

January 13, 2022

Re: Submission of a Revised manuscript to the World Journal of Stem Cells

Dear Editors and Editorial Committee,

Thank you very much for giving us the opportunity to revise our invited review (Manuscript number: 74043)

We would be grateful for the consideration of our revised manuscript “The important factor of sex hormone affecting differentiation and commitment of mesenchymal stem cells” (by Yuanlong Gu, Wei Shen, Zhipeng Li, Bo Zhou, Zijun Lin and Lianping He) for publication in the World Journal of Stem Cells. The authors have read and complied with author guidelines, and they all have seen and approved this manuscript for publication. None of the authors had a conflict of interest to disclose concerning this manuscript.

We are grateful to the Editors for their precious contributions and comments. We have revised our manuscript accordingly for grammar, style, structure and we hope that you will now find it suitable for publication in the World Journal of Gastroenterology. In case of final acceptance, we agree to make this manuscript open-access.

The changes in the manuscript are identified in track change mode. Below you can find a point-by-point reply to the reviewers. We used red to denote revised or inserted text.

Thank you for your precious time.

We are looking forward to receiving your decision in due time.

Corresponding Author:

Yuanlong Gu, MMed, Chief Doctor, Interventional Oncology, Municipal Hospital Affiliated to Taizhou University, Municipal Hospital Affiliated to Taizhou University, No.381 Zhongshan East Road, Jiaojiang District, Taizhou City, Zhejiang Province, Taizhou 318000, Zhejiang, China. ylg@tzc.edu.cn

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Minor revision

Specific Comments to Authors: In this paper the authors wrote a letter to the Editor in which commented the article written by Alessio N, published on world journal of stem cell. Alessio et al in their paper studied why skinny people (SP) intake excess calories than the body needs while present normal body composition. They summarized these reasons as the existence of some factors in the human serum of SP that promote the differentiation of mesenchymal stromal cells (MSCs) towards brown adipocytes. The authors of the letter to the Editor point out several important weakness of the published paper and hypothesizes an important role of sexual hormones in the results found by Alessio N. et al. I think that authors are right. However, the abstract is confusing and should be rewritten describing the most important comments within the Letter to the editor. Moreover, they should describe better and more deeply the study design and the results of the published paper of Alessio N. et al.

Response:

Thank you for acknowledging the content of our article and providing valuable review comments. We humbly accept your suggestion and are aware of the flaws in this summary. The previous abstracts did not do a good job of highlighting the research design and results of the paper published by Alessio N. et al., nor did they highlight the critical points of this paper. Therefore, we rewrite the abstract. The content is as follows:

Abstract

The original study by Alessio N. et al, found that skinny people (SP) serum may promote the formation of brown adipocytes, but not the differentiation of white adipocytes. This finding may explain why SPs do not tend to become obese despite consuming more calories than the body's normal needs. More

importantly, they found that circulating factors in SP serum could promote the expression of UCP-1 protein, thereby reducing fat accumulation. In this study, only male serum samples were selected to avoid the interference of sex hormones in this experiment, but adult males also contain estrogen, which mainly comes from the direct production of testes; at the same time, adult females also secrete androgens, and females have androgens are mainly secreted from the adrenal cortex. We believe that the method of excluding sex hormone interference by sex selection alone may be flawed, so we make some comments on the article and debate the statistical method of the article.

Reviewer #2:

Scientific Quality: Grade D (Fair)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The manuscript arouses considerable interest for the topic it deals with. However, there are some observations to highlight from a technical point of view. In particular, it would be interesting to consider the age of each female and male sample in order to obtain more specific data. Furthermore, it would be clearer to describe the process of differentiation and commitment of the MSCs also through images with associated captions. From a point of view of materials and methods, the manuscript highlights few methods of analysis. In particular, as regards the statistical method, the manuscript lacks a descriptive part of this method and the meaning of the statistical parameters it takes into consideration, such as variance. Finally, as far as the reference bibliography is concerned, the manuscript has few references and of those that exist, some are much less recent and should be replaced with other more recent references that can best support the issue being addressed.

