Research Article

Myostatin Upregulation in Patients in the Chronic Phase of Severe Burn Injury Leads to Muscle Cell Catabolism

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Keywords

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Abstract

Background: Burn injury leads to a hypercatabolic response and ultimately muscle wasting with drastic implications for recovery of bodily functions, patient's quality of life (QoL), and long-term survival. Several treatment options target the body's initial stress response, but pharmacological approaches to specifically address muscle protein metabolism have only been poorly investigated. **Objective:** The aim of this study was to assess the role of myostatin and follistatin in burn injury and its possible implications in muscle wasting syndrome. **Methods:** We harvested serum from male patients within 48 h and again 9–12 months after severe burn injury (>20% of total body surface area). By means of myoblast cultures, immunohistochemistry, immunoblotting, and scratch assay, the role of myostatin and its implications in post-burn muscle metabolism and myoblast proliferation and differentiation was analyzed. **Results:** We were able to show increased proliferative and myogenic capacity, decreased myostatin, decreased SMAD 2/3, and elevated follistatin concentrations in human skeletal myoblast cultures with serum conditioned medium of patients in the acute phase of burn injury and conversely a reversed situation in patients in the chronic phase of burn injury. Thus, there is a biphasic response to burn trauma, initiated by an anabolic state and followed by long-term hypercatabolism. **Conclusion:** We conclude that the myostatin signaling pathway plays an important regulative role in burn-associated muscle wasting and that blockade of myostatin could prove to be a valuable treatment approach improving the rehabilitation process, QoL, and long-term survival after severe burn injury.

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Introduction

Thermal injury results in a hypercatabolic and hypermetabolic response with drastic implications for recovery of body functions and the patient's quality of life (QoL). After severe burn injury, metabolic rates have been shown to be 40–80% above normal levels and remain elevated for up to 2 years after initial injury [1–3]. Post-burn hypermetabolism is due to ATP-consuming reactions, such as increased protein turnover, hepatic gluconeogenesis and cycling of glucose and fatty acids, mitochondrial thermogenesis, and adrenergic stress [1].

After thermal injury, muscle serves as a primary energy source and muscle hypercatabolism results due to an imbalance of protein degradation and synthesis, with breakdown rates dramatically surpassing protein synthesis rates [1, 2]. The resulting muscle cachexia leads to an erosion of lean body mass, which in turn is associated with decreased wound healing, immunodepression, and increased mortality [1–3]. Moreover, it severely impacts the patient's QoL and leads to increased rehabilitation time and even care dependency in young and old patients alike.

Besides treatment of wounds, modulation of temperature, and substitution of sufficient nutrients, other treatment options include β -blockers, such as propranolol, as well as recombinant growth hormone and testosterone analogs to reduce burn-induced hypermetabolism [1]. However, preventing excessive cachexia and loss of lean body mass is essential to guarantee a successful long-term rehabilitation of burn victims. Early mobilization and exercise training has been shown to be beneficial in restoring lean body mass and muscle strength [1]. However, pharmacological treatments to support muscle regeneration and prevent muscle loss on a molecular level are still poorly investigated.

Myostatin, as a member of the transforming growth factor beta superfamily, is a central inhibitor of skeletal muscle growth by binding to activin receptor type II (ActIIIB) [4, 5]. Myostatin has been shown to decrease muscle mass by promoting protein degradation and inhibiting protein synthesis in muscle cells [6]. Furthermore it has well-known anti-osteogenic effects causing increased bone resorption and inhibition of osteogenic differentiation [4]. Follistatin antagonizes activin A, GDF11, and myostatin and is thus a nonselective inhibitor. It leads to an increase in muscle mass as well as an increase in osteogenic differentiation and bone mineralization [4]. Increased signaling by the ActIIIB pathway has already been demonstrated to play an important role in cancer- and AIDS-induced cachexia and muscle atrophy [7–9]. Zhou et al. [7] have shown prolonged survival, prevention of excessive muscle wasting, and increased muscle growth in cancer cachexia patients upon blockade of the ActIIIB pathway.

The potential role of myostatin and its antagonist follistatin in severe burns has not yet been properly investigated. We hypothesize that myostatin and follistatin play an important role in muscle metabolism in patients following thermal injury and aim to further investigate their role in burn-induced muscle cachexia and their possible implications for future treatment options.

