The role and underlying mechanisms of irisin in exercise-mediated cardiovascular protection (98448)

Reviewer comments:

Thank you for the opportunity to review this manuscript, titled "**The role and underlying mechanisms of irisin in exercise-mediated cardiovascular protection**". The submitted manuscript is of interest and can add to the research area.

A recent review, titled 'The Role of FNDC5/Irisin in Cardiovascular Disease' (**https://doi.org/10.3390/cells13030277**), highlights, in a very succinct manner, the role of irisin in CVD, hence, this publication has a lot of link with the paper in hand. However, as this paper has a focus on irisin in exercise-mediated cardiovascular protection, this paper is of relevance. However, it is clear that the story being told is weak and needs improvement. More clear and concise language is required, and authors need to home in on major points of the review. The reader should walk away after reading this paper and know how different types of exercise influence irisin expression (local and global), what concentration of irisin is needed to elicit a response, how it elicits this response in tissues associated with CV system (common pathways, target genes) and the protective mechanisms that ensue. Currently this isn't the case.

An overhaul of the paper is required, and more scientific clarity is required.

Please see major points and individual points below:

- For all in text references, please leave a space between the last word and the reference. Please double check throughout and ensure formatting is correct.
- Figure 1: when describing the tissues that release FNDC5/irisin, avoid writing other organs' as it is ambiguous. Just state 'Primary expression sites: skeletal muscle … etc…'. This notion applies to the whole manuscript too.
- Visual clarity of figures needs to be improved.
- The figure legends for all figures are not descriptive enough, and do not help the reader. For example, figure 2: what do the colours represent? What degree of expression? Much more information is need in all figures.
- A lot of terms are abbreviated when they don't need to be – it just overcomplicates the prose.
- Subsections do not reflect subtitles, more concise writing is required.
- Avoid continuous listing of points, i.e., XX et al showed this, XX et al showed this, etc etc. Keep the reader intrigued and offer an enjoyable reading experience.
- Use tables and figures more effectively – also figure and table legends need much more detail and require all abbreviated words described.

Individual points:

17-18: First line of the abstract is too long winded – the first line the reader sees needs to be clearer and more concise. Consider something like:
“Irisin, a product of the post-translational processing of fibronectin type III domain-containing protein 5 (FNDC5), is a novel myokine which is upregulated during exercise”.

Also, avoid starting each sentence with ‘Irisin’, if possible, as it sounds just like you are listing things instead of telling a story

19: Try more concise wording: “facilitates white adipose tissue browning”

25: Instead of cytokine, potentially change to myokine

29-30: sentence structure / grammar needs to be addressed.
e.g.: “statistics from a worldwide cohort indicate a 57.2% and 52.6% incidence of CVD, in women and men, respectively”

31-35: sentence structure / grammar needs to be addressed.

34: “and an effective non-drug therapeutic strategy to treat CVD”, consider: “and a non-invasive therapeutic strategy to manage and potentially treat CVD”

36: ‘myogenic cytokine’ suggests in this sentence that irisin is inducing its affect via a myogenic-dependent manner. Irisin is known to promote myogenesis and hence be ‘pro-myogenic’, however, in the context of this sentence ‘myokine’ is more suitable

38-40. check wording here, ‘Irisin expression is dependent on peroxisome proliferator-activated receptor γ coactivator 1α’ may be more concise and appropriate

48: grammar, please don’t start a sentence with ‘And’, reword sentence.

49-50: delete: ‘Hoping that this article can bring inspiration to their research.’

55-56: additional and more recent references may be required here (lira et al, 2010).

57-59: Please check grammar and wording of this sentence (e.g., two full stops at end of sentence), also need to provide a reference for this:
“Additionally, the presence of PGC1α in muscle tissue has been shown to trigger a rise in the levels of fibronectin type III domain-containing protein 5 (FNDC5), a membrane-based protein that undergoes cleavage and release, resulting in the formation of a novel hormone known as irisin..”

60: change ‘autocrine/paracrine’ to ‘autocrine and/or paracrine’ (autocrine and paracrine are not synonymous).

64: spelling check: encoding

72-78: Grammar needs checking throughout this section. Full stop to be removed before (figure 1). Also, references need to be checked (formatting off).

78: at some stage, give refence to: **https://doi.org/10.3390/cells13030277**

80-82: repetitive wording, please make sentence more concise.

