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Review

Mechanisms and Clinical Applications of Glucocorticoid Steroids in Muscular Dystrophy

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Abstract. Glucocorticoid steroids are widely used as immunomodulatory agents in acute and chronic conditions. Glucocorticoid steroids such as prednisone and deflazacort are recommended for treating Duchenne Muscular Dystrophy where their use prolongs ambulation and life expectancy. Despite this benefit, glucocorticoid use in Duchenne Muscular Dystrophy is also associated with significant adverse consequences including adrenal suppression, growth impairment, poor bone health and metabolic syndrome. For other forms of muscular dystrophy like the limb girdle dystrophies, glucocorticoids are not typically used. Here we review the experimental evidence supporting multiple mechanisms of glucocorticoid action in dystrophic muscle including their role in dampening inflammation and myofiber injury. We also discuss alternative dosing strategies as well as novel steroid agents that are in development and testing, with the goal to reduce adverse consequences of prolonged glucocorticoid exposure while maximizing beneficial outcomes.

Keywords: Glucocorticoid steroids, muscular dystrophy, neuromuscular diseases, molecular signaling, immunomodulation, metabolism, muscle physiology

GLUCOCORTICOID STEROIDS ACT THROUGH THE GLUCOCORTICOID RECEPTOR TO REGULATE GENE EXPRESSION

Glucocorticoid steroids are endogenous hormones that coordinate basal and stress responses by directing tissue-specific transcriptional programs. In humans, the primary endogenous glucocorticoid is cortisol, while in mice, corticosterone is the predominant form. The two compounds share the basic four ring steroid structure and are closely related differing by a single hydroxyl group. However, the two compounds differ in synthetic pathways where only corticosterone is a precursor to the mineralocorticoid, aldosterone. Cortisol and corticosterone are produced by the adrenal cortex in response to stress and circadian stimuli (Fig. 1). Activated by the corticotropin releasing hormone (CRH), the adrenocorticotropic hormone (ACTH) from the anterior pituitary stimulates the adrenal gland to secrete cortisol. In turn, cortisol activates the glucocorticoid receptor (GR) to antagonize production of CRH by the hypothalamus and ACTH by the pituitary gland in a negative feedback loop [1, 2]. Circulating endogenous glucocorticoid levels peak just prior to the beginning of the active phase each day. Synthetic glucocorticoids are classified as short- or long-acting depending upon the duration of ACTH suppression they elicit [3], although

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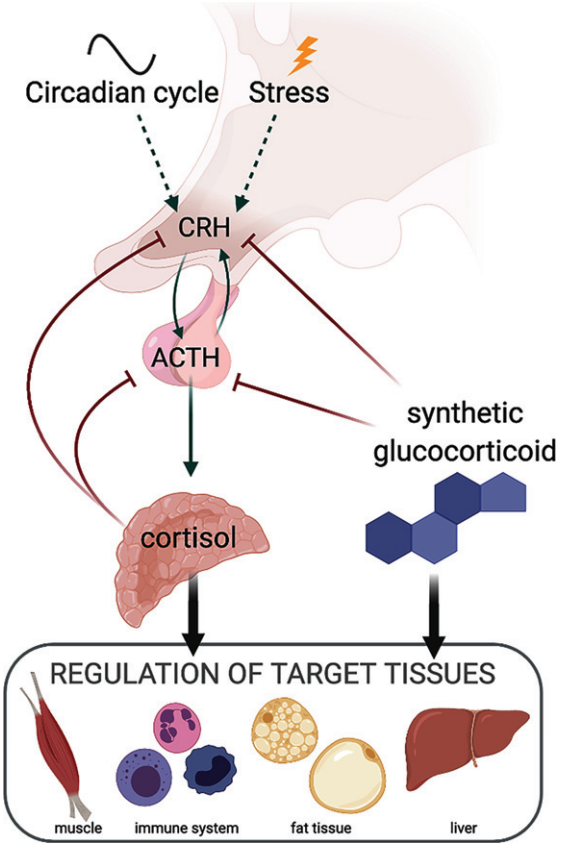

Fig. 1. Diagram summarizing relationships between endogenous and synthetic glucocorticoids and the hypothalamic-pituitary-adrenal axis.

most have a serum half-life of approximately 1–3 hrs [4–6]. The most commonly prescribed synthetic glucocorticoids include dexamethasone, deflazacort, and prednisone, and these agents are widely used clinically to treat autoimmune and other conditions.

