

Irisin regulates the phosphorylation of glucocorticoid receptor Ser212 and Ser234 and mediates glucocorticoid-induced muscle atrophy in mice

Corresponding Author: Professor Yanjun Dong

This manuscript has been previously submitted at another journal. This document only contains information relating to versions considered at Communications Biology.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

In this manuscript, Yang and colleagues report on irisin-driven regulation of the glucocorticoid-induced pro-atrophic program in muscle.

Strengths:

- Interesting new role for irisin in the muscle response to metabolic strength
- Focus on GR phosphorylation sites

Weaknesses to address:

Major:

- C2C12 myoblasts are not a faithful epigenetic/transcriptional model for in vivo muscle remodeling (see for instance PMID: 31589602), hence all the C2C12-based findings should be considered suggestive and moved to supplement.
- Mechanistic validation of the claimed novel links between irisin and GR regulation should be tested in vivo. Experiments with chemical or pharmacological inhibitors (like the ones presented here) are not rigorous enough to support the claim. Experiments should be performed in muscle in vivo with serine-mutant constructs of GR, as well as with integrin or JNK/ERK pathway silencing through knock-down.
- Muscle mass and myofiber CSA analyses should be compounded by myofiber typing and muscle function analyses, at the very minimum.
- If the irisin benefits in muscle in high-fat diet (HFD) are dependent on the counteraction of the muscle glucocorticoid program, can those effects be blocked by adrenalectomy?

Minor:

- Rationale for using the hPCK9 mouse? Are the findings replicable in a WT mouse with high-fat diet?
- Details and rationale for using recombinant irisin instead of commercial irisin should be explicit and at the start of the Results section

Reviewer #2

(Remarks to the Author)

In this study the authors have examined GR in mediating the catabolic, anti-anabolic actions of high dose glucocorticoids in murine models. They report that nuclear translocation is dependant on dephosphorylation of ser212/ser234 on the GR and that the exercise induced hormone irisin prevents these deleterious processes by inhibiting dephosphorylation of ser212/ser234. Generally, the data are relatively robust and supportive in the models used. The findings and experimental design underpinning them would be suited to the journal. However, currently I have issues with the formatting and writing style of the introduction and elements of the discussion. The authors need to carefully review and amend these sections for clarity and to improve accuracy of some of their statements.

Major

Graphical Abstract

Line 29: Some confusion with graphical abstract. States 'In the presence of ligands, the glucocorticoid receptor (GR) undergoes basal phosphorylation at Ser212 and Ser23'. Actually looks like in absence of ligand it is phosphorylated. The ligand (GC) then drives dephosphorylation

Line 34: You need to introduce irisin properly.

Abstract

Line 47: You need to introduce irisin properly.

Introduction needs significant work in writing style. Sentences are short and feel clipped. Abbreviation such as GC for glucocorticoid are not introduced in text. There are some major factual errors or miscommunications. For example, states 'GC is as an endogenous hormone released in response to pathological conditions and as an exogenous drug used for the treatment of inflammation.' Actually, GCs are regulated in normal physiology by HPA axis and then are influenced in disease settings. These currently create a very confusing narrative. Some aspects of sentence require more clear explanation and clarification. For example line 69 indicates that GCs mediate muscle wasting across multiple disease settings. However, whilst the refs all agree that GCs contribute, it is unlikely they are the sole driver of muscle wasting in disease in the absence of exogenous therapeutic dosing.

A more comprehensive discussion of GCs action on muscle metabolism is required. GCs also influence levels of growth hormone, IGF1 and androgens and estrogens. Maybe separate by systemic actions, and local metabolic regulation. The manuscript should completely differentiate between mechanisms relating to autophagy, UPS and anabolic AKT/MTor signalling and impacts on differentiation and myoblast proliferation.

Results:

Line 168. Elevated serum glucocorticoid levels are a major contributor to chronic stress-induced muscle wasting[37]. Tone down. Is a contributor. Not necessarily major depending on the setting.

Can authors comment on general half life of Irisin in their model in regard to therapeutic regimen. Should roughly be around 1-2 hours. This study gave once daily. Please comment

Fig 4. There are a huge number of genes changing in Dex/irisin vs dex in the volcano plot. How do we know these aren't being changed as a result of alternative transcriptional mechanism including residual monomer tethering trans repression monomer response element binding? However, I do accept the argument that GR translocation is attenuated and so genomic reduced.

Line 308 states: 'IGF-I and MSTN are responsible for skeletal muscle anabolism and catabolism[9, 39]'. They do contribute to these processes but other factors also influence. Just really need to watch how terminology is being used throughout.

Greater clarity around animal numbers in all experimental groups is required

Discussion:

Authors need to consider wider limitations to the models, including dose/frequency of delivery of irisin. Should irisin be administered prior to injury in aging model. Also potential for different efficacy in exogenous excess versus endogenous/disease HPA GC excess.

Have the authors considered if dissociated steroids may play a role here. Recently FDA approved compounds like vamorolone, which have improved profiles of muscle and bone side effects have looked promising for certain inflammatory settings. Presumably, they may also circumvent aspects of Ser212/Ser234 phosphorylation and GR nuclear localisation?

Reviewer #3

(Remarks to the Author)

The manuscript by Yang et al examines regulation of glucocorticoid receptor (GR) activation and its downstream effects on skeletal muscle mass. It has been well documented that excess glucocorticoids (GCs) induce skeletal muscle atrophy. Further, the role of GR activation in mediating the effects of glucocorticoids on skeletal muscle mass have also been reported (223547783, 23733748, 25254959). The current study extends previous studies by examining the role of GR phosphorylation at Ser212 and Ser234 on mediating the effects of GCs on skeletal muscle mass. Further, the ability of irisin to regulate the genomic effects of GCs was investigated. In vitro experiments on myotubes demonstrated that irisin could regulate the phosphorylation status of the GR. The results suggest that the effects of GCs on muscle can be mediated through regulation of the phosphorylation status of the GR, however, additional experiments in vivo demonstrating that the effects of irisin are mediated by GR phosphorylation would strengthen the conclusions.

One limitation of the study is that neither protein synthesis or protein degradation were measured directly. The authors use MuRF1 and Atrogin1 as markers of global protein degradation. These E3 ligases are excellent markers of muscle atrophy, but should not be used as markers of global protein degradation. Also, decreases in Akt phosphorylation alone should not be used as an indicator of decreases in protein synthesis.

MuRF1 and Atrogin1 protein expression was quantified by western blots. What is the specificity of the antibodies used against MuRF1 and Atrogin1? Many of the antibodies against MuRF1 are non-specific in mouse tissues. In several of the figures there are multiple bands for MuRF1 and Atrogin1. Which band is quantified?

Figure 1J: were these data generated from regenerated fibers? The histograms in Figure 1I suggest regeneration in the irisin +aging muscle is reduced relative to the aging alone tissues.

Lines 215-216: The data presented in figure 1 does not show that irisin ameliorates age-induced atrophy. The experiment

examined regeneration in adult vs aged mice. The data presented in I and J from the aged mice are not convincing.

Line 443: Sarcopenia is usually used to imply age-related muscle loss. The examples provided in this sentence are not age-related. A more appropriate term might be "muscle atrophy"

In a several places, additional references to original research articles, as opposed to reviews, are recommended. In general, the authors fail to acknowledge previous work that has demonstrated the role of GR activation in mediating the effects of glucocorticoids on skeletal muscle mass.

Lines 44-46: the genomic effects of GCs have been demonstrated in genetically modified mice: see 223547783, 23733748, 25254959.

Line 79: ref 8 is a review article. The original articles are: 18612045, 21807613.

