Steroids 201 (2024) 109337

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Uphill running preferred over downhill running for recovery from glucocorticoid-induced muscle atrophy

Masanobu Murao [a,b,*], Tetsuo Imano [a,c], Yoshinobu Sato [a,d], Masaaki Nakajima [a]

[a] Research institute of health and welfare, Kibi international university, 8 Iga-machi, Takahashi-city, Okayama 716-8508, Japan
[b] Rehabilitation unit, Kyoto university hospital, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto-city, Kyoto 606-8507, Japan
[c] Department of physical therapy, Hiroshima international and medical welfare collage, 14-22 Hijiyamahonmachi, Minami-ku, Hiroshima-city, Hiroshima 732-0816, Japan
[d] Studio TAIKA, 28-6 Shinyuno, Kannabe-cho, Fukuyama-city, Hiroshima 720-2122, Japan

1. Introduction

Glucocorticoid (GC)-induced skeletal muscle atrophy is a significant side effect that commonly occurs with long-term administration of high doses of GC, leading to a notable impairment of activities of daily living (ADL) [1]. This type of muscle atrophy is characterized by proximal muscle atrophy and a dominance of type II fibers, resulting in a substantial decline in muscle strength and limitations of key ADL actions, such as walking and standing [1–3].

In clinical practice, pharmacotherapy and physical therapy are used to counteract GC-induced muscle atrophy [3–5]. Recent advances in pathological chemistry have provided insights into GC-induced muscle atrophy, which is believed to be associated with decreased insulin-like growth factor 1 (IGF-1) and increased myostatin [4,5]. Unfortunately, studies investigating the potential of exercise therapy to prevent and suppress GC-induced muscle atrophy in rats are limited, as are those investigating the relationship between exercise therapy and the dynamics of IGF-1 or myostatin [6–16]. However, recent findings suggest that downhill running may increase the expression of irisin [17–19], a myokine associated with IGF-1 and myostatin. Downhill running is characterized by lower metabolic costs and greatekr loading on antigravity muscles [20,21]. Assessing changes in the intramuscular levels of these myokines and the skeletal muscle mass for each exercise mode is crucial for the development of exercise therapy protocols for GC-induced muscle atrophy.

Although previous studies have investigated treadmill running therapy initiated before or during GC treatment [7,9,10,12–14], there is a lack of research examining the effects of running during the recovery period after GC treatment. Muscle weakness resulting from GC-induced muscle atrophy often becomes evident after treatment, prompting the prescription of rehabilitation interventions once symptoms appear. By investigating the effects of the intervention on muscles that have already undergone atrophy, the optimal timing of the rehabilitation intervention can be determined.

This study aimed to determine the most effective running mode for recovery from GC-induced muscle atrophy.

2. Experimental

2.1. Animals

A total of 32 adult male 10-week-old Wistar rats (CLEA Japan Inc., Osaka, Japan) were used. All animals were maintained at a 12:12 h light/dark cycle and standard laboratory temperature of 23 °C. The rats had free access to water and food. The experimental protocol was approved by the University Animal Care and Use Committee (A-19-03).

2.2. Experimental protocol (Fig. 1)

The rats were randomly divided into four groups: a) vehicle group (VEH, n = 8); b) dexamethasone (DEX)-injected and sedentary group (n = 8); c) DEX-injected and 15° uphill (UH) running group (n = 8); and d) DEX-injected and −15° downhill (DH) running group (n = 8). All animals underwent treadmill habituation-run consecutively for two days at 10 m/min for 10 min before DEX or saline injection.

The VEH group was allowed six weeks of free movement after five consecutive days of intraperitoneal administration of 1 ml/kg^{-1} saline, starting from the beginning of the experiment. Conversely, 600 μg/kg^{-1} of DEX (Sigma-Aldrich, Missouri, USA) diluted in an equal volume (1 ml/kg^{-1}) of saline was administered in the remaining groups via i.p. injection for five consecutive days in order to induce muscle atrophy, as seen in previous studies [22–24]. Following treatment, the DEX group was allowed six weeks of free movement within their cages. Fig. 1.

The UH and DH groups ran on an inclined treadmill (MK-680;

* Corresponding author at: Research institute of health and welfare, Kibi international university, 8 Iga-machi, Takahashi-city, Okayama 716-8508, Japan.
E-mail address: candy_notfound@yahoo.co.jp (M. Murao).

https://doi.org/10.1016/j.steroids.2023.109337
Received 15 August 2023; Received in revised form 1 November 2023; Accepted 14 November 2023
Available online 15 November 2023

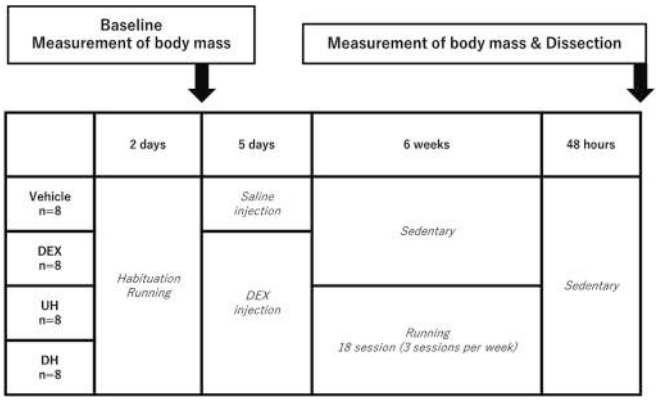

Fig. 1. Experimental protocol. DEX, dexamethasone injection and sedentary group; UH, dexamethasone injection and uphill running group; DH, dexamethasone injection and downhill running group.