In response to ligand binding, GR drives transcriptional changes to directly alter gene expression in target cells and tissues. Upon ligand binding, GR translocates into the nucleus where it binds glucocorticoid response elements (GREs) in DNA either by itself or in concert with co-factors to regulate gene expression. GRE binding by GR can lead to activation or repression of target genes, so-called "trans-activation" and "trans-repression" functions of the GR as reviewed in [7], depending on GR interactions with co-factors. As a member of the nuclear receptor superfamily, GR interacts with a diverse group of coactivators and co-repressors, orchestrating tissue-specific transcriptional responses [8–10]. In muscle, few specific GR co-factors have been identified, and the best studied is FOXO1, which mediates steroid-induced atrophy [11]. Traditionally, GR is thought to bind DNA as either homodimer or monomer in conjunction with co-factors, but a recent study suggested GR formed a tetramer, as two dimers, after binding DNA [12]. The significance of this conformation requires further study to determine whether it regulates precise transcriptional processes. Because GRs function within complexes, GR binding to a GRE, on its own, is not a strong predictor of GR-dependent gene regulation. The likelihood of an occupied GRE driving transcriptional regulation of a gene increases the closer that the GRE is to the gene's transcriptional start site [13, 14]. GR binding sites can also work concordantly, with clusters of GREs mediating GR-dependent transcription [14]. GR binding can further control gene expression by modulating the epigenetic landscape around its target genes [15–17]. This epigenetic remodeling is likely a crucial component of GR-induced gene regulation, although more investigation is required to better decipher how loss of chromatin-modifying co-factors impacts expression of GR target genes.

In addition to GR, glucocorticoids can interact with structurally similar nuclear receptors including the mineralocorticoid receptor (MR) and the androgen receptor (AR) [18]. In the presence of glucocorticoid, these receptors can form heterodimers with stronger transactivation capacity than the individual receptors [19, 20]. Endogenous corticosteroids bind MR with 5- to 10-fold higher affinity than GR [21], so it is likely that basal circulating cortisol binds MR preferentially with GR occupancy during circadian peaks or stress [22, 23]. Synthetic glucocorticoids such as deflazacort [24], prednisone [25], and dexamethasone [26] have less affinity for MR [27]. AR is also structurally similar to GR, and the two proteins can form heterodimers [28]. AR and GR have substantial overlap in their agonist-dependent interactomes, indicating shared regulatory features [29]. AR and GR are known to interact in non-muscle tissues, and AR has critical roles in skeletal muscle development and function. However, the physiological effects of AR-GR heterodimerization are not fully understood, as data supports both competitive inhibition [28] and coordination [29].

Glucocorticoids as anti-inflammatory agents

Glucocorticoids both suppress proinflammatory signaling and activate anti-inflammatory responses [30, 31] (Fig. 2). Glucocorticoids inhibit the inflam-

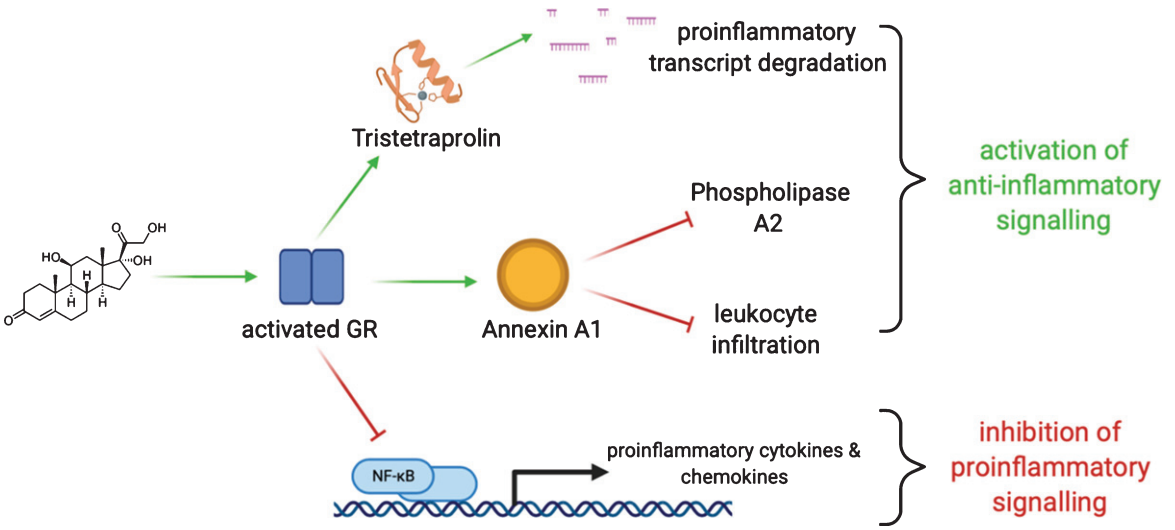

Fig. 2. Glucocorticoids act through the glucocorticoid receptor (GR). GR activation promotes degradation of transcripts mediating proinflammatory signals through, among other mechanisms, RNA-binding proteins like tristetrapolin. GR activation also stimulates the expression of annexin A1 which serves to orchestrate termination of inflammation and avoid adverse prolonged activation. GR activation also acts directly to limit the action of key proinflammatory mediators.

