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Insulin signaling in skeletal muscle during inflammation and/or immobilisation

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Abstract

Background: The decline in the downstream signal transduction pathway of anabolic hormone, insulin, could play a key role in the muscle atrophy and insulin resistance observed in patients with intensive care unit acquired weakness (ICUAW). This study investigated the impact of immobilisation via surgical knee and ankle fixation and inflammation via *Corynebacterium parvum* injection, alone and in combination, as risk factors for altering insulin transduction and, therefore, their role in ICUAW.

Results: Muscle weight was significantly decreased due to immobilisation [estimated effect size (95% CI) -0.10 g (-0.12 to -0.08); $p < 0.001$] or inflammation [estimated effect size (95% CI) -0.11 g (-0.13 to -0.09); $p < 0.001$] with an additive effect of both combined ($p = 0.024$). pAkt was only detectable after insulin stimulation [estimated effect size (95% CI) 85.1-fold (76.2 to 94.0); $p < 0.001$] irrespective of the group and phosphorylation was not impaired by the different perturbations. Nevertheless, the phosphorylation of GSK3 observed in the control group after insulin stimulation was decreased in the immobilisation [estimated effect size (95% CI) -40.2 (-45.6 to -34.8)] and inflammation [estimated effect size (95% CI) -55.0 (-60.4 to -49.5)] groups. The expression of phosphorylated GS (pGS) was decreased after insulin stimulation in the control group and significantly increased in the immobilisation [estimated effect size (95% CI) 70.6-fold (58.8 to 82.4)] and inflammation [estimated effect size (95% CI) 96.7 (85.0 to 108.5)] groups.

Conclusions: Both immobilisation and inflammation significantly induce insulin resistance, i.e., impair the insulin signaling pathway downstream of Akt causing insufficient GSK phosphorylation and, therefore, its activation which caused increased glycogen synthase phosphorylation, which could contribute to muscle atrophy of immobilisation and inflammation.

Keywords: Intensive care unit acquired weakness, Immobilisation, Inflammation, Muscle atrophy, Insulin signaling

Background

In approximately 40% of Intensive Care Unit (ICU) patients an acquired Weakness (ICUAW) can be detected [1]. Depending on the constellation of risk factors, such as immobilisation, hyperglycaemia, or severity of systemic inflammation, the incidence of ICUAW diagnosis can increase to 86% [2]. ICUAW has a profound short—as well as

long-term impact on patients such as delayed weaning of mechanical ventilation and/or deferred discharge from the hospital. Mortality and morbidity in ICUAW patients has been shown to be dramatically increased up to 5 years after discharge [3].

Muscle atrophy and insulin resistance are two main contributing pathophysiologic factors identified in ICUAW. These factors are highly interdependent making it difficult to investigate them separately in humans [4, 5]. The two triggers for insulin resistance that are invariably present during critical illness are inflammation and immobilisation. Mobilization on the other hand has been shown to increase insulin sensitivity in critically ill [6].

Current management recommendations for ICUAW aim at minimising immobilisation and insulin resistance [7]. Early protocol-based mobilization has been shown to improve functionality and counteract muscle atrophy as well as insulin resistance [6, 8, 9]. Nevertheless, evidence has not always been uniform and some studies failed to demonstrate the association [10]. Although controversial, intensive insulin therapy has been shown to have a protective effect regarding the development of neuromuscular dysfunction [11]. While early mobilization has been implemented in clinical routine, intensive insulin therapy is still under discussion due to multiple large scaled randomized-controlled trials showing increased mortality or serious adverse events [12].

Weber-Carstens and colleagues investigated the insulin signaling pathway in critically ill patients. They observed a significant insulin resistance using a euglycemic hyperinsulinemic clamp and at the end of the insulin signaling pathway insufficient translocation of GLUT4 transporters to the sarcolemma. Since they investigated the insulin signaling pathway up to the point of Akt phosphorylation and were not able to identify any aberrant signaling, the insulin signaling dysfunction seems to be downstream of Akt, which was not investigated in this study [5]. Contrary to these observations, in an animal burn model, phosphorylation of Akt and its ability to transduce anabolic signals downstream was significantly impaired [13]. Thus, further investigations to examine the roles of immobilisation and inflammation could point to the primary aetiologic factor leading to muscle wasting in critical illness.

Hence, this study investigated the effect of immobilisation and inflammation alone and their combination in decreasing the anabolic response to exogenous insulin by examining signaling changes downstream of Akt. The insulin transduction pathway was studied in skeletal muscle in an established rat model of immobilisation and/or inflammation, previously described by us [14].

Methods

All experiments described in the study protocol were approved and performed according to the guidelines by governmental veterinary and animal protection office (Regierung von Oberbayern, TVA 55.2-1-54-2532-121-2015; approval date 12th of August 2015).

Animal model and study design

Details of the methodology are described in the appendix. In brief, male Sprague Dawley rats (Charles River, Germany) were initially (day 0) randomized within a double hit model to either control, immobilisation alone, inflammation alone or the combination of immobilisation and inflammation as described previously [14, 15]. After 12 days of

perturbation, the rats were further randomized to receive either an insulin or a saline bolus injection (see Fig. 1).

Immobilisation

On day 0, all animals received general anaesthesia and underwent Kirschner wire immobilisation of the ankle and knee joint at 90° of one leg. In rats of “sham-immobilisation” group, wires were immediately removed to control for effects of the surgery. All animals received 0.01 mg/kg buprenorphine i.p. once to ensure sufficient analgesia and comfort without affecting the insulin signaling pathway.

Systemic inflammation

All animals randomly received an injection of either 56 mg/kg heat-inactivated *Corynebacterium parvum* (Hoffmann-La Roche, Switzerland) or 0.5 ml saline via the dorsal penile vein on days 0, 4, and 8. Sustained systemic inflammation was confirmed by measuring venous methaemoglobin levels via routine blood gas analysis on days 0, 4, 8, and 12 [16]. Body weight was measured at the same time points to evaluate the effect of the systemic inflammation.