Line 86: the effect of GR transcriptional responses on GC-induced muscle atrophy have been reported. 223547783, 23733748, 25254959

Line 467: 223547783

Muscle histology methods (lines 666-674): How many fibers were analyzed in each muscle? The description is a bit confusing: was it 100 fibers total or 100 fibers in each of 5 fields for a total of 500 fibers? The number of mice analyzed was on the low side n=3 or 4.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

My points were partially addressed, although several key aspects of the manuscript remain insufficiently addressed in my opinion.

Reviewer #2

(Remarks to the Author)

I am happy that the authors have engaged with and addressed the corrections and comments relating to the manuscript from this reviewer. I have no further comments and am happy to approve this output for publication.

Reviewer #3

(Remarks to the Author)

The authors have addressed several of my concerns. However, the following concerns still remain:

The cross-sectional area measurements were made on only 100 fibers per muscle. This is a very small representation of the muscles analyzed. Was there a bias toward a specific type of fiber?

The specificity of the MuRF1 antibody was not determined. Two bands were identified on the gels and both bands were analyzed.

The figure legend and paragraph headings still say that iris ameliorates age-induced atrophy. These headings need to be revised since the data do not support this conclusion.

There are a number of grammatical and spelling errors that should be corrected.

Version 2:

Reviewer comments:

Reviewer #3

(Remarks to the Author)

The authors have addressed by concerns.

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Response to reviewers' comments

(COMMSBIO-25-0472-T)

Reviewer #1 (Remarks to the Author):

In this manuscript, Yang and colleagues report on irisin-driven regulation of the glucocorticoid-induced pro-atrophic program in muscle.

Strengths:

- Interesting new role for irisin in the muscle response to metabolic strength
- Focus on GR phosphorylation sites

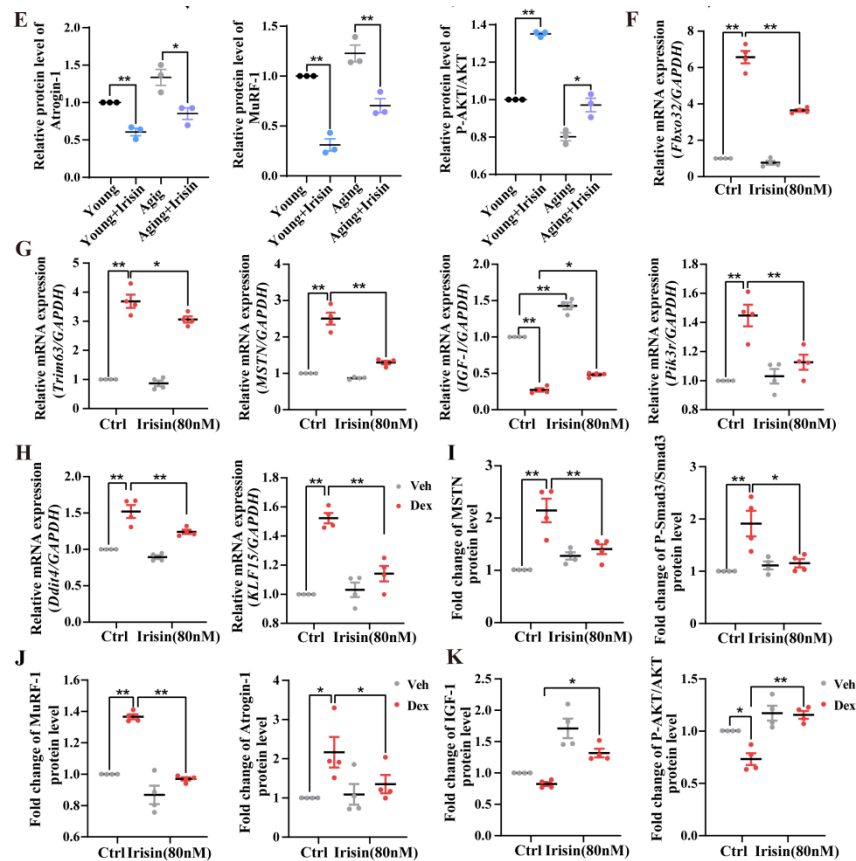
Weaknesses to address:

Major:

- 1. C2C12 myoblasts are not a faithful epigenetic/transcriptional model for in vivo muscle remodeling (see for instance PMID: 31589602), hence all the C2C12-based findings should be considered suggestive and moved to supplement.

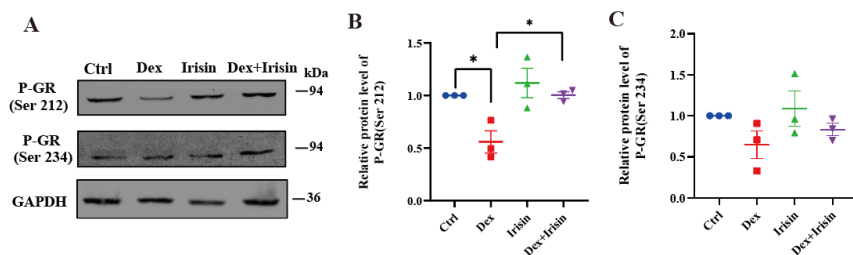
Response: Thank you very much for recognizing our work, We fully acknowledge that C2C12 cells, as an in vitro model, cannot completely replicate the transcriptional and epigenetic landscape of skeletal muscle in vivo. As highlighted in PMID: 31589602, there are indeed significant differences in enhancer usage and transcriptional regulation between native skeletal muscle tissue and cultured myotubes^[1]. Nevertheless, C2C12 myotubes remain one of the most widely used and broadly accepted cellular models for studying skeletal muscle atrophy and its underlying mechanisms^[2-4]. We fully considered this limitation during the design of our experiment. All key findings obtained from C2C12 cells were further validated in vivo mice model. Specifically, for transcriptomic analysis, we performed RNA sequencing using mice skeletal muscle tissue. A subset of differentially expressed genes identified from the in vivo RNA-seq data was selected for qPCR validation in C2C12 myotubes to preliminarily explore potential regulatory mechanisms. These validation results have been provided in Supplementary **Figure S1F-H**. Furthermore, we conducted in vivo experiments involving dexamethasone, irisin, and their combination. As shown in **Supplementary Figure S4**, irisin effectively prevented the dexamethasone-induced reduction in GR phosphorylation at Ser212 and Ser234 sites, thereby supporting the mechanistic insights observed in vitro. Taken together, the data derived from C2C12 experiments provide mechanistic support and serve as an integral part of our overall research framework. We sincerely appreciate your thoughtful feedback and constructive suggestions.

Figure S1F-H



Supplementary Figure S4

Supplemental
figure 4



References

1. Ramachandran K, Senagolage MD, Sommars MA, Futtner CR, Omura Y, Allred AL, et al. Dynamic enhancers control skeletal muscle identity and reprogramming. *PLoS Biol.* 2019;17:e3000467. doi:10.1371/journal.pbio.3000467
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3. Zhang HJ, Wang BH, Wang X, Huang CP, Xu SM, Wang JL, et al. Handelin alleviates cachexia- and aging-induced skeletal muscle atrophy by improving protein homeostasis and inhibiting inflammation. *J Cachexia Sarcopenia Muscle.* 2024;15:173-88. doi:10.1002/jcsm.13381
4. Song J, Liu J, Cui C, Hu H, Zang N, Yang M, et al. Mesenchymal stromal cells ameliorate diabetes-induced muscle atrophy through exosomes by enhancing AMPK/ULK1-mediated autophagy. *J Cachexia Sarcopenia*

-2. Mechanistic validation of the claimed novel links between irisin and GR regulation should be tested in vivo. Experiments with chemical or pharmacological inhibitors (like the ones presented here) are not rigorous enough to support the claim. Experiments should be performed in muscle in vivo with serine-mutant constructs of GR, as well as with integrin or JNK/ERK pathway silencing through knock-down.