matory cascades that cause acute tissue damage through the binding of GR to transcription factors NF-kB and AP-1, which inhibits their activity [32, 33]. In monocytes, dexamethasone is known to increase transcription and protein synthesis of the NF-kB inhibitor, IkBα [34]. Similarly, dexamethasone activates glucocorticoid-induced leucine zipper (GILZ), which inhibits AP-1 to lower downstream cytokine synthesis [35, 36]. Glucocorticoid-mediated *trans*-repression of NF-kB and AP-1 acts on multiple downstream gene targets, including genes encoding inflammatory cytokines and chemokines such as IL-6, IL-12, IL-1, TNFα, and COX-2 [37, 38]. Activated GR not only binds NF-kB to prevent its activation, but it is also known to displace the NF-kB coactivator CBP from the DNA-binding subunits of NF-kB, preventing its transcriptional activity, and adding an additional layer of immunosuppression [39, 40]. Similar to NF-kB, activated GR binds AP-1 to prevent its DNA binding and activity [32, 41]. A partial reduction in immune cells, especially T cell infiltration, into muscle has been observed in steroid-treated human DMD and *mdx* muscle [42–44].

Lipocortin 1 (also known as Annexin A1) is a strong anti-inflammatory effector whose expression is driven by glucocorticoids [45]. High levels of annexin A1 suppress proinflammatory genes including IL-6, COX-2, and iNOS [46–49]. In the presence of glucocorticoids, annexin A1 is upregulated in myeloid cells where it dynamically hinders inflammatory responses. Annexin A1 is known to suppress phospholipase A2 to prevent synthesis of inflammatory eicosanoids [50–53]. Furthermore, enhanced production of annexin A1 in neutrophils inhibits leukocyte transmigration thereby limiting acute tissue injury [48, 49, 54, 55]. Glucocorticoids can also dictate annexin A1 localization within the cell [56, 57]. After exposure to dexamethasone, annexin A1 translocates to the plasma membrane, where it is then secreted to promote leukocyte detachment [52, 57]. Through these mechanisms, annexin A1 has been implicated in quelling acute inflammation to limit local tissue injury.

Metabolic modulation by glucocorticoids

Glucocorticoids also promote the "fight or flight" stress response through metabolic modulation [58]. Acutely, glucocorticoids trigger the liberation of glucose, amino acids, and fatty acids into circulation so these substrates are available for rapid energy production to fuel stress responses [59–61]. Glucocorticoids broadly stimulate metabolic cascades via enhanced cAMP signaling and PKA activation to amplify energy-producing pathways [62–64]. Substrate availability is orchestrated by extensive upregulation of gluconeogenesis and degradation of hepatic glycogen to generate glucose production. At the same time, lipolysis generates free fatty acids and glycerol, and proteolysis liberates amino acids to drive energy

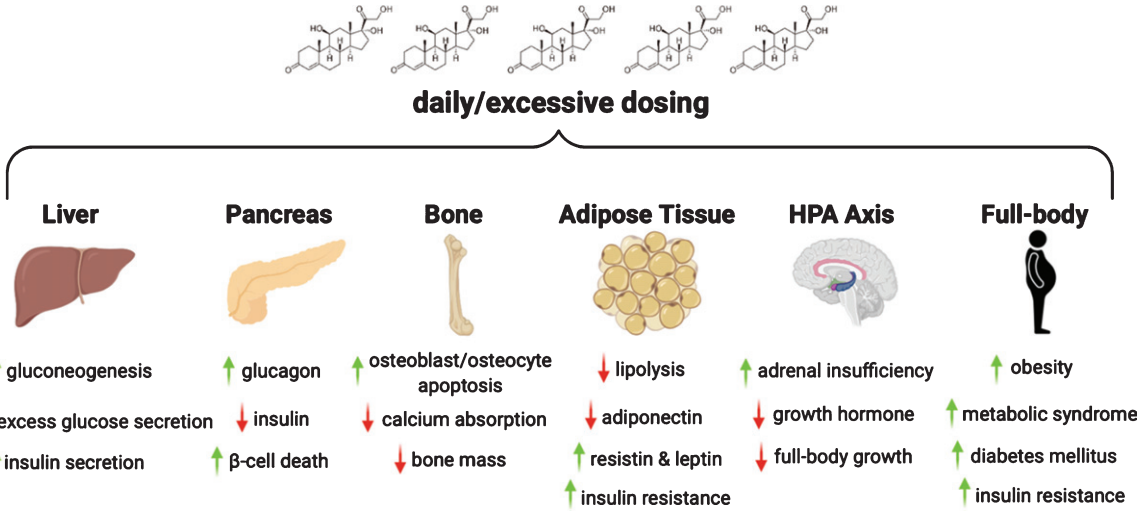

Fig. 3. Chronic intake of glucocorticoids results in glucocorticoid excess which, in turn, has adverse effects on liver, pancreas, bone, adipose tissue and hypothalamus-pituitary-adrenal axis. In DMD, chronic steroid use through puberty also leads to growth suppression and poor bone health. Long term steroid use is linked to metabolic syndrome and insulin resistance.

availability [65–68]. Chronic stimulation of these pathways, for example, from chronic use of synthetic glucocorticoids cause adverse metabolic effects with tissue-specific outcomes, including within the liver, pancreas, adipose tissue, and skeletal muscle [69–73].

