Published in final edited form as:

Curr Opin Clin Nutr Metab Care. 2010 July ; 13(4): 423–428. doi:10.1097/MCO.0b013e32833a5107.

CORTICOSTEROIDS AND MUSCLE WASTING ROLE OF TRANSCRIPTION FACTORS, NUCLEAR COFACTORS, AND HYPERACETYLATION

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Abstract

Purpose of review—The purpose of this review is to discuss novel insight into mechanisms of glucocorticoid-regulated muscle wasting, in particular the role of transcription factors and nuclear cofactors. In addition, novel strategies that may become useful in the treatment or prevention of glucocorticoid-induced muscle wasting are reviewed.

Recent findings—Studies suggest that glucocorticoid-induced upregulation of the transcription factors FOXO1 and C/EBP β and downregulation of MyoD and myogenin are involved in glucocorticoid-induced muscle wasting. In addition, glucocorticoid-induced hyperacetylation caused by increased expression of the nuclear cofactor p300 and its histone acetyl transferase activity and decreased expression and activity of histone deacetylases (HDACs) plays an important role in glucocorticoid-induced muscle proteolysis and wasting. Other mechanisms may also be involved in glucocorticoid-induced muscle wasting, including insulin resistance and store-operated calcium entry. Novel potential strategies to prevent or treat glucocorticoid-induced muscle wasting include the use of small molecule HDAC activators, dissociated glucocorticoid receptor agonists, and 11 β -hydroxysteroid dehydrogenase type 1 inhibitors.

Summary—An increased understanding of molecular mechanisms regulating glucocorticoid-induced muscle wasting will help develop new strategies to prevent and treat this debilitating condition.

Keywords

Muscle wasting; glucocorticoids; FOXO1; C/EBP β ; MyoD; myogenin; p300; histone deacetylases

Introduction

Loss of muscle mass is commonly seen in patients with sepsis, severe injury, and cancer [1,2,3*]. Muscle wasting in these conditions has severe clinical consequences, including muscle weakness and fatigue, delayed ambulation with increased risk for thromboembolic and pulmonary complications, prolonged need for ventilatory support and extended stay in the intensive care unit. In patients with cancer, cachexia and muscle wasting contribute significantly to poor quality of life and may also negatively impact the effectiveness of chemotherapy [3*,4].

Glucocorticoids are important mediators of muscle wasting in many catabolic conditions. In addition, patients treated with corticosteroids for various disease states, such as arthritis, asthma, and chronic obstructive pulmonary disease, frequently suffer from muscle wasting as a side-effect of the treatment [5]. Understanding mechanisms of corticosteroid-regulated loss of muscle mass, therefore, has important clinical implications and may help develop new treatment strategies to prevent this debilitating condition.

Mechanisms involved in glucocorticoid-regulated muscle wasting have been reviewed in previous reports from our [6,7] and other laboratories [8*]. In the present review, we discuss novel aspects of molecular mechanisms regulating corticosteroid-induced loss of muscle mass. In particular, we review recent evidence that the expression and activity of transcription factors and nuclear cofactors involved in the modulation of muscle mass may be regulated by glucocorticoids. In addition, potential strategies to prevent or treat glucocorticoid-induced muscle wasting are discussed.

Forkhead Box O (FOXO) transcription factors

Recent evidence suggests that glucocorticoid-induced muscle wasting may at least in part reflect regulation of transcription factors in muscle wasting. Members of the FOXO transcription factor family (FOXO1, FOXO3a, and FOXO4) regulate genes in the ubiquitin-proteasome proteolytic pathway, in particular the ubiquitin ligases atrogin-1/MAFbx and MuRF1 [9–11], as well as autophagy-related genes [12,13], myostatin [14,15], and cathepsin L [16]. Previous studies provided evidence that glucocorticoids may upregulate the expression and activity of FOXO transcription factors [9,10,17].

In a recent study we found that glucocorticoids are involved in sepsis-induced upregulation of FOXO1 in skeletal muscle [18]. In those experiments, sepsis induced by cecal ligation and puncture in rats, resulted in an approximately 7-fold increase in FOXO1 mRNA levels and an approximately 3-fold increase in FOXO1 protein levels. The increase in FOXO1 expression was reduced by treating rats with the glucocorticoid receptor antagonist RU38486 suggesting that the sepsis-induced upregulation of FOXO1 was at least in part regulated by glucocorticoids. Further support for a role of glucocorticoids in the regulation of FOXO1 expression was found in the same study by treating rats with dexamethasone. This treatment resulted in a 2- to 3-fold increase in FOXO1 mRNA levels accompanied by a 3- to 4-fold increase in atrogin-1 and MuRF1 mRNA levels. Importantly, in the same report, silencing the FOXO1 gene by transfecting cultured myotubes with FOXO1 siRNA reduced protein degradation and partly inhibited the dexamethasone-induced increase in atrogin-1 and MuRF1 expression. In contrast, silencing FOXO3a with siRNA technique did not influence the dexamethasone-induced activation of atrogin-1 and MuRF1 expression.