Muromachi Inc., Tokyo, Japan) for 30 min (15 min running/5 min rest/15 min running) × 3 sessions/week × 6 weeks (a total of 18 sessions). UH rats ran at a + 15°-incline at 12 m/min for the first week, 14 m/min for the second week, and 16 m/min for the third and subsequent weeks. To incur the same metabolic cost as UH as per the regression expression of Schlagowski et al. [25], DH rats ran at a −15°-incline at 20 m/min for the first week, 26 m/min for the second week, and 30 m/min for the third and subsequent weeks.

2.3. Tissue collection and storage

Forty-eight hours after the end of the intervention period, animals were sacrificed by an overdose i.p. injection of pentobarbital sodium. Soleus (SOL), medial gastrocnemius (GAS), and extensor digitorum longus (EDL) muscles were harvested from the left hind limb and promptly weighed to determine wet weight. The gastrocnemius muscle uniquely satisfied the following conditions: it was susceptible to GC-induced atrophy [26] and it exhibited eccentric contraction induced by downhill running [27,28]. The soleus muscle, which primarily consists of Type I fibers, is minimally affected by DEX [29,30]. The EDL is a Type II fiber-dominant muscle [31]; however, since it acts in ankle dorsiflexion, its eccentric contraction is not accentuated by downhill running. We provided data on the wet weights of these muscles to demonstrate that the effect of DEX is consistent with the findings of previous studies.

GC administration leads to a decrease in both whole-body and skeletal muscle mass [14,29]. Thus, normalization using significantly reduced whole-body mass at autopsy as the denominator may underestimate the decline in skeletal muscle mass since both the numerator and denominator decreased simultaneously. Using the baseline whole-body mass as the denominator, which aligned with the normal growth curve for all groups, enabled us to compare pure skeletal muscle atrophy. Baseline whole-body mass was used as a reference for standardizing the muscle wet weight in the results.

The left GAS was fixed in 10 % phosphate-buffer for 24 h. It was further subjected to sucrose replacement treatment before being embedded in an OCT compound and stored at −80 °C for future use. The GAS obtained from the right hindlimb was placed in a screw tube and stored at −80 °C until it was needed.

2.4. Image analysis

Frozen blocks of the GAS obtained from the left hind limb were sectioned to a thickness of 10 μm (transverse section) using a cryostat. Subsequently, the sections were stained using the nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) staining method following the standard procedure. The resulting cross-sectional images were digitized and imported to a computer. The thinning, staining, digitization, and import processes were performed by the Center for Anatomical, Pathological, and Forensic Medical Research at Kyoto University Graduate School of Medicine in Kyoto, Japan.

For each muscle section, five random cross-sectional images were captured at × magnification of 200x. Using ImageJ software developed by the United States National Institutes of Health, 100 fibers were randomly selected and quantified for each fiber type (Type I, Type IIA, and Type IIB) from each muscle section. Additionally, the cross-sectional areas of the fibers were measured to further analyze the muscle samples. Similar to the muscle wet weight, the baseline whole-body mass was employed as the denominator for the normalization of skeletal muscle CSA.

2.5. Biochemical analysis

The GAS obtained from the right hind limb was thawed and homogenized. The total protein content of the extracted samples was analyzed using the Bradford method. Enzyme-linked immunosorbent assays (ELISA) were conducted on the extracts using commercially available kits for measuring irisin (Phoenix Pharmaceuticals Inc., Burlingame, CA, Cat. No. EK-067–29), myostatin (R&D Systems Inc., McKinley, USA, Cat. No. DGDF80), and IGF-1 (R&D Systems Inc., McKinley, USA, Cat. No.MG100). The assays were performed in duplicates, adhering to the instructions provided in the respective kit protocols. The mean value of duplicate wells was used for analysis. Microplate Leaders SH-1200 (Corona Electric Co., Ltd., Ibaraki, Japan) and SF-6 (Corona Electric Co., Ltd., Ibaraki, Japan) software were used for the calculations. The concentration of myokines in the GAS was determined by dividing it by the total protein concentration in the GAS.

2.6. Statistical analysis

Statistical analyses were performed using JMP PRO 17.0.0 software (SAS Institute Inc., Cary, NC, USA). All data are presented as means ± SE. The level of statistical significance was set at $\alpha = 0.05$. A one-way ANOVA test was used to examine the main effects among the groups for each outcome. Tukey's test was performed as a post-hoc test.

3. Results

3.1. Whole-body mass (Fig. 2)

The whole-body masses of all groups at baseline and at dissection are listed in Table 1. The body masses of the UH and VEH groups were significantly restored compared with those of the DEX group ($p < .05$). No significant differences were observed between the DH and DEX groups. Fig. 2.

3.2. Skeletal muscle wet weight

The wet weight of each skeletal muscle is listed in Table 1. The percentage change of the SOL wet weight was significantly higher in the UH and DH groups than that in the DEX group ($p < .05$). The DH group percentage change was also significantly higher than that of the VEH group ($p < .05$) (Fig. 3A).

The GAS wet weight in the UH and DH groups was significantly higher than that in the DEX group, similar to the weight differences seen with the SOL ($p < .05$). The GAS wet weight in the DH group was also significantly higher than that in the VEH group ($p < .05$) (Fig. 3B).