Glucocorticoids exert their metabolic effects on multiple tissues and organs. In liver, the effects of glucocorticoids on gluconeogenesis and glycogen content can unfavorably shift hepatic metabolism when chronically stimulated. Persistent gluconeogenesis and glucose production directly cause hyperglycemia and lead to insulin resistance, a hallmark of the metabolic syndrome and diabetes mellitus. Glucocorticoid use can lead to clinical diabetes in non-diabetics [74, 75]. Furthermore, glucocorticoids exacerbate glucose control in those who have diabetes mellitus, often resulting in a greater need for insulin [74, 76, 77]. Prolonged hyperglycemia and insulin resistance promote lipogenesis in hepatic tissue, which can lead to non-alcoholic fatty liver disease and steatosis [75, 78].

In the pancreas, glucocorticoids stimulate glucagon production from α-cells [79]. Additionally, glucocorticoids suppress pancreatic β-cell activity, lowering rates of insulin secretion [80]. Similar to liver, the pancreas exhibits maladaptive responses when chronically exposed to glucocorticoids. Glucocorticoid-mediated inhibition of insulin production occurs concomitant with the inhibition of glucose uptake by other tissues, raising overall serum glucose [81]. The block of insulin production is partially attributed to the cytotoxic effects that glucocorticoids exert on β-cells [73, 82]. It has been shown that chronic dexamethasone exposure is associated with oxidative stress and pro-apoptotic effects in β-cells [83].

Adipose tissue is also a glucocorticoid target. With acute glucocorticoid exposure, enhanced lipolysis occurs, increasing glycerol and free fatty acids [62, 84]. In response to chronic glucocorticoid exposure, insulin-responsive lipolysis decreases and adipogenesis increases [62, 84–86]. Moreover, chronic glucocorticoid use alters adipokine levels, decreasing adiponectin [70] and increasing resistin and leptin secretion, which can influence food intake and insulin responses [87, 88]. Collectively, this hormonal dysregulation contributes to the onset of glucocorticoid-induced obesity and diabetes.

Synthetic glucocorticoids are powerful regulators of systemic metabolism. The effect of acute glucocorticoid exposure is adaptive since it supplies multiple substrates for enhanced energy production. However, chronic excess glucocorticoid levels produce adverse metabolic reprogramming, mediated through multiple peptide hormone pathways (Fig. 3). As a consequence, chronic, long-term glucocorticoids result in insulin resistance, fatty liver, obesity, and even diabetes mellitus. In the setting of muscular dystrophy, where muscle mass is already depleted, these consequences can be further exacerbated.

Effects of glucocorticoids on muscle

Glucocorticoids are pleiotropic regulators of striated muscle function. Genetic ablation of GR exclusively in heart and skeletal muscle significantly alters metabolism and striated muscle performance [89, 90]. Glucocorticoids are prohibited as performance enhancing drugs, although the supporting evidence and the mechanistic rationale are equivocal [91–93]. An important open question is how glucocorticoids regulate muscle contractility. Several studies have pointed to a direct activating role of glucocorticoids in store-operated calcium entry (SOCE). In cultured myotubes, dexamethasone increased SOCE by 15–25% [94]. In isolated rat ventricular cardiomyocytes, a short-term pretreatment with dexamethasone increased contractile force, calcium transient amplitude, and SOCE magnitude through serum and glucocorticoid-regulated kinase 1 (SGK1) [95]. Genetic ablation of GR in murine hearts decreased t-tubule system density and increased the distance between ryanodine receptors and L-type calcium channels [96]. Conversely, dexamethasone decreased the physical distance and improved synchrony of intracellular calcium release through GR-mediated activation of the autophagic flux [96]. These steroid-driven changes in contractility are highly relevant in neuromuscular disease settings. Eight weeks of prednisolone dosing in 5-month-old *mdx* mice increased specific force of the extensor digitorum longus muscle by 26% [97]. Thus, glucocorticoids appear to improve muscle contractility, especially after acute short term exposure.