On day 12, after 6–12 h of fasting, animals were secondarily randomised to receive either 0.65 IU/kg insulin or an equivalent volume of saline via laparotomic portal vein injection [13, 17]. For this procedure, animals received general anaesthesia with isoflurane. Tibialis anterior muscles were harvested 5 min after the injection and weighed to evaluate the effect of systemic inflammation on muscle mass. Animals were subsequently euthanised. Muscles were immediately snap frozen in liquid nitrogen and stored at −80 °C until analysis later.

Protein analysis by immunoblot

Expression of protein kinase b (Akt), phosphorylated protein kinase b (pAkt), glycogen-synthase-kinase-3-beta (GSK), phosphorylated glycogen-synthase-kinase-3-beta (pGSK), glycogen synthase (GS) and phosphorylated glycogen synthase (pGS) were assessed semiquantitatively using the Western blot technique. The primary antibodies were used

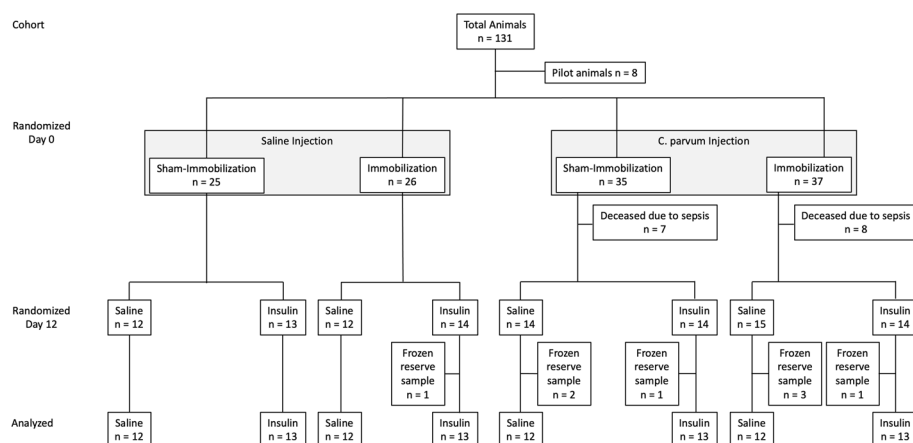


Fig. 1 Study scheme. Single-hit and double-hit models of systemic inflammation and/or immobilisation with and without insulin

in a 1:5,000 dilution and the secondary antibodies in a 1:10,000 dilution (see Additional file 1: Table S1). Bands were visualized with the Bio-Rad Molecular Imager[®] Chemi-Doc[™] XRS+ (Bio-Rad Laboratories, Inc., USA) and analysed with ImageLab (Bio-Rad Laboratories, Inc., USA). Expression values were normalised to total protein [18].

Statistical analysis

Data are shown as mean and standard deviation for continuous variables as well as count and percentage for categorical variables. Relative amount of phosphorylated protein was determined as the quotient between total amount of phosphorylated protein and non-phosphorylated protein within the same group. A linear mixed model was applied to analyse the main effects of immobilisation, inflammation, side and insulin treatment on methaemoglobin concentrations, body weight, muscle weight or protein concentration of operated/contralateral legs. If the main factor (i.e., immobilisation, inflammation, side, and insulin) proved to be significant, post-hoc analyses were then performed to investigate differences between or within the experimental groups. Effect sizes are reported as estimated marginal means. Significance level was set at $p < 0.05$. Statistical analysis was performed with SPSS Statistics 27 (IBM, USA).

Results

In total, 131 rats were included into the experiment. Eight animals were used for establishing the experimental procedures in the laboratory, so that 123 animals were randomized into the four groups. 15 animals with systemic inflammation (8 with and 7 without concomitant immobilisation) had a rapidly deteriorating general condition and had to be euthanised as per the approved protocol. Therefore, 108 animals were available for the final analysis: 12 samples per group in the saline groups and 13 in the insulin groups ($n = 100$) were analysed, resulting in at least 10 analysable western blot samples per group and leg side. A total of 8 samples were kept as frozen reserve samples (see Fig. 1).

Methaemoglobin (Meth-Hb)

Met-Hb as a surrogate parameter for inflammation showed an increase over time only after injection of *Corynebacterium parvum* ($p < 0.001$) without an interaction to immobilisation ($p = 0.201$) (Fig. 2). On day 12, the difference between animals having received *Corynebacterium parvum* injection (inflammation as well as immobilisation and inflammation) and those that did not (control and immobilisation) was estimated to be 4.2% (95% CI: 3.6 to 4.8).

Body weight

Body weight on day 0, before perturbations was 224.0 [12.5] g. There was a significant main effect for time ($p < 0.001$) and inflammation ($p < 0.001$) with a significant interaction of time with inflammation ($p < 0.001$). The inflammation (220.6 [15.3] g; $p < 0.001$ for both groups) and immobilisation superimposed on inflammation (221.2 [15.9] g; $p < 0.001$ for both groups) group showed a significantly decreased body weight compared to the control (276.0 [20.0] g) as well as immobilisation (260.8 [16.5] g) group on day 12