Response: Thank you very much for your insightful comment. As your suggestion, we conducted in vivo experiments in mice treated with dexamethasone (Dex) and irisin. We examined the phosphorylation status of GR at Ser212 and Ser234 in skeletal muscle tissue. The results showed that irisin increased phosphorylation at both sites in Dex treatment condition, consistent with that in vitro findings and further supporting the regulatory role of irisin in modulating GR activity. We have added this results in Supplementary Figure S4.

We agree with your suggestion that it would be better to validate the in vitro results using transgenic mice. Therefore, we searched for relevant transgenic mice but were unable to obtain them. We then attempted to overexpress GR Ser212 and Ser234 sites by directly infecting muscle tissue with lentivirus. However, after two months of optimization, the transfection efficiency still could not meet the experimental requirements. Unfortunately, we were unable to complete this part of the experiment.

Currently, we have used overexpression systems and inhibitors in vitro to demonstrate that the phosphorylation of GR Ser212 and Ser234 sites is the primary target for Irisin's inhibition of glucocorticoid genomic effects. Additionally, we have examined key components of this pathway in vivo. Although we were unable to use knockout mice, in this revision, we have supplemented the in vivo validation by including the detection of GR Ser212 and Ser234 phosphorylation to strengthen our findings. We have also addressed the limitations of this part of the study in the discussion section 684-695.

pages 32 lines 684-696: In summary, we have used overexpression systems and inhibitors in vitro to demonstrate that glucocorticoid genomic effects are the main pathway through which glucocorticoids induce muscle atrophy in mice; Increased glucocorticoid receptor (GR) Ser212 and Ser234 site phosphorylation reduces glucocorticoid-induced muscle atrophy in mice; Irisin reduce glucocorticoid genomic effects via GR Ser212/Ser234 phosphorylation in mice muscle atrophy. Additionally, we have examined key components of this pathway in vivo. This revealed the mechanisms of irisin crosstalk with glucocorticoid genomic and non-genomic effects, Although we were unable to conduct experiments using gene knockout mice, the relevant research still provides an important basis for further exploration. And, this is expected to expand our understanding and potentially guide therapeutic strategies for many diseases.

-3 Muscle mass and myofiber CSA analyses should be compounded by myofiber typing and muscle function analyses, at the very minimum.

Response: We sincerely thank the reviewer for this valuable comment. Myofiber cross-sectional area (CSA) analysis is currently one of the most widely used and direct methods for evaluating skeletal muscle atrophy^[5]. In our study, we therefore focused on CSA measurements, muscle wet weight analysis, and the expression of key atrophy-related proteins to comprehensively assess the degree of muscle atrophy.

Although we did not directly perform fiber typing or functional assays in this study, we deliberately selected muscles with distinct and representative fiber-type compositions for muscle mass analysis. Specifically, the tibialis

anterior (TA) and gastrocnemius (Gas) are mixed muscles predominantly composed of type IIa and IIb fibers, with a small proportion of type I fibers; the soleus (Sol) consists mainly of oxidative type I fibers; and the extensor digitorum longus (EDL) is primarily composed of fast-twitch type IIb fibers. Our results showed that Dex and irisin treatment significantly influenced the mass of TA, Gas, and EDL, while the mass of Sol remained largely unaffected. These findings suggest that the effects of Dex and irisin are likely more pronounced in muscles enriched in type II fibers. Furthermore, our findings are supported by previous studies reporting that Dex and irisin exert greater effects on type IIb fast-twitch fibers, while their impact on type I fibers appears to be limited^[6]. This aligns well with our observations and reinforces our interpretation.

This point was also addressed in the discussion section in page 25 lines 532-546: In muscle weight measurement, we deliberately selected muscles with distinct and representative fiber-type compositions for muscle mass analysis. Specifically, the tibialis anterior (TA) and gastrocnemius (Gas) are mixed muscles predominantly composed of type IIa and IIb fibers, with a small proportion of type I fibers; the soleus (Sol) consists mainly of oxidative type I fibers; and the extensor digitorum longus (EDL) is primarily composed of fast-twitch type IIb fibers. Our results showed that Dex and irisin treatment significantly influenced the mass of TA, Gas, and EDL, while the mass of Sol remained largely unaffected. These findings suggest that the effects of Dex and irisin are likely more pronounced in muscles enriched in type II fibers. Furthermore, our findings are supported by previous studies reporting that Dex and irisin exert greater effects on type IIb fast-twitch fibers, while their impact on type I fibers appears to be limited. This aligns well with our observations and reinforces our interpretation.

References

5. Stouth DW, vanLieshout TL, Mikhail AI, Ng SY, Raziee R, Edgett BA, et al. CARM1 drives mitophagy and autophagy flux during fasting-induced skeletal muscle atrophy. *Autophagy*. 2024;20:1247-69. doi:10.1080/15548627.2023.2288528
6. Hunt LC, Graca FA, Pagala V, Wang YD, Li Y, Yuan ZF, et al. Integrated genomic and proteomic analyses identify stimulus-dependent molecular changes associated with distinct modes of skeletal muscle atrophy. *Cell Rep*. 2021;37:109971. doi:10.1016/j.celrep.2021.109971

-4 If the irisin benefits in muscle in high-fat diet (HFD) are dependent on the counteraction of the muscle glucocorticoid program, can those effects be blocked by adrenalectomy?

Response: We thank the reviewer for this insightful question. High-fat diet (HFD)-induced skeletal muscle atrophy is a complex process involving multiple factors^[7]. While glucocorticoids (GCs) have been identified as important mediators contributing to muscle wasting under HFD conditions, this does not necessarily imply that GC signaling is the sole driver of HFD-induced muscle atrophy^[8, 9]. There is no clear or systematic evidence showing whether adrenalectomy (ADX) can block HFD-induced muscle atrophy. It is important to note that ADX not only eliminates GC production, but also disrupts the secretion of other adrenal hormones such as mineralocorticoids and catecholamines, both of which are known to play roles in skeletal muscle atrophy and metabolism^[10, 11]. Therefore, the use of ADX introduces multiple physiological confounders, making it difficult to attribute any observed effects solely to the loss of GCs.

In addition, previous studies have demonstrated that irisin attenuates skeletal muscle atrophy in other metabolic disease models such as diabetes, suggesting that its protective effects are not entirely dependent on GC, and may involve additional signaling pathways^[12]. The central finding of our study is the identification of a novel mechanism through which irisin through integrin $\alpha\text{V}\beta 5$ receptors to modulate GC signaling.

References

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12. Zhou T, Wang S, Pan Y, Dong X, Wu L, Meng J, et al. Irisin Ameliorated Skeletal Muscle Atrophy by Inhibiting Fatty Acid Oxidation and Pyroptosis Induced by Palmitic Acid in Chronic Kidney Disease. *Kidney Blood Press Res.* 2023;48:628-41. doi:10.1159/000533926

-5 Rationale for using the *hPCSK9* mouse? Are the findings replicable in a WT mouse with high-fat diet?

Response: In our previous studies, we demonstrated that administration of irisin improved skeletal muscle condition in wild-type (WT) mice^[13]. Furthermore, other studies have reported the protective effects of irisin on skeletal muscle in obese mice^[14]. These findings together provide a solid experimental and theoretical basis for the beneficial role of irisin in skeletal muscle under WT conditions.