Patients and Methods

Patient Recruitment

Serum harvest and experiments were performed prospectively in accordance with the ethics committee of Ruhr University Bochum after approval between September 2015 and March 2017. Informed consent was obtained from male patients to rule out any hormonal bias. Male patients with severe burn injury (>20% of total body surface area) between the age of 18 and 75 years were included in the patient group within the first 48 h after injury. All patients with severe burn injuries admitted to our institution were asked to be included into the study between 2015 and 2017. In the control group, male probands between the age of 18 and 75 years without any acute, malignant, infectious, or autoimmune disease were included. To measure

the long-term effects of severe burns on myostatin signaling, serum was collected from patients with severe burns between 9 and 12 months of burn injury according to the inclusion and exclusion criteria named above. After acquisition of informed consent, 7.5 mL of full blood was collected into serum tubes. After centrifugation with 3,000 rpm for 10 min, serum was obtained.

Myoblast Culturing

For myotube differentiation, human skeletal myoblasts (HSkMs) were obtained (ThermoFisher Scientific, Waltham, MA, USA). The proliferation medium consisted of Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO, USA) with fetal calf serum (15%), fetuin (bovine, 50 µg/mL), epidermal growth factor (10 ng/mL), basic fibroblast growth factor (1 ng/mL), insulin (10 µg/mL), and dexamethasone (0.4 µg/mL). After initial proliferation, differentiation to myotubes was initiated for 5 days before immunofluorescence and Western blot were performed according to standard protocols [10].

Patient serum conditioned medium contained 7.5% fetal calf serum and 7.5% serum from healthy patients, patients in the acute phase of burn injury, and patients in the chronic phase of burn injury, respectively.

Immunofluorescence

For immunofluorescence stainings of myogenin (mouse, monoclonal, SantaCruz Biotechnology, Santa Cruz, CA, USA, sc-52903, 1:100, myogenin [M-225]), follistatin (mouse, monoclonal, SantaCruz Biotechnology, sc-365003, 1:100, follistatin [C-8]), GDF11 (mouse, monoclonal, Santa Cruz Biotechnology, sc-393335, 1:100, GDF11), myostatin (rabbit, polyclonal, SantaCruz Biotechnology, sc-6885-R, 1:50, GDF8), caspase 3 (H-277) (rabbit, polyclonal, SantaCruz Biotechnology, sc-7184, 1:50, caspase 3), GSK-3-beta (3D10) (mouse, monoclonal, Cell Signaling Technology, Boston, MA, USA, 9832S, 1:50, GSK-3-beta), and SMAD 2/3 (rabbit, Cell Signaling Technology, 8828, 1:200, SMAD 2/3) sections and chamber slides were incubated at 58 °C for 1 h and subsequently rehydrated and incubated with 0.125% proteinase K for 15 min at 37 °C. After a short washing step with phosphate-buffered saline, sections and cells on chamber slides were permeabilized with 0.1% Tween 20 for 4 min and treated with blocking solution for 1 h. Incubation with primary antibodies followed overnight in blocking solution at 4 °C. After washing with phosphate-buffered saline, a rabbit or goat biotinylated secondary antibody followed by the AB reagent was used for detection. For mouse monoclonal antibodies, conjugated mouse IgGκ-binding proteins were used instead of the secondary antibody. All sections were counterstained with DAPI. Sections were subsequently mounted with Fluoromount Aqueous Mounting Medium (Sigma Aldrich). Images for immunofluorescence were taken with a fluorescence microscope (Olympus IX3 series).