84-85: include a note of the proteases that cleave FNDC5, important in the context of mechanistic activation. Consider this publication https://doi.org/10.1186/s13578-020-00413-3 for more information

87: Although literature states ‘100% identical’, I think this language is too weighted. Please consider rewording with ‘Irisin is highly conserved between species, with human and mouse homology being almost 100%’

89: not skeleton muscle, re word: skeletal muscle. This applies to figure 3 too.

90: ‘small amounts’ is too colloquial. Consider ‘residual levels’

93: figure 2 needs to be improved, with appropriate figure legend (what expression level do the colours represent?)

96: be consistent with referencing style. Seeing this makes me worried that references may not be formatted properly. Please double check all references.

98: what previous studies? Reference required. Consider integrating with subsequent sentence

104-131: After reading this subsection (**4 The protective role of irisin in the cardiovascular system)**, I have not left with a general understanding of the protective role of irisin in CV system… I recommend, re-reading and re-writing this section. What specific modalities promote CV protection? Major pathways that are activated? Phenotypic benefits of irisin signalling?

105-107: grammar and sentence structure needs to be improved here.

110: reference formatting, check full stops

112: the word ‘settled’ indicates this area of research has been ‘completed’, which is not the case, please addressing sentence wording

114-115: “Currently, the role of irisin in the body is the focus of attention, especially through exercise to induce its secretion.” This sentence doesn’t make any sense, please address

118: grammar: The -> A

120-121: refer to previous comment on homology

126: “But circulating irisin levels are not always equal to the expression of irisin in skeletal muscle.” Repetition of this point, state once and expand with discussion

127-129: More detail needed re study parameters here… what was the control group?

129-130: wording here is odd, please address

132: You don’t discuss the function of irisin in this subsection, you merely dip in and out of the association of irisin with myocardial dysfunction and disease. What is the function of irisin in myocardial protection??

133-139: This section doesn’t make sense. I’m lost regarding the information on aliphatic acid (energy source in adult vs fetus?)? The refence used here (Flori et al) doesn’t clarify or support the points you’re making.

140-147: If you are not regularly using abbreviated phrases, then there is no real need to abbreviate all terminology e.g., you abbreviate heart failure, then you do it again for acute heart failure. Its only a small point, but it makes things confusing with so many abbreviated terms.

155-203: This section is much better as you describe different models, how irisin regulates signalling pathways, and the benefits of this. A good summary.

169: formatting, superscript required for $ApoE^{-/-}$

209: spelling, secret -> secrete

209-211: “Skeletal muscle can synthesize and secret myokines, which can act on other organs, such as adipose tissue, brain, and liver, by auto-, para-, and endocrine manners”, this sentence doesn’t make sense. The listed tissues here suggest the only route of muscle-to-tissue cross-talk is via endocrine signalling. Please elaborate…

220-242: Check grammar and sentence structure in this section. Also, I see no reference to the tables you have generated in this section. Moreover, when exercise induced an increase in irisin, what was the new concentrations (circulation and/or muscle)? Is this increase similar to published beneficial effects of increased irisin i.e., does the increase in irisin have the capacity to promote a beneficial effect on myocardial function etc.

257: format reference

287: might? This wording seems odd

300: Dependent on context, LC3/P62 signalling drives autophagy, which at too high of a level could be disastrous to skeletal muscle function. Just a point to consider.

304: ‘activated the PI3K/Akt signaling pathway’, in what organ / tissue? More clarity needed

305: ‘ALCAT1 knockout mice’, why this mouse model?? Whats the significance?

322: muscle needed (gastrocnemius muscle)

336: ‘short duration and transient’ these terms are nearly synonymous with each other

340: check reference format

361: lucubrate, interesting choice of word, consider ‘decipher’

362-363: repetition, please address

374-376: repetition, please address

377: from your research what is a common pathway that links exercise induced irisin production and cardiovascular protection / amelioration of CVD? I feel there is a degree of dissidence throughout the review process. You highlight a lot of points, but don’t really succeed in brining them together and offering your opinion on potential links etc.

401: ‘Exercise can promote irisin production and thus plays a protective role in CVD.’ This is such a strong statement, the wording here is too cut-throat. Use phrases like, ‘is potentially associated with’

401-411: The conclusion introduces new points (e.g., glucolipid metabolism), at this stage we do not need to introduce any new points, we just need to summarise the document in a clear and concise manner – the conclusion need to be addressed