In this study, we chose the *hPCSK9* mouse model because, in addition to its common use in cardiovascular research, it provides a more stable and rapid way to induce hypercholesterolemia^[15]. Based on this, we aimed to further evaluate the effects of irisin in a model that better reflects metabolic disturbance, in order to assess its potential protective role against skeletal muscle atrophy under high cholesterol conditions.

References

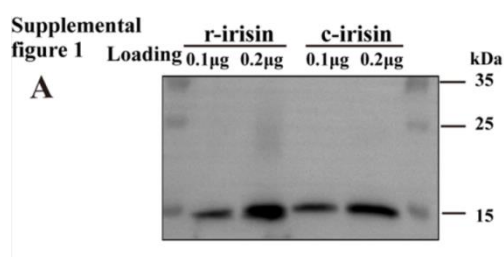
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15. Carreras A, Pane LS, Nitsch R, Madeyski-Bengtson K, Porritt M, Akcakaya P, et al. In vivo genome and base editing of a human *PCSK9* knock-in hypercholesterolemic mouse model. *BMC Biol.* 2019;17:4. doi:10.1186/s12915-018-0624-2

-6 Details and rationale for using recombinant irisin instead of commercial irisin should be explicit and at the start of the Results section

Response: We appreciate the reviewer's helpful suggestion. At the beginning of our study, we purchased

commercial irisin (c-irisin) from Phoenix Pharmaceuticals (067-17), which was produced using a prokaryotic expression system. After reviewing the literature, we found that several published studies investigating the effects of irisin on skeletal muscle also used irisin produced through prokaryotic expression, indicating that this system is widely accepted and effective for such research^[16, 17].

However, due to the high cost of commercial irisin and the large amount required for our animal experiments, we decided to produce recombinant irisin (r-irisin) in-house using the same expression system. To ensure the reliability and comparability of our results, we validated our r-irisin against the c-irisin. Western blot analysis showed that the banding patterns of r-irisin and c-irisin were consistent (Figure S1A), suggesting structural similarity. Furthermore, r-irisin exhibited comparable biological activity to c-irisin in muscle-related functional assays, supporting its functional equivalence. In response to the reviewer's comment, we have added this rationale at the beginning of the results, on pages 11 lines 216-221 in the revised manuscript.



pages 11 lines 216-221: Initially, we purchased commercial irisin (c-irisin), which is expressed in a prokaryotic system, from Phoenix Pharmaceuticals. As many other published studies also use irisin expressed in a prokaryotic system we adopted the same approach for our own preparation in order to reduce costs and ensure a sufficient supply for in vivo experiments

References

- Guo M, Yao J, Li J, Zhang J, Wang D, Zuo H, et al. Irisin ameliorates age-associated sarcopenia and metabolic dysfunction. *Journal of Cachexia, Sarcopenia and Muscle*. 2023;14:391-405. doi:<https://doi.org/10.1002/jcsm.13141>
- Reza MM, Sim CM, Subramaniam N, Ge X, Sharma M, Kambadur R, et al. Irisin treatment improves healing of dystrophic skeletal muscle. *Oncotarget*. 2017;8:98553-66. doi:10.18632/oncotarget.21636

Reviewer #2 (Remarks to the Author):

In this study the authors have examined GR in mediating the catabolic, anti-anabolic actions of high dose glucocorticoids in murine models. They report that nuclear translocation is dependant on dephosphorylation of ser212/ser234 on the GR and that the exercise induced hormone irisin prevents these deleterious processes by inhibiting dephosphorylation of ser212/ser234. Generally, the data are relatively robust and supportive in the models used. The findings and experimental design underpinning them would be suited to the journal. However, currently I have issues with the formatting and writing style of the introduction and elements of the discussion. The authors need to carefully review and amend these sections for clarity and to improve accuracy of some of their statements.

Response: Thank you very much for recognizing our work, we have revised our manuscript according to your comments. Many thanks for your support and constructive suggestions.

Major

Graphical Abstract

-1.Line 29: Some confusion with graphical abstract. States 'In the presence of ligands, the glucocorticoid receptor (GR) undergoes basal phosphorylation at Ser212 and Ser23'. Actually looks like in absence of ligand it is phosphorylated. The ligand (GC) then drives dephosphorylation.

Response: We thank the reviewer for pointing out this issue. We have revised the graphical abstract description as follows: "In the absence of ligands, the glucocorticoid receptor (GR) undergoes basal phosphorylation at Ser212 and Ser234. When glucocorticoids (GCs) bind to GR, they promote dephosphorylation at these sites." [Please see pages 2 lines 29 in the revised manuscript.](#)

[pages 2 lines 29](#) : In the absence of ligands, the glucocorticoid receptor (GR) undergoes basal phosphorylation at Ser212 and Ser234. When glucocorticoids (GCs) bind to GR, they promote dephosphorylation at these sites, facilitating the translocation of the GR to the nucleus. This promotes the transcription of genes involved in protein degradation (Trim63, Fbxo32, Myostatin) and inhibits those involved in protein synthesis (IGF-1).

-2.Line 34: You need to introduce irisin properly.

Response: Thank you for the suggestion. As recommended, we have added a brief introduction to irisin in the graphical abstract text: "Irisin is a myokine secreted by skeletal muscle in response to exercise and has been shown to play a beneficial role in regulating muscle mass and metabolism." [Please see pages 2 lines 35-36 in the revised manuscript.](#)

[pages 2 lines 35-37](#) : Irisin is a myokine secreted by skeletal muscle in response to exercise and has been shown to play a beneficial role in regulating muscle mass and metabolism.

Abstract

-3.Line 47: You need to introduce irisin properly.

Response: Thank you for the suggestion. As recommended, we have added a brief introduction to irisin in the abstract text: "Irisin is a myokine induced by exercise and has been shown to exert multiple beneficial effects on on muscle mass and metabolism regulation." [Please see pages 3 lines 47-49 in the revised manuscript.](#)

[pages 3 lines 47-49](#): Irisin is a myokine induced by exercise and has been shown to exert multiple beneficial effects on on muscle mass and metabolism regulation.

-4.Introduction needs significant work in writing style. Sentences are short and feel clipped. Abbreviation such as GC for glucocorticoid are not introduced in text. There are some major factual errors or miscommunications. For example, states ‘GC is as an endogenous hormone released in response to pathological conditions and as an exogenous drug used for the treatment of inflammation.’ Actually, GCs are regulated in normal physiology by HPA axis and then are influenced in disease settings. These currently create a very confusing narrative. Some aspects of sentence require more clear explanation and clarification. For example line 69 indicates that GCs mediate muscle wasting across multiple disease settings. However, whilst the refs all agree that GCs contribute, it is unlikely they are the sole driver of muscle wasting in disease in the absence of exogenous therapeutic dosing.

Response: We sincerely thank the reviewer for the valuable comments and suggestions. In the revised manuscript, we have modified the writing style of the section introducing glucocorticoids (GCs) to improve clarity and scientific accuracy. Specifically, we have added the full term “glucocorticoids” upon first mention, and rephrased the relevant description to better reflect the physiological regulation of GCs. We have also corrected the previous description of the role of GCs in skeletal muscle atrophy. [These revisions can be found on page 4, lines 70–84 of the revised manuscript.](#)

[pages 4 lines 70-84](#) : Glucocorticoids (GCs) are endogenous steroid hormones secreted by the adrenal cortex under the control of the hypothalamic-pituitary-adrenal (HPA) axis, and their levels fluctuate in response to both physiological and pathological stress. In clinical practice, GCs are also widely used as exogenous drugs for the treatment of inflammatory and autoimmune diseases. Persistent elevation of GC levels, whether caused by endogenous overproduction or long-term pharmacological administration, can disrupt muscle protein homeostasis by reducing the rate of protein synthesis and increasing the rate of protein degradation. These changes ultimately lead to skeletal muscle atrophy and weakness. Previous reports have shown that GCs induce muscle atrophy under conditions of chronic stress such as aging, cancer, diabetes, and chronic kidney diseases. Although GCs may not be the sole factor responsible for muscle atrophy in these disease settings, their role in promoting catabolic pathways in skeletal muscle is well recognized.