Immunoblotting

Whole-cell lysates were prepared using ice-cold NP40 lysis buffer (25 mM TRIZMA base, 150 mM NaCl, 10 mM Na₄P₂O₇, 25 mM β-glycerophosphate, 10% glycerol, 0.75% NP40, 25 mM NaF, pH 7.2), and RIPA lysis buffer (1% NP40, 1% CHAPS, 0.1% SDS, 0.15 M NaCl, 10 mM Na phosphate, 2 mM EDTA, 50 mM NaF, pH 7.2), respectively, containing 1:100 protease inhibitor cocktail set I (Calbiochem, Darmstadt, Germany) and Na orthovanadate (0.2 mM). Protein content was determined by using the Bio-Rad DC protein assay kit (Bio-Rad, Hercules, CA, USA). Immunoblotting was performed as described before [11]. Briefly, proteins were separated by SDS-PAGE and transferred to nitrocellulose membrane. The membranes were blocked in 5% skim milk powder (Fluka, Buchs, Switzerland) dissolved in TBST (50 mM TRIZMA base, 150 mM NaCl, pH 7.5 adjusted with HCl, 0.1% Tween 20) for 1 h at room temperature and probed overnight with appropriate primary antibodies, diluted in 5% bovine serum albumin or skim milk powder as recommended by the provider, followed by incubation for 1 h in HRP-conjugated secondary antibody diluted in 5% skim milk. Primary antibodies against phospho-GSK-3-beta (9832S) and β-actin (4967S) were obtained from Cell Signaling Technology, myostatin (sc-6885-R), caspase 3 (sc-7184), GDF11 (sc-393335), and pSMAD (8828S) from SantaCruz Biotechnology. Antibodies were visualized by ECL Western blot detection reagents (Amersham, Buckinghamshire, UK), quantified by densitometric scanning using Adobe Photoshop CC (Adobe, Mountain View, CA, USA), and normalized against loading controls.

Scratch Assay

In order to measure the proliferative and migrative capacity of myoblasts, cells were seeded with 13,000 cells per chamber in a two-well silicone insert (Ibdi, Munich, Germany) with a defined cell-free gap (500 µm). After 48 h, inserts were removed and cells were incubated accordingly with 7.5% of patient serum or 7.5%

of fetal calf serum additional to standard 7.5% fetal calf serum. Images were taken 10 and 24 h after removal of the insert with a bright-field microscope (Zeiss Axiovert 100, Zeiss, Oberkochen, Germany) and the following settings with Axiovision 4.8: objective 2.5×, exposure time 614 ms, dimensions 3,900 × 3,090 Px, scanned color.

Statistical Analysis

Statistical significance was assessed by Student's *t* test or by Mann-Whitney test, according to data distribution. Statistical significances were set at $p < 0.05$. Calculation of significant correlation was done with the Pearson test. All analyses were performed using the SPSS Statistics for Windows software (version 21.0; IBM, Armonk, USA), considering a critical significance value of 5%.

Results

Serum conditioned medium of patients in the acute phase of burn injury decreased myostatin levels and increased follistatin levels in HSkM culture, while conditioned medium of patients in the chronic phase of burn injury reversed this effect.

To evaluate the myogenic effect of measured serum concentration of myostatin and follistatin, sera of healthy individuals as well as patients in the acute and chronic phase of burn injury were applied to HSkMs (7.5% fetal calf serum, 7.5% patient serum). After 72 h of incubation, immunofluorescence against myostatin, follistatin, GDF11, myostatin, downstream target SMAD 2/3, and myogenic factor myogenin was performed. While follistatin revealed higher levels in the myoblast culture of serum conditioned medium of patients in the acute phase of burn injury (3.4-fold increase compared to healthy serum conditioned myoblasts, $p < 0.001$), serum conditioned medium of patients in the chronic phase of burn injury showed a decrease compared to healthy individuals. The effect was reversed for myostatin levels, showing decreased concentrations in serum myoblast culture of patients in the acute phase of burn injury (1.9-fold decrease compared to healthy serum conditioned medium conditioned myoblasts, $p = 0.004$), while conditioned cultures of patients in the chronic phase of burn injury showed an increase compared to serum conditioned myoblast culture of patients in the acute phase (3.3-fold increase compared to the serum conditioned medium of patients in the acute phase of burn injury, $p < 0.001$). Myogenin concentrations, which are indicative of muscle differentiation, were elevated in serum conditioned myoblast culture of patients in the acute phase of burn injury (1.9-fold increase compared to healthy serum conditioned medium, $p = 0.03$), but reduced in serum conditioned myoblast culture of patients in the chronic phase compared to serum conditioned culture in patients in the acute phase and healthy subjects (8.9-fold decrease, $p < 0.001$). SMAD 2/3 as a downstream target of myostatin was decreased in serum conditioned myoblast culture of patients in the acute phase of burn injury, but increased in serum conditioned myoblast culture of patients in the chronic phase as compared to serum conditioned myoblast culture of healthy subjects. GDF11 showed a similar behavior as myostatin, a significant increase in serum of healthy subjects and serum myoblast culture of patients in the chronic phase of burn injury compared to healthy controls. The serum of patients in the acute phase of burn injury showed decreased GDF11 levels compared to healthy controls (2.4-fold compared to serum conditioned myoblast culture of healthy subjects, $p = 0.04$) (Fig. 1).

