(mainly PDE4), resulting in increased cAMP levels and stimulation of a Ca^{2+}-dependent pathway involving CamKKβ (Park et al., 2012). Some groups suggest that SIRT1 functions much later in the pathway, after AMPK stimulates NAD^+ production through increased transcription of NAD^+ synthetic enzymes (Um et al., 2010). Regardless of the proposed mechanism, the transcriptional coactivator PGC1α is the ultimate recipient of the signaling pathway. Using C2C12 cells, Price et al. showed that lower doses of resveratrol increased SIRT1-dependent phosphorylation of AMPK, while a higher dose led to SIRT1-independent activation of AMPK. Interestingly, the two doses displayed opposite trends; higher resveratrol decreased NAD^+ and ATP levels, while the lower dose led to increases in both metabolites, though the NAD^+ change was not evident until 12 hr. These results convincingly demonstrate that different dosages of resveratrol can elicit varied responses.

Most importantly, the work by Price et al. provides strong evidence that SIRT1 is a critical player in mediating the effects of resveratrol on mitochondrial biogenesis and the switch to more oxidative muscle fibers (Price et al., 2012). Given the pleiotropic effects of resveratrol, the molecular mechanism remains in dispute. When and how are SIRT1 and PDE4 involved in the resveratrol-dependent activation of AMPK? How does resveratrol concentration differentially affect the reported targets and their associated signaling pathways? A careful time course analysis of all the implicated factors is essential. For example, if SIRT1 is directly activated by resveratrol, then upon treatment, deacetylation of LKB1 should precede or coincide with AMPK phosphorylation. Similarly, does SIRT1-dependent deacetylation of PGC1α occur prior to, coincident with, or after initial AMPK activation? It will be important to dissect the initial signaling events from those of the metabolically reprogrammed state. Increased NAD^+ synthesis might to be a long-term adaption to drive sustained sirtuin function or to replenish NAD^+ levels as a result of an initial surge in sirtuin activity. The results from Price et al. strengthen the biological link between resveratrol and SIRT1-dependent processes, and provide a backdrop to further studies that resolve the mechanism.

REFERENCES

Barger, J.L., Kayo, T., Vann, J.M., Arias, E.B., Wang, J., Hacker, T.A., Wang, Y., Raederstorff, D., Morrow, J.D., Leeuwenburgh, C., et al. (2008). PLoS ONE *3*, e2264. 10.1371/journal.pone.0002264.

Borra, M.T., Smith, B.C., and Denu, J.M. (2005). J. Biol. Chem. *280*, 17187–17195.

Donmez, G., and Guarente, L. (2010). Aging Cell *9*, 285–290.

Howitz, K.T., Bitterman, K.J., Cohen, H.Y., Lamming, D.W., Lavu, S., Wood, J.G., Zipkin, R.E., Chung, P., Kisielewski, A., Zhang, L.L., et al. (2003). Nature *425*, 191–196.

Kaeberlein, M., McDonagh, T., Heltweg, B., Hixon, J., Westman, E.A., Caldwell, S.D., Napper, A., Curtis, R., DiStefano, P.S., Fields, S., et al. (2005). J. Biol. Chem. *280*, 17038–17045.

Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., et al. (2006). Cell *127*, 1109–1122.

Park, S.J., Ahmad, F., Philp, A., Baar, K., Williams, T., Luo, H., Ke, H., Rehmann, H., Taussig, R., Brown, A.L., et al. (2012). Cell *148*, 421–433.

Pearson, K.J., Baur, J.A., Lewis, K.N., Peshkin, L., Price, N.L., Labinskyy, N., Swindell, W.R., Kamara, D., Minor, R.K., Perez, E., et al. (2008). Cell Metab. *8*, 157–168.

Price, N.L., Gomes, A.P., Ling, A.J.Y., Duarte, F.V., Martin-Montalvo, A., North, B.J., Agarwal, B., Ye, L., Ramadori, G., Teodoro, J.S., et al. (2012). Cell Metab. *15*, this issue, 675–690.

Um, J.H., Park, S.J., Kang, H., Yang, S., Foretz, M., McBurney, M.W., Kim, M.K., Viollet, B., and Chung, J.H. (2010). Diabetes *59*, 554–563.