-5.A more comprehensive discussion of GCs action on muscle metabolism is required. GCs also influence levels of growth hormone, IGF1 and androgens and estrogens. Maybe separate by systemic actions, and local metabolic regulation. The manuscript should completely differentiate between mechanisms relating to autophagy, UPS and anabolic AKT/MTor signalling and impacts on differentiation and myoblast proliferation.

Response: We sincerely thank the reviewer for this insightful comment. In the revised manuscript, we have expanded our discussion on the role of glucocorticoids (GCs) in skeletal muscle metabolism. [These revisions can be found in the revised manuscript on page 5, lines 85–104.](#)

[pages 5 lines 85-104](#) : In addition to their direct effects on protein synthesis and degradation, glucocorticoids (GCs) also exert systemic regulatory effects on skeletal muscle metabolism. For instance, GCs suppress the hypothalamic-pituitary-growth hormone (GH) axis and reduce circulating levels of GH and insulin-like growth factor-1 (IGF-1), both of which are essential for promoting muscle anabolism. GCs are also known to inhibit the synthesis of sex steroid hormones, including androgens and estrogens, which further impairs muscle maintenance. Locally within skeletal muscle, GCs activate proteolytic mechanisms by engaging the ubiquitin proteasome system (UPS) and the autophagy lysosome pathway to promote protein degradation. Simultaneously, they suppress

anabolic signaling through pathways such as PI3K-AKT-mTOR, leading to reduced protein synthesis. Moreover, GCs impair muscle regenerative processes by inhibiting satellite cell activation, myoblast proliferation, and differentiation. Together, these systemic and local mechanisms disrupt skeletal muscle homeostasis and contribute to muscle atrophy under a variety of physiological and pathological conditions.

Results:

-6.Line 168. Elevated serum glucocorticoid levels are a major contributor to chronic stress-induced muscle wasting^[37]. Tone down. Is a contributor. Not necessarily major depending on the setting.

Response: Thank you for pointing out this. We have changed our describe. [Please see pages 10 lines 200 in the revised manuscript.](#)

[pages 10 lines 200:](#) Elevated serum glucocorticoid levels are an important contributor to chronic stress-induced muscle wasting

-7.Can authors comment on general half life of Irisin in their model in regard to therapeutic regimen. Should roughly be around 1-2 hours. This study gave once daily. Please comment.

Response: We thank the reviewer for this valuable comment. Irisin has been reported to have a very short half-life in vivo, in our previous studies, we administered irisin intraperitoneally at a dose of 0.5 mg/kg every other day. To assess the effectiveness of this dosing regimen, we collected serum on days 8 and 14 and measured irisin levels using ELISA. The results showed that circulating irisin levels remained approximately twice the physiological baseline at both time points, indicating that this dosing strategy was sufficient to maintain a biologically active concentration over time^[1]. In addition, several published studies have employed a similar dosing frequency and reported consistent biological effects^[2, 3]. Therefore, we consider the administration schedule of irisin used in this study to be well-supported.

This point was also addressed in the discussion section in page 24 lines 507-517: Irisin has been reported to have a very short half-life in vivo, in our previous studies, we administered irisin intraperitoneally at a dose of 0.5 mg/kg every other day. To assess the effectiveness of this dosing regimen, we collected serum on days 8 and 14 and measured irisin levels using ELISA. The results showed that circulating irisin levels remained approximately twice the physiological baseline at both time points, indicating that this dosing strategy was sufficient to maintain a biologically active concentration over time. In addition, several published studies have employed a similar dosing frequency and reported consistent biological effects. Therefore, we consider the administration schedule of irisin used in this study to be well-supported.

References

1. Yang J, Wang K, An Y, Wu R, Li J, Wang H, et al. Mst1/2 Is Necessary for Satellite Cell Differentiation to Promote Muscle Regeneration. *Stem Cells*. 2022;40:74-87. doi:10.1093/stmcls/sxab010.
2. Reza MM, Sim CM, Subramaniam N, Ge X, Sharma M, Kambadur R, et al. Irisin treatment improves healing of dystrophic skeletal muscle. *Oncotarget*. 2017;8:98553-66. doi:10.18632/oncotarget.21636
3. Guo M, Yao J, Li J, Zhang J, Wang D, Zuo H, et al. Irisin ameliorates age-associated sarcopenia and metabolic dysfunction. *J Cachexia Sarcopenia Muscle*. 2023;14:391-405. doi:10.1002/jcsm.13141

-8.Fig 4. There are a huge number of genes changing in Dex/irisin vs dex in the volcano plot. How do we know

these aren't being changed as a result of alternative transcriptional mechanism including residual monomer tethering trans repression monomer response element binding? However, I do accept the argument that GR translocation is attenuated and so genomic reduced.

Response: We sincerely appreciate the reviewer's insightful comments. Although a large number of differentially expressed genes were observed in the volcano plot comparing the Dex/Irisin and Dex groups, this study focused on key genes closely associated with skeletal muscle atrophy. We fully acknowledge that the observed transcriptional changes may not be limited to GR-related mechanisms, and that other transcriptional regulatory pathways may also be involved, as the reviewer correctly pointed out.

However, given the observed reduction in GR nuclear translocation and the changes in GR phosphorylation status, we prioritized investigating the classical GR-mediated genomic pathway as the primary mechanism underlying the effects of irisin. In this study, we specifically elucidated how irisin modulates GC genomic effects through regulation of GR phosphorylation. Nonetheless, we fully recognize the possibility of alternative transcriptional mechanisms, and we greatly appreciate the reviewer's suggestion, which offers a valuable direction for future exploration of irisin's broader regulatory effects.

This point was also addressed in the discussion section in page 26 lines 577-590:. In our study, we sequenced the transcriptome of Gas from Dex and irisin-treated mice, although a large number of differentially expressed genes were observed in the volcano plot comparing the Dex/Irisin and Dex groups, this study focused on key genes closely associated with skeletal muscle atrophy. and our transcriptome results showed that irisin could significantly repress protein degradation-related genes and activate protein synthesis-related genes. Furthermore, we also verified in vitro and in vivo by qRT-PCR consistent with the transcriptome results. Therefore, irisin might ameliorate GC-induced muscle atrophy by regulating the genomic effects of GCs. It should be noted that the observed transcriptional changes may not be limited to GR-related mechanisms, and that other transcriptional regulatory pathways may also be involved, which offers a valuable direction for future exploration of Irisin's broader regulatory effect.

-9.Line 308 states: 'IGF-I and MSTN are responsible for skeletal muscle anabolism and catabolism[9, 39].'. They do contribute to these processes but other factors also influence. Just really need to watch how terminology is being used throughout.

Response: Thank you for pointing out this. We have changed our describe "IGF-I and MSTN are responsible for skeletal muscle anabolism and catabolism" to "IGF-I and MSTN are key regulators involved in skeletal muscle anabolism and catabolism". [Please see pages 17 lines 350-351 in the revised manuscript.](#)
[pages 17 lines 350-351:](#) IGF-I and MSTN are key regulators involved in skeletal muscle anabolism and catabolism

-10.Greater clarity around animal numbers in all experimental groups is required

Response: We thank the reviewer for this helpful suggestion. In the revised manuscript, we have clearly indicated the number of animals used in each experimental group in the figure legends.

