

Defining the STATus quo in muscle hypertrophy.

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Skeletal muscle is required for locomotion, breathing, and postural support, thus maintenance of skeletal muscle mass is necessary to sustain quality of life. With aging and/or diseases such as Type 2 diabetes, cardiovascular disease, and chronic obstructive pulmonary disorder, muscle atrophy is commonly associated with increased risk of morbidity and mortality (2). Muscle mass is maintained by the balance of anabolic and catabolic processes, with decreased rates of muscle protein synthesis and higher rates of protein degradation present in these disease states (2). Although there are well-established methods for inducing muscular hypertrophy like resistance training, the specific molecular mechanisms dictating muscle growth are not entirely understood.

Recently, an elegant study performed by Perez-Schindler et al (3) examined the role of signal transducer and activator of transcription-3 (STAT3) in load-induced muscle hypertrophy in humans and mouse models. The investigators collected skeletal muscle biopsies from healthy male subjects that had performed a single bout of resistance exercise. STAT3 phosphorylation (pSTAT3) was then examined in the muscle samples, with no differences detected across groups. Concurrently, the authors utilized a synergist ablation (SA) surgery on skeletal muscle specific STAT3 knockout (KO) mice to induce mechanical overload and hypertrophy of the plantaris muscle. Despite lacking STAT3 in the skeletal muscle of the KO mice, no difference in plantaris mass or fiber cross-sectional area were observed when compared to the WT mice. They observed similar decreases in myostatin, a negative regulator of muscle mass, and similar increases in activation of canonical muscle growth pathways (mTORC1) in KO mice compared to WT mice (3). Together, these findings suggest that STAT3 localized to the mature skeletal muscle cell is not playing a necessary role in load-induced skeletal muscle hypertrophy.

Current evidence would suggest that STAT3 is involved in skeletal muscle hypertrophy through a pathway dependent on the interleukin-6 family of cytokines (IL-6, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), etc...). Both acute resistance exercise and resistance training were shown to upregulate IL-6 mRNA expression in skeletal muscle of Wistar rats, accompanied with increased STAT3 phosphorylation and upregulation of STAT3 dependent genes (1). Genetic deletion of LIF blunted the hypertrophic response of the plantaris and soleus muscles with SA; however, pSTAT3

levels similarly increased when compared to WT mice (6). This suggests the possibility that the IL-6 family members play a role in load-induced hypertrophy that does not depend on pSTAT3 in the muscle. Serrano et al (4) has shown that genetic deletion of IL-6 attenuated muscle hypertrophy in the same overload model, which suggests that the IL-6 cytokine family is critical. However, Serrano et al suggested IL-6 was affecting satellite cell (SC) dynamics in that they found reduced skeletal muscle STAT3 activation in the satellite cells. This was associated with reduced mRNA expression for genes that regulate proliferation, migration, and differentiation of satellite cells (4). From this, they conclude that the IL-6/STAT3 axis is critical for muscle hypertrophy, mediated by SC proliferation; however, the study performed by Perez-Schindler et al (3) would not be able to directly test this hypothesis since Cre-driver would not be activated in SC, thus allowing normal STAT3 activation in the SC.

In the human component of the study, Perez-Schindler et al did not show any differences in pSTAT3 following a single bout of resistance exercise (3). A previous study has shown that 2 hrs following a single bout of resistance exercise, pSTAT3 is significantly increased (8), indicating that the timing of sample collection is possibly a critical factor. Another consideration to make is that Toth et al (7) found no significant differences in pSTAT3 in skeletal muscle homogenates at various time points following resistance exercise, but when pSTAT3 was quantified by immunofluorescence, there was a significant elevation in SCs positive for pSTAT3 at 1 hour, 3 hours, and 24 hours post exercise. Thus, measuring pSTAT3 in whole muscle homogenates would not account for localization differences in STAT3 activation. Therefore, while Perez-Schindler et al. did not observe any changes in pSTAT3 in whole muscle homogenates, examination at the level of the SC may have been necessary to detect differences in STAT3 activation.

Interestingly, STAT3 activation has also been implicated in diseases or conditions characterized by muscle wasting. Phosphorylation of STAT3 has been shown to activate caspase-3, myostatin, and the ubiquitin-proteasome system, while loss of STAT3 activation was shown to decrease activity of these pathways (5). Thus, with the development of this unique STAT3 KO model, the authors are poised to dissect the role of STAT3 under multiple different contexts, allowing for a better elucidation for the role

of STAT3 in muscle biology. For example, perhaps the STAT3 KO model is resistant to muscle atrophy in models of cancer cachexia or diseases that results in significant loss of muscle mass.

In conclusion, the study from Perez-Schindler et al clearly demonstrates that STAT3 activation is not necessary for load-induced muscle hypertrophy, clearing up a number of equivocal findings in the literature concerning STAT3. However, some questions remain concerning the role of STAT3 in skeletal muscle, and the model described in this paper provides scientists in the muscle biology field a unique opportunity to further delineate the role that STAT3 plays in skeletal muscle across numerous conditions.

Disclosures

No conflict of interest, financial or otherwise, are declared by the authors

Author Contributions

A.J.A. and E.E.S. drafted the manuscript; A.J.A. and E.E.S. edited and revised the manuscript; A.J.A. and E.E.S. approved the final version of the manuscript.

References

1. **Begue G, Douillard A, Galbes O, Rossano B, Vernus B, Candau R, Py G.** Early Activation of Rat Skeletal Muscle IL-6/STAT1/STAT3 Dependent Gene Expression in Resistance Exercise Linked to Hypertrophy. *PLoS One* 8, 2013.
2. **Cohen S, Nathan J a, Goldberg AL.** Muscle wasting in disease: molecular mechanisms and promising therapies. *Nat Rev Drug Discov* 14: 58–74, 2015.
3. **Pérez-Schindler J, Esparza M, McKendry J, Breen L, Philp A, Schenk S.** Overload-mediated skeletal muscle hypertrophy is not impaired by loss of myofiber STAT3. *Am. J. Physiol. - Cell Physiol.* (2017). doi: 10.1152/ajpcell.00100.2017.
4. **Serrano AL, Baeza-Raja B, Perdiguero E, Jardí M, Muñoz-Cánoves P.** Interleukin-6 Is an

Essential Regulator of Satellite Cell-Mediated Skeletal Muscle Hypertrophy. *Cell Metab* 7: 33–44, 2008.

5. **Silva KAS, Dong J, Dong Y, Dong Y, Schor N, Twardy DJ, Zhang L, Mitch WE.** Inhibition of Stat3 activation suppresses caspase-3 and the ubiquitin-proteasome system, leading to preservation of muscle mass in cancer cachexia. *J Biol Chem* 290: 11177–11187, 2015.
6. **Spangenburg EE, Booth FW.** Leukemia inhibitory factor restores the hypertrophic response to increased loading in the LIF(-/-) mouse. *Cytokine* 34: 125–130, 2006.
7. **Toth KG, McKay BR, de Lisio M, Little JP, Tarnopolsky MA, Parise G.** IL-6 induced STAT3 signalling is associated with the proliferation of human muscle satellite cells following acute muscle damage. *PLoS One* 6, 2011.
8. **Trenerry MK, Carey K a, Ward AC, Cameron-Smith D.** STAT3 signaling is activated in human skeletal muscle following acute resistance exercise. *J Appl Physiol* 102: 1483–1489, 2007.