate mitochondrial biogenesis through the control of PGC1α expression in mammalian tissues.[1,2,4,6,7,13,23] Changes in the levels or ratio of metabolic indices [blue boxes] are sensed by specific protein-modifying enzymes (i.e., kinases, phosphatases). These proteins in turn activate diverse signaling cascades [gold circles] that ultimately affect the expression of PGC1α [pink oval]. Proteins involved in the NO-dependent activation of PGC1α are highlighted in mauve. Increased expression and/or activity of PGC1α stimulates mitochondrial biogenesis by potentiating the activity of relevant transcription factors (e.g., NRFs, PPARs). The relative roles of various signaling inputs in the overall regulation of PGC1α levels under both basal and adaptive conditions remain unknown. Also unclear is whether different tissues rely on these signaling pathways to varying degrees in regulating PGC1α expression. Solid lines denote established signaling pathways, dashed lines represent putative signaling cascades, and red lines denote protein-modifying enzymes that regulate eNOS activity and mitochondrial biogenesis.

undoubtedly require a comprehensive molecular genetic approach, which includes the generation of tissue-specific single and double knockouts of the relevant NOS genes.

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