Myostatin and caspase 3 were decreased in myotubes differentiated from HSkMs in serum conditioned medium of patients in the acute phase of burn injury, while GSK-3-β was increased.

Next, we sought to determine the impairment of sera from patients in the chronic phase of burn injury on HSkM differentiation. Analogously to the method described above, sera from healthy subjects and patients in the acute and chronic phase of burn injury were utilized. Cells

Fig. 1. Immunohistological reactions of HSKMs in fetal calf serum (control group) and HSKMs treated with sera of healthy individuals, with sera of patients in the acute and chronic phase of burn injury, and with sera of patients in the chronic phase of burn injury treated with supplementation of follistatin (MSTN-AB). Immunofluorescence showed decreased levels of myostatin, GDF11, SMAD 2/3, and caspase 3 and increased levels of follistatin and myogenin in serum conditioned media of patients in the acute phase of burn injury and reversely in serum conditioned media of patients in the chronic phase. Supplementation of follistatin led to decreased expression of myostatin, SMAD 2/3, and caspase 3 and to increased levels of myogenin compared to unsupplemented sera. In follistatin-treated serum conditioned cells of patients in the chronic phase of burn injury, there were increased levels of GDF11 compared to sera of patients in the acute phase and decreased levels compared to unsupplemented sera of patients in the chronic phase of burn injury. Results are shown as means $\pm$ SEM. * p < 0.05, ** p < 0.01, *** p < 0.001 (two-sample t test). FCS, fetal calf serum; HSKMs, human skeletal myoblasts.

Fig. 2. Immunoblotting of HSkMs in fetal calf serum (control group) and HSkMs treated with sera of healthy individuals and patients in the acute and chronic phase of burn injury after differentiation (7 days). Immunoblotting revealed decreased myostatin concentrations in serum conditioned culture of patients in the acute phase of burn injury and a reversed situation in serum conditioned culture of patients in the chronic phase ($p < 0.001$). GDF11 showed similar tendencies, although differences between sera of patients in the acute and chronic phase of burn injury were not significant. GSK-3-beta was increased in conditioned cells of patients in the acute phase of burn injury compared to conditions in healthy subjects and patients in the chronic phase ($p < 0.01$). The situation was reversed for expression of caspase 3 ($p < 0.01$). Results are shown as means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (two-sample t test). HSkMs, human skeletal myoblasts.

were first differentiated for 7 days and then prepared for immunoblotting. Caspase 3, as a marker of muscle atrophy, as well as GSK-3-beta, as a marker of muscle hypertrophy, were used to characterize muscle cell properties [12–14]. We were able to demonstrate decreased myostatin protein concentration in serum conditioned HSkM culture of patients in the acute phase of burn injury compared to the control (only fetal calf serum) and the serum conditioned medium of healthy subjects. On the contrary, an elevation of myostatin was shown in serum conditioned cells of patients in the chronic phase compared to serum of patients in the acute phase of burn injury (1.5-fold increase, $p < 0.001$). GDF11 was decreased in conditioned cells of healthy subjects and patients in the acute phase of burn injury compared to patients in the chronic phase, though not significantly. GSK-3-beta was increased in conditioned cells of patients in the acute phase compared to conditions in healthy subjects and patients in the

Fig. 3. Scratch assay (400 µm) of HSkMs in fetal calf serum (control group) and HSkMs treated with sera of healthy individuals and patients in the acute and chronic phase of burn injury. Up to 24 h after burn injury, there was an increased proliferative capacity in serum conditioned cells of patients in the acute phase of burn injury and a decreased proliferation in serum conditioned cells of patients in the chronic phase after 24 h. Supplementation of myostatin to serum of patients in the acute phase of burn injury decreased myoblast proliferation, while supplementation of follistatin (MSTN-AB) decreased the proliferative capabilities of the cells. Results are shown as means $\pm$ SEM of change compared to the initial gap size. # $p < 0.05$. FCS, fetal calf serum; HSkMs, human skeletal myoblasts.

chronic phase of burn injury (1.4-fold increase, $p < 0.01$). Caspase 3 was utilized to determine cell atrophy and was shown to be increased in conditioned cells of patients in the chronic phase compared to conditions in healthy subjects and acute phase of burn injury (1.7-fold, $p < 0.01$) (Fig. 2).