Nonetheless, prolonged and especially high-dose intake of glucocorticoids promotes muscle wasting and weakness. In a mechanical model of muscle contusion in rats, a high single-dose of 25 mg/kg methylprednisolone resulted in significant benefits in force recovery in the short term (24 hours), but promoted weakness and tissue disorganization at later time points (7–14 days) [98]. Daily administration (60–1,200 μg/kg) of dexamethasone for 5 days induced a rapid dose- and GR-dependent induction of myostatin, promoting loss of muscle mass and myosin type II in rats [99]. Chronic glucocorticoid intake upregulates atrogenes like *Fbxo32* and *Trim63*, which is reduced after muscle-specific GR ablation [100]. Glucocorticoid-induced muscle atrophy leads to sustained and unbalanced activation of the FOXO3 transcription factor. Dexamethasone increases FOXO3 phosphorylation and activity in muscle [67], and FOXO3 inhibition prevents glucocorticoid-induced atrophy in cultured myotubes [101]. Another proposed mechanism linking sustained GR activation to loss of muscle mass is the cross-inhibition between GR and the insulin-responsive anabolic factor mTORC1 [102]. In healthy human subjects, six days of daily prednisone at 0.8 mg/kg was sufficient to induce an acute state of muscle insulin resistance and depressed protein anabolism [103]. The long-term wasting effects are relevant for dystrophic muscle. Chronic continuous exposure to prednisone using sub-cutaneous pellets in food or water in *mdx* mice for 50 weeks resulted in early benefits for approximately ∼2 months and then showed exacerbation of dystrophic progression and weakness [104]. Thus, the atrophy-inducing effects from chronic daily glucocorticoids may counteract the benefits from anti-inflammatory and pro-performance effects.

GLUCOCORTICOIDS IN DYSTROPHIC MUSCLE

The chronic and ongoing injury state seen in DMD is one of the targets of glucocorticoids [105]. A comparative study of deflazacort versus prednisone in *mdx* mice studied the response to cardiotoxin-mediated injury and found that both drugs increased fiber diameter in tibialis anterior and diaphragm. In addition, deflazacort increased many features of regeneration [106]. The same group evaluated glucocorticoids in *mdx* limb muscles using NMR and found increased levels of taurine and creatine, metabolic biomarkers of muscle energy [107]. Glucocorticoids drive functional improvement in dystrophic muscle through activation of transcription factors like KLF15. KLF15 is a GR-activated factor shown to mediate nutrient utilization in glucocorticoid-treated *mdx* muscle [108]. Genetic manipulation of *Klf15* showed that increased KLF15 is beneficial to dystrophic muscle [108]. KLF15, glucocorticoids, and branched chain amino acids may also be relevant to other models of neuromuscular diseases. For example, prednisone, *Klf15* overexpression, and BCAA supplementation improved pathophysiology of a murine model of spinal muscular atrophy [109].

Glucocorticoids also play a direct role in the susceptibility of the sarcolemma to injury. Glucocorticoid exposure accelerated sarcolemmal resealing and repair cap formation at the site of sarcolemmal

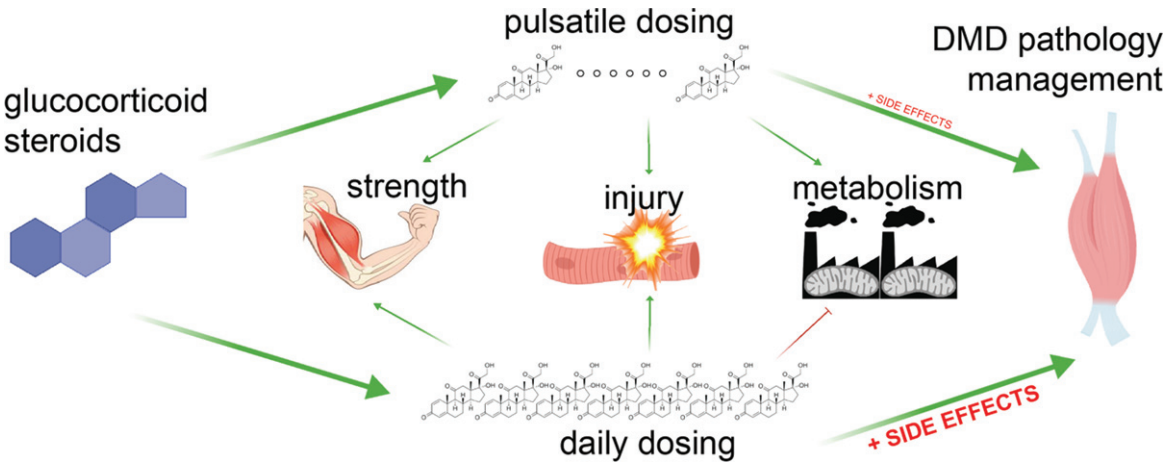

Fig. 4. Daily glucocorticoids improve DMD pathology but induce untoward metabolic side effects. Studies in dystrophic mice and DMD patients suggest that the metabolic benefits of intermittent glucocorticoids can reduce side effects, while maintaining benefits.