In the EDL, there were no significant differences among all groups.

3.3. Cross sectional areas of the medial gastrocnemius muscle (Fig. 4)

The CSA of the GAS are listed in Table 1. For type I fibers, the UH group showed a significantly greater ratio of the CSA/Baseline body

Table 1

Variables	Vehicle		DEX		DEX + Uphill runnning		DEX + Downhill running	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body mass								
Baseline (g)	300.6	5.7	338.7	3.9	284.9	2.2	301.3	5.6
Dissection (g)	419.6	10.7	425.0	10.8	380.8	5.2	399.8	8.4
Percent changes in body mass								
at Dissection/at Baseline (%)	139.5	1.6	125.4	2.3	133.7	1.9	123.8	1.7
Skeletal muscle wet weight/Baseline body mass								
Soleus (%)	0.054	0.001	0.048	0.002	0.062	0.002	0.065	0.003
GAS (%)	0.327	0.009	0.287	0.007	0.334	0.004	0.318	0.007
Extensor digitorum longus (%)	0.056	0.002	0.050	0.002	0.056	0.001	0.056	0.002
Myokine levels in the GAS								
Irisin (ng/g)	5.59	0.44	5.17	0.46	7.37	0.67	7.28	0.55
IGF-1 (pg/g)	99.14	24.43	185.9	18.1	129.95	13.81	130.60	16.02
Myostatin (pg/g)	42.75	2.33	40.33	2.49	38.92	3.08	31.20	1.42
CSA of the GAS								
Type I (cm^2)	1317.4	76.3	1314.0	73.5	1377.9	66.9	1160.4	62.4
Type IIa (cm^2)	2371.1	170.5	2431.1	132.0	2601.9	106.3	2182.2	85.5
Type IIb (cm^2)	3439.9	206.7	3274.7	137.2	3427.3	172.7	3113.2	166.8
Ratio of CSA of the GAS/Baseline body mass								
Type I	4.38	0.28	3.88	0.22	4.84	0.23	3.85	0.19
Type IIa	7.89	0.57	7.18	0.41	9.13	0.39	7.24	0.31
Type IIb	11.44	0.70	9.67	0.43	12.03	0.56	10.33	0.60

GAS, medial gastrocnemius; CSA, cross sectional area

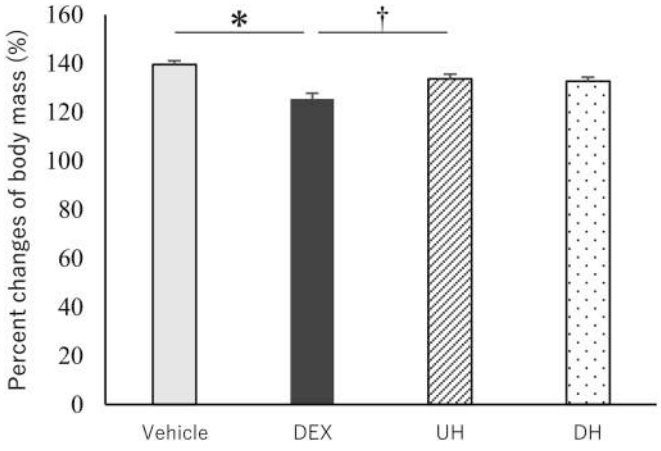

Fig. 2. Percentage changes of body mass. DEX, dexamethasone injection and sedentary group; UH, dexamethasone injection and uphill running group; DH, dexamethasone injection and downhill running group. *: Significant difference vs. vehicle group ($p < .05$); †: Significant difference vs. dexamethasone injection and sedentary group ($p < .05$).

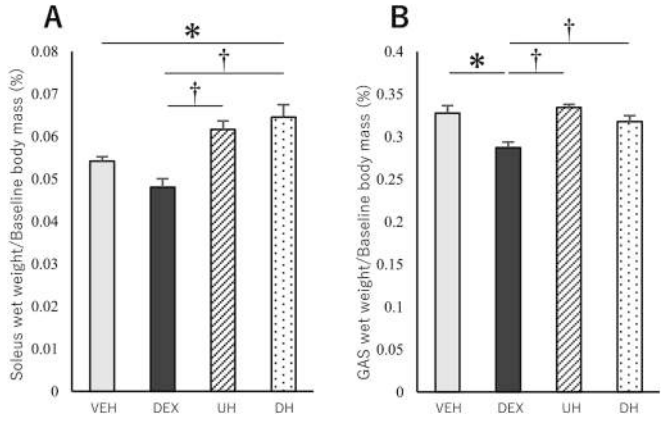

Fig. 3. Skeletal muscle wet weight relative to baseline body mass. A) Soleus; B) Medial gastrocnemius. GAS, medial gastrocnemius; VEH, vehicle group; DEX, dexamethasone injection and sedentary group; UH, dexamethasone injection and uphill running group; DH, dexamethasone injection and downhill running group. *: Significant difference vs. vehicle group ($p < .05$); †: Significant difference vs. dexamethasone injection and sedentary group ($p < .05$).

mass than the DEX and DH groups ($p < .05$) (Fig. 4, Fig. 5). No significant differences were observed between the other groups. .