Taken together, our recent observations [18] suggest that glucocorticoid-induced activation of ubiquitin-proteasome-dependent muscle proteolysis may at least in part reflect upregulation of FOXO1.

CCAAT/enhancer binding protein (C/EBP) β

In previous studies from our laboratory, the expression and DNA binding activity of the transcription factors C/EBP β and δ were increased in skeletal muscle during sepsis [19]. This response to sepsis was inhibited by RU38486, suggesting that the activation of C/EBP β and δ was regulated by glucocorticoids. Further evidence for a role of glucocorticoids in the regulation of C/EBP β and δ was found in subsequent experiments in which treatment of rats in vivo or of cultured myotubes in vitro with dexamethasone resulted in increased C/EBP β and δ expression, DNA binding activity and transcriptional activation [20]. When electrophoretic mobility shift assay (EMSA) was performed, supershift analysis suggested

that C/EBP β may be particularly sensitive to glucocorticoids. A more detailed review of the potential role of C/EBP transcription factors in glucocorticoid-induced muscle wasting was provided recently [7].

In more recent experiments (unpublished observations), we have found more direct evidence of a link between glucocorticoid-induced activation of C/EBP β and muscle wasting. In those experiments, silencing of C/EBP β with siRNA technique inhibited the dexamethasone-induced increase in protein degradation and atrogin-1/MAFbx and MuRF1 expression and prevented the atrophy noticed in dexamethasone-treated myotubes.

MyoD and myogenin

MyoD is a transcription factor that regulates muscle differentiation and development. It is also required for regeneration and self-renewal of skeletal muscle satellite cells [21]. The transcriptional activities of MyoD are negatively regulated by a family of inhibitors of DNA-binding (Id) proteins among which Id1 is the most important factor with regard to MyoD binding [22].

Muscle wasting is characterized by decreased levels of MyoD, at least in part reflecting ubiquitin-proteasome-dependent degradation of the transcription factor [23,24]. Studies suggest that TNF can reduce the MyoD protein abundance in skeletal muscle cells secondary to NF- κ B activation and that these effects of TNF may play a role in muscle wasting and cachexia [24,25].

Importantly for this review, a recent study suggests that glucocorticoids can stimulate the degradation of MyoD and that this effect of glucocorticoids may be a mechanism of glucocorticoid-induced muscle wasting [26*]. In those experiments, treatment of cultured C2C12 muscle cells (a mouse skeletal muscle cell line) with dexamethasone resulted in increased proteasome-dependent degradation of MyoD. Interestingly, the dexamethasone-induced MyoD degradation took place in the nucleus by the N-terminus dependent mechanism [27] whereas cytoplasmic MyoD degradation was not regulated by dexamethasone suggesting that MyoD is differentially regulated in different cellular compartments. The results in that study [26] were interpreted as indicating that glucocorticoid-dependent nuclear degradation of MyoD via the N-terminus pathway is an important mechanism of muscle protein catabolism. In the same study, degradation of Id1 was not influenced by dexamethasone further tipping the balance toward reduced MyoD activity in dexamethasone-treated muscle cells.

Myogenin is an additional myogenic transcription factor that is involved in muscle differentiation and development [28]. Unlike MyoD, it is not known if myogenin levels change during muscle atrophy. A recent study suggests, however, that glucocorticoid-induced muscle wasting may in fact be characterized by reduced myogenin levels [29*]. In those experiments, treatment of cultured C2C12 myotubes with dexamethasone resulted in a relatively rapid (within 6–12 hours) decline in myogenin protein levels whereas the amounts of myogenin mRNA did not change. Because the proteasome inhibitor MG132 stabilized myogenin levels, the dexamethasone-induced decline of myogenin protein was interpreted as being caused by ubiquitin-proteasome-dependent degradation of the transcription factor. This interpretation was further supported by increased expression of atrogin-1/MAFbx and evidence of a physical interaction between myogenin and atrogin-1/MAFbx in the nuclei of the dexamethasone-treated muscle cells (an observation that is important because it adds myogenin to the list of potential substrates for atrogin-1/MAFbx).