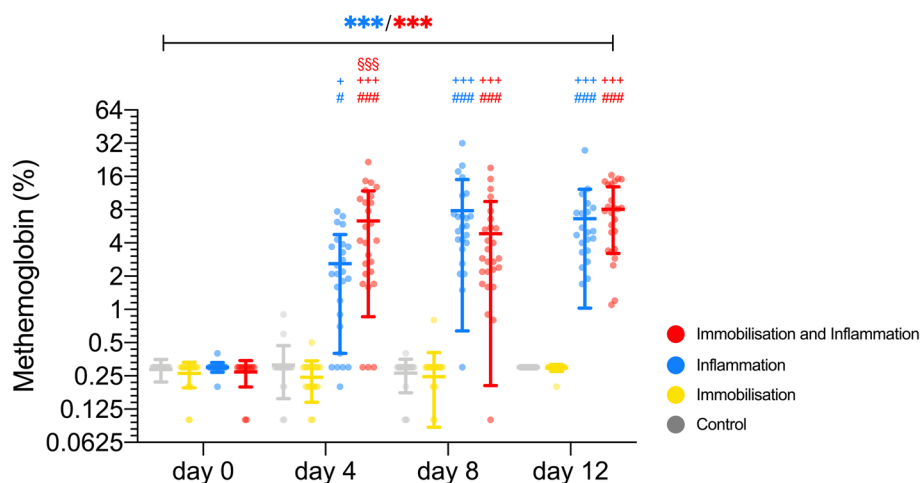


Fig. 2 Methaemoglobin (MetHb) blood levels in %. The control (mean [SD]; day 0: 0.29 [0.06], day 4: 0.31 [0.16], day 8: 0.26 [0.09], day 12: 0.30 [0.00]; $p=0.380$) and immobilisation (mean [SD]; day 0: 0.26 [0.08], day 4: 0.25 [0.09], day 8: 0.25 [0.16], day 12: 0.30 [0.02]; $p=0.293$) group showed no change in methaemoglobin blood levels over time while in the inflammation (mean [SD]; day 0: 0.28 [0.08], day 4: 2.65 [2.31], day 8: 7.07 [5.73], day 12: 6.50 [5.09]; $p<0.001$) as well as immobilisation and inflammation (mean [SD]; day 0: 0.28 [0.05], day 4: 6.22 [5.57], day 8: 4.77 [4.62], day 12: 8.06 [4.75]; $p<0.001$) groups a significant increase over time was present. This led to significantly increased values in the inflammation group in comparison with the control and immobilisation group on day 4 ($p=0.035/p=0.027$) as well as on day 8 ($p<0.001$ for both groups) and 12 ($p<0.001$ for both groups). The immobilisation and inflammation group showed an increase over all three other groups on day 4 ($p<0.001$ for all three groups) and over the control and immobilisation groups on day 8 ($p<0.001$ for both groups) and 12 ($p<0.001$ for both groups). The scale of the y-axis has been Log2 transformed. The number of identical symbols at the top of the error bars indicate the significance level: one <0.05 ; two <0.01 and three <0.001 . *showed significant difference over time with the colour of the symbol indicating the respective group. # showed significant difference to the control group at the specific time point. + Significant difference to the immobilisation group at the specific time point. \$ showed significant difference to the inflammation group at the specific time

(Fig. 3). The body weight standardized to the muscle weight is presented in the Appendix (Additional file 1: Additional Results and Additional file 1: Fig. S1).

Muscle weight

There was a significant effect of immobilisation ($p<0.001$), inflammation ($p<0.001$) and their interaction immobilisation with inflammation ($p=0.024$), as well as the side examined—ipsilateral versus contralateral ($p<0.001$), with an interaction of immobilisation \times side ($p<0.001$), but not inflammation \times side ($p=0.07$) on muscle weight. There was no effect of insulin ($p=0.89$) on muscle weight.

The effect of immobilisation alone was estimated to be -0.10 g (95% CI -0.12 to -0.08), of inflammation -0.11 g (95% CI -0.13 to -0.09) and of surgery -0.13 g (95% CI -0.15 to -0.12) (Fig. 4).

Phosphorylation of key enzymes of the insulin signaling pathway

Phosphorylated protein kinase b (pAkt)/total protein kinase b (Akt)

Immobilisation [estimated effect size (95% CI) 9.1-fold (0.2 to 17.9); $p=0.045$] and insulin [estimated effect size (95% CI) 85.1-fold (76.2 to 94.0); $p<0.001$] had an independent effect on Akt phosphorylation. Post-hoc, no differences between the groups

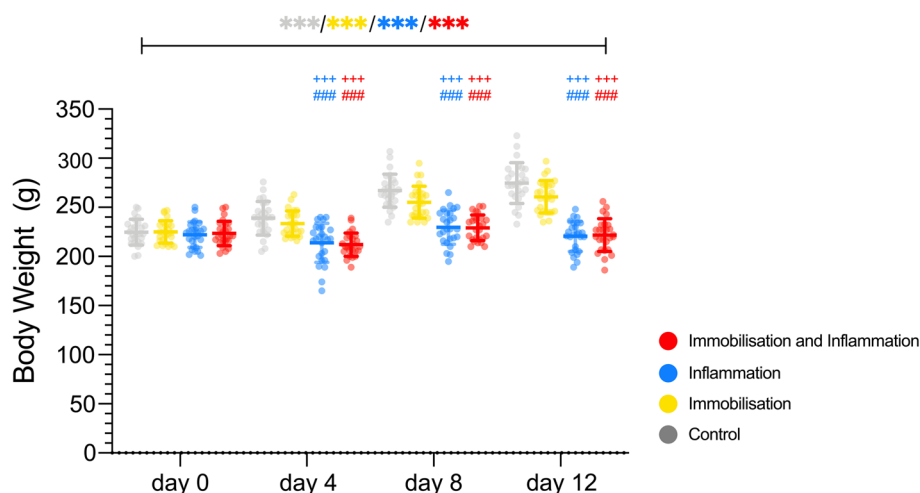


Fig. 3 Body weight in gram. The control (mean [SD]; day 0: 224.9 [13.3], day 4: 239.5 [17.3], day 8: 267.8 [16.7], day 12: 276.0 [20.0]; $p < 0.001$), immobilisation (mean [SD]; day 0: 225.0 [11.4], day 4: 233.5 [12.9], day 8: 255.2 [16.3], day 12: 260.8 [16.5]; $p < 0.001$), inflammation (mean [SD]; day 0: 222.2 [13.0], day 4: 214.1 [19.5], day 8: 229.8 [17.5], day 12: 220.6 [15.3]; $p < 0.001$) as well as immobilisation and inflammation (mean [SD]; day 0: 223.8 [12.8], day 4: 213.0 [13.6], day 8: 230.0 [14.4], day 12: 221.2 [15.9]; $p < 0.001$) group showed a significant change over time. The increase over time in the control and immobilisation group led to significantly higher values in comparison with either the inflammation or immobilisation and inflammation group on days 4, 8 and 12 ($p < 0.001$ for all three timepoints and 3 comparisons). The number of identical symbols at the top of the error bars indicate the significance level: one < 0.05 ; two < 0.01 and three < 0.001 . *Significant difference over time with the colour of the symbol indicating the respective group. #Significant difference to the control group at the specific time point. +Significant difference to the immobilisation group at the specific time point. §Significant difference to the inflammation group at the specific time point

could be observed irrespective of the administration of insulin or saline. Insulin led to a significant increase in Akt phosphorylation in all groups but did not significantly differ from each other (Figs. 5a, b, 6).