injury in normal myofibers and also in multiple models of muscular dystrophy including *mdx* mice and two models of limb girdle muscular dystrophy, *Dysf-null* and *Sgcg-null* mice [110, 111]. The effects of membrane stabilization in *mdx* mice and LGMD-2B patient (*DYSF* mutation) cells were particularly striking for vamorolone, a novel compound designed to minimize GR trans-activation while retaining anti-inflammatory action [112, 113]. Although the causal relationship between anti-inflammatory and anti-injury effects are still unclear, the benefits of glucocorticoids on membrane repair likely extend beyond dystrophinopathies.

Because of the adverse consequences of chronic steroid use, multiple approaches are being pursued to reduce these side effects, such as novel compounds (e.g. vamorolone) and alternative dosing strategies. These alternative dosing strategies have primarily relied on having intervals (days) where steroids are not given. In both pilot and longitudinal studies of DMD patients receiving alternative dosing strategies, benefit was observed without the same magnitude of side effects [114, 115]. An intermittent regimen of oral prednisolone for two consecutive days per week was tested in *mdx* mice; treated *mdx* mice showed an increased strength over time and improved survival between 80 and 104 weeks of age [116]. Intermittent injection of prednisone or deflazacort at a minimal dose of once-weekly comparably benefitted sarcolemmal repair, fibrosis, and immune infiltrations as daily steroids in short term experiments [110, 111]. Once-weekly versus daily prednisone induced opposite epigenetic and metabolic programs in dystrophic *mdx* muscle. While the daily prednisone activated the GR-FOXO axis and drove muscle atrophy and insulin resistance, once-weekly prednisone activated the GR-KLF15-MEF2C axis and enhanced insulin sensitivity, nutrient uptake, and oxidative catabolism in dystrophic muscle, leading to long-term benefits (32–40 weeks) in both *mdx* and *Dysf-null* mice. Therefore, glucocorticoid dosing strategies can improve muscle physiology while minimizing adverse consequences (Fig. 4).

Clinical use of glucocorticoids in Duchenne muscular dystrophy

Glucocorticoid steroids are considered standard of care in DMD [117]. Early clinical trials of prednisone demonstrated a clear benefit compared to natural history. In a double-blind, randomized clinical trial of daily prednisone, DMD patients in both low and high dose groups improved muscle strength and function [118]. Follow-up studies confirmed long-term benefits on ambulation and pulmonary function [119, 120]. In a prospective multi-center cohort study of 440 DMD patients were followed for ten years (NCT-00468832), steroid treatment improved upper and lower extremity muscle strength across all ages and prolonged life expectancy. In 2016, glucocorticoids were recommended to treat DMD patients by the American Academy of Neurology (AAN) [121] and by a Cochrane review [122], but specific dosing recommendations were not given.

Although steroids improve outcome in DMD, it remains unclear if prednisone or deflazacort is

superior. A multi-center double-blind randomized study compared prednisone (daily 0.75 mg/kg) or deflazacort (daily 0.9 mg) for 12 months ($N = 18$). Using a natural history cohort as steroid-naïve control, the study reported similar muscle benefits with both steroids, with deflazacort causing less weight gain than prednisone [123]. A larger study of 340 DMD boys indicated boys on deflazacort were able to maintain ambulation longer at the cost of increased adverse effects including short stature, Cushingoid appearance, and cataracts compared to prednisone [124]. The Finding the Optimum Regimen For Duchenne Muscular Dystrophy (FOR-DMD) study randomized 196 DMD boys to daily deflazacort 0.9 mg/kg, daily deflazacort 1.2 mg/kg, and daily prednisone 0.75 mg/kg, and reported that patients on all regimens showed similar muscle benefits, but deflazacort had less weight gain and fewer behavior-related adverse events as compared to prednisone (NCT-01603407). A *post-hoc* analysis from the placebo arm of the DMD ACT trial (ataluren) reported patients on deflazacort had improved 6 minute walk distances (6MWD) and 4 stair climb times (4SCT) compared to prednisone [125]. A similar *post hoc* analysis from the placebo groups of the phase 3 ataluren trial and tadalafil studies ($N = 231$) showed that deflazacort improved 6MWD and rise-from-supine time significantly more than prednisone, while no difference was found in 10-meter run and North Star Ambulatory Assessment (NSAA) scores between the steroid treated groups [126]. A retrospective study ($N = 330$) over 13 years reported that patients on deflazacort ambulated for a longer period of time (15.6 years) then on prednisone (13.5 years). It is unclear if the prolonged benefit is due to earlier average age at initiation (deflazacort, 6.5 y; prednisone 8.1 y) or steroid type. Additional reported benefits from deflazacort included increased lean body mass, lower weight gain, and decreased risk of scoliosis [127].