In type IIA fibers, the UH group showed significantly greater ratio of the CSA/Baseline body mass than the DEX and DH groups ($p < .05$), similar to that of type I fibers. No significant differences were observed among the other groups. Fig. 5

In type IIB fibers, the UH group showed a significantly greater ratio of the CSA/Baseline body mass than that of the DEX group ($p < .05$). No significant differences were observed between the other groups. Fig. 5

3.4. Myokine levels in the medial gastrocnemius muscle

Only one sample each of the VEH, DEX, and UH groups with a coefficient of variation of > 20 % for total protein in the GAS was available, which serves as the denominator for myokine levels; as such, these samples were excluded from the analysis. The UH group showed significantly higher intramuscular irisin levels than that of the DEX group ($p < .05$) (Fig. 6A). The DH group also showed a trend toward higher intramuscular irisin levels than that of the DEX group; however, the difference was not significant ($p = .052$). No significant differences were observed between the other groups.

Muscle myostatin levels in the DH group were significantly lower than in the DEX group (Fig. 6B); however, there were no significant differences among the other groups.

One-way ANOVA revealed no significant differences in muscle IGF-1 levels between the groups.

4. Discussion

This study has three main findings. First, inclined running commenced after DEX injection and effectively restored significant losses in muscle wet weight caused by DEX to levels comparable to those in healthy development, irrespective of whether it was uphill or downhill running. Second, uphill running specifically induces hypertrophy of GAS Type IIB fibers, which are typically affected by GC-induced muscle atrophy. Finally, similar to myostatin and IGF-1, irisin may serve as an important mediator of muscle hypertrophy in GC-induced muscle atrophy in vivo.

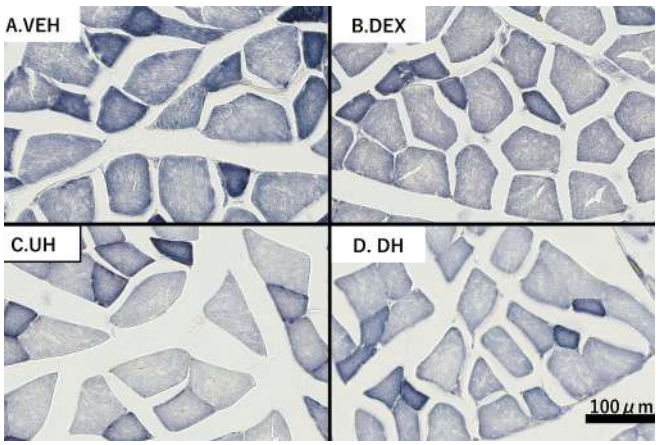

Fig. 4. Typical nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR)-staining images of medial gastrocnemius. A, vehicle group; B, dexamethasone injection and sedentary group; C, dexamethasone injection and uphill running group; D, dexamethasone injection and downhill running group.

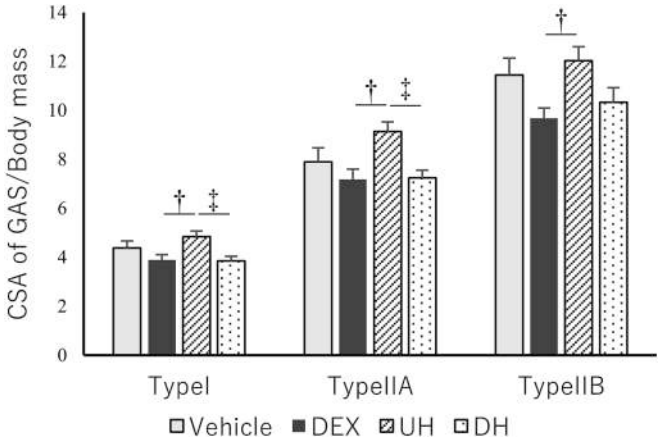

Fig. 5. Cross sectional area of the medial gastrocnemius for each fiber type relative to baseline body mass. CSA, cross sectional area; GAS, medial gastrocnemius; DEX, dexamethasone injection and sedentary group; UH, dexamethasone injection and uphill running group; DH, dexamethasone injection and downhill running group. †: Significant difference vs. dexamethasone injection and sedentary group ($p < .05$); ‡: Significant difference vs. dexamethasone injection and uphill running group ($p < .05$).

4.1. Timing of initiation of exercise therapy for GC-induced muscle atrophy

Previous studies explored the potential of treadmill running as an intervention to mitigate muscle atrophy [7,9–14]. Notably, different exercise protocols and GC treatment regimens have been employed in these studies [7,9–14], leading to varying outcomes. Hickson et al. [7] conducted a 12-week preventive exercise intervention with administration of GC limited to the last 10 days of the intervention period. Conversely, other studies opted for the concurrent administration of GC and exercise interventions [12–14]. Additionally, variations in the type of GC administered, route of administration, and dosage further contributed to the diversity of findings among the studies [7,9–14].

In the present study, DEX was injected before the exercise protocol to mimic muscle atrophy, which is commonly observed after high-dose GC therapy. Consequently, despite the occurrence of muscle atrophy induced by GC, exercise therapy promotes the histological recovery of skeletal muscle. These findings provide valuable insights into the potential of exercise therapy as a recovery strategy following high-dose GC therapy-induced muscle atrophy. By focusing on atrophied muscles, our study addresses a critical aspect of clinical relevance, as patients undergoing high-dose GC therapy often experience muscle wasting as a side effect. Identifying exercise conditions that facilitate recovery in this specific context can significantly affect clinical decision making and patient outcomes.