Response:

We thank you for your affirmation of the value of our article, and at the same

time humbly accept your comments on our review. We have supplemented statistical methods and updated the reference content. The detailed modification is as follows:

REFERENCE

- 1 Alessio N, Squillaro T, Monda V, Peluso G, Monda M, Melone MA, Galderisi U, Di Bernardo G. Circulating factors present in the sera of naturally skinny people may influence cell commitment and adipocyte differentiation of mesenchymal stromal cells. *World J Stem Cells* 2019; 11: 180-195 [PMID: DOI: 10.4252/wjsc.v11.i3.180]
- 2 Hong L, Sultana H, Paulius K, Zhang G. Steroid regulation of proliferation and osteogenic differentiation of bone marrow stromal cells: A gender difference. *J Steroid Biochem Mol Biol* 2009; 114: 180-185 [PMID: DOI: 10.1016/j.jsbmb.2009.02.001]
- 3 Alrabadi N, Al-Rabadi GJ, Maraqa R, Sarayrah H, Alzoubi KH, Alqudah M, Al-U'datt DaG. Androgen effect on body weight and behaviour of male and female rats: Novel insight on the clinical value. *Andrologia* 2020; 52: e13730 [PMID: DOI: 10.1111/and.13730]
- 4 Herbst KL, Bhasin S. Testosterone action on skeletal muscle. *Curr Opin Clin Nutr Metab Care* 2004; 7: 271-277 [PMID, <https://pubmed.ncbi.nlm.nih.gov/15075918>]
- 5 Nelson LR, Bulun SE. Estrogen production and action. *J Am Acad Dermatol* 2001; 45: S116-S124 [PMID, <https://pubmed.ncbi.nlm.nih.gov/11511861>]
- 6 Rubinow KB. Estrogens and body weight regulation in men. *Adv Exp Med Biol* 2017; 1043: 285-313 [PMID: DOI: 10.1007/978-3-319-70178-3_14]
- 7 An R, Shen J, Bullard T, Han Y, Qiu D, Wang S. A scoping review on economic globalization in relation to the obesity epidemic. *Obes Rev* 2020; 21: e12969 [PMID: DOI: 10.1111/obr.12969]
- 8 Chang E, Varghese M, Singer K. Gender and sex differences in adipose tissue. *Current diabetes reports* 2018; 18: 69 [PMID: PMC6525964 DOI: 10.1007/s12020-018-0069-2]

10.1007/s11892-018-1031-3

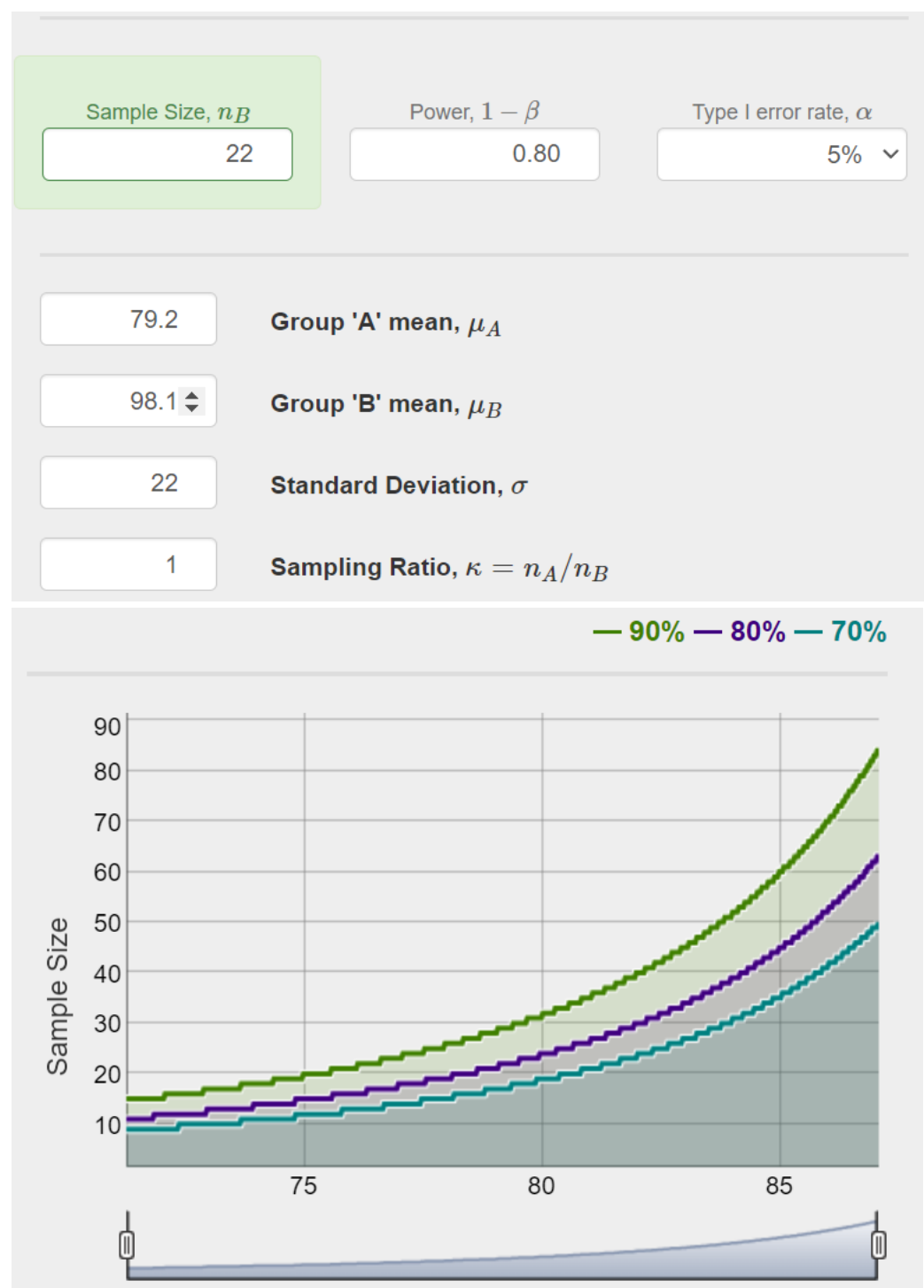
9 Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. *Endocr Rev* 2013; 34: 309-338 [PMID: DOI: 10.1210/er.2012-1055]