-11.Discussion:

Authors need to consider wider limitations to the models, including dose/frequency of delivery of irisin. Should irisin be administered prior to injury in aging model. Also potential for different efficacy in exogenous excess

versus endogenous/disease HPA GC excess.

Response: We sincerely thank the reviewer for this insightful and constructive comment. Regarding the dosing frequency and administration schedule of irisin, we referred to both our previous studies and relevant literature^[4, 5, 6]. Accordingly, we selected an intraperitoneal injection regimen administered every other day.

In addition, with respect to the timing of irisin administration in the mouse model, several studies have adopted a pre-injury or pre-intervention administration strategy^[7, 8]. This approach is intended to improve the baseline metabolic condition of aged skeletal muscle and enhance its regenerative capacity in response to subsequent injury.

As for the model limitations, we primarily used exogenous glucocorticoid (GC) administration to induce muscle atrophy in order to establish a clear and controllable experimental system. This allowed us to focus specifically on the direct effects of GCs on skeletal muscle. We fully recognize that in actual pathological conditions such as chronic inflammation, cancer, or aging, endogenous GC levels are regulated by the hypothalamic–pituitary–adrenal (HPA) axis and may present more dynamic and complex changes. Therefore, in this study, we deliberately excluded other disease-related factors and selected an exogenous GC-induced model to better define the direct role of GCs in muscle atrophy and to investigate the regulatory mechanism of irisin under this specific background.

This point was also addressed in the discussion section in page 23 lines 481-491:we primarily used exogenous glucocorticoid (GC) administration to induce muscle atrophy in order to establish a clear and controllable experimental system. This allowed us to focus specifically on the direct effects of GCs on skeletal muscle. We fully recognize that in actual pathological conditions such as chronic inflammation, cancer, or aging, endogenous GC levels are regulated by the hypothalamic–pituitary–adrenal (HPA) axis and may present more dynamic and complex changes. Therefore, in this study, we deliberately excluded other disease-related factors and selected an exogenous GC-induced model to better define the direct role of GCs in muscle atrophy and to investigate the regulatory mechanism of irisin under this specific background.

References

4. Yang J, Wang K, An Y, Wu R, Li J, Wang H, et al. Mst1/2 Is Necessary for Satellite Cell Differentiation to Promote Muscle Regeneration. *Stem Cells*. 2022;40:74-87. doi:10.1093/stmcls/sxab010
5. Reza MM, Sim CM, Subramaniam N, Ge X, Sharma M, Kambadur R, et al. Irisin treatment improves healing of dystrophic skeletal muscle. *Oncotarget*. 2017;8:98553-66. doi:10.18632/oncotarget.21636
6. Guo M, Yao J, Li J, Zhang J, Wang D, Zuo H, et al. Irisin ameliorates age-associated sarcopenia and metabolic dysfunction. *J Cachexia Sarcopenia Muscle*. 2023;14:391-405. doi:10.1002/jcsm.13141
7. Reza MM, Subramaniam N, Sim CM, Ge X, Sathiakumar D, McFarlane C, et al. Irisin is a pro-myogenic factor that induces skeletal muscle hypertrophy and rescues denervation-induced atrophy. *Nat Commun*. 2017;8:1104. doi:10.1038/s41467-017-01131-0
8. Daneshvar N, Tatsumi R, Peeler J, Anderson JE. Premature satellite cell activation before injury accelerates myogenesis and disrupts neuromuscular junction maturation in regenerating muscle. *Am J Physiol Cell Physiol*. 2020;319:C116-c28. doi:10.1152/ajpcell.00121.2020

-12. Have the authors considered if dissociated steroids may play a role here. Recently FDA approved compounds like vamorolone, which have improved profiles of muscle and bone side effects have looked promising for certain inflammatory settings. Presumably, they may also circumvent aspects of Ser212/Ser234 phosphorylation and GR nuclear localisation?

Response: We sincerely thank the reviewer for this insightful and forward-looking suggestion. Although our current study did not directly assess the effects of dissociated steroids or selective glucocorticoid receptor modulators (SGRMs) such as vamorolone, we fully recognize their potential to avoid classical GR dimerization and GRE-driven transcriptional activation, which may help reduce GC-related side effects on muscle and bone, as reported in previous studies^[9]. In our study, we observed that irisin modulates the genomic effects of GCs by regulating the dephosphorylation of GR at Ser212 and Ser234, thereby inhibiting its nuclear translocation. Whether irisin can also selectively regulate GR activity in a manner similar to SGRMs remains unknown. This presents an interesting and valuable direction for future research.

References

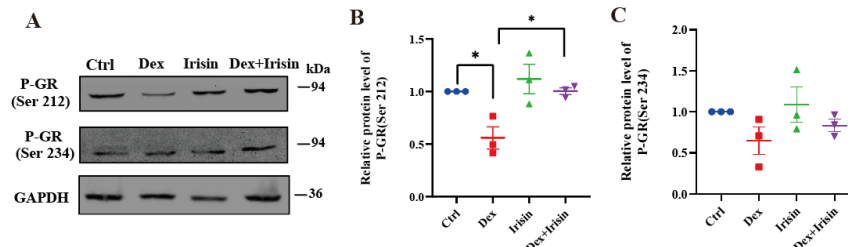
9. Crastin A, Shanker A, Sagmeister MS, Taylor A, Lavery GG, Raza K, et al. Vamorolone: a novel metabolism resistant steroid that suppresses joint destruction in chronic polyarthritis with reduced systemic side effects. *Rheumatology*. 2025;doi:10.1093/rheumatology/keaf129

Reviewer #3 (Remarks to the Author):

-1.The manuscript by Yang et al examines regulation of glucocorticoid receptor (GR) activation and its downstream effects on skeletal muscle mass. It has been well documented that excess glucocorticoids (GCs) induce skeletal muscle atrophy. Further, the role of GR activation in mediating the effects of glucocorticoids on skeletal muscle mass have also been reported (223547783, 23733748, 25254959). The current study extends previous studies by examining the role of GR phosphorylation at Ser212 and Ser234 on mediating the effects of GCs on skeletal muscle mass. Further, the ability of irisin to regulate the genomic effects of GCs was investigated. In vitro experiments on myotubes demonstrated that irisin could regulate the phosphorylation status of the GR. The results suggest that the effects of GCs on muscle can be mediated through regulation of the phosphorylation status of the GR, however, additional experiments in vivo demonstrating that the effects of irisin are mediated by GR phosphorylation would strengthen the conclusions.

Response: Thank you very much for recognizing our work, we have revised our manuscript according to your comments and added additional experiments to prove our claims. As your suggestion, we conducted in vivo experiments in mice treated with dexamethasone (Dex) and irisin. We examined the phosphorylation status of GR at Ser212 and Ser234 in skeletal muscle tissue. The results showed that irisin increased phosphorylation at both sites in Dex treatment condition, consistent with that in vitro findings and further supporting the regulatory role of irisin in modulating GR activity. We have added this results in Supplementary Figure S4. Below are our responses to the review's comments. Many thanks for your support and constructive suggestions.

Supplemental
figure 4



One limitation of the study is that neither protein synthesis or protein degradation were measured directly. The authors use MuRF1 and Atrogin1 as markers of global protein degradation. These E3 ligases are excellent markers of muscle atrophy, but should not be used as markers of global protein degradation. Also, decreases in Akt phosphorylation alone should not be used as an indicator of decreases in protein synthesis.