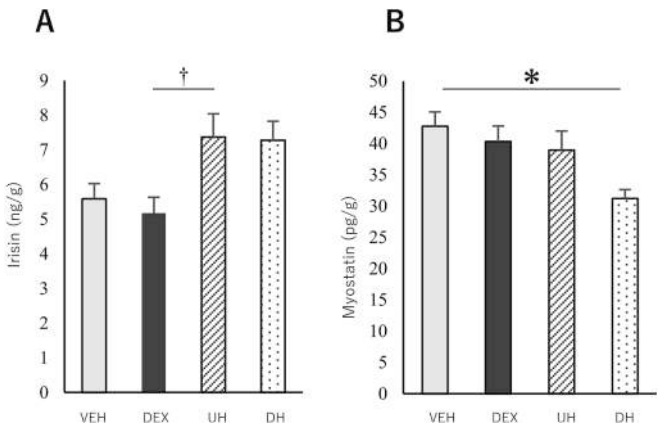

Fig. 6. Myokine concentration in each skeletal muscle. A, Irisin; B, Myostatin. VEH, vehicle group; DEX, dexamethasone injection and sedentary group; UH, dexamethasone injection and uphill running group; DH, dexamethasone injection and downhill running group. *: Significant difference vs. vehicle group ($p < .05$); †: Significant difference vs. dexamethasone injection and sedentary group ($p < .05$).

4.2. Differences between uphill and downhill running for recovery from GC-induced muscle atrophy

Although few studies have explored the relationship between differences in incline running and myokine expression, which regulates skeletal muscle mass, recent evidence suggests that transient downhill running may increase the expression of irisin and its precursor FNDC5 in both blood and muscle tissues [17–19]. With this in mind, we hypothesized that downhill running would facilitate recovery from GC-induced muscle atrophy. However, contrary to our expectations, the results of our study demonstrated that uphill running promoted recovery from GC-induced muscle atrophy. Previous studies have also indicated that moderate-load exercises more effectively suppress GC-induced muscle atrophy compared to high-load exercises [14], underscoring the significance of load adjustment and skeletal muscle tissue damage. This study focused on equalizing the metabolic load between the UH and DH groups; therefore, the rats were not matched for mechanical power (inclination and running distance). Downhill running, which was performed with higher mechanical power than that in the UH group, may have imposed significant stress on the vulnerable GAS owing to its weakened state after DEX administration [32,33]. Furthermore, downhill running has been associated with the initiation and advancement of knee osteoarthritis [34,35]. These findings may have important implications for patients prescribed rehabilitation after experiencing various illnesses and reduced physical strength.

4.3. Contribution of myokine in exercises promoting recovery from GC-induced muscle atrophy

It has been extensively documented that high doses of GC elevate myostatin levels in vivo and decrease IGF-1 levels, leading to increased protein catabolism and reduced skeletal muscle atrophy [4,5,22]. However, there are only a few reports on the relationship between GC treatment and irisin, which has emerged as a significant regulator of skeletal muscle mass. Chang et al. [36] reported that irisin treatment counteracted GC-induced atrophy in C2C12 muscle stem cells using in vitro methods. The findings of the current study, which showed

accelerated recovery from GC-induced muscle atrophy and increased irisin levels in the UH and DH groups, support this report.

Notably, prior research on irisin treatment in skeletal muscle atrophy models, such as non-weight-bearing [37], denervation-induced muscle atrophy [38], and dystrophic muscle [39], has shown partial amelioration of all types of muscle atrophy. In addition, irisin contributes to the regulation of muscle mass by regulating the expression of IGF-1 and myostatin [36,40]. Most studies demonstrating the suppression of muscle atrophy by GC were conducted prior to the discovery of irisin [7,9–14]. Our findings underscore the need for more comprehensive investigations to elucidate the extent of irisin involvement in this context.

While the Irisin levels in the GAS of both the UH and DH groups did not exhibit significant differences, there was a notable restoration in the CSA of Type I and Type IIA fibers in the UH group compared to that in the DH group. This discrepancy could be attributed to the distinctive nature of GC-induced muscle atrophy, wherein type IIB fibers are particularly susceptible to atrophy [5], and the variation in neuroeffectors that primarily engage Type II fibers during downhill running [41], rather than the influence of irisin. Therefore, additional research is required to ascertain the extent to which irisin contributes to muscle hypertrophy following GC treatment.

IGF-1 and myostatin secretion are thought to be regulated by resistance training rather than endurance exercises, such as treadmill running [42,43]. Myostatin was suppressed in the DH group at higher mechanical powers, although this was not reflected in the CSA results. In future experimental setups, it may be worthwhile to consider comparisons of exercises matched for mechanical power rather than metabolic costs.

4.4. Limitations

This study had several limitations. Regrettably, myokine levels were only assessed at a single time point, specifically at six weeks post-DEX administration. As a result, irisin levels in the DEX and VEH groups did not exhibit significant differences, possibly due to the spontaneous recovery of irisin and myostatin levels, which would ideally have decreased once these levels were restored. Indeed, previous studies have reported that in vivo irisin levels decrease [44] and myostatin levels increase [4] after the administration of GC. A study design that incorporates tissue assessments at multiple time points is necessary to elucidate the temporal variability of myokines. Some researchers have expressed concerns regarding commercial ELISA kits for irisin [45,46]. Hence, based on previous studies, we opted for a kit deemed to possess high validity [47]. There was a difference in weight between the groups at baseline even when they were randomly assigned.