In addition to the immunoblotting experiments, protein measurements were supplemented by immunocytochemistry of HSkMs. We were able to find decreased levels of myostatin and GDF11 in sera of patients in the acute phase of burn injury as compared to the control group and GDF11 and caspase 3 compared to the healthy group. In addition, increased levels of myostatin, GDF11, caspase 3, and SMAD 2/3 in sera of patients in the chronic phase of burn injury were confirmed compared to patients in the acute phase (Fig. 2).

The proliferative capacity of myoblasts was increased in serum conditioned medium of patients in the acute phase of burn injury, but decreased 9–12 months after burn injury.

To analyze further effects of elevated follistatin and decreased myostatin levels upon burn injury, myoblast proliferation was monitored through a scratch assay. While sera obtained from patients in the acute phase of burn injury (up to 48 h after injury) increased the proliferative capacity of myoblasts compared to fetal calf serum and sera from healthy control patients, sera from patients in the chronic phase of burn injury decreased it. Supplementation of myostatin (2 µg/mL) to serum of patients in the acute phase of burn injury decreased the proliferation significantly, while supplementation of follistatin (2 µg/mL) completely antagonized this effect (Fig. 3).

Discussion

The aim of this study was to investigate the role of myostatin and follistatin in muscle wasting syndrome associated to burn injury. We were able to show decreased proliferative and myogenic capacity, elevated myostatin, elevated SMAD 2/3, and decreased follistatin concentrations in HSkM cultures with serum conditioned medium of patients in the chronic phase of burn injury, suggesting an accentuated catabolic situation. In contrast, cultures with serum conditioned medium of patients in the acute phase of burn injury showed increased proliferative and myogenic capacity, decreased myostatin, decreased SMAD 2/3, but increased follistatin concentrations, thus indicating an anabolic state. Additionally, we were able to show an increase in GDF11 and caspase 3, a marker of muscle atrophy, in cells treated with serum of patients in the chronic phase of burn injury after a differentiation period of 7 days and reversed results in cells treated with serum of patients in the acute phase of burn injury. GSK-3-beta, a marker of muscle hypertrophy, was elevated in cells treated with serum of patients in the acute phase of burn injury.

Muscle wasting in burn injury is thought to be due to a misbalance of protein degradation and protein synthesis. This is a result of the hypermetabolic response to severe stress leading to a surge of plasma catecholamine and corticosteroid levels, tachycardia, and increased cardiac work and oxygen consumption. In order to sustain the increased metabolic rate, muscle protein is degraded much faster than it is synthesized [2, 3].

Interestingly, our data suggest an initial attempt of the body to counterregulate the fast muscle degradation with decreased levels of myostatin and enhanced levels of follistatin. Yousuf et al. [15] also demonstrated a biphasic bodily response to acute burn injury. However, they described an initial degenerative situation 2 days after burn and a compensatory pro-regenerative response 7–14 days after burn with an early decrease in myofiber cross-sectional area and loss of body weight followed by a compensatory increase. Concomitantly, they demonstrated an increase in Murf1 and NF-κB, mediators of muscle protein degradation, and a decrease in Pax7-positive cells, regulating satellite cell proliferation, 2 days following burn trauma with a reversed situation 7–14 days after burn. These findings seem to contradict our results demonstrating a pro-regenerative response 48 h after burn injury. However, as myostatin also promotes lipolysis and inhibits adipogenesis, increased muscle growth and simultaneous loss of body fat can still lead to an overall loss of body weight [16]. Moreover, although key transcription factors are downregulated in the presence of myostatin, Amthor et al. [17] demonstrated that the expression levels of Pax7 remain unaffected in the presence of myostatin. A more recent study by Wang and McPherron [18] even showed that application of myostatin inhibitors does not lead to a general activation of satellite cells. Thus, degenerative response as shown by Yousuf et al. [15] does not necessarily contradict our findings. For further investigation, a more comprehensive study is needed including identification of

muscle-secreted proteins such as myostatin and follistatin, activation of satellite cells, and expression of key transcription factors as well as clinical observations such as loss of body weight.