-2.Response: We sincerely thank the reviewer for this valuable comment. We fully acknowledge that direct measurement of protein synthesis and degradation rates would provide greater precision in evaluating muscle protein metabolism. In this study, we used widely accepted methods to assess skeletal muscle atrophy, including muscle weight measurement, cross-sectional area (CSA) analysis, and the expression levels of atrophy-related markers MuRF-1 and Atrogin-1^[10]. Although these two E3 ligases do not fully reflect global protein degradation, they are key components of the UPS and are widely used as markers of muscle atrophy^[11]. Likewise, while AKT phosphorylation alone may not directly represent protein synthesis, it is a central node in the PI3K/AKT/mTOR pathway and provides useful insight into anabolic signaling^[12].

Our study focuses on the phenotype and mechanism of skeletal muscle atrophy, aiming to determine whether irisin regulates GR phosphorylation and thereby affects its genomic actions on atrophy-related gene expression.

Although we did not directly measure protein synthesis or degradation rates, the markers used are widely accepted and provide sufficient evidence to support our conclusions.

This point was also addressed in the discussion section in 546-552.

References

10. Sun Y, Wei X, Zhao T, Shi H, Hao X, Wang Y, et al. Oleanolic acid alleviates obesity-induced skeletal muscle atrophy via the PI3K/Akt signaling pathway. *FEBS Open Bio.* 2024;14:584-97. doi:10.1002/2211-5463.13780
11. Souza ALG, Alves ALR, Martinez CG, Sousa JC, Kurtenbach E. Biomarkers of Skeletal Muscle Atrophy Based on Atrogenes Evaluation: A Systematic Review and Meta-Analysis Study. *Int J Mol Sci.* 2025;26:doi:10.3390/ijms26083516
12. Sasako T, Umehara T, Soeda K, Kaneko K, Suzuki M, Kobayashi N, et al. Deletion of skeletal muscle Akt1/2 causes osteosarcopenia and reduces lifespan in mice. *Nat Commun.* 2022;13:5655. doi:10.1038/s41467-022-33008-2

-3. MuRF1 and Atrogin1 protein expression was quantified by western blots. What is the specificity of the antibodies used against MuRF1 and Atrogin1? Many of the antibodies against MuRF1 are non-specific in mouse tissues. In several of the figures there are multiple bands for MuRF1 and Atrogin1. Which band is quantified?

Response: Thank you for pointing out this. For MuRF-1, we used the antibody provided by [Abcam \(ab183094\)](#). According to the manufacturer's datasheet, this antibody detects a single band at approximately 40 kDa in C2C12 cells. However, in our experiments, we observed two closely migrating bands around 40 kDa in both mouse skeletal muscle tissue and C2C12 myotube, and the expression trends of both bands were consistent across all treatment groups. One possible explanation is the use of a relatively high concentration (12%) separating gel. Therefore, we included both bands in the quantification of MuRF1 protein expression. For Atrogin-1, we used another antibody from [Abcam \(ab168372\)](#). As shown in the product datasheet, this antibody detects a single band at approximately 42 kDa in mouse skeletal muscle, which is consistent with our observations, indicating good specificity. We will include the product links for the antibodies.

-4. Figure 1J: were these data generated from regenerated fibers? The histograms in Figure 1I suggest regeneration in the irisin + aging muscle is reduced relative to the aging alone tissues.

Response: Thank you for pointing out this. In our study, the fiber size distribution data shown in Figure 1J were indeed derived from centrally nucleated regenerating myofibers following CTX injection. As shown in Figure 1K, we further quantified the average cross-sectional area (CSA) of these centrally nucleated fibers, and the results showed that the CSA in the irisin-treated group was higher than control group in both young and aged mice. However, the overall difference was not very prominent, which may explain why the “irisin + aging” group does not appear clearly larger than the aging group in the representative images in Figure 1I.

The rationale for including the aging mouse model in our study was based on our Figure 1B result that aging is associated with elevated serum GC levels. We aimed to further evaluate the potential role of irisin under this condition. Although we observed some improvement in muscle regeneration in aged mice, the overall effect was not significant. In addition, irisin did not show a clear effect on GC-induced protein degradation. Therefore, we speculate that in aged mice, irisin may act more through promoting muscle regeneration, such as satellite cell activation and proliferation. We will further investigate this mechanism in our future studies.

-5.Lines 215-216: The data presented in figure 1 does not show that irisin ameliorates age-induced atrophy. The experiment examined regeneration in adult vs aged mice. The data presented in I and J from the aged mice are not convincing.

Response: Thank you very much for recognizing our work. We have revised the relevant description in the manuscript. Specifically, we now state that Irisin did not significantly decreased the protein levels of Atrogin-1 and MuRF-1 levels in aging mouse muscles, but it increased AKT phosphorylation, suggesting a role in promoting protein synthesis rather than inhibiting degradation. [Please see pages 12 lines 245-248 in the revised manuscript.](#) In addition, we have removed the inappropriate statement that "irisin ameliorates age-induced muscle atrophy" from the main text.

[pages 12 lines 250-253](#) :Irisin did not significantly decreased the protein levels of Atrogin-1 and MuRF-1 levels in aging mouse muscles, but it increased AKT phosphorylation, suggesting a role in promoting protein synthesis rather than inhibiting degradation

-6.Line 443: Sarcopenia is usually used to imply age-related muscle loss. The examples provided in this sentence are not age-related. A more appropriate term might be “muscle atrophy”

Response: As your suggestion, we have replaced the term “sarcopenia” with “muscle atrophy”. Thanks for your positive suggestion.

In a several places, additional references to original research articles, as opposed to reviews, are recommended. In general, the authors fail to acknowledge previous work that has demonstrated the role of GR activation in mediating the effects of glucocorticoids on skeletal muscle mass.

Lines 44-46: the genomic effects of GCs have been demonstrated in genetically modified mice: see 223547783, 23733748, 25254959.

Line 79: ref 8 is a review article. The original articles are: 18612045, 21807613.

Line 86: the effect of GR transcriptional responses on GC-induced muscle atrophy have been reported. 223547783, 23733748, 25254959

Line 467: 223547783

Response: Thank you very much for recognizing our work, As your suggestion, we have carefully reviewed and updated the references to ensure that original studies are cited where applicable.

-7.Muscle histology methods (lines 666-674): How many fibers were analyzed in each muscle? The description is a bit confusing: was it 100 fibers total or 100 fibers in each of 5 fields for a total of 500 fibers? The number of mice analyzed was on the low side n=3 or 4.

Response: We thank the reviewer for this helpful comment. In the revised manuscript, we have clarified the histological analysis method. Specifically, For each mouse, 5 random fields were selected from transverse sections of the TA muscle, and a total of 100 myofibers were measured across the 5 fields. In the HFD model, 5 mice were analyzed per group, while in the dexamethasone treatment model, 6 mice were analyzed per group. The corresponding description has been revised in the Methods section (lines 781-785).

Thank you for pointing out this. In the high-fat diet (HFD) model, 5 mice per group were used for body weight monitoring and histological analysis, while 3mice were used for western blot (WB) experiments. In the

dexamethasone (Dex) treatment model, 6 mice per group were used for body weight, muscle weight, and histological analysis, and 4 mice per group were used for WB. These sample sizes have now been clearly stated in the Methods section and figure legends.

pages 36 lines 781-785 : For each mouse, 5 random fields were selected from transverse sections of the TA muscle, and a total of 100 myofibers were measured across the 5 fields. In the HFD model, 5 mice were analyzed per group, while in the dexamethasone treatment model, 6 mice were analyzed per group

Response to reviewers' comments

Reviewer #1 (Remarks to the Author):

My points were partially addressed, although several key aspects of the manuscript remain insufficiently addressed in my opinion.