Phosphorylated glycogen synthase kinase 3 beta (pGSK3)/glycogen synthase kinase 3 beta (GSK3)

All main factors (immobilisation, inflammation, insulin, side) were highly significant ($p < 0.001$). Similarly, were the interaction terms immobilisation \times side, inflammation \times side, immobilisation \times inflammation \times side ($p < 0.001$) and insulin \times side highly significant ($p = 0.008$). The estimated effect sizes were -40.2 (95% CI -45.6 to -34.8) for immobilisation, -55.0 (95% CI -60.4 to -49.5) for inflammation and 22.7 (95% CI 17.3 to 28.2) for insulin. These effects lead to significantly reduce amounts of pGSK3 in the immobilisation and inflammation group in comparison with all others groups. The additive nature of the two interventions is displayed in the significant reduction in the operated compared to the contralateral leg within the immobilisation and inflammation group. The control group on the other hand as well as the contralateral leg of the immobilisation and also the inflammation group showed a significant increase in pGSK3 after insulin stimulation (Figs. 5c, d, 6).

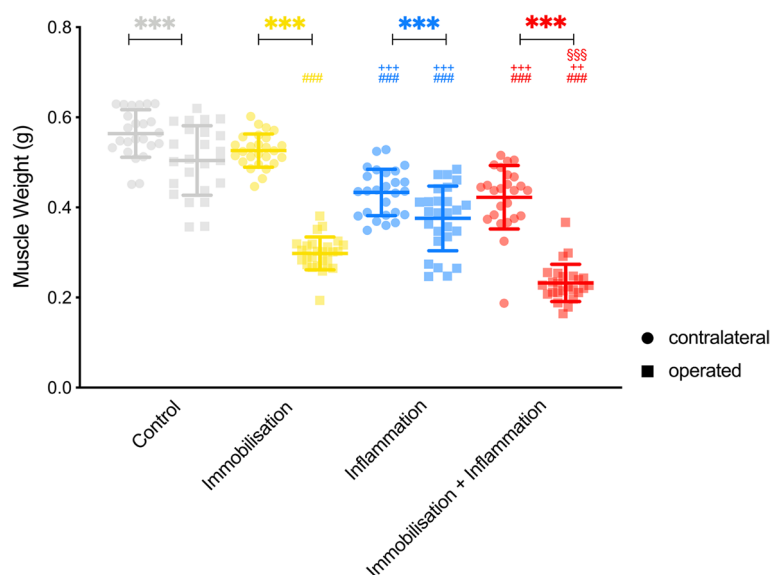


Fig. 4 Muscle weight in gram. In all 4 groups a significant difference in muscle weight between the operated and contralateral leg was present ($p < 0.001$ for all four groups). The control group (mean [SD]; contralateral: 0.56 [0.05]; operated: 0.50 [0.08]) had significantly higher muscle weights in the contralateral and operated leg in comparison with the inflammation (mean [SD]; contralateral: 0.43 [0.05]; $p < 0.001$; operated: 0.38 [0.07]; $p < 0.001$) as well as immobilisation and inflammation group (mean [SD]; contralateral: 0.42 [0.07]; $p < 0.001$; operated: 0.23 [0.04]; $p < 0.001$). Meanwhile, only in the operated leg a significant difference between the control and immobilisation group (mean [SD]; operated: 0.30 [0.04]; $p < 0.001$) was present. The immobilisation group showed a significantly higher muscle weight in the contralateral leg (mean [SD]; contralateral: 0.53 [0.04]) in comparison with the inflammation ($p < 0.001$) as well as immobilisation and inflammation group ($p < 0.001$) while showing a lower muscle weight in the operated leg in comparison with the inflammation group ($p < 0.001$) but a higher muscle weight in the operated leg in comparison with the immobilisation and inflammation group ($p = 0.001$). When comparing the inflammation group to the immobilisation and inflammation group a significantly higher muscle weight in the operated leg was present ($p < 0.001$). The number of identical symbols at the top of the error bars indicate the significance level: one < 0.05 ; two < 0.01 and three < 0.001 . *Significant difference between the operated and contralateral leg within the same group. #Significant difference to the control group for the respective leg. +Significant difference to the immobilisation group for the respective leg. ⁵Significant difference to the inflammation group for the respective leg

Phosphorylated glycogen synthase (pGS)/glycogen synthase (GS)

A highly significant ($p < 0.001$) effect for immobilisation {estimated effect size [95% CI 70.6-fold (58.8 to 82.4)]}, inflammation {estimated effect size [95% CI 96.7 (85.0 to 108.5)]} and insulin {estimated effect size [95% CI -23.6 (-35.4 to -11.8)]} as main factors could be observed, while only the interaction term immobilisation \times inflammation was significant ($p < 0.001$). The immobilisation with inflammation group showed significantly increased amounts of pGS in comparison with all other groups without a difference between the muscles. Furthermore, no response to insulin stimulation was evident. In contrast, the control group as well as the contralateral leg of the immobilisation group had a significant reduction of pGS with insulin stimulation (Figs. 5e, f, 6).