Previous studies have shown increased metabolic rates (10–40% above average) in burn patients 6–12 months after injury [1, 2, 19, 20]. Thus, net muscle degradation still outweighs muscle growth months after the initial injury, leading to muscle wasting syndrome. This corresponds to the persistent catabolic state with increased levels of myostatin shown in our patients in the chronic phase of burn injury. Consequently, the initial anabolic response to burn injury could indicate a failed attempt to counteract the hypermetabolic response, ultimately leading to muscle degradation.

Besides thermoregulation, adequate substitution of nutrients, glucose management, and surgical intervention, there are multiple pharmacological treatment approaches targeting the body's stress response to severe burn injury. Beta-blockers such as propranolol, recombinant growth hormone, and testosterone and its analogs (e.g., oxandrolone) have been shown to significantly reduce resting metabolic rate and muscle protein degradation and to enhance accretion of lean body mass as well as wound healing and muscle protein synthesis in burn victims 1 year after injury [1, 19].

However, only few studies have investigated treatment approaches to specifically target muscle metabolism after burn. The mechanism of muscle wasting in catabolic diseases has been widely investigated and involves many different factors. In muscle, the ATP-dependent ubiquitin-proteasome system is primarily responsible for degradation of muscle proteins, which is activated by low levels of phosphorylated Akt, which in turn involves increased levels of glucocorticoids as well as low insulin and IGF-1 levels [21]. Caspase 3 is another important protease-catalyzing muscle protein degradation [21] which is also initiated by decreased phosphorylated Akt. Moreover, impaired satellite cell function, involved in muscle protein synthesis and preservation, has been shown in chronic kidney disease and cancer associated with muscle wasting [21]. As the mechanisms initiating muscle wasting are extremely complex, the question arises whether reversing or even preventing this process can be accomplished by one single drug rather than a more comprehensive treatment approach.

Interestingly, upon blockade of the Akt/mTOR pathway in cancer patients, increased levels of phosphorylated Akt have been demonstrated to lead to reduced activation of the ubiquitin-proteasome system and thus significantly decelerated muscle protein degradation [7, 21, 22]. Moreover, there is evidence that systemically administered myostatin can singularly induce muscle wasting [23]. Elevated levels of myostatin have been shown in many diseases associated with muscle wasting, e.g., AIDS, renal failure, heart failure, cancer, chronic kidney diseases, and chronic obstructive pulmonary disease [7–9, 21]. In burn patients, Lang et al. [24] showed increased levels of myostatin 24 h after thermal injury, which is in accordance with our study.

Even though our hospital holds one of the largest burn centers in the country, severe burn injuries are still quite rare. Thus, the limitations of this study include a small number of cases. It would have been interesting to further subcategorize the severity of the burn injury depending on the percentage of the injured total body surface area. Moreover, a comparison between male and female subjects to account for the influence of hormonal factors in the muscle metabolism of burn victims would have provided us with further insight. As we aimed to generate a homogenous group of subjects, we did not account for our patients' physical activity, which could have differed substantially considering the wide range of age from 18 to 75 years.

Muscle wasting severely impacts the QoL of burn victims and drastically hinders long-term rehabilitation and survival. Moreover, it may lead to long-term care dependency even

in young patients. Thus, prevention of muscle loss is of enormous importance in the treatment of patients after burn injury.

Given the considerable impact of a blockade of the myostatin/activin signaling pathway on preventing muscle protein degradation and promoting muscle growth, we conclude that blockade of myostatin could be a valuable treatment approach in patients with burn injury. Moreover, it is a great advantage that myostatin inhibitors can be administered systemically, so that treatment would not involve a complex manipulation of genes but would be feasible pharmacologically.

Conclusion

In our study, we were able to show decreased levels of myostatin and increased levels of follistatin in patients in the acute phase of burn injury and reversely 9–12 months after burn. These findings imply a biphasic response to burn trauma and highlight the important role of the myostatin signaling pathway in muscle wasting. As muscle wasting in patients with burn injuries has poor prognostic implications, increasing morbidity and mortality, treatment with myostatin inhibitors could prove to be beneficial in improving long-term survival and patient's QoL and in accelerating the rehabilitation process. However, further studies are needed to investigate the exact role of myostatin and its inhibition after burn injury in animal studies.

Statement of Ethics

The authors certify that they complied with the ethical guidelines for authorship and publishing. The study was performed in accordance with the ethics committee of Ruhr University Bochum after approval (approval number: 16-5619-BR). All subjects gave written consent.

Disclosure Statement

The authors have no conflicts of interest to declare.

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