1.describe the muscle fiber type analysed (original major point #3 from reviewer #1)

Response: We sincerely appreciate the reviewer's further comments and valuable suggestions. Regarding the description of muscle fiber types, in our experiments we mainly selected the tibialis anterior (TA) for sectioning and cross-sectional area (CSA) analysis. The TA is a typical mixed muscle, composed predominantly of type IIa and IIb fibers, with a smaller proportion of type I fibers. In our CSA analysis, we therefore included all mixed fibers within the TA rather than focusing on a single fiber type, which allowed us to more comprehensively reflect the overall degree of muscle atrophy.

With respect to the number of fibers analyzed, we applied different strategies depending on the experimental model. In the aged mouse muscle injury model, our primary objective was to evaluate the effect of irisin injection on muscle regenerative capacity after injury. Accordingly, CSA analysis specifically focused on centrally nucleated regenerating fibers within the injured region. To ensure comparability across groups, the CSA of 100 centrally nucleated fibers per TA muscle was measured, obtained from 5 randomly selected fields, to assess the effect of irisin on regenerating fiber size.

In contrast, in the high-fat diet (HFD) mouse model and the Dex-induced muscle atrophy model, we fully adopted the reviewer's suggestion and substantially increased the number of fibers analyzed to improve representativeness and reliability. In the HFD model, approximately 500 fibers per TA muscle were measured, with 3 mice in total, yielding approximately 1500 fibers (Figure 1E and 1F), while in the Dex model, approximately 500 fibers per TA muscle were measured, with 5 mice in total, yielding approximately 2500 fibers (Figure 2E and 2F). The corresponding raw data have been included in Supplementary Data 1 for further reference, and the experimental methods have been revised accordingly.

Figure 1E and 1F

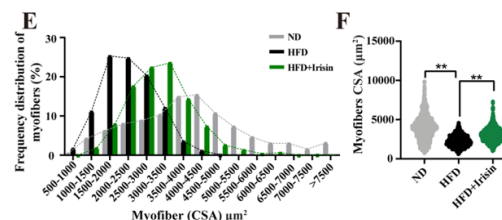
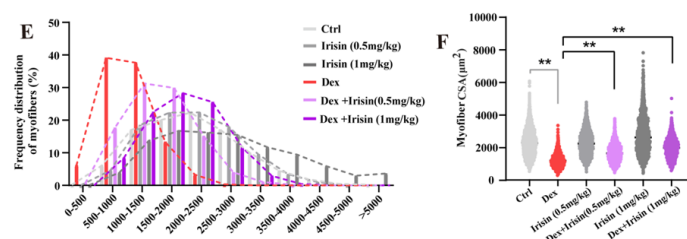


Figure 2E and 2F



Reviewer #2 (Remarks to the Author):

I am happy that the authors have engaged with and addressed the corrections and comments relating to the manuscript from this reviewer. I have no further comments and am happy to approve this output for publication.

Response: We sincerely thank the reviewer for their positive evaluation.

Reviewer #3 (Remarks to the Author):

The authors have addressed several of my concerns. However, the following concerns still remain:

1. The cross-sectional area measurements were made on only 100 fibers per muscle. This is a very small representation of the muscles analyzed. Was there a bias toward a specific type of fiber?

Response: We sincerely appreciate the reviewer's further comments and valuable suggestions. Regarding the description of muscle fiber types, in our experiments we mainly selected the tibialis anterior (TA) for sectioning and cross-sectional area (CSA) analysis. The TA is a typical mixed muscle, composed predominantly of type IIa and IIb fibers, with a smaller proportion of type I fibers. In our CSA analysis, we therefore included all mixed fibers within the TA rather than focusing on a single fiber type, which allowed us to more comprehensively reflect the overall degree of muscle atrophy.

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Figure 1E and 1F

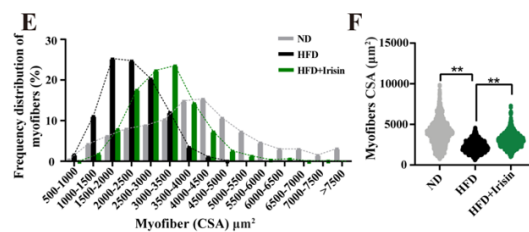
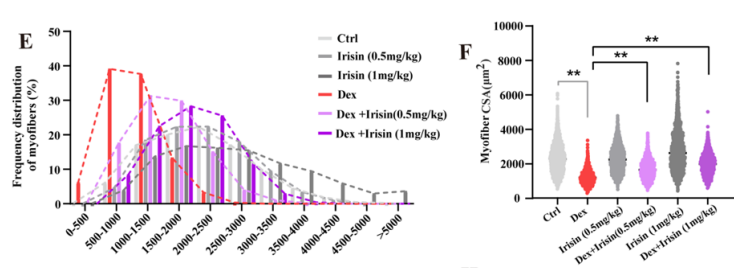
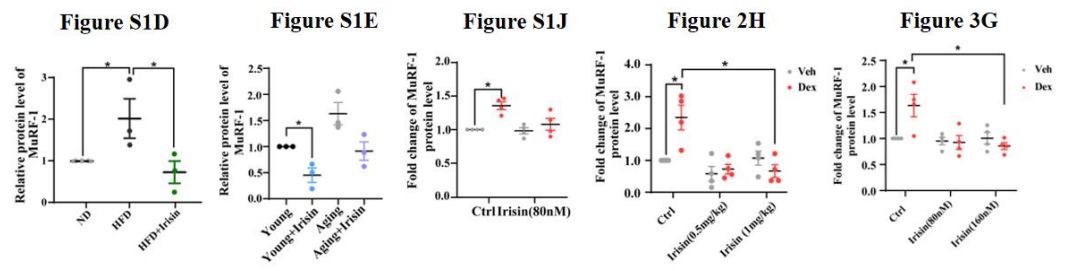


Figure 2E and 2F



2.The specificity of the MuRF1 antibody was not determined. Two bands were identified on the gels and both bands were analyzed.

Response: We sincerely appreciate the reviewer’s insightful comments regarding the specificity of the MuRF1 antibody. In our study, we used the MuRF1 antibody (ab183094, rabbit polyclonal), which consistently detected two closely migrating bands at approximately 40 kDa. According to the manufacturer’s reference data, the antibody also yields two bands in this region, with the upper band interpreted as the specific signal and the lower band as non-specific(see [antibody datasheet](#) below). To ensure accuracy and comparability across experiments, we restricted our quantitative analyses of MuRF1 to the upper band and the results remained consistent with our previous findings. The corresponding raw data are provided in the Supplementary Data, and in the revised figures(S1D, S1E, S1J, 2H, 3G) we now present only the upper band for clarity and accuracy.



3. The figure legend and paragraph headings still say that irisin ameliorates age-induced atrophy. These headings need to be revised since the data do not support this conclusion.

Response: We thank the reviewer for this valuable comment. As suggested, we have revised the paragraph headings and figure legends to state “Irisin ameliorates HFD-induced muscle atrophy” instead of referring to age-induced atrophy. In addition, we have modified the conclusion to more accurately reflect the results in aged mice: “Taken together, these results indicate that irisin alleviates HFD-induced muscle atrophy. In aged mice, irisin promoted an increase in the CSA of regenerating myofibers after injury, but its effect on age-related muscle atrophy was limited.” (Revised manuscript, lines 249–252).

4. There are a number of grammatical and spelling errors that should be corrected.

Response: We greatly appreciate the reviewer for pointing out this issue. We have carefully checked the manuscript for grammatical and spelling errors, and all corrections have been made. The revised manuscript highlights the changes in red for clarity.