Muscling In on PGC-1α for Improved Quality of Life in ALS

Ashu Johri[1,*] and M. Flint Beal[1,*]
[1]Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York-Presbyterian Hospital, New York, NY 10065, USA
*Correspondence: johri.ashu@gmail.com (A.J.), fbeal@med.cornell.edu (M.F.B.)
DOI 10.1016/j.cmet.2012.04.015

Impaired activity of peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α has been implicated in the pathophysiology of several neurodegenerative disorders. In this issue, Da Cruz et al. (2012) show improved muscle function, but not survival, with increased PGC-1α activity in muscle in a mouse model of amyotrophic lateral sclerosis.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that targets motor neurons in the brain and spinal cord, resulting in muscle weakness, atrophy, and eventual death. In about 20% of familial ALS cases, the disease is associated with mutations in the gene that encodes copper-zinc superoxide dismutase (SOD1), which

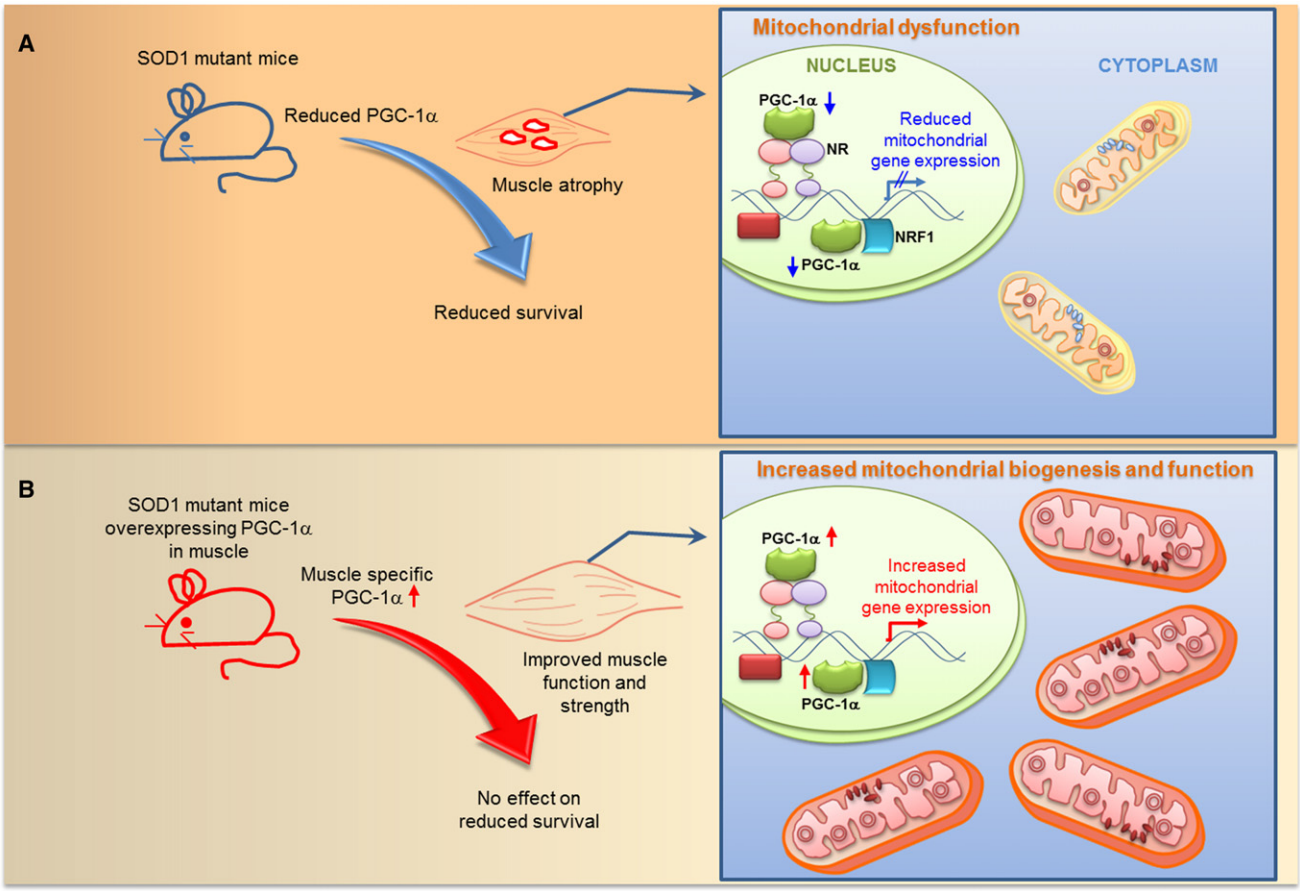

Figure 1. Improving Muscle Function Alone Is Not Sufficient to Combat ALS

(A) PGC-1α normally binds to the nuclear receptors (NR), such as the nuclear respiratory factor-1 (NRF-1), and modulates the expression of genes involved in mitochondrial biogenesis and function (right panel). The SOD1 mutant mice have reduced activity of PGC-1α, as well as mitochondrial dysfunction and muscle atrophy (left panel). Previous studies proposed that ALS originates in muscle, and from there it spreads to axons through neuromuscular junctions—and that in ALS neuronal death is a secondary consequence of degeneration of muscle starting from neuromuscular junctions.