Steroids dosing is an active area of research interest with the hope to maximize the benefits/side effects ratio. In DMD steroid treatment is better than no steroid treatment, but the optimal dose and dosing regimen remains unclear [128]. Current dosing regimens include daily, 10 days on followed by 10 days off (10/10), and twice per week (two consecutive weekend days of high dose). Daily dosing causes many side effects including but not limited to weight gain, bone fractures, behavioral disturbances, and Cushingoid features [122]. The 10/10 regimen was initially reported to maintain muscle benefits with fewer side effects and no change in quality of life ($N = 17$), although the study lacked a daily treated control cohort [129]. A later study compared 10/10 to daily dosing ($N = 25$/group) for two years and reported that patients on daily steroids remained ambulatory for longer but had shorter stature, higher BMI, and higher rates of vertebral fractures than patients on 10/10 [130]. Two other studies looking at weekend high dose steroids in DMD showed similar trends. A year-long randomized study 4–10 y patients ($N = 64$; prednisone daily at 0.75 mg/kg versus high dose weekend at 10 mg/kg) showed no significant differences between treatment groups, concluding that weekend prednisone dosing was as effective as daily dosing [114]. A later study in infants/toddlers treated with weekend 10 mg/kg prednisone for 12 months ($N = 23$; 0.4–2.4 y) reported a slight improvement on the Bayley-III gross motor-scaled score and excessive weight gain for 56% of patients [115]. Recently, we reported data from a retrospective cross-sectional study ($N = 24$) comparing daily versus weekend steroid use over five years in groups with comparable body mass indices. Despite similar cumulative doses, DMD patients in the weekend steroid cohort showed lower levels of glycemia, insulinemia, and fat mass and higher lean mass than patients in the daily group [131]. Additionally, morning cortisol levels were higher in the weekend cohort than in the daily cohort, consistent with the concept of lower suppression of the hypothalamic-pituitary-adrenal axis with intermittent dosing [131]. Thus, intermittent steroid dosing appears to mediate steroid benefit with a lower side effect profile, even in the chronic setting.

Efforts to generate novel glucocorticoid derivatives are ongoing and seek to reduce side effects or improve targeted aspects of glucocorticoid function, such as activation of GR, as reviewed in [132, 133]. Examples of novel synthetic derivatives include CpdX, an anti-inflammatory GR agonist, as reviewed in [134, 135], and vamorolone (also known as VBP-15), a dissociative GR ligand and MR antagonist that improves membrane stability [112, 136]. Vamorolone was developed to help alleviate insulin resistance by selecting compounds with Δ9–11 and R1/R3 modifications to promote NF-KB activity and reduce a specific transcriptional cascade mediated by the glucocorticoid receptor [24, 112, 137, 138]. A phase IIA trial ($N = 48$; 4–7 y and steroid-naïve at start) tested daily vamorolone at multiple doses including 2.0 and 6.0 mg/kg/day and reported no evidence of insulin resistance or adrenal suppression after two

weeks of treatment [136]. In the open label extension phase of the study, clinical improvement in time-to-stand (primary endpoint) was reported for the 2.0 and 6.0 mg/kg doses, which also associated with BMI Z-score averages comparable to the prednisone control group (0.493 vs. 0.543) [139]. Vamorolone is currently in a phase IIB clinical trial (NCT-03439670; 4–7 y, ambulatory and steroid-naïve at start), which will compare daily vamorolone 2.0 and 6.0 mg/kg to daily prednisone 0.75 mg/kg/day or placebo for 48 weeks.

Cardiomyopathy is a leading cause of death in DMD, and effects of glucocorticoids on the dystrophic heart require more study. Retrospective studies of steroid use in DMD found significant benefits to onset of cardiomyopathy and systolic function decline [140, 141]. In the study comparing weekend to daily glucocorticoid regimens, no differences were found between treatments on electrocardiography parameters, myocardial thickness or fractional shortening [131], suggesting intermittent dosing might match daily dosing for cardiac benefits. No data are yet available for vamorolone trials in DMD boys, although heart rate has been included as secondary outcome measure in the ongoing trial (NCT03439670). Despite some promising indications, dedicated clinical studies are still required to define the longterm cardiovascular effects of glucocorticoid steroids in DMD.