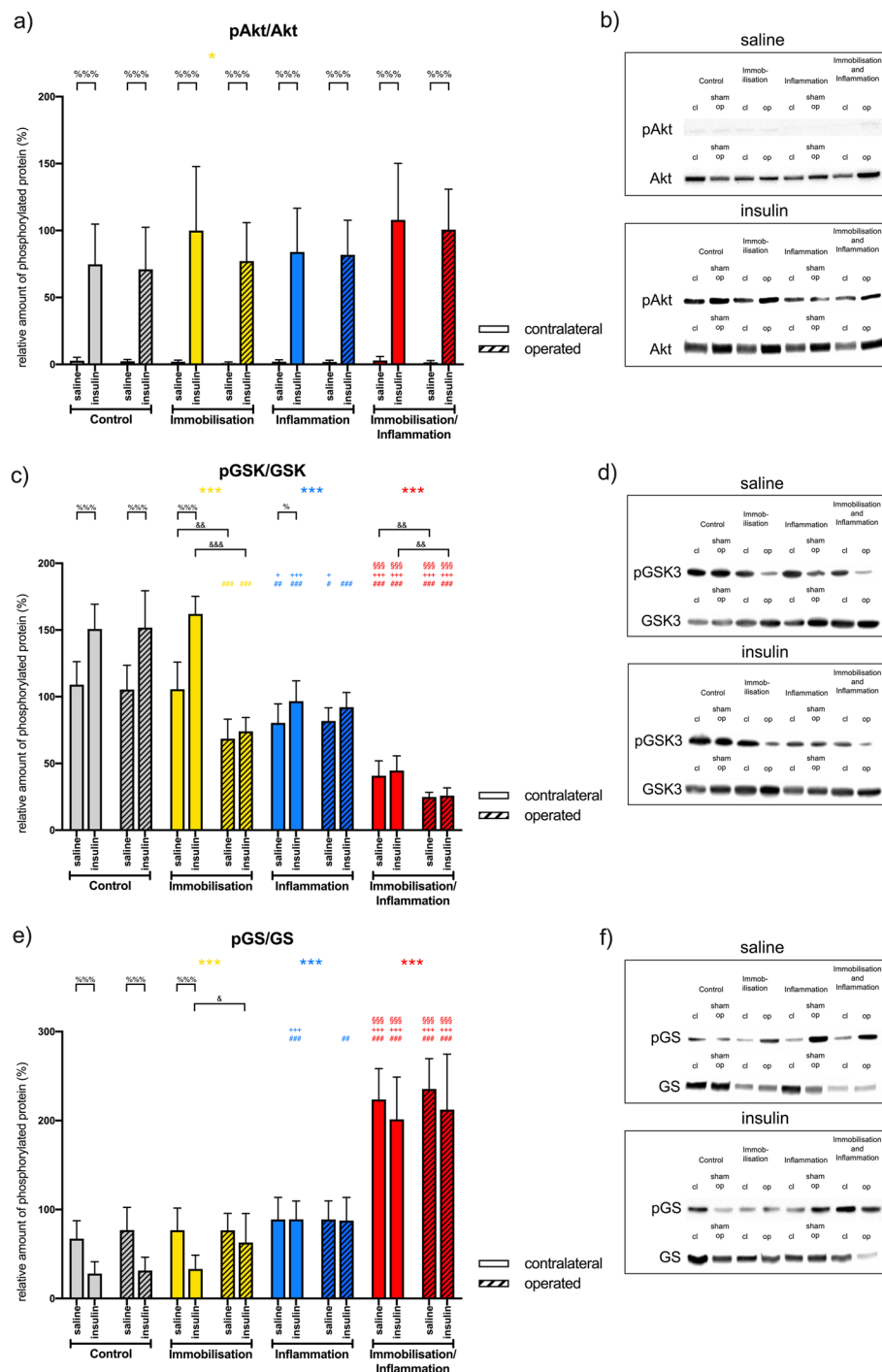


Fig. 5 Relative amount as well as western blots of phosphorylated key enzymes of the insulin signaling pathway. The number of identical symbols at top of each box indicate the significance level: one < 0.05; two < 0.01 and three < 0.001. Expression values were normalised to total protein. Protein kinase b = Akt; phosphorylated Protein kinase B = pAkt; glycogen synthase kinase 3 beta (GSK3); phosphorylated glycogen synthase kinase 3 beta (pGSK3); Glycogen synthase = GS; phosphorylated Glycogen synthase (pGS); contralateral = cl; operated = op; sham-operated = sham op. *Significant the main effect of the factors immobilisation, inflammation as well as immobilisation and inflammation. #Significant difference to the control group for the respective leg. +Significant difference to the immobilisation group for the respective leg. [§]Significant difference to the inflammation group for the respective leg. %Significant difference of the insulin group to the saline group for the respective group. [¶]Significant difference of the operated leg to the contralateral leg for the respective group

