PGC-1α reduces proteasome and lysosome activity and attenuates myotube protein degradation

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Introduction. Skeletal muscle adapts to physiological demands by altering its metabolism and modifying its size (Schiaffino *et al.,* 2007). Regulation of muscle size is determined by two opposite phenomena, hypertrophy and atrophy. The ubiquitin proteasome pathway (UPP) is seen as the predominate pathway involved in muscle protein degradation (Solomon & Goldberg, 1996), however, recently the lysosomal pathway has been shown to play a key role in myotube protein degradation (Zhao *et al.,* 2007). Peroxisome Proliferator - activated receptor gamma, co-activator 1 alpha (PGC1α), a key protein involved in oxidative metabolism, has been shown to attenuate atrophy in mice, in part, *via* inhibiting the Forkhead (FoXO) transcriptional regulation of atrogin-1 and MuRF1 (Sandri *et al.,* 2006), two key members of the UPP (Bodine *et al.,* 2001). Whether PGC-1α influences proteasomal and lysosomal activity is unknown.

Methods. Mouse C2C12 myotubes were infected for 48 h with an adenovirus (Adv) containing green fluorescent protein (GFP-Adv) or human PGC-1α (hPGC-1α-Adv). After infection, myotubes were treated with or without dexamethasone (DEX) (10 μM) for 24 h and the release of [^{3}H]-tyrosine into the media was used as a measure of protein degradation. Proteasomal chymotrypsin- and caspase-like activities, as well as lysosomal cathepsin protease activity was measured *via* fluorometric analysis of their cleaved fluorescent peptide substrates, Suc-LLVY-AMC, Z-Leu-Leu-Glu-MCA and Z-Phe-Arg-AMC·HCl, respectively. Expression of PGC-1α, atrogin-1 and MuRF1 mRNA were measured *via* quantitative PCR.

Results. When compared to the GFP-Adv control group, over expressing hPGC-1α in C2C12 myotubes attenuated basal as well as DEX induced protein degradation by 18% and 33%, respectively. PGC-1α blunted proteasomal chymotrypsin- and caspase-like activities, and lysosomal cathepsin activity by 42%, 43% and 64%, respectively and reduced atrogin-1 and MuRF1 mRNA levels by 50% and 74%, respectively.

Conclusions. Our results demonstrate that PGC-1α attenuates both basal and DEX induced protein degradation, in part, *via* reducing the activities of the proteasome and lysosome. The reductions in atrogin-1 and MuRF1 support previous observations and suggest a possible mechanism influencing the reduced proteasomal activity. Further investigations are required to determine how PGC-1α attenuates proteasomal and lysosomal activity.

Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuela DM, DeChiara TM, Stitt TN, Yancopoulos GD & Glass, DJ. (2001) *Science*, **294:** 1704-8.

Sandri M, Lin J, Handschin C, Yang W, Arany ZP, Lecker SH, Goldberg AL & Spiegelman BM. (2006) *Proceedings of the National Academy of Sciences USA*, **103:** 16260-5.

Schiaffino S, Sandri M & Murgia M. (2007) *Physiology (Bethesda),* **22:** 269-78.

Solomon V & Goldberg AL. (1996). *Journal of Biological Chemistry* **271:** 26690-7.

Zhao J, Brault JJ, Schild A, Cao P, Sandri M, Schiaffino S, Lecker SH & Goldberg AL. (2007) *Cell Metabolism* **6:** 472-83.