Nuclear cofactors

Gene transcription is regulated not only by transcription factors but by a complex interaction between other nuclear proteins as well, including various nuclear cofactors. In recent experiments, we found that the expression and activity of p300, a versatile nuclear cofactor with histone acetyl transferase (HAT) activity [30], were upregulated in dexamethasone-treated cultured myotubes [31,32]. p300 is analogous to CREB binding protein (CBP) and the properties of these factors (p300/CBP) are almost identical. Interestingly, co-immunoprecipitation provided evidence for a glucocorticoid-induced physical interaction between p300 and C/EBP β [31], suggesting that C/EBP β acetylation may be involved in glucocorticoid-induced muscle wasting. Indeed, recent experiments in our laboratory suggest that treatment of cultured myotubes with dexamethasone increases cellular levels of acetylated C/EBP β (unpublished observations). When cultured myotubes were transfected with p300 siRNA or a p300 expression plasmid that was mutated in its HAT activity domain (and lacked HAT activity), the dexamethasone-induced increase in protein degradation was blocked, providing strong evidence for a role of p300/HAT expression and activity in glucocorticoid-induced muscle wasting. A recent study by Tobimatsu et al [33*] confirmed our observation of p300 involvement in glucocorticoid-induced muscle atrophy. Interestingly, when p300 activity was reduced by overexpressing CREB-binding protein (CBP)/p300-interacting transactivator with ED-rich tail 2 (Cited2) which binds to the cysteine-histidine-rich region 1 of p300, dexamethasone-induced myotube atrophy and expression of atrogen-1/MAFbx and MuRF1 were reduced [33*], providing additional strong molecular evidence for a role of p300 in glucocorticoid-induced muscle wasting.

Of note, acetylation of chromatin and other cellular proteins (including transcription factors) is regulated not only by HAT activity but by histone deacetylase (HDAC) activity as well. Importantly, in our previous studies, treatment of cultured muscle cells with dexamethasone did not only increase p300/HAT but also inhibited the expression and activity of HDAC3 and 6 [32]. Taken together, these changes further support the role of hyperacetylation in glucocorticoid-induced muscle wasting. Importantly, when hyperacetylation was induced by treating myotubes with the HDAC inhibitor trichostatin A (TSA), protein degradation was increased to the same extent as that caused by dexamethasone, providing the first evidence that hyperacetylation may induce muscle proteolysis [32].

Because the experiments performed in our laboratory [31,32] and by others [33] were conducted in vitro in cultured myotubes, it will be important in future studies to determine whether p300 and HDACs play a similar role in glucocorticoid-induced muscle wasting in vivo. Recent (unpublished) observations in our laboratory do indeed suggest that glucocorticoids regulate muscle acetylation in vivo as well. For example, treatment of rats with dexamethasone increased p300/HAT and reduced HDAC6 expression and activity and in additional experiments, treatment of rats with TSA stimulated muscle proteolysis and upregulated atrogen-1/MAFbx expression.

The role of hyperacetylation in glucocorticoid-regulated muscle wasting is important because it suggests that prevention of hyperacetylation may be a therapeutic strategy to reduce the loss of muscle mass in catabolic patients and in individuals treated with corticosteroids. Recent experiments in which p300/HAT expression and activity were manipulated at the molecular level support that notion [32,33]. More important from a clinical standpoint is the recent development of small molecules that reduce acetylation by activating HDACs [34].

Other novel insight into mechanisms regulating glucocorticoid-induced muscle wasting

Although the present review is focused on the role of transcription factors, nuclear cofactors, and hyperacetylation in glucocorticoid-induced muscle wasting, additional important knowledge related to corticosteroids and muscle atrophy has been generated recently. Some of that information is reviewed briefly here. Novel insights into the glucocorticoid actions on skeletal muscle glucose and protein metabolism were reviewed recently with a special emphasis on the linkage between glucocorticoid-induced insulin resistance and protein catabolism [35*]. The role of impaired insulin signaling in glucocorticoid-induced muscle wasting was also highlighted in a recent study by Hu et al [36*].

Previous studies from our and other laboratories suggest that calcium uptake and concentrations are increased in skeletal muscle during sepsis and in other muscle wasting conditions as well, including denervation, burn injury, and muscular dystrophy [37–39]. These observations are significant because calcium is an important regulator of muscle protein breakdown [40]. In a recent study, we found evidence that glucocorticoids may increase muscle calcium levels secondary to stimulated store-operated calcium entry (SOCE) and that this effect of glucocorticoids was regulated by increased phospholipase A2 activity [41]. Additional experiments in the same study suggested that dexamethasone-induced protein degradation was calcium- and SOCE-dependent, suggesting that inhibition of SOCE may be a therapeutic tool to prevent glucocorticoid-induced muscle wasting.

Prevention and treatment of glucocorticoid-induced muscle wasting

Although treatment of glucocorticoid-induced muscle catabolism with anabolic hormones has been described in several previous studies, recent reports have shed new light on mechanisms involved in this effect of anabolic hormones, in particular testosterone. For example, Zhao et al [42*] reported recently that testosterone blocked dexamethasone-induced muscle protein degradation secondary to inhibited expression of atrogen-1/MAFbx both in vivo in dexamethasone-treated rats and in vitro in dexamethasone-treated cultured C2C12 muscle cells. In another study, the attenuation of dexamethasone-induced muscle atrophy by testosterone at least in part reflected improved signaling in pathways downstream of IGF-1/insulin associated with muscle protein synthesis [43*]. Recent studies suggest that growth hormone releasing peptide-2 (GHRP-2), β 2-agonists, and cAMP phosphodiesterase inhibitors (increasing muscle levels of cAMP) may also be able to prevent glucocorticoid-induced muscle wasting [44,45*].

Based on the recent discovery that hyperacetylation may be involved in glucocorticoid-induced muscle wasting [31,32], it may be speculated that small molecule HDAC activators [34] may become useful in preventing and treating muscle wasting (as discussed above).

Other potential treatment modalities that have not yet been reported in the context of glucocorticoid-induced muscle wasting but may be tested in the near future include two novel classes of agents, i.e., dissociated glucocorticoid receptor (GR) agonists and 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) inhibitors. Dissociated GR agonists are designed to induce glucocorticoid-regulated transrepression pathways while minimizing transactivation activity, the latter being responsible for metabolic side-effects (probably including muscle wasting) of glucocorticoids [46,47]. Although speculative at this point, it is possible that dissociated GR ligands will become useful in the future to prevent loss of muscle mass in conditions characterized by glucocorticoid-regulated muscle wasting and in patients being treated with glucocorticoids.

The action of glucocorticoids in peripheral tissues is regulated by the enzymes 11 β -HSD1 and 11 β -HSD2 that convert inactive to active corticosteroid and active to inactive corticosteroid, respectively [48]. In order to reduce tissue levels of active glucocorticoids, several new classes of 11 β -HSD1 inhibitors are presently being developed [reviewed in ref #35*]. Although these drugs have been tested mainly in the context of glucocorticoid-regulated insulin resistance and treatment of diabetes, it may be speculated that they will become useful in the treatment of glucocorticoid-induced muscle wasting as well. Interestingly, in a recent study, treatment of cultured C2C12 myotubes with retinoic acid, the carboxylic form of vitamin A, resulted in downregulated expression and activity of 11 β -HSD1 and made the myotubes resistant to the effects of glucocorticoids [49*].

Summary and conclusions

Muscle wasting in various catabolic conditions is at least in part mediated by glucocorticoids. In addition, loss of muscle mass is a serious side-effect of treatment with corticosteroids. Potential mechanisms regulating glucocorticoid-induced muscle wasting are summarized in Fig 1. Although multiple mechanisms are probably involved, upregulation of the expression and activity of certain transcription factors (FOXO1 and C/EBP β) and downregulation of other transcription factors (MyoD and myogenin) are important mechanisms of glucocorticoid-induced muscle wasting. Glucocorticoid-induced hyperacetylation caused by increased p300/HAT and reduced HDAC expression and activity also plays an important role in glucocorticoid-induced muscle wasting. Other mechanisms contributing to glucocorticoid-induced muscle atrophy include insulin resistance and increased muscle calcium levels, in part reflecting activated SOCE. Novel therapeutic strategies that may become useful in the future to prevent glucocorticoid-regulated muscle wasting include treatment with HDAC activators, dissociated GR agonists, and 11 β -HSD1 inhibitors.

Acknowledgments

Supported in part by NIH grants R01 DK37908 and R01 NR08545. ZA was supported by the Department of Clinical Medicine, "Sapienza", University of Rome, Rome, Italy

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Fig. 1.

Potential mechanisms involved in glucocorticoid-induced muscle wasting. Although regulation of certain transcription factors and nuclear cofactors is emphasized in this review, other mechanisms are probably involved as well. Studies suggest that the expression and activity of FOXO transcription factors and C/EBP β are upregulated by glucocorticoids and that hyperacetylation caused by increased p300/HAT and decreased HDAC expression and activity may contribute to transcription factor activation. Proteasome-dependent degradation may contribute to reduced expression and activity of the “anabolic transcription factors” MyoD and myogenin, further accentuating the loss of muscle mass. Although there is evidence that glucocorticoid-induced hyperacetylation stimulates muscle protein degradation, the role of hyperacetylation in the regulation of the ubiquitin-proteasome pathway and of autophagic/lysosomal protein degradation is not known at present (as indicated by the question mark).