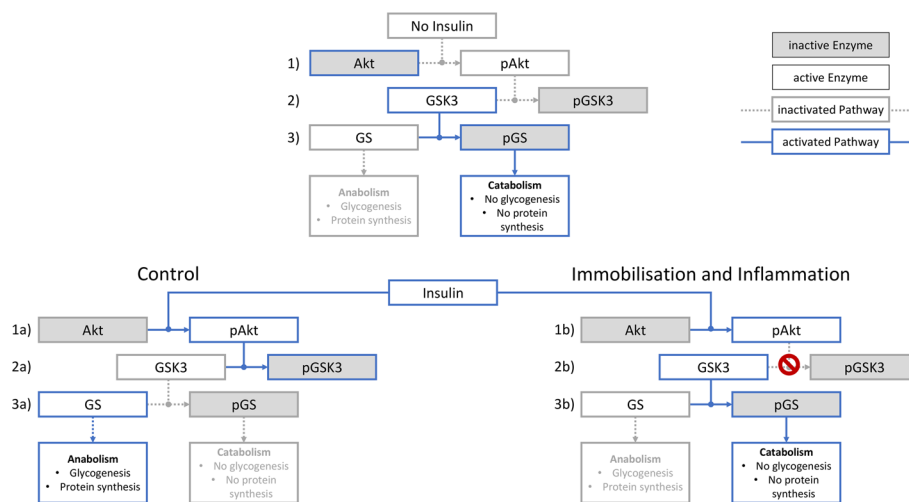


Fig. 6 Impairment of the Akt–GSK3–GS pathway in skeletal muscle. No insulin (Control and intervention groups with saline): (1) Akt in its deactivated state. (2) No inhibition through Akt, hence GSK 3 remains activated. (3) This leads to deactivation of GS via phosphorylation. → Catabolism is enabled Control (Control group with insulin): (1a) Insulin leads to activation of Akt to phosphorylated Akt. (2a) This causes deactivation of GSK3 to pGSK3. (3a) Consequently, GS remains activated. → Anabolism is enabled. Immobilisation and Inflammation (Intervention group with insulin): (1b) Insulin leads to activation of Akt to pAkt. (2b) GSK3 remains activated as inhibition via pAkt is impaired. (3b) This results in deactivation of GS to pGS. → Catabolism is enabled

Discussion

In a rat double-hit model combining two main risk factors for ICUAW (immobilisation and inflammation), we found a significant muscle atrophy as well as an impaired insulin signaling pathway. The insulin signaling pathway was intact up to the level of Akt phosphorylation. Although Akt was phosphorylated, the function of pAKT seemed to be impaired as the downstream effect on GSK3 phosphorylation was impaired resulting in an increased GS phosphorylation and its deactivation. None of these mechanisms showed a response to insulin stimulation indicating severe insulin resistance.

Immobilisation and inflammation lead to muscle atrophy, with an additive effect of both conditions combined. These findings are in line with previously published reports using the same model and demonstrate an effective ICUAW model [14, 15]. Furthermore, the persistent elevation in Methaemoglobin after injection of *Corynebacterium parvum* are indicative of a sufficient inflammation [15].

We also observed a decrease in muscle weight in the contralateral leg of the immobilisation group compared to controls as well as a significant decrease due to the sham operation in the intraindividual comparison, which can be interpreted as decreased mobility due to either the contralateral immobilisation or surgical pain.

The insulin signaling pathway showed significant alterations due to inflammation and immobilisation. Akt/pAkt is a key mediator in muscle atrophy and insulin resistance. Previous research has shown that overexpression of Akt leads to muscle hypertrophy and can prevent atrophy, while the Akt phosphorylation is downregulated during muscle atrophy [19–21]. Furthermore, it has been shown that a dysfunctional kinase activity of Akt reduced GLUT4 translocation by 87%, which resulted in severe insulin resistance

similar to critically ill patients [5, 22, 23]. Activation of Akt via phosphorylation after insulin stimulation was unhampered in our study confirming recent findings in critically ill patients [5, 24]. This indicates that insulin signaling up to the point of Akt phosphorylation was intact and that the defect is likely downstream of pAkt. Nevertheless, there are conflicting results regarding pAkt in critically ill patients as well as animals, which show a downregulation of pAkt due to inflammation [5, 13, 25]. The exact role of pAkt/Akt still needs further investigation.

Regulation of muscle mass downstream of Akt is mediated via the kinase mTOR-s6 kinase-1 (s6K1) pathway. [19, 26, 27]. We focussed on GSK and observed a significant decrease in phosphorylation due to immobilisation and inflammation, which is in line with the findings in critically ill patients [25]. This decrease in phosphorylation leads to an insufficient deactivation after stimulation with insulin pointing towards an increased GSK3 activity during critical illness.

GSK3 has been established as relevant mediator in ubiquitin–proteasome-mediated muscle atrophy that also mediates atrophy during critical illness [28, 29]. Hence, inhibition of GSK3 leads to muscle sparing during models of critical illness [30]. The observed increase in active GSK3 with insufficient deactivation confirmed its involvement in muscle atrophy during critical illness and suggests that insulin resistance originates from insufficient phosphorylation via Akt.

GSK3 has been shown to inhibit Adenosine monophosphate-activated protein kinase (AMPK) as part of the anabolic insulin pathway [31]. AMPK initiates catabolic processes in energy depleted cells such as muscle atrophy through induction of muscle protein degradation as well as inhibition of muscle protein synthesis [32–34]. This helps replenish the energy stores by promoting glucose uptake via GLUT4 [35]. Insufficient AMPK deactivation reflected in an increased pAMPK/AMPK-ratio has been observed in critically ill patients and would also explain the phenotype of ICUAW with muscle atrophy [35, 36]. Inhibition of AMPK via GSK3 would, therefore, be a plausible mechanism. On the other hand would an increased activation of AMPK potentially reverse the insufficient GLUT4 translocation and, therefore, improve glucose uptake into skeletal muscle as it has also been evidenced to be disrupted during critical illness [5].

Active GSK3 prevents dephosphorylation and activation of GS leading to insufficient glycogen synthesis, which is confirmed within our data as we observed a significantly increased content of pGS in animals submitted to immobilisation and inflammation while showing no response to insulin stimulation [37]. A low muscle glycogen has been shown to correlate with fatigability measured as exercise time until exhaustion [38]. Our results of insufficient GSK3 phosphorylation and the consecutive phosphorylation and deactivation of GS propose a mechanism for the low muscle glycogen content that has been observed in the muscle of critically ill patients [39]. This could explain recent observations showing that even though a regain of muscle mass can be observed after critical illness, patients still suffer from impaired muscle function confirming that ICUAW extends beyond muscle atrophy [40]. Depletion of glycogen as the main fuel source would be one explanation for fatigue and muscle dysfunction [41].

While our investigation adds new insights to the pathophysiology of ICUAW there are, of course, certain limitations. First, the results stem from an animal model that cannot account for all risk factors present during an intensive care therapy and, therefore,

need to be confirmed in a translational approach in the future. Moreover, there are multiple pathways involved in synthesis and degradation of muscle as well as in the glucose homeostasis, respectively, insulin resistance, which were not all investigated in this study.