Currenlty, serum biomarkers and muscle imaging are being investigated to define longitudinal predictive biomarkers of responsiveness to steroids and side effect development. A study using aptamer technology for serum protein identification found seventeen DMD-associated potential biomarkers that responded to steroid intake, including lumican and osteomodulin [142]. A multi-center study found that glucocorticoid use partially counteracted the effects of disease progression on the circulating levels of malate dehydrogenase 2 and ankyrin repeat domain 2 proteins [143]. Objective measures of treatment response and overall muscle health are critical to the success of clinical trials to provide adjuncts to functional testing. In addition to chemical measures, muscle imaging using magnetic resonance is emerging as a promising biomarker [144]. In a year long MRI study, there was less intramuscular fat in steroid-treated versus steroid-naïve DMD ($N = 30$) [145]. Longitudinal MRI and magnetic resonance spectroscopy were used to quantify DMD disease progression and the effect of glucocorticoids in rapidly (vastus lateralis) versus slowly (soleus) degenerating muscles [146]. These data indicate several potential pharmacodynamic biomarkers that can be used to optimize steroid dosing.

Potential of glucocorticoids in other conditions and new treatments

Although there is relatively good data on the use of glucocorticoids in DMD, the use of these agents in other forms of muscular dystrophy, like the milder Becker Muscular Dystrophy (BMD) is less well studied. Examining glucocorticoids in BMD takes on new importance given the anticipated clinical outcomes from gene therapy with micro-dystrophins. There have been few clinical trials assessing glucocorticoid effects in BMD. One study tested daily prednisone for 6 months in a limited number of BMD patients ($N = 6$) and reported significant improvement in overall motor disability and myofiber necrosis [147], but further and larger studies are still required to consolidate these encouraging trends. Similarly, there are few trials examining the effect of steroids in the limb-girdle muscular dystrophies (LGMDs); the rare nature of these disorders challenges having sufficiently powered clinical trials. In a case report, two siblings with β-sarcoglycan limb-girdle muscular dystrophy (LGMD) showed clinical improvement in quantitative muscle testing with 22 months of 0.9 mg/kg/day deflazacort [148]. Prior case reports had shown similar benefits of prednisone in patients with δ-sarcoglycan LGMD [149, 150]. A double-blind placebo-controlled cross-over trial in dysferlin-mediated LGMD tested deflazacort (daily 1.0 mg/kg in month one, followed by 1 mg/kg every other day in months two through six) versus placebo (NCT-00527228; $N = 25$). Deflazacort associated with decreased muscle strength, per % CIDD score, and Neuromuscular Symptom Score, and the study concluded that off-label use of deflazacort is not warranted in LGMD-2B [151]. Currently, there are two clinical trials testing glucocorticoids in LGMD patients. In one study (NCT-03783923; enrolling $N = 100$) daily deflazacort 0.6 mg/kg will be tested versus placebo in FKRP related LGMD for 26 weeks with 4SCT as primary outcome. In another study (NCT-04054375; enrolling $N = 30$), once-weekly prednisone 0.75 mg/kg will be tested in patients with all forms of LGMD, with safety as primary endpoint and muscle function improvement as a secondary outcome. Clinical case studies and animal models point to some efficacy of glucocorticoids in a broader range of muscular dystrophies. The results of

open label clinical trials will help shed light on potential and disease-specific strategies of glucocorticoid use beyond DMD.

Gene correction/replacement therapies are advancing. At least two oligonucleotides for skipping mutated exons of the *DMD* gene have been approved by the FDA (Exondys51, Vyondys53). Gene therapy to express mini-dystrophin in DMD muscle is being evaluated in ongoing clinical trials (NCT00428935, NCT02376816 and NCT03362502). Moreover, gene therapy to correct the endogenous mutant dystrophin through CRISPR/Cas9 has shown promising results in pre-clinical large animal models [152]. With the progress in virally-mediated gene therapies, it is likely that life expectancy, symptom development and duration of treatment will change considerably for DMD. It is expected that the gene therapies will significantly slow disease progression, therefore creating the clinical need for long term management. In this respect, it will be imperative to advance our precise understanding of mechanisms and biomarkers of glucocorticoid steroid action in dystrophic muscle physiology. This knowledge will be key to maximize steroid efficacy in supporting genetic/functional rescue, while minimizing the adverse effects associated with virtually life-long intake of these drugs.

CONCLUSIONS

Glucocorticoid steroids are powerful agents to regulate inflammation, metabolism and muscle physiology. Their clinical use in neuromuscular diseases will be further informed by integrative studies that evaluate the effect of these agents on immune cells, muscle tissue, and metabolic homeostasis. It will be critical to adapt glucocorticoid regimens to specific subtypes of neuromuscular disease, perhaps tailored using imaging or serum biomarkers. Therefore, a deeper understanding of direct versus indirect mechanisms of action will be critical to revise and expand to optimize the use of glucocorticoids in DMD and possibly in other neuromuscular conditions.

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CONFLICT OF INTEREST STATEMENT

MQ and EMM are listed as co-inventors on a patent application related to intermittent glucocorticoid use filed by Northwestern University.

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