10 Takao K, Iizuka K, Liu Y, Sakurai T, Kubota S, Kubota-Okamoto S, Imaizumi T, Takahashi Y, Rakhat Y, Komori S, Hirose T, Nonomura K, Kato T, Mizuno M, Suwa T, Horikawa Y, Sone M, Yabe D. Effects of chrebp deficiency on adrenal lipogenesis and steroidogenesis. *The Journal of endocrinology* 2021; 248: 317-324 [PMID: DOI: 10.1530/joe-20-0442]

11 Schnabl K, Westermeier J, Li Y, Klingenspor M. Opposing actions of adrenocorticotrophic hormone and glucocorticoids on ucp1-mediated respiration in brown adipocytes. *Frontiers in physiology* 2018; 9: 1931 [PMID: PMC6344423 DOI: 10.3389/fphys.2018.01931]

Statistical method

The authors of this study collected serum from a total of 12 adult males and divided them into two groups, NP and SP, with 6 samples in each group. We used PS (Power and Sample size calculation) software (HyLown Consulting LLC • Atlanta, GA) for sample size estimation. With this method, we estimated the sample size of Triglycerides according to a type II error β of 0.2, that is, a power of 0.8 ($1-\beta$) and a first type error (α) of 5%. According to the data in table 1 normal and skinny patient parameters, the mean values of triglycerides in the NP and SP groups were 79.2 mmol/L and 98.1 mmol/L, respectively, sample ratio is 1, standard deviation(σ) is 22, after calculation, the theoretical conservative estimate of the sample size of each group should be at least 22 cases, while the sample size of each group in this experiment is only 6 cases, so we believe that the statistical power of this experiment is low, and it is recommended that the author supplement the samples of each group content.



Reviewer #3:

Scientific Quality: Grade A (Excellent)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors: The letter focus on conclusions taken by the work of Alessio et al (2019) in respect to MSCs differentiation in fat cells.

Therefore, the title of the letter should point specifically differentiation in this cell type, instead of a general capacity. Authors refers to mesenchymal stem cells as pluripotent cells, however, MSCs are widely described as multipotent cells.

Response:

Thank you for your recognition of our article, we humbly accept your comments on the revision of our article title, and ow the article title is changed from “The important factor of sex hormone affecting differentiation and commitment of mesenchymal stem cells” to “Skinny people serum factor promotes the differentiation of multipotent stem cells into brown adipose tissue.”

We normalized the use of proper nouns and changed the text "mesenchymal stem cells" to "multipotent stem cells"

Reviewer #4:

Scientific Quality: Grade E (Do not publish)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Rejection

Specific Comments to Authors: The submitted Letter to the Editor, aims at providing a critical reading of an article published in 2019 in the World Journal of Stem Cells, entitled:” Circulating factors present in the sera of naturally skinny people may influence cell commitment and adipocyte differentiation of mesenchymal stromal cells”, contributed by Alessio N, et al. In this article, the Authors start from the background assumption that “Research on physiopathology of obesity may receive new hints from studies on skinny people (SP)”. The Authors are aware of the fact that “These are individuals who show a poor or null gaining of body weight, in spite of high-calorie intake, by far exceeding the body requirements”. The declared aim of the study was “To evaluate how circulating factors present in the SP sera may affect adipogenesis of mesenchymal stromal cells (MSCