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Review

Glucocorticoid-induced apoptosis and cellular mechanisms of myopathy

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ABSTRACT

Glucocorticoid-induced myopathy is a common side effect of chronic glucocorticoid therapy. Several mechanisms are currently being examined as ways in which glucocorticoid-induced myopathy occurs. These include apoptotic signaling through mitochondrial-mediated and Fas-mediated apoptosis, the role of the proteosome, the suppression of the IGF-1 signaling, and the role of ceramide in glucocorticoid-induced apoptosis and myopathy. It is difficult to differentiate which mechanism may be the initiating event responsible for the induction of apoptosis; however, all of the mechanisms play a vital role in glucocorticoid-induced myopathy.

Contents

1. Introduction

Glucocorticoids are immunosuppressants that are clinically used to reduce acute inflammation and swelling. Millions of people take glucocorticoids for chronic therapy to treat diseases such as rheumatoid arthritis, asthma, organ transplants, and primary or secondary adrenal insufficiency (Addison's disease). Common side effects of glucocorticoids include insomnia, nervousness, gastrointestinal upset, arthralgias, immunosuppression, edema and myopathy [1].

With over 50 years of use [2], glucocorticoids are one of the common medications known to cause myopathy, especially with prolonged high doses. The incidence of muscle weakness and myopathy can reach as high as 50% of persons receiving long-term glucocorticoid therapy [3,4]. Characteristics of myopathy include muscle atrophy and weakness, insulin resistance, oxidative stress, and mitochondrial dysfunction. Steroid-induced myopathy is proximal and symmetrical and may involve both upper and lower extremities. Histological changes may include type II specific atrophy of muscle fibers, loss of myosin filaments in sarcomeres with preservation of thin filaments and Z-bands, and necrosis [5]. Steroid myopathy is more commonly associated with the use of fluorinated steroids, such as dexamethasone, betamethasone, and triamcinolone, but can also be caused from nonfluorinated steroids, such as prednisolone and hydrocortisone [6].

While mechanisms of glucocorticoid-induced myopathy are not completely understood they are being slowly elucidated. Apoptosis may be a contributing mechanism and therefore is the focus of this review. Glucocorticoid-induced apoptosis has been shown to involve mitochondrial-mediated and Fas-mediated signaling.

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It has also been shown that the proteosome, the suppression of the IGF-1 signaling, and the accumulation of ceramide may play important roles in glucocorticoid-induced apoptosis and myopathy. Most of what we know about glucocorticoid-induced apoptosis comes from data regarding immune cells. There are far less data specifically focused on glucocorticoid-induced apoptosis in skeletal muscle. Using the data from immune cells and evidence that glucocorticoid-induced apoptosis in skeletal muscle may be by a similar mechanism to that of immune cells, we have hypothesized that myopathy may involve activation of Fas signaling leading to caspase-8 activation via generation of ceramide. Activation of mitochondrial-mediated signaling may be a mechanism to amplify the apoptotic signal. Moreover, the proteosome and IGF-1 signaling play a role in regulating this apoptotic process by increasing the apoptotic potential. The role of these apoptotic pathways and signaling molecules in glucocorticoid-induced apoptosis in skeletal muscle and immune cells will be discussed. Better understanding of these mechanisms may help to develop therapies to preserve muscle mass and function in those patients on chronic glucocorticoid therapy.

2. Apoptotic signaling

Apoptosis is an evolutionary conserved process by which individual cells of a multicellular organism commit suicide. Although apoptosis is important in maintaining health, excessive or inadequate apoptosis can contribute to disease pathophysiology. Apoptosis is executed by specific cellular signaling pathways and is therefore characterized by specific biochemical and morphological events. Some of these identifying features of apoptosis include chromatin condensation and DNA fragmentation into mono- and oligonucleosomes, cellular shrinkage, translocation of phosphatidylserine to the outer leaflet of the plasma membrane, and membrane blebbing forming apoptotic bodies which are engulfed by macrophages or neighboring cells.

Apoptosis is mediated by the activation of a variety of cysteine proteases, known as caspases. Caspases normally exist in an inactivated state called procaspases but can be activated by proteolytic cleavage and subsequent heterodimerization. Initiation of apoptosis involves activation of a caspase cascade in which "initiator" caspases (i.e., caspase-8, caspase-9, caspase-12) first become activated and then cleave and activate "effector" caspases (i.e., caspase-3, caspase-6, caspase-7). The effector caspases carry out the proteolytic events that result in cellular breakdown and demise. There are 14 known mammalian caspases (i.e., caspase-1–caspase-14), which participate in the apoptotic process depending on the stimulus and respective signaling pathway activated and/or cell type undergoing apoptosis.

Mitochondria play a central role in initiating apoptosis (see Fig. 1). Upon stimulation, mitochondria can release cytochrome *c* into the cytosol which forms a complex, known as the apoptosome, with procaspase-9, Apaf-1, and dATP. Once the apoptosome is formed, procaspase-9 can cleave and activate itself. The active enzyme, caspase-9, can cleave and activate effector caspases such as procaspase-3, which leads to the typical morphological features of apoptosis. The Bcl-2 family of proteins regulates the release of cytochrome *c*. This family consists of a number of proteins, which are anti-apoptotic or pro-apoptotic. For example, Bcl-2 and Bcl-X_L protect against cytochrome *c* release and are therefore anti-apoptotic while Bax, Bak, Bad, and Bid favor cytochrome *c* release and are therefore pro-apoptotic. The ratio and interaction of the anti-apoptotic and pro-apoptotic Bcl-2 family proteins determines the fate of cytochrome *c* release from the mitochondria. Often the Bcl-2/Bax ratio is used as an indicator of apoptotic potential where a high ratio protects against apoptosis and a low ratio favors apoptosis. Another regulatory mechanism of apoptosis involves the inhibition of caspases by inhibitor of apoptosis proteins (IAPs). The IAPs (i.e., XIAP, cIAP1, cIAP2) can bind to and inhibit activity of caspase-9 and -3. Lastly, the mitochondria can release additional proteins, along with cytochrome *c*, to relieve the inhibition exerted by the IAPs so indeed apoptosis can be executed. These proteins include Smac/Diablo and Omi/HtrA2.

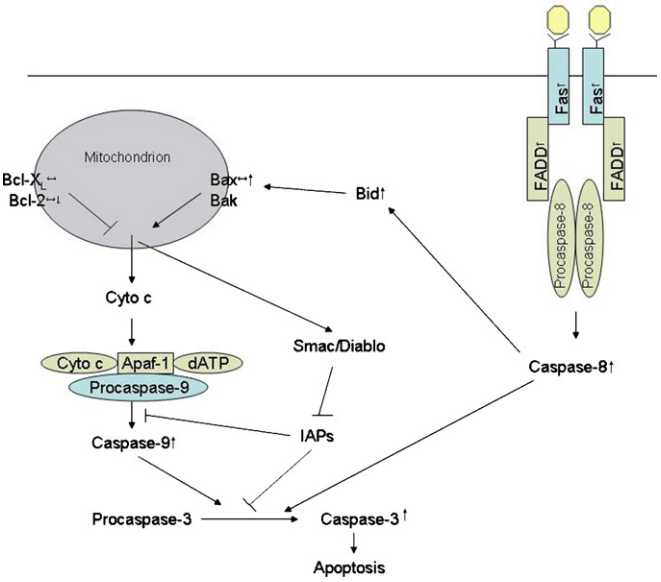

Fig. 1. Simplified scheme of mitochondrial-mediated and Fas-mediated apoptosis. Cytochrome *c* (Cyto *c*) release from the mitochondrion leads to formation of the apoptosome (Cyto *c*, dATP, Apaf-1, and procaspase-9) and activation of procaspase-9. Active caspase-9 cleaves and activates procaspase-3 which leads to apoptosis. Bax and Bak favor Cyto *c* release while Bcl-2 and Bcl-X_L inhibit Cyto *c* release. IAPs inhibit apoptosis by inhibiting activation of procaspase-9 and -3. Smac/Diablo is released from the mitochondrion and inhibits the IAPs, relieving their inhibitory effect on apoptosis. Fas-mediated apoptosis involves recruitment of FADD to the cytoplasmic domain of the Fas receptor which then recruits Procaspase-8. Once activated, caspase-8 can then activate caspase-3 directly and/or activate Bid which then activates the mitochondrial-mediated signaling pathway. Arrows signify a stimulatory effect and blunt lines signify an inhibitory effect. Superscript arrows represent the known effects of glucocorticoid treatment in skeletal muscle.

Mitochondria can also release pro-apoptotic proteins that are involved in caspase-independent apoptosis. Mitochondria can release apoptosis inducing factor (AIF) and endonuclease G (EndoG) which translocate to the nucleus to induce chromatin condensation and DNA fragmentation in a caspase-independent manner.

Several other apoptotic pathways require an alternate initiator caspase to initiate the caspase cascade (i.e., caspase-8). Receptor-mediated pathways can be activated by tumor necrosis factor-alpha (TNF-α) or Fas ligand (FasL) binding to their cognate receptors and induce apoptosis by the activation of procaspase-8, which cleaves and activates procaspase-3 to initiate the caspase cascade. For example, caspase-8 can be activated by the recruitment of Fas associated protein with death domain (FADD) to the intracellular domain of the Fas receptor, which then recruits procaspase-8 leading to its activation (see Fig. 1). Once apoptosis is initiated via caspase-8 the release of cytochrome *c*, and other pro-apoptotic proteins, from the mitochondria and activation of the mitochondrion-mediated signaling may occur, but is downstream from caspase-8 activation. Active caspase-8 cleaves Bid, which then stimulates Bax and Bak activity resulting in cytochrome *c* release. Some cell types require activation of the mitochondrion-mediated signaling via Bid to execute apoptosis and others do not.

3. Mechanisms of glucocorticoid-induced apoptosis and myopathy

It has been consistently shown that glucocorticoids induce apoptosis in skeletal muscle as evidence by the activation of caspase-3, DNA fragmentation, and/or translocation of phosphatidylserine [7–12]. The signaling pathways responsible are slowly being unraveled. Evidence suggests that the mitochondrial-mediated apoptotic signaling is activated by glucocorticoid treatment, but it is not clear if its activation is the initiating event in the induction of apoptosis or if it is activated as a result of an alternative upstream apoptotic signaling pathway, such as receptor-mediated apoptotic signaling (see Fig. 1). In the latter case, activation of the mitochondrial-mediated signaling may function to amplify the apoptotic signal, however, not required for the execution of apoptosis in response to glucocorticoids. Glucocorticoid-induced modulation of the ubiquitin–proteasome system, IGF-1 signaling, and ceramide play an important role in the induction of apoptosis and myopathy.

3.1. The role of mitochondrial-mediated signaling in glucocorticoid-induced apoptosis

Glucocorticoid treatment leads to activation of the mitochondrial-mediated apoptotic signaling pathway in a variety of cell types including skeletal muscle [8,13–18]. It was shown that protein levels of Bax and Bad were elevated while levels of Bcl-2 and Bcl-X_L were unaffected by treatment in rat soleus [8]. Lim et al. reported that glucocorticoid treatment decreased the protein levels of Bcl-2 with little effect on Bax in the soleus muscle of mice [9]. In either case, the ratio between anti-apoptotic and pro-apoptotic Bcl-2 members in skeletal muscle was decreased potentiating apoptosis, as also shown to be the case in other cell types. For example, in acute lymphoblastic leukemia (ALL) cell lines and *ex vivo* samples taken from ALL patients that are responsive to dexamethasone treatment, dexamethasone was shown to increase the activation of Bax and Bak and down-regulate Bcl-2 and Bcl-X_L [16]. This was not the case in *ex vivo* samples from dexamethasone-resistant ALL patients [16]. In the latter case, *ex vivo* samples treated with dexamethasone did not show any changes in the Bcl-2 family proteins [16]. Glucocorticoid treatment also increases the expression of pro-apoptotic Bcl-2 proteins and decreases the expression of anti-apoptotic Bcl-2 proteins in thymocytes [19,20]. Hoijman et al. showed that 3 h of glucocorticoid treatment of primary thymocytes lead to an increase in Bax and a decrease in Bcl-X_L protein content, while there was no change in Bcl-2 content [20]. These data suggest that activation of the pro-apoptotic and down-regulation of anti-apoptotic Bcl-2 proteins occurs during glucocorticoid-induced apoptosis.

Mitochondrial cytochrome *c* release from mitochondria in glucocorticoid-treated skeletal muscle cells has not been measured, however it has been shown to be elevated in treated thymocytes [14,15,20,21]. Hoijman et al. reported a correlation between the protein levels of Bax and the release of cytochrome *c* [20]. Caspase-9 was also activated in glucocorticoid-treated thymocytes [13,14]. It has also been shown that glucocorticoid treatment results in elevated protein levels of cleaved caspase-9 in rat soleus muscle [8]. These data indicate that glucocorticoid-induced apoptosis leads to the activation of mitochondrial-mediated signaling in both skeletal muscle and thymocytes, presumably via cytochrome *c* release. However, in multiple myeloma cells glucocorticoid treatment leads to activation of caspase-9 independent of cytochrome *c* and Apaf-1 [22]. Glucocorticoid treatment leads to Smac/Diablo, but not cytochrome *c*, release from mitochondria which then binds to XIAP causing dissociation from and activation of caspase-9 [22]. Inhibition of caspase-9 in these cells significantly attenuated apoptosis, but did not completely abolish it. Taken together, the mechanism of caspase-9 activation in response to glucocorticoid treatment may vary depending on cell type, but appears to be dependent on the release of pro-apoptotic proteins (e.g., cytochrome *c*, Smac/Diablo) from the mitochondria. In thymocytes it appears that cytochrome *c* release does occur in response to glucocorticoid treatment. Future studies will determine if this is also the case in skeletal muscle.

3.2. The role of Fas-mediated signaling in glucocorticoid-induced apoptosis

Although glucocorticoid treatment is associated with activation of mitochondrial-mediated signaling, there is evidence to suggest that it is activated via receptor-mediated mechanisms. Along with activation of caspase-9, caspase-8 has been shown to be activated in the soleus muscle of glucocorticoid-treated rats [7,8]. Furthermore, the protein levels of Bid, FADD, and Fas were also elevated [7,8]. Typically, Fas-ligand receptor binding induces formation of this complex. Once the complex is formed, procaspase-8 molecules cleave and activate each other. Fas is expressed in pathophysiological skeletal muscle, but not in normal healthy muscle cells, making muscle cells more susceptible to Fas-induced apoptosis. From these data, it is reasonable to hypothesize that a potential mechanism of glucocorticoid-induced apoptosis in skeletal muscle may be activation of caspase-8 via formation of the death-inducing complex consisting of Fas, FADD, and procaspase-8. Caspase-8 activates caspase-3 and Bid, with the latter leading to activation of the mitochondrial-mediated signaling as a mechanism of amplification of the apoptotic signal. Thus, activation of mitochondrial-mediated signaling is not obligatory for apoptotic cell death in response to glucocorticoid treatment.

Although this hypothesis has not been tested in skeletal muscle, it has been tested in glucocorticoid-treated thymocytes. The data support that activation of mitochondrial-mediated signaling is not obligatory for glucocorticoid-induced apoptosis in thymocytes [13,14]. It was shown that dexamethasone induces the release of cytochrome *c* and the activity of caspase-8, -9, and -3 [13,14]. Using various caspase inhibitors, inhibition of caspase-9 did not prevent activation of caspase-3, cytochrome *c* release, or apoptosis [13,14]. However, inhibition of caspase-8 did prevent the release of cytochrome *c* and caspase-3 activation [14] and did significantly attenuate apoptosis, although it did not completely abolish apoptosis [13]. These data are consistent with the effects of dexamethasone in caspase-9 null mice in which apoptosis of thymocytes is not completely inhibited, suggesting that mitochondrial-mediated apoptotic signaling is not required for apoptosis to occur [23,24]. Moreover, Bid is not necessary for glucocorticoid-induced apoptosis in thymocytes, as shown in Bid deficient knockout mice [25]. The data support the hypothesis that caspase-8 may be important in activating mitochondrial-mediated signaling as a mechanism of amplification, but may not be the required and initiating event in glucocorticoid-induced apoptosis.

How is caspase-8 activated? Marchetti et al. showed that the glucocorticoid-induced caspase-8 activity in thymocytes correlated with the formation a death-inducing complex with FADD and caspase-8 [14]. It has previously been reported that formation of this complex can activate caspase-8 and induce Fas-mediated apoptosis in a Fas-ligand independent mechanism [26]. It was also determined that formation of the death-inducing complex and activation of caspase-8 was dependent upon activation of acidic sphingomyelinase (aSMase) and production of ceramide [13,14]. Marchetti et al. showed that activation of aSMase in thymocytes is mediated by protein kinase C (PKC) and phosphatidylinositol-dependent phospholipase C (PI-PLC) [14]. Glucocorticoid-induced apoptosis of L6 muscle cells was also shown to be dependent on PKC and PLC activity, as well as phospholipase A_2 (PLA_2) [10]; evidence

that the mode of glucocorticoid-induced apoptosis in myocytes may be similar to that in thymocytes.

In summary, glucocorticoid-induced apoptosis may be due to the synthesis of ceramide via activation of aSMase leading to formation of the death-inducing complex and activation of caspase-8 which, in turn, results in apoptosis via direct cleavage of caspase-3 and indirectly via activation of the mitochondrial-mediated pathway. Since caspase-8 inhibition did not completely abolish apoptosis there may be other apoptotic signaling pathways involved.

3.3. Role of the proteasome in glucocorticoid-induced apoptosis and myopathy

Glucocorticoids stimulate activity of the proteasome in skeletal muscle causing significant atrophy via myofibrillar protein degradation [27]. Glucocorticoids stimulate the expression of several proteins involved in the ubiquitin–proteasome system, including ubiquitin, conjugating enzymes, ubiquitin ligases, and subunits of the proteasome [28,29]. For example, dexamethasone treatment increases the expression of the E3 ubiquitin ligase MuRF1 (muscle RING finger protein 1) in differentiated myotubes which was shown to be responsible for degradation of myosin heavy chain (MYH) [30]. MuRF1 (−/−) mice experience significantly less dexamethasone-induced MYH depletion compared to wild-type mice [30].

The catabolic action of glucocorticoids appears to be mediated via the FOXO transcription factors. Overexpression of FOXO causes induction of genes involved in the ubiquitin–proteasome system and muscle atrophy [31]. Furthermore, glucocorticoid-induced atrophy was prevented by overexpressing a dominant-negative form of FOXO-3a [31]. Thus, activation of the ubiquitin–proteasome system induced by glucocorticoid treatment is mediated through FOXO transcription factors and plays a central role in myofibrillar protein degradation leading to significant atrophy of muscle fibers.

Aside from myofibrillar protein degradation, activation of the ubiquitin–proteasome system has also been implicated as an important mediator of glucocorticoid-induced apoptosis [13,15,32]. Tonomura et al. showed that inhibition of the proteasome partially inhibited H_2O_2 production, alterations in mitochondrial membrane potential, cytochrome *c* release, caspase-3 activity, and apoptosis in dexamethasone treated thymocytes [15]. Furthermore, proteosome inhibitors also prevented DNA fragmentation and phosphatidylserine exposure on glucocorticoid-treated thymocytes [33]. Lepine et al. also showed that proteasome inhibition partially attenuated alterations in mitochondrial membrane potential and significantly interfered with activation of caspase-8, -9, and -3 [13]. Data suggest that activation of the proteasome is upstream from disruption of the mitochondrial transmembrane potential and caspase activation. Stabilization of the mitochondrial transition pore and inhibition of caspases by pharmacological agents did not prevent proteasome activation [33,34]. Overexpression of Bcl-2, inhibition of protein synthesis, and antioxidant supplementation did prevent proteasome activation [34].

The proteins targeted for degradation by the proteasome in glucocorticoid-induced apoptosis are currently being unveiled. Some targets of the proteasome during apoptosis induction include proteins that regulate the cell cycle such as c-Fos [35], p27Kip1 [36], and ornithine decarboxylase [37] and proteins that inhibit caspase activity such as XIAP and cIAP1 [32]. Inhibiting degradation of each of these proteins has shown to attenuate apoptosis [32,35–37].

The ubiquitin–proteosome system is also responsible for regulating myogenic transcription factors (MRFs) and their negative regulators, the family of inhibitors of DNA-binding (Id) proteins, which are involved in muscle differentiation and development [38]. MyoD is a central MRF that turns on the transcriptional program for differentiation and development. The Id proteins inhibit the action of MyoD and inhibit differentiation of myoblasts into myotubes to potentiate proliferation of myoblasts. It has also been shown that the Id proteins may be involved in the induction of apoptosis of cardiac and skeletal muscle cells [39–41]. Skeletal muscle apoptosis associated with unloading-induced muscle atrophy involves Id2 [40]. It was found that muscle unloading lead to increased protein content of cytoplasmic Id2 with no change in nuclear levels of Id2 [40]. The cytoplasmic content of Id2 positively correlated with pro-apoptotic markers such as the TUNEL index, Bax, AIF, and p53 and negatively correlated with anti-apoptotic markers such as Bcl-2. Interestingly, nuclear levels of Id2 negatively correlated with pro-apoptotic markers [40]. These data suggest that the Id proteins may have different functions depending on cellular localization in skeletal muscle. Nuclear localization may be associated with proliferation, while cytoplasmic localization may be associated with apoptosis induction. In cardiac myocytes, overexpression of Id1 causes apoptosis [39]. In addition, overexpression of Id1 in C2C12 myoblasts lead to decreased cell viability, however, the mode of cell death was not determined [41]. These data suggest that the Id family may play an important role in determining the life or death of a cell.

It is known that glucocorticoids lead to a decreased content of MyoD and an increased content of Id1 [38]. Other Id proteins have not been investigated. Glucocorticoids differentially regulate degradation of these proteins by N-terminal ubiquitination leading to degradation of MyoD, but not Id1, in differentiated myotubes to promote muscle protein catabolism and possibly apoptosis [38]. In undifferentiated myoblasts, Id1 localized to the nucleus, but in differentiated myotubes Id1 is localized to the cytoplasm [42], therefore, it is possible that increased cytoplasmic levels of Id1 induced by glucocorticoid treatment may potentiate apoptosis in skeletal muscle cells, similarly to Id2, and also possible that overexpression of Id1 has the same effect in skeletal muscle cells as that shown in cardiac myocytes.

These data suggest that the proteasome may play an important role in glucocorticoid-induced apoptosis by degrading cell-cycle and anti-apoptotic regulatory proteins, thereby, increasing the apoptotic potential.

3.4. Suppression of IGF-I signaling in glucocorticoid-induced apoptosis and myopathy

The loss of muscle mass in glucocorticoid treatment is, in part, due to suppression of IGF-1 signaling [43]. In fact, it has been shown that systemic administration of IGF-1 and IGF-1 gene transfer can attenuate glucocorticoid-induced muscle atrophy [43–45]. IGF-I has an anabolic effect in skeletal muscle by increasing rate of protein synthesis [46] and by stimulating satellite cell proliferation and differentiation for growth and repair [47]. IGF-1 signaling plays an anti-catabolic role in skeletal muscle by suppressing the activity of the ubiquitin–proteasome system and also by suppressing apoptosis [12].

Many of the effects of IGF-1 are mediated through phosphorylation of Akt. First, Akt phosphorylation leads to inhibition of FOXO transcription factors and, therefore, expression of proteins involved in the ubiquitin–proteasome system. Secondly, activation of Akt suppresses apoptosis by inhibiting Bad [48] and also prevents cleavage of procaspase-9 [49], both via phosphorylation. Thirdly, Akt phosphorylates and inhibits pro-apoptotic Forkhead transcription factor, FKHRL1, which is thought to regulate the expression of many pro-apoptotic genes, such as Fas ligand [50]. Indeed, Akt phosphorylation has been found to be suppressed in glucocorticoid-treated muscle tissue, as much as 50% [8,12,51]. Thus, decreased IGF-I signaling induced by glucocorticoids may potentiate apoptosis by relieving inhibition on Bad, procaspase-9, and FKHRL1 and also by

increased degradation of XIAP and cIAP1 via upregulaton of the ubiquitin–proteasome system.

Factors that are responsible for glucocorticoid-induced suppression of IGF-1 signaling are under investigation. Dexamethasone inhibited IGF-I-mediated Akt phosphorylation in L6 myoblasts under conditions of metabolic stress, which led to enhanced apoptosis via the induction of the PI3K inhibitor, p85α. p85α interferes with PI3K activation via IGF-I, thereby, inhibiting Akt phosphorylation [12]. Others have shown that inhibition of IGF-1 signaling may also be due to reduced muscle content of IGF-1 [43] and insulin-receptor substrate-1 (IRS-1) [52] or increased levels of myostatin [27] and ceramide [53], both of which inhibit Akt phosphorylation.

In summary, suppression of IFG-1 signaling by glucocorticoids contributes to myopathy by suppressing anabolic processes and stimulating catabolic processes such as proteolysis and apoptosis.

3.5. The role of ceramide in glucocorticoid-induced apoptosis and myopathy

Ceramide is a sphingosine-based lipid second messenger produced by *de novo* synthesis and sphingomyelin degradation. Glucocorticoid treatment increases the production of ceramide in a variety of cell types, including skeletal muscle [13,14,53] and may be a central player in many of the pathological effects associated with glucocorticoid-induced myopathy, including insulin resistance, atrophy, and apoptosis.

Ceramide is known to interfere with IGF-1/insulin signaling resulting in decreased expression and decreased phosphorylation and activation of Akt [51,53]. Due to the many protein targets of Akt, reduced signaling has a plethora of consequences. First, ceramide production and reduced Akt signaling contributes to insulin resistance. A strong negative correlation exists between ceramide content in human skeletal muscle and insulin sensitivity [54]. It has also been shown that infusion of ceramide into cultured myotubes inhibits insulin signaling [55,56]. Skeletal muscle accounts for approximately 80% of whole body insulin-stimulated glucose disposal [57]. A blunted insulin response and suppressed Akt activation in skeletal muscle leads to decreased glucose uptake due to reduced Glut4 translocation and decreased glycogen synthesis via insufficient glycogen synthase activity [51]. Using physiologic levels of insulin, a blunted response in glucocorticoid-treated rats lead to a 40% reduction in glucose uptake and a 70% reduction in glycogen synthesis in the soleus muscle [51]. Akt expression was reduced by 50% [51].

Secondly, ceramide production and reduced Akt signaling can contribute to myofiber atrophy. Ceramide inhibits IGF-1 induced protein synthesis and differentiation which would inhibit muscular growth and repair [58]. Addition of ceramide and inducers of intracellular ceramide production inhibit IGF-1 induced protein synthesis and expression of myogenin and MyoD, which leads to reduced differentiation of myoblasts and fusion into myotubes [58]. The same treatment was shown to inhibit protein synthesis in myotubes [58]. Ceramide was also shown to inhibit amino acid uptake into skeletal muscle [59]. Furthermore, ceramide was shown to induce G(2) cell cycle arrest in rhabdomyosarcoma cells via the rapid induction of p21, consequently resulting in apoptosis at a later time point [60]. Ceramide was also shown to stimulate the ubiquitin–proteasome system [61]. In summary, ceramide accumulation likely contributes to glucocorticoid muscle atrophy by attenuating growth and repair via inhibition of amino acid uptake, suppression of IGF-1 induced protein synthesis and differentiation, and the induction of cell cycle arrest while also stimulating myofibrillar protein degradation via the ubiquitin–proteasome system.

Thirdly, ceramide production can induce apoptosis in a variety of cell types, including muscle cells [13,14,60,62]. As previously mentioned, ceramide can induce apoptosis in rhabdomyosarcoma cell lines, following cell cycle arrest [60]. It was shown that overexpression of Bcl-2 could prevent apoptosis induced by exogenous ceramide treatment, but did not prevent induction of p21 and cell cycle arrest. Inhibition of p21 attenuated apoptosis [60]. The data suggests that the actions of Bcl-2 are downstream of p21 induction. Ceramide has also been shown to induce apoptosis in myotubes [62]. Glucocorticoids cause an increase in circulating free fatty acids (FFAs) and accumulation in insulin-dependent tissues, such as skeletal muscle [53,63]. The intramyocellular lipid accumulation can lead to apoptosis via the production of ceramide, as well as insulin insensitivity previously described [62]. Apoptosis was confirmed via caspase-3 activation, phosphatidylserine exposure, and positive TUNEL staining. Interestingly, inhibition of caspase-3 not only attenuated apoptosis but also restored insulin sensitivity [62]. It was noted that cytochrome *c* release from mitochondria and caspase-9 activation is associated with ceramide-induced apoptosis, but it was not determined if these events were required for apoptosis to occur [62]. The authors noted that exposure of myotubes to FFAs also lead to endoplasmic reticulum (ER) stress [62]. It is known that ER stress and calcium dyshomeostasis can induce apoptosis via activation of calpain and caspase-12. It is also known that glucocorticoid-induced apoptosis is associated with elevated intracellular calcium [10,18]. Calcium sequestration and calpain inhibition attenuated glucocorticoid-induced apoptosis in L6 muscle cells [10]. It is possible that apoptosis was mediated through calpain activation of caspase-12. However, the role of caspase-12 in glucocorticoid-induced apoptosis has not been studied.

Ceramide is also known to induce oxidative stress and mitochondrial dysfunction; characteristics of glucocorticoid-induced myopathy. Glucocorticoids have been shown to decrease the expression of endogenous antioxidants and increase ROS production in cultured L6 muscle cells [10]. Furthermore, biopsied muscle taken from patients undergoing chronic glucocorticoid treatment showed decreased activity of complex I of the electron transport chain and increased oxidative damage to nuclear and mitochondrial DNA in a dose-dependent manner [64]. Ceramide has been shown to inhibit complex I in brain mitochondria [65]. There was also a strong correlation between the dose of glucocorticoids and the production of lactate at rest and during aerobic exercise in these patients, suggesting that the treatment may cause mitochondrial dysfunction [64]. It has also been reported that mitochondrial enlargement and aggregation can occur with glucocorticoid treatment [66].

In summary, chronic glucocorticoid treatment causes myopathy which is characterized by oxidative stress, mitochondrial dysfunction, insulin resistance, muscle atrophy due to impaired growth regeneration and excessive proteolysis, and apoptosis. Ceramide has been shown to play a role in all of these processes and, therefore, may be a central mediator of glucocorticoid-induced myopathy. However, more research is needed to verify this hypothesis.

4. Perspective

Aside from its central role in apoptosis, recent data suggests that caspase-3 also has a non-apoptotic role in skeletal muscle [67]. Research has shown that caspase-3 plays a role in muscle proteolysis in catabolic states by cleaving the actomyosin complex resulting in 14-kDa actin fragments in which the ubiquitin–proteasome system is then responsible for degrading. The role of caspase-3 in muscle proteolysis yields a 125% increase in protein degradation by the ubiquitin–proteasome system [67]. Thus, caspase-3 may play a dual role in glucocorticoid-induced myopathy; death of individual muscle fibers via apoptosis, as well as, muscle proteolysis leading to atrophy of muscle fibers.

Type II muscle fibers appear to be more susceptible to the atrophying effects of glucocorticoids compared to type I fibers [6]. The cause is currently not known. Furthermore, there are currently no studies that investigate the differential effects of glucocortoicoids on apoptotic adaptations in various fiber types.

5. Conclusion

Glucocorticoids are pharmacologically used to suppress inflammation and the immune response. These effects are mediated through the induction of apoptosis of thymocytes and other immune cells. Glucocorticoids cause significant myopathy, among many other detrimental side effects, but are continually used to treat patients due to the targeted effectiveness. It is important to elucidate the mechanisms of glucocorticoid-induced myopathy to hopefully find ways to alleviate this adverse effect of the drug. Glucocorticoid-induced myopathy is characterized by significant muscular atrophy which is due to suppressed protein synthesis and growth, enhanced proteolysis of myofibrillar proteins, and the induction of apoptosis.

Glucocorticoid-induced apoptosis in skeletal muscle is associated with activation of the mitochondrial and receptor-mediated signaling pathways. It has also been shown that the proteasome, suppression of the IGF-1 signaling, and generation of ceramide play a role as well. It is difficult to discern a single clear cut signaling pathway responsible for the induction of apoptosis. It is likely that multiple signaling pathways work together to orchestrate cell death. A possible scheme may involve the activation of Fas signaling leading to caspase-8 activation via the generation of ceramide, as shown in thymocytes. Activation of the mitochondrial-mediated signaling pathway may be a mechanism to amplify the apoptotic signal. The proteasome may play a role by degrading anti-apoptotic proteins, such as XIAP or cIAP1, and cell cycle proteins. Suppression of IGF-1 signaling may play a role by enhancing the ubiquitin–proteasome system and by leading to the dephosphorylation and activation of pro-apoptotic proteins. More research focusing on glucocorticoid-induced apoptosis of skeletal muscle is required to confirm the mechanism and will be vital in the development of successful preventative measures of glucocorticoid-induced myopathy in suffering patients.

References

[1] J.P. Seale, M.R. Compton, Side-effects of corticosteroid agents, Med. J. Aust. 144 (1986) 139–142.
[2] R.J. Zoorob, D. Cender, A different look at corticosteroids, Am. Fam. Physician 58 (1998) 443–450.
[3] R.A. Covar, D.Y. Leung, D. McCormick, J. Steelman, P. Zeitler, J.D. Spahn, Risk factors associated with glucocorticoid-induced adverse effects in children with severe asthma, J. Allergy Clin. Immunol. 106 (2000) 651–659.
[4] S.L. Bowyer, M.P. LaMothe, J.R. Hollister, Steroid myopathy: incidence and detection in a population with asthma, J. Allergy Clin. Immunol. 76 (1985) 234–242.
[5] F. Mastagllia, Drug induced myopathies, Pract. Neurol. 6 (2006) 4–13.
[6] J. Owczarek, M. Jasinska, D. Orszulak-Michalak, Drug-induced myopathies. An overview of the possible mechanisms, Pharmacol. Rep. 57 (2005) 23–34.
[7] M.C. Lee, J.S. Lee, M.J. Lee, J.H. Lee, H.I. Kim, Fas mediates apoptosis in steroid-induced myopathy of rats, Neuropathol. Appl. Neurobiol. 27 (2001) 396–402.
[8] M.C. Lee, G.R. Wee, J.H. Kim, Apoptosis of skeletal muscle on steroid-induced myopathy in rats, J. Nutr. 135 (2005) 1806S–1808S.
[9] J.H. Lim, D.Y. Kim, M.S. Bang, Effects of exercise and steroid on skeletal muscle apoptosis in the mdx mouse, Muscle Nerve 30 (2004) 456–462.
[10] A. Orzechowski, M. Jank, B. Gajkowska, T. Sadkowski, M.M. Godlewskia, A novel antioxidant-inhibited dexamethasone-mediated and caspase-3-independent muscle cell death, Ann. N.Y. Acad. Sci. 1010 (2003) 205–208.
[11] Y. Oshima, Y. Kuroda, M. Kunishige, T. Matsumoto, T. Mitsui, Oxidative stress-associated mitochondrial dysfunction in corticosteroid-treated muscle cells, Muscle Nerve 30 (2004) 49–54.
[12] J.R. Singleton, B.L. Baker, A. Thorburn, Dexamethasone inhibits insulin-like growth factor signaling and potentiates myoblast apoptosis, Endocrinology 141 (2000) 2945–2950.
[13] S. Lepine, B. Lakatos, M.P. Courageot, H. Le Stunff, J.C. Sulpice, F. Giraud, Sphingosine contributes to glucocorticoid-induced apoptosis of thymocytes independently of the mitochondrial pathway, J. Immunol. 173 (2004) 3783–3790.
[14] M.C. Marchetti, B. Di Marco, G. Cifone, G. Migliorati, C. Riccardi, Dexamethasone-induced apoptosis of thymocytes: role of glucocorticoid receptor-associated Src kinase and caspase-8 activation, Blood 101 (2003) 585–593.
[15] N. Tonomura, K. McLaughlin, L. Grimm, R.A. Goldsby, B.A. Osborne, Glucocorticoid-induced apoptosis of thymocytes: requirement of proteasome-dependent mitochondrial activity, J. Immunol. 170 (2003) 2469–2478.
[16] E. Laane, T. Panaretakis, K. Pokrovskaja, E. Buentke, M. Corcoran, S. Soderhall, M. Heyman, J. Mazur, B. Zhivotovsky, A. Porwit, D. Grander, Dexamethasone-induced apoptosis in acute lymphoblastic leukemia involves differential regulation of Bcl-2 family members, Haematologica 92 (2007) 1460–1469.
[17] M.M. Conradie, H. de Wet, D.D. Kotze, J.M. Burrin, F.S. Hough, P.A. Hulley, Vanadate prevents glucocorticoid-induced apoptosis of osteoblasts in vitro and osteocytes in vivo, J. Endocrinol. 195 (2007) 229–240.
[18] M.C. Davis, K.S. McColl, F. Zhong, Z. Wang, M.H. Malone, C.W. Distelhorst, Dexamethasone-induced inositol 1,4,5-trisphosphate receptor elevation in murine lymphoma cells is not required for dexamethasone-mediated calcium elevation and apoptosis, J. Biol. Chem. (2008).
[19] S. Lepine, J.C. Sulpice, F. Giraud, Signaling pathways involved in glucocorticoid-induced apoptosis of thymocytes, Crit. Rev. Immunol. 25 (2005) 263–288.
[20] E. Hoijman, L. Rocha Viegas, M.I. Keller Sarmiento, R.E. Rosenstein, A. Pecci, Involvement of Bax protein in the prevention of glucocorticoid-induced thymocytes apoptosis by melatonin, Endocrinology 145 (2004) 418–425.
[21] C. Hegardt, G. Andersson, S.M. Oredsson, Spermine prevents cytochrome c release in glucocorticoid-induced apoptosis in mouse thymocytes, Cell Biol. Int. 27 (2003) 115–121.
[22] D. Chauhan, T. Hideshima, S. Rosen, J.C. Reed, S. Kharbanda, K.C. Anderson, Apaf-1/cytochrome c-independent and Smac-dependent induction of apoptosis in multiple myeloma (MM) cells, J. Biol. Chem. 276 (2001) 24453–24456.
[23] R. Hakem, A. Hakem, G.S. Duncan, J.T. Henderson, M. Woo, M.S. Soengas, A. Elia, J.L. de la Pompa, D. Kagi, W. Khoo, J. Potter, R. Yoshida, S.A. Kaufman, S.W. Lowe, J.M. Penninger, T.W. Mak, Differential requirement for caspase 9 in apoptotic pathways in vivo, Cell 94 (1998) 339–352.
[24] K. Kuida, T.F. Haydar, C.Y. Kuan, Y. Gu, C. Taya, H. Karasuyama, M.S. Su, P. Rakic, R.A. Flavell, Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9, Cell 94 (1998) 325–337.
[25] X.M. Yin, K. Wang, A. Gross, Y. Zhao, S. Zinkel, B. Klocke, K.A. Roth, S.J. Korsmeyer, Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis, Nature 400 (1999) 886–891.
[26] O. Micheau, E. Solary, A. Hammann, M.T. Dimanche-Boitrel, Fas ligand-independent, FADD-mediated activation of the Fas death pathway by anticancer drugs, J. Biol. Chem. 274 (1999) 7987–7992.
[27] H. Gilson, O. Schakman, L. Combaret, P. Lause, L. Grobet, D. Attaix, J.M. Ketelslegers, J.P. Thissen, Myostatin gene deletion prevents glucocorticoid-induced muscle atrophy, Endocrinology 148 (2007) 452–460.
[28] S.C. Bodine, E. Latres, S. Baumhueter, V.K. Lai, L. Nunez, B.A. Clarke, W.T. Poueymirou, F.J. Panaro, E. Na, K. Dharmarajan, Z.Q. Pan, D.M. Valenzuela, T.M. DeChiara, T.N. Stitt, G.D. Yancopoulos, D.J. Glass, Identification of ubiquitin ligases required for skeletal muscle atrophy, Science 294 (2001) 1704–1708.
[29] W.E. Mitch, A.L. Goldberg, Mechanisms of muscle wasting. The role of the ubiquitin–proteasome pathway, N. Engl. J. Med. 335 (1996) 1897–1905.
[30] B.A. Clarke, D. Drujan, M.S. Willis, L.O. Murphy, R.A. Corpina, E. Burova, S.V. Rakhilin, T.N. Stitt, C. Patterson, E. Latres, D.J. Glass, The E3 ligase MuRF1 degrades myosin heavy chain protein in dexamethasone-treated skeletal muscle, Cell Metab. 6 (2007) 376–385.
[31] M. Sandri, C. Sandri, A. Gilbert, C. Skurk, E. Calabria, A. Picard, K. Walsh, S. Schiaffino, S.H. Lecker, A.L. Goldberg, Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy, Cell 117 (2004) 399–412.
[32] Y. Yang, S. Fang, J.P. Jensen, A.M. Weissman, J.D. Ashwell, Ubiquitin protein ligase activity of IAPs and their degradation in proteasomes in response to apoptotic stimuli, Science 288 (2000) 874–877.
[33] T. Hirsch, B. Dallaporta, N. Zamzami, S.A. Susin, L. Ravagnan, I. Marzo, C. Brenner, G. Kroemer, Proteasome activation occurs at an early, premitochondrial step of thymocyte apoptosis, J. Immunol. 161 (1998) 35–40.
[34] B. Dallaporta, M. Pablo, C. Maisse, E. Daugas, M. Loeffler, N. Zamzami, G. Kroemer, Proteasome activation as a critical event of thymocyte apoptosis, Cell Death Differ. 7 (2000) 368–373.
[35] H. He, X.M. Qi, J. Grossmann, C.W. Distelhorst, c-Fos degradation by the proteasome. An early, Bcl-2-regulated step in apoptosis, J. Biol. Chem. 273 (1998) 25015–25019.
[36] G. Gil-Gomez, A. Berns, H.J. Brady, A link between cell cycle and cell death: Bax and Bcl-2 modulate Cdk2 activation during thymocyte apoptosis, EMBO J. 17 (1998) 7209–7218.
[37] E. Grassilli, F. Benatti, P. Dansi, A.M. Giammarioli, W. Malorni, C. Franceschi, M.A. Desiderio, Inhibition of proteasome function prevents thymocyte apoptosis: involvement of ornithine decarboxylase, Biochem. Biophys. Res. Commun. 250 (1998) 293–297.
[38] L. Sun, J.S. Trausch-Azar, L.J. Muglia, A.L. Schwartz, Glucocorticoids differentially regulate degradation of MyoD and Id1 by N-terminal ubiquitination to promote muscle protein catabolism, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 3339–3344.
[39] K. Tanaka, J.B. Pracyk, K. Takeda, Z.X. Yu, V.J. Ferrans, S.S. Deshpande, M. Ozaki, P.M. Hwang, C.J. Lowenstein, K. Irani, T. Finkel, Expression of Id1 results in apoptosis of cardiac myocytes through a redox-dependent mechanism, J. Biol. Chem. 273 (1998) 25922–25928.
[40] P.M. Siu, S.E. Alway, Id2 and p53 participate in apoptosis during unloading-induced muscle atrophy, Am. J. Physiol. Cell Physiol. 288 (2005) C1058–1073.

[41] Y. Jen, H. Weintraub, R. Benezra, Overexpression of Id protein inhibits the muscle differentiation program: in vivo association of Id with E2A proteins, Genes Dev. 6 (1992) 1466–1479.
[42] L. Sun, J.S. Trausch-Azar, A. Ciechanover, A.L. Schwartz, Ubiquitin–proteasome-mediated degradation, intracellular localization, and protein synthesis of MyoD and Id1 during muscle differentiation, J. Biol. Chem. 280 (2005) 26448–26456.
[43] O. Schakman, H. Gilson, V. de Coninck, P. Lause, J. Verniers, X. Havaux, J.M. Ketelslegers, J.P. Thissen, Insulin-like growth factor-I gene transfer by electroporation prevents skeletal muscle atrophy in glucocorticoid-treated rats, Endocrinology 146 (2005) 1789–1797.
[44] M. Fournier, Z.S. Huang, H. Li, X. Da, B. Cercek, M.I. Lewis, Insulin-like growth factor I prevents corticosteroid-induced diaphragm muscle atrophy in emphysematous hamsters, Am. J. Physiol. Regul. Integr. Comp. Physiol. 285 (2003) R34–43.
[45] F.M. Tomas, The anti-catabolic efficacy of insulin-like growth factor-I is enhanced by its early administration to rats receiving dexamethasone, J. Endocrinol. 157 (1998) 89–97.
[46] T.H. Bark, M.A. McNurlan, C.H. Lang, P.J. Garlick, Increased protein synthesis after acute IGF-I or insulin infusion is localized to muscle in mice, Am. J. Physiol. 275 (1998) E118–123.
[47] A. Musaro, C. Giacinti, G. Borsellino, G. Dobrowolny, L. Pelosi, L. Cairns, S. Ottolenghi, G. Cossu, G. Bernardi, L. Battistini, M. Molinaro, N. Rosenthal, Stem cell-mediated muscle regeneration is enhanced by local isoform of insulin-like growth factor 1, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 1206–1210.
[48] T.F. Franke, D.R. Kaplan, L.C. Cantley, PI3K: downstream AKTion blocks apoptosis, Cell 88 (1997) 435–437.
[49] M.H. Cardone, N. Roy, H.R. Stennicke, G.S. Salvesen, T.F. Franke, E. Stanbridge, S. Frisch, J.C. Reed, Regulation of cell death protease caspase-9 by phosphorylation, Science 282 (1998) 1318–1321.
[50] A. Brunet, A. Bonni, M.J. Zigmond, M.Z. Lin, P. Juo, L.S. Hu, M.J. Anderson, K.C. Arden, J. Blenis, M.E. Greenberg, Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor, Cell 96 (1999) 857–868.
[51] J. Buren, Y.C. Lai, M. Lundgren, J.W. Eriksson, J. Jensen, Insulin action and signalling in fat and muscle from dexamethasone-treated rats, Arch. Biochem. Biophys. (2008).
[52] M.J. Saad, F. Folli, J.A. Kahn, C.R. Kahn, Modulation of insulin receptor, insulin receptor substrate-1, and phosphatidylinositol 3-kinase in liver and muscle of dexamethasone-treated rats, J. Clin. Invest. 92 (1993) 2065–2072.
[53] W.L. Holland, J.T. Brozinick, L.P. Wang, E.D. Hawkins, K.M. Sargent, Y. Liu, K. Narra, K.L. Hoehn, T.A. Knotts, A. Siesky, D.H. Nelson, S.K. Karathanasis, G.K. Fontenot, M.J. Birnbaum, S.A. Summers, Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance, Cell Metab. 5 (2007) 167–179.
[54] M. Straczkowski, I. Kowalska, A. Nikolajuk, S. Dzienis-Straczkowska, I. Kinalska, M. Baranowski, M. Zendzian-Piotrowska, Z. Brzezinska, J. Gorski, Relationship between insulin sensitivity and sphingomyelin signaling pathway in human skeletal muscle, Diabetes 53 (2004) 1215–1221.
[55] J.A. Chavez, T.A. Knotts, L.P. Wang, G. Li, R.T. Dobrowsky, G.L. Florant, S.A. Summers, A role for ceramide, but not diacylglycerol, in the antagonism of insulin signal transduction by saturated fatty acids, J. Biol. Chem. 278 (2003) 10297–10303.
[56] J.A. Chavez, S.A. Summers, Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes, Arch. Biochem. Biophys. 419 (2003) 101–109.
[57] A.D. Baron, G. Brechtel, P. Wallace, S.V. Edelman, Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans, Am. J. Physiol. 255 (1988) E769–774.
[58] K. Strle, S.R. Broussard, R.H. McCusker, W.H. Shen, R.W. Johnson, G.G. Freund, R. Dantzer, K.W. Kelley, Proinflammatory cytokine impairment of insulin-like growth factor I-induced protein synthesis in skeletal muscle myoblasts requires ceramide, Endocrinology 145 (2004) 4592–4602.
[59] R. Hyde, E. Hajduch, D.J. Powell, P.M. Taylor, H.S. Hundal, Ceramide down-regulates System A amino acid transport and protein synthesis in rat skeletal muscle cells, FASEB J. 19 (2005) 461–463.
[60] D.C. Phillips, J.T. Hunt, C.G. Moneypenny, K.H. Maclean, P.P. McKenzie, L.C. Harris, J.A. Houghton, Ceramide-induced G2 arrest in rhabdomyosarcoma (RMS) cells requires p21Cip1/Waf1 induction and is prevented by MDM2 overexpression, Cell Death Differ. 14 (2007) 1780–1791.
[61] C.A. Calatayud, L.A. Pasquini, J.M. Pasquini, E.F. Soto, Involvement of the ubiquitin-mediated proteolytic system in the signaling pathway induced by ceramide in primary astrocyte cultures, Dev. Neurosci. 27 (2005) 397–407.
[62] S.M. Turpin, G.I. Lancaster, I. Darby, M.A. Febbraio, M.J. Watt, Apoptosis in skeletal muscle myotubes is induced by ceramides and is positively related to insulin resistance, Am. J. Physiol. Endocrinol. Metab. 291 (2006) E1341–1350.
[63] M. Novelli, A. Pocai, C. Chiellini, M. Maffei, P. Masiello, Free fatty acids as mediators of adaptive compensatory responses to insulin resistance in dexamethasone-treated rats, Diab. Metab. Res. Rev. 24 (2008) 155–164.
[64] T. Mitsui, H. Azuma, M. Nagasawa, T. Iuchi, M. Akaike, M. Odomi, T. Matsumoto, Chronic corticosteroid administration causes mitochondrial dysfunction in skeletal muscle, J. Neurol. 249 (2002) 1004–1009.
[65] J. Yu, S.A. Novgorodov, D. Chudakova, H. Zhu, A. Bielawska, J. Bielawski, L.M. Obeid, M.S. Kindy, T.I. Gudz, JNK3 signaling pathway activates ceramide synthase leading to mitochondrial dysfunction, J. Biol. Chem. (2007).
[66] M. Marolda, V. Palma, F.S. Camporeale, M. Carandente, M. Cioffi, A.V. Orsini, A. Gentile, Steroid myopathy: clinical and immunohistochemical study of a case, Ital. J. Neurol. Sci. 12 (1991) 409–413.
[67] J. Du, X. Wang, C. Miereles, J.L. Bailey, R. Debigare, B. Zheng, S.R. Price, W.E. Mitch, Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions, J. Clin. Invest. 113 (2004) 115–123.