(B) Da Cruz et al. (2012) now disprove the speculation that muscle alone is responsible for neuronal death in ALS. Increasing PGC-1α activity in muscle in a mouse model of ALS caused by a SOD1 mutation results in increased mitochondrial biogenesis, reduced muscle atrophy, and improved strength and performance. Total area of mitochondria per myofiber was significantly increased by 3- to 4-fold (right panel). Increased PGC-1α activity improves muscle strength throughout illness; however, it does not extend survival (left panel). This study shows that improving PGC-1α activity in muscle is an attractive palliative therapy for ALS.

aggregates and causes mitochondrial dysfunction.

Da Cruz et al. (2012) show that increasing peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α) activity in muscle in a transgenic mouse model of ALS caused by a mutation in SOD1 is able to sustain muscle function throughout the disease course; however, survival was not extended. This surprising result directly addresses a controversy that has arisen in the field of ALS research, which was whether the expression of mutant SOD1 in muscle alone is sufficient to cause the illness (Figure 1A). Prior work showed that muscle-restricted expression of mutant SOD1 in mice produced muscle damage and atrophy (Dobrowolny et al., 2008; Wong and Martin, 2010) and in one study caused degeneration of motor neurons (Wong and Martin, 2010). It was therefore suggested that muscle is the primary target of mutant SOD1 and that toxicity is caused by mitochondrial dysfunction, leading to the degeneration of the neuromuscular junctions (NMJs—sites on muscle fibers where nerves attach and signal the fibers to contract), axons, and motor neurons.

Mitochondrial damage and dysfunction occur in human ALS patients and in SOD1 mutant transgenic mice. Furthermore, degenerating mitochondria in the perinuclear region are seen in ALS transgenic mice with either TDP43 or FUS mutations, and VCP mutations impair mitochondrial calcium homeostasis. Mitochondrial abnormalities are among the earliest signs of disease onset in transgenic mouse models with SOD1 mutations, and expressing mutant SOD1 confined to mitochondria can cause the disease. An impairment of mitochondrial fusion and retrograde transport occurs in mutant SOD1 transgenic mice (Magrane et al., 2012). Misfolded SOD1 mutant protein binds onto the cytoplasmic surface of mitochondria, where it binds to Bcl-2, the voltage dependent anion channel (VDAC), and the TOM complexes, impairing protein import into mitochondria.

PGC-1α functions as a molecular rheostat, modulating the activity of genes

involved in mitochondrial biogenesis and antioxidant defenses. Of late, pharmacologic/transcriptional activation of the PGC-1α pathway has emerged as an attractive approach to ameliorate mitochondrial dysfunction in all major neurodegenerative disorders. Reduced levels of PGC-1α occur in brain tissue of patients with Alzheimer's disease, Parkinson's disease, and Huntington's disease (HD). Increased expression of PGC-1α reduces Aβ plaques in vitro, improves survival of transgenic mouse models of ALS, and prevents atrophy of striatal neurons in transgenic mouse models of HD. Liang et al. (2011) showed an age-dependent decrease in PGC-1α in SOD1-G93A mice. Moreover, they showed that increased expression of PGC-1α slowed the progression of ALS, moderately extended life span, improved motor performance, and decreased motor neuron death (Liang et al., 2011). The Pasinetti lab also showed that PGC-1α overexpression, using a neuron-specific enolase promoter, significantly improves motor function and survival of SOD1-G93A mice (Zhao et al., 2011).

PGC-1α improves muscle function by activation of mitochondrial biogenesis, resulting in more type I oxidative muscle fibers, which are rich in mitochondria, as well as enhancing the number of acetylcholine receptors at the NMJs. Da Cruz and colleagues crossed the G37R SOD1 mutant ALS mice to transgenic mice, which have an 8-fold increase in PGC-1α selectively expressed in muscle by using a muscle creatine kinase promoter (Lin et al., 2002; Da Cruz et al., 2012). The downstream mitochondrial genes controlled by PGC-1α were increased, and there was an increase in acetylcholine receptor clustering, which is expected to enhance NMJs and neuromuscular transmission. There was increased mitochondrial biogenesis, and the total area of mitochondria per myofiber was significantly increased by 3- to 4-fold, and fine limb muscles showed increased resistance to fatigue (Figure 1B).