5. Conclusion

Uphill running may be a suitable intervention for promoting muscle volume recovery in cases of GC-induced muscle atrophy. Inclined running exercises are suitable for people who do not have joint problems, such as in their knees, or those with some other type of limitation.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are indebted to the laboratory members who supported our experiments.

The authors thank the Center for Anatomical, Pathological, and Forensic Medical Research, Kyoto University Graduate School of Medicine for preparing the microscope slides.

References

[1] A.L. Goldberg, H.M. Goodman, Relationship between cortisone and muscle work in determining muscle size, J. Physiol. 200 (3) (1969) 667–675, https://doi.org/10.1113/jphysiol.1969.sp008715.
[2] A.F. Clark, P.J. Vignos Jr., Experimental corticosteroid myopathy: effect on myofibrillar ATPase activity and protein degradation, Muscle. Nerve. 2 (4) (1979) 265–273, https://doi.org/10.1002/mus.880020405.
[3] A.G. Macedo, T.A.F. Almeida, D.A. Massini, V.F. De Paula, D.M. De Oliveira, D.M. Pessôa Filho, Effects of exercise training on glucocorticoid-induced muscle atrophy: Literature review, Steroids. 195 (2023) 109240, https://doi.org/10.1016/j.steroids.2023.109240.
[4] O. Schakman, H. Gilson, J.P. Thissen, Mechanisms of glucocorticoid-induced myopathy, J. Endocrinol. 197 (1) (2008) 1–10, https://doi.org/10.1677/JOE-07-0606.
[5] O. Schakman, S. Kalista, C. Barbe, A. Loumaye, J.P. Thissen, Glucocorticoid-induced skeletal muscle atrophy, Int J Biochem Cell Biol. 45 (10) (2013) 2163–2172, https://doi.org/10.1016/j.biocel.2013.05.036.
[6] P.F. Gardiner, B. Hibl, D.R. Simpson, R. Roy, V.R. Edgerton, Effects of a mild weight-lifting program on the progress of glucocorticoid-induced atrophy in rat hindlimb muscles, Pflugers Archiv: Eur. J. Physiol. 385 (2) (1980) 147–153, https://doi.org/10.1007/BF00588695.
[7] R.C. Hickson, J.R. Davis, Partial prevention of glucocorticoid-induced muscle atrophy by endurance training, Am. J. Physiol. 241(3) (1981) E226-232, https://doi.org/10.1152/ajpendo.1981.241.3.E226.
[8] F.F. Horber, J.R. Scheidegger, B.E. Grunig, F.J. Frey, Evidence that prednisone-induced myopathy is reversed by physical training, J. Clin. Endocrinol. Metab. 61 (1) (1985) 83–88, https://doi.org/10.1210/jcem-61-1-83.
[9] S.M. Czerwinski, T.G. Kurowski, T.M. O'Neill, R.C. Hickson, Initiating regular exercise protects against muscle atrophy from glucocorticoids, J. Appl. Physiol. 63 (4) (1987) 1504–1510, https://doi.org/10.1152/jappl.1987.63.4.1504.
[10] M.T. Falduto, R.C. Hickson, A.P. Young, Antagonism by glucocorticoids and exercise on expression of glutamine synthetase in skeletal muscle, FASEB. J. 14 (1989) 2623–2628, https://doi.org/10.1096/fasebj.3.14.2574120.
[11] M.T. Falduto, S.M. Czerwinski, R.C. Hickson, Glucocorticoid-induced muscle atrophy prevention by exercise in fast-twitch fibers, J. Appl. Physiol. 69 (3) (1990) 1058–1062, https://doi.org/10.1152/jappl.1990.69.3.1058.
[12] K. Nakago, M. Senda, M. Touno, Y. Takahara, H. Inoue, Influence of exercise on muscle fibers in rats with steroid myopathy, Acta. Med. Okayama. 53 (6) (1999) 265–270, https://doi.org/10.18926/AMO/31621.
[13] M. Okita, T. Yoshimura, J. Nakano, M. Watabe, T. Nagai, K. Kato, K. Eguchi, Effects of treadmill exercise on muscle fibers in mice with steroid myopathy, J. Jpn. Phys. Ther. Ass. 4 (1) (2001) 25–27, https://doi.org/10.1298/jjpta.4.25.
[14] K. Uchikawa, H. Takahashi, K. Hase, Y. Masakado, M. Liu, Strenuous exercise-induced alterations of muscle fiber cross-sectional area and fiber-type distribution in steroid myopathy rats, Am. J. Phys. Med. Rehabil. 87 (2) (2008) 126–133, https://doi.org/10.1097/PHM.0b013e31815869d0.
[15] A.G. Macedo, A.L. Krug, N.A. Herrera, A.S. Zago, J.W. Rush, S.L. Amaral, Low-intensity resistance training attenuates dexamethasone-induced atrophy in the flexor hallucis longus muscle, J. Steroid. Biochem. Mol. Biol. 143 (2014) 357–364, https://doi.org/10.1016/j.jsbmb.2014.05.010.
[16] A.L. Krug, A.G. Macedo, A.S. Zago, J.W. Rush, C.F. Santos, S.L. Amaral, High-intensity resistance training attenuates dexamethasone-induced muscle atrophy, Muscle. Nerve. 53 (5) (2016) 779–788, https://doi.org/10.1002/mus.24906.
[17] Y. Tsuchiya, S. Mizuno, K. Goto, Irisin response to downhill running exercise in humans, J. Exerc. Nutrition. Biochem. 22 (2) (2018) 12–17, https://doi.org/10.20463/jenb.2018.0011.
[18] M. Murao, T. Imano, J. Akiyama, T. Kawakami, M. Nakajima, Effect of single bout downhill running on the serum irisin concentrations in rats, Growth. Factors. 37 (5–6) (2020) 1–6, https://doi.org/10.1080/08977194.2020.1742118.
[19] M. Dehghani, M. Kargarfard, F. Rabiee, M.H. Nasr-Esfahani, K. Ghaedi, A comparative study on the effects of acute and chronic downhill running vs uphill running exercise on the RNA levels of the skeletal muscles PGC1-α, FNDC5 and the adipose UCP1 in BALB/c mice, Gene. 679 (2018) 369–376, https://doi.org/10.1016/j.gene.2018.09.024.
[20] R.B. Armstrong, M.H. Laughlin, L. Rome, C.R. Taylor, Metabolism of rats running up and down an incline, J. Appl. Physiol. 55 (2) (1983) 518–521, https://doi.org/10.1152/jappl.1983.55.2.518.
[21] V. Chavanelle, P. Sirvent, G. Ennequin, K. Caillaud, C. Montaurier, B. Morio, N. Boisseau, R. Richard, Comparison of oxygen consumption in rats during uphill (concentric) and downhill (eccentric) treadmill exercise tests, J. Sport. Sci. Med. 13 (3) (2014) 689–694.
[22] K. Ma, C. Mallidis, S. Bhasin, V. Mahabadi, J. Artaza, N. Gonzalez-Cadavid, J. Arias, B. Salehian, Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression, Am. J. Physiol. Endocrinol. Metabol. 285(2) (2003) E363-371, https://doi.org/10.1152/ajpendo.00487.2002.
[23] D. Yamamoto, T. Maki, E.H. Herningtyas, N. Ikeshita, H. Shibahara, Y. Sugiyama, S. Nakanishi, K. Iida, G. Iguchi, Y. Takahashi, H. Kaji, K. Chihara, Y. Okimura, Branched-chain amino acids protect against dexamethasone-induced soleus muscle