Conclusion

Immobilisation and inflammation lead to an impairment in the insulin signaling pathway downstream of Akt causing an insufficient phosphorylation and deactivation of GSK after an insulin stimulus. This could directly contribute to muscle atrophy, and furthermore, phosphorylated and deactivated GS could hamper glycogen disposition and promote muscle fatigue.

Abbreviations

ICUAW	Intensive care unit acquired weakness
ICU	Intensive care unit
Akt	Protein kinase b
pAkt	Phosphorylated protein kinase b
GSK	Glycogensynthase-kinase-3-beta
pGSK	Phosphorylated glycogensynthase-kinase-3-beta
GS	Glycogen synthase
pGS	Phosphorylated glycogen synthase
MethHb	Methaemoglobin

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40635-023-00503-9>.

Additional file 1: Appendix.

Acknowledgements

Not applicable.

Author contributions

JJG has contributed to the analysis and interpretation of the data as well as drafting and revision of the manuscript. TG has contributed to the acquisition and analysis of the data as well as revision of the manuscript. HL has contributed to the conception and design of the work, acquisition and interpretation of the data as well as revision of the manuscript. JAJM has contributed to the conception and design of the work, acquisition and interpretation of the data as well as revision of the manuscript. MB has contributed to the conception and design of the work, acquisition and interpretation of the data as well as revision of the manuscript. SJS has contributed to the conception and design of the work, acquisition, analysis and interpretation of the data as well as drafting and revision of the manuscript. All authors have approved the submitted version of the manuscript and have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature. All authors read and approved the final manuscript.

Funding

Open Access funding enabled and organized by Projekt DEAL. Julius J. Grunow is participant in the BIH-Charité Junior Clinician Scientist Program funded by the Charité-Universitätsmedizin Berlin and the Berlin Institute of Health.

Availability of data and materials

The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All experiments described in the study protocol were approved and performed according to the guidelines by governmental veterinary and animal protection office (Regierung von Oberbayern, TVA 55.2-1-54-2532-121-2015; approval date 12th of August 2015).

Consent for publication

Not applicable.

Competing interests

Dr. Schaller reports grants from Reactive Robotics GmbH (Munich, Germany), grants and non-financial support from STIMIT AG (Biel, Switzerland), Liberate Medical LLC (Crestwood USA), ESICM (Geneva, Switzerland), grants, personal fees and non-financial support from Fresenius Kabi Deutschland GmbH (Bad Homburg, Germany), personal fees from Springer Verlag GmbH (Vienna Germany) for educational purposes, non-financial support from National and international societies (and their congress organizers) in the field of anesthesiology and intensive care medicine, outside the submitted work. Dr. Schaller held stocks in small amounts from Rhön-Klinikum AG and holds stocks in small amounts from Alphabeth Inc., Bayer AG and Siemens AG; these holdings have not affected any decisions regarding his research or this study.

Received: 20 November 2022 Accepted: 20 February 2023

Published online: 27 March 2023

References

1. Fan E, Cheek F, Chlan L, Gosselink R, Hart N, Herridge MS et al (2014) An official American Thoracic Society Clinical Practice guideline: the diagnosis of intensive care unit-acquired weakness in adults. *Am J Respir Crit Care Med* 190:1437–1446
2. Appleton RT, Kinsella J, Quasim T (2015) The incidence of intensive care unit-acquired weakness syndromes: a systematic review. *J Intensive Care Soc* 16:126–136
3. Van Aerde N, Meersseman P, Debaveye Y, Wilmer A, Gunst J, Casaer MP et al (2020) Five-year impact of ICU-acquired neuromuscular complications: a prospective, observational study. *Intensive Care Med* 46:1184–1193
4. Wollersheim T, Woehlecke J, Krebs M, Hamati J, Lodka D, Luther-Schroeder A et al (2014) Dynamics of myosin degradation in intensive care unit-acquired weakness during severe critical illness. *Intensive Care Med* 40:528–538
5. Weber-Carstens S, Schneider J, Wollersheim T, Assmann A, Bierbrauer J, Marg A et al (2013) Critical illness myopathy and GLUT4: significance of insulin and muscle contraction. *Am J Respir Crit Care Med* 187:387–396
6. Patel BK, Pohlman AS, Hall JB, Kress JP (2014) Impact of early mobilization on glycemic control and ICU-acquired weakness in critically ill patients who are mechanically ventilated. *Chest* 146:583–589
7. Hermans G, De Jonghe B, Bruyninckx F, Van den Berghe G (2014) Interventions for preventing critical illness polyneuropathy and critical illness myopathy. *Cochrane Database Syst Rev* 2014:CD006832
8. Grunow JJ, Goll M, Carbon NM, Liebl ME, Weber-Carstens S, Wollersheim T (2019) Differential contractile response of critically ill patients to neuromuscular electrical stimulation. *Crit Care* 23:308
9. Schaller SJ, Anstey M, Blobner M, Edrich T, Grabitz SD, Gradwohl-Matis I et al (2016) Early, goal-directed mobilisation in the surgical intensive care unit: a randomised controlled trial. *Lancet* 388:1377–1388
10. Carbon NM, Engelhardt LJ, Wollersheim T, Grunow JJ, Spies CD, Mardian S et al (2022) Impact of protocol-based physiotherapy on insulin sensitivity and peripheral glucose metabolism in critically ill patients. *J Cachexia Sarcopenia Muscle* 13:1045–1053
11. Van den Berghe G, Schoonheydt K, Becx P, Bruyninckx F, Wouters PJ (2005) Insulin therapy protects the central and peripheral nervous system of intensive care patients. *Neurology* 64:1348–1353
12. Van den Berghe G, Schetz M, Vlasselaers D, Hermans G, Wilmer A, Bouillon R et al (2009) Clinical review: Intensive insulin therapy in critically ill patients: NICE-SUGAR or Leuven blood glucose target? *J Clin Endocrinol Metab* 94:3163–3170
13. Sugita H, Kaneki M, Sugita M, Yasukawa T, Yasuhara S, Martyn JA (2005) Burn injury impairs insulin-stimulated Akt/PKB activation in skeletal muscle. *Am J Physiol Endocrinol Metab* 288:E585–591
14. Schaller SJ, Nagashima M, Schonfelder M, Sasakawa T, Schulz F, Khan MAS et al (2018) GTS-21 attenuates loss of body mass, muscle mass, and function in rats having systemic inflammation with and without disuse atrophy. *Pflugers Arch* 470:1647–1657
15. Fink H, Helming M, Unterbuchner C, Lenz A, Neff F, Martyn JA et al (2008) Systemic inflammatory response syndrome increases immobility-induced neuromuscular weakness. *Crit Care Med* 36:910–916
16. Krafte-Jacobs B, Brilli R, Szabo C, Denenberg A, Moore L, Salzman AL (1997) Circulating methemoglobin and nitrite/nitrate concentrations as indicators of nitric oxide overproduction in critically ill children with septic shock. *Crit Care Med* 25:1588–1593
17. Sugita M, Sugita H, Kim M, Mao J, Yasuda Y, Habiro M et al (2012) Inducible nitric oxide synthase deficiency ameliorates skeletal muscle insulin resistance but does not alter unexpected lower blood glucose levels after burn injury in C57BL/6 mice. *Metabolism* 61:127–136
18. Gurtler A, Kunz N, Gomolka M, Hornhardt S, Friedl AA, McDonald K et al (2013) Stain-Free technology as a normalization tool in Western blot analysis. *Anal Biochem* 433:105–111
19. Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R et al (2001) Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* 3:1014–1019
20. Blaauw B, Canato M, Agatea L, Toniolo L, Mammucari C, Masiero E et al (2009) Inducible activation of Akt increases skeletal muscle mass and force without satellite cell activation. *FASEB J* 23:3896–3905
21. Pallafacchina G, Calabria E, Serrano AL, Kalhovde JM, Schiaffino S (2002) A protein kinase B-dependent and rapamycin-sensitive pathway controls skeletal muscle growth but not fiber type specification. *Proc Natl Acad Sci USA* 99:9213–9218
22. Thong FS, Bilan PJ, Klip A (2007) The Rab GTPase-activating protein AS160 integrates Akt, protein kinase C, and AMP-activated protein kinase signals regulating GLUT4 traffic. *Diabetes* 56:414–423
23. Zisman A, Peroni OD, Abel ED, Michael MD, Mauvais-Jarvis F, Lowell BB et al (2000) Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat Med* 6:924–928

24. Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P et al (1996) Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J* 15:6541–6551
25. Constantin D, McCullough J, Mahajan RP, Greenhaff PL (2011) Novel events in the molecular regulation of muscle mass in critically ill patients. *J Physiol* 589:3883–3895
26. Rommel C, Bodine SC, Clarke BA, Rossman R, Nunez L, Stitt TN et al (2001) Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. *Nat Cell Biol* 3:1009–1013
27. Welsh GI, Miller CM, Loughlin AJ, Price NT, Proud CG (1998) Regulation of eukaryotic initiation factor eIF2B: glycogen synthase kinase-3 phosphorylates a conserved serine which undergoes dephosphorylation in response to insulin. *FEBS Lett* 421:125–130
28. Verhees KJ, Schols AM, Kelders MC, Op den Kamp CM, van der Velden JL, Langen RC (2011) Glycogen synthase kinase-3beta is required for the induction of skeletal muscle atrophy. *Am J Physiol Cell Physiol* 301:C995–C1007
29. Scheffold JC, Wollersheim T, Grunow JJ, Luedi MM, Z'Graggen WJ, Weber-Carstens S (2020) Muscular weakness and muscle wasting in the critically ill. *J Cachexia Sarcopenia Muscle* 11:1399–1412
30. Dugo L, Collin M, Allen DA, Patel NS, Bauer I, Mervaala EM et al (2005) GSK-3beta inhibitors attenuate the organ injury/dysfunction caused by endotoxemia in the rat. *Crit Care Med* 33:1903–1912
31. Suzuki T, Bridges D, Nakada D, Skiniotis G, Morrison SJ, Lin JD et al (2013) Inhibition of AMPK catabolic action by GSK3. *Mol Cell* 50:407–419
32. Nakashima K, Yakabe Y (2007) AMPK activation stimulates myofibrillar protein degradation and expression of atrophy-related ubiquitin ligases by increasing FOXO transcription factors in C2C12 myotubes. *Biosci Biotechnol Biochem* 71:1650–1656
33. Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A et al (2004) Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* 117:399–412
34. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS et al (2008) AMPK phosphorylation of rap-1 mediates a metabolic checkpoint. *Mol Cell* 30:214–226
35. Kurth-Kraczek EJ, Hirshman MF, Goodyear LJ, Winder WW (1999) 5' AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. *Diabetes* 48:1667–1671
36. Puthucherry ZA, Astin R, McPhail MJW, Saeed S, Pasha Y, Bear DE et al (2018) Metabolic phenotype of skeletal muscle in early critical illness. *Thorax* 73:926–935
37. McManus EJ, Sakamoto K, Armit LJ, Ronaldson L, Shpiro N, Marquez R et al (2005) Role that phosphorylation of GSK3 plays in insulin and Wnt signalling defined by knockin analysis. *EMBO J* 24:1571–1583
38. Bergstrom J, Hermansen L, Hultman E, Saltin B (1967) Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71:140–150
39. Millan IS, Hill J, Wischmeyer P (2015) Measurement of skeletal muscle glycogen status in critically ill patients: a new approach in critical care monitoring. *Crit Care* 19:P400
40. Dos Santos C, Hussain SN, Mathur S, Picard M, Herridge M, Correa J et al (2016) Mechanisms of chronic muscle wasting and dysfunction after an intensive care unit stay. A pilot study. *Am J Respir Crit Care Med* 194:821–830
41. Nielsen J, Schroder HD, Rix CG, Ortenblad N (2009) Distinct effects of subcellular glycogen localization on tetanic relaxation time and endurance in mechanically skinned rat skeletal muscle fibres. *J Physiol* 587:3679–3690

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