These mice consistently showed a significant increase in running performance on a closed running wheel. At presymptomatic stages, the muscles showed no morphologic differences; however, at symptomatic ages, when the fiber size and distribution of gastrocnemius muscle was decreased sharply in the SOD1 G37R mice, the expression of PGC-1α prevented muscle atrophy. Even at end stage, muscle atrophy was significantly reduced. The percentage of denervated NMJs, however, was unaffected, and there was no effect on numbers of α-motor neurons, and on astroglial and microglial activation. These findings show that the disease is not a consequence of a dying back of axons following damage to NMJs in muscle. This finding differs from the findings of Wong and Martin, who observed that expression of mutant SOD1—confined to muscle using a skeletal muscle actin promoter—lead to NMJ abnormalities, distal axonopathy, and motor neuron degeneration (Wong and Martin, 2010). They found that overexpression of both wild-type as well as G37R and G93A mutant SOD1 were equally toxic to motor neurons, a curious result. The authors took care to exclude expression of the mutant SOD1 outside muscle, with western blots and immunocytochemistry, but did not show absence of mutant SOD1 mRNA in tissue or motor neurons. The preponderance of evidence favors the conclusion from the Da Cruz et al. (2012) study, which shows that muscle is not a primary target for mutant SOD1 toxicity and that improving muscle function alone is not sufficient to delay the disease onset and to increase survival, although it produces symptomatic benefits throughout the course of the illness.

Where does this leave us? Increasing PGC-1α expression levels appears to be worthwhile as a palliative treatment to maintain strength and mobility. Two pharmacologic approaches to increase PGC-1α are to treat with fibrates or thioglitazones. Fibrates are predominantly PPARα activators, which have been used to treat hyperlipidemia. Bezafibrate exerts beneficial effects in a mouse model of mitochondrial myopathy (Wenz et al., 2008). We showed that bezafibrate activates PGC-1α, increases numbers of mitochondria, produces neuroprotective effects, improves muscle pathology, and extends survival in a transgenic mouse model of HD (Johri et al., 2012).

Pharmacologic approaches increasing PGC-1α in both neurons and muscle may, therefore, improve survival. The importance of mitochondrial dysfunction in the pathogenesis of ALS was greatly strengthened by a recent phase 2 clinical trial in ALS patients with dexpramipexole, which localizes to mitochondria where it exerts antioxidant effects (Cudkowicz et al., 2011). It produced dose-dependent improvement on the ALS functional rating scale and increased survival. This finding, if confirmed in an ongoing phase 3 clinical trial, will be a major advance in developing neuroprotective drugs for the treatment of ALS and will place mitochondria at the forefront of the illness.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grant [P01AG14930] and by the Huntington Disease Society of America coalition for the cure to M.F.B.

REFERENCES

Cudkowicz, M., Bozik, M.E., Ingersoll, E.W., Miller, R., Mitsumoto, H., Shefner, J., Moore, D.H., Schoenfeld, D., Mather, J.L., Archibald, D., et al. (2011). Nat. Med. *17*, 1652–1656.

Da Cruz, S., Parone, P.A., Lopes, V.S., Lillo, C., McAlonis-Downes, M., Lee, S.K., Vetto, A.P., Petrosyan, S., Marsala, M., Murphy, A.N., et al. (2012). Cell Metab. *15*, this issue, 778–786.

Dobrowolny, G., Aucello, M., Rizzuto, E., Beccafico, S., Mammucari, C., Boncompagni, S., Belia, S., Wannenes, F., Nicoletti, C., Del Prete, Z., et al. (2008). Cell Metab. *8*, 425–436.

Johri, A., Calingasan, N.Y., Hennessey, T.M., Sharma, A., Yang, L., Wille, E., Chandra, A., and Beal, M.F. (2012). Hum. Mol. Genet. *21*, 1124–1137.

Liang, H., Ward, W.F., Jang, Y.C., Bhattacharya, A., Bokov, A.F., Li, Y., Jernigan, A., Richardson, A., and Van Remmen, H. (2011). Muscle Nerve *44*, 947–956.

Lin, J., Wu, H., Tarr, P.T., Zhang, C.Y., Wu, Z., Boss, O., Michael, L.F., Puigserver, P., Isotani, E., Olson, E.N., et al. (2002). Nature *418*, 797–801.

Magrane, J., Sahawneh, M.A., Przedborski, S., Estevez, A.G., and Manfredi, G. (2012). J. Neurosci. *32*, 229–242.

Wenz, T., Diaz, F., Spiegelman, B.M., and Moraes, C.T. (2008). Cell Metab. *8*, 249–256.

Wong, M., and Martin, L.J. (2010). Hum. Mol. Genet. *19*, 2284–2302.

Zhao, W., Varghese, M., Yemul, S., Pan, Y., Cheng, A., Marano, P., Hassan, S., Vempati, P., Chen, F., Qian, X., et al. (2011). Mol. Neurodegener. *6*, 51.