atrophy in rats, Muscle. Nerve. 41 (6) (2010) 819–827, https://doi.org/10.1002/mus.21621.
[24] N. Shimizu, N. Yoshikawa, N. Ito, T. Maruyama, Y. Suzuki, S. Takeda, J. Nakae, Y. Tagata, S. Nishitani, K. Takehana, M. Sano, K. Fukuda, M. Suematsu, C. Morimoto, H. Tanaka, Crosstalk between glucocorticoid receptor and nutritional sensor mTOR in skeletal muscle, Cell. Mmetabol. 13 (2) (2011) 170–182, https://doi.org/10.1016/j.cmet.2011.01.001.
[25] A.I. Schlagowski, M.E. Isner-Horobeti, S.P. Dufour, L. Rasseneur, I. Enache, E. Lonsdorfer-Wolf, S. Doutreleau, A. Charloux, F. Goupilleau, I. Bentz, A. L. Charles, B.Y. Kouassi, J. Zoll, B. Geny, F. Favret, Mitochondrial function following downhill and/or uphill exercise training in rats, Muscle. Nerve. 54 (5) (2016) 925–935, https://doi.org/10.1002/mus.25144.
[26] B.Y. Wang, A.W. Hsiao, H.T. Shiu, N. Wong, A.Y. Wang, C.W. Lee, O.K. Lee, W. Y. Lee, Mesenchymal stem cells alleviate dexamethasone-induced muscle atrophy in mice and the involvement of ERK1/2 signalling pathway, Stem Cell Res Ther 14 (1) (2023) 195, https://doi.org/10.1186/s13287-023-03418-0.
[27] X.G. Liu, Y.J. Zhou, T.C. Liu, J.Q. Yuan, Effects of low-level laser irradiation on rat skeletal muscle injury after eccentric exercise, Photomed. Laser. Surg. 27 (6) (2009) 863–869, https://doi.org/10.1089/pho.2008.2443.
[28] N.L. Totou, S.S. de Moura, F.A.D. Martins Júnior, F.B. de Sousa, D.B. Coelho, E. C. de Oliveira, R.A.S. Dos Santos, L.K. Becker, W.G. de Lima, Oral administration of angiotensin-(1–7) decreases muscle damage and prevents the fibrosis in rats after eccentric exercise, Exl. Physiol. 106 (8) (2021) 1710–1719, https://doi.org/10.1113/EP089308.
[29] A.G. Macedo, A.L. Krug, L.M. Souza, A.M. Martuscelli, P.B. Constantino, A.S. Zago, J.W. Rush, C.F. Santos, S.L. Amaral, Time-course changes of catabolic proteins following muscle atrophy induced by dexamethasone, Steroids. 107 (2016) 30–36, https://doi.org/10.1016/j.steroids.2015.12.016.
[30] P.B. Constantino, T.J. Dionisio, F. Duchatsch, N.A. Herrera, J.O. Duarte, C. F. Santos, C.C. Crestani, S.L. Amaral, Exercise attenuates dexamethasone-induced hypertension through an improvement of baroreflex activity independently of the renin-angiotensin system, Steroids. 128 (2017) 147–154, https://doi.org/10.1016/j.steroids.2017.10.007.
[31] Y. Morimoto, Y. Kondo, H. Kataoka, Y. Honda, R. Kozu, J. Sakamoto, J. Nakano, T. Origuchi, T. Yoshimura, M. Okita, Heat treatment inhibits skeletal muscle atrophy of glucocorticoid-induced myopathy in rats, Physiol. Res. 64 (6) (2015) 897–905, https://doi.org/10.33549/physiolres.932942.
[32] H. Takekura, N. Fujinami, T. Nishizawa, H. Ogasawara, N. Kasuga, Eccentric exercise-induced morphological changes in the membrane systems involved in excitation-contraction coupling in rat skeletal muscle, J. Pphysiol. 533 (Pt 2) (2001) 571–583, https://doi.org/10.1111/j.1469-7793.2001.0571a.x.
[33] R.B. Armstrong, R.W. Ogilvie, J.A. Schwane, Eccentric exercise-induced injury to rat skeletal muscle, J. Appl. Physiol. 54 (1) (1983) 80–93, https://doi.org/10.1152/jappl.1983.54.1.80.
[34] G.P. Morais, C. Chemerka, A. Masson, R.A. Seerattan, A.L. da Rocha, R. Krawetz, A. S.R. da Silva, W. Herzog, Excessive downhill training leads to early onset of knee osteoarthritis, Osteoarthritis, Cartilage. 29 (6) (2021) 870–881, https://doi.org/10.1016/j.joca.2021.03.016.
[35] C. Li, S. Inoue, J. Hatakeyama, H. Jiang, D. Takamura, O. Shirado, H. Moriyama, Effects of uphill and downhill walking on post-traumatic osteoarthritis development in mice, J. Physiol. 601 (10) (2023) 1781–1795, https://doi.org/10.1113/JP284337.
[36] J.S. Chang, I.D. Kong, Irisin prevents dexamethasone-induced atrophy in C2C12 myotubes, Pflugers. Arch. 472 (4) (2020) 459–502, https://doi.org/10.1007/s00424-020-02367-4.
[37] G. Colaianni, T. Mongelli, C. Cuscito, P. Pignataro, L. Lippo, G. Spiro, A. Notarnicola, I. Severi, G. Passeri, G. Mori, G. Brunetti, B. Moretti, U. Tarantino, S.C. Colucci, J.E. Reseland, R. Vettor, S. Cinti, M. Grano, Irisin prevents and restores bone loss and muscle atrophy in hind-limb suspended mice, Sci. Rep. 7 (1) (2017) 2811, https://doi.org/10.1038/s41598-017-02557-8.
[38] M.M. Reza, N. Subramaniyam, C.M. Sim, X. Ge, D. Sathiakumar, C. McFarlane, M. Sharma, R. Kambadur, Irisin is a pro-myogenic factor that induces skeletal muscle hypertrophy and rescues denervation-induced atrophy, Nat. Commun. 8 (1) (2017) 1104, https://doi.org/10.1038/s41467-017-01131-0.
[39] M.M. Reza, C.M. Sim, N. Subramaniyam, X. Ge, M. Sharma, R. Kambadur, C. McFarlane, Irisin treatment improves healing of dystrophic skeletal muscle, Oncotarget 8 (58) (2017) 98553–98566, https://doi.org/10.18632/oncotarget.21636.
[40] G. Colaianni, S. Cinti, S. Colucci, M. Grano, Irisin and musculoskeletal health, Ann. NY. Acad. Sci. 1402 (1) (2017) 5–9, https://doi.org/10.1111/nyas.13345.
[41] J. Douglas, S. Pearson, A. Ross, M. McGuigan, Chronic adaptations to eccentric training: asystematic review, Sport. Med. 47 (5) (2017) 917–941, https://doi.org/10.1007/s40279-016-0628-4.
[42] M. Khalafi, B. Aria, M.E. Symonds, S.K. Rosenkranz, The effects of resistance training on myostatin and follistatin in adults: a systematic review and meta-analysis, Physiol. Behav. 269 (2023), 114272, https://doi.org/10.1016/j.physbeh.2023.114272.
[43] J. Fink, B.J. Schoenfeld, K. Nakazato, The role of hormones in muscle hypertrophy, Phys. Sportsmed. 46 (1) (2018) 129–134, https://doi.org/10.1080/00913847.2018.1406778.
[44] M.A. Mohammed, M.O. Mahmoud, A.S. Awaad, G.M. Gamal, D. Abdelfatah, Alpha lipoic acid protects against dexamethasone-induced metabolic abnormalities via APPL1 and PGC-1 α up regulation, Steroids. 144 (2019) 1–7, https://doi.org/10.1016/j.steroids.2019.01.004.
[45] E. Albrecht, F. Norheim, B. Thiede, T. Holen, T. Ohashi, L. Schering, S. Lee, J. Brenmoehl, S. Thomas, C.A. Drevon, H.P. Erickson, S. Maak, Irisin - a myth rather than an exercise-inducible myokine, Sci. Rep. 5 (2015) 8889, https://doi.org/10.1038/srep08889.
[46] I.G. Fatouros, Is irisin the new player in exercise-induced adaptations or not? A 2017 update, Clin. Chem. Lab. Med. 56 (4) (2018) 525–548, https://doi.org/10.1515/cclm-2017-0674.
[47] N. Perakakis, G.A. Triantafyllou, J.M. Fernandez-Real, J.Y. Huh, K.H. Park, J. Seufert, C.S. Mantzoros, Physiology and role of irisin in glucose homeostasis, Nature. Rev. Endocrinol. 13 (6) (2017) 324–337, https://doi.org/10.1038/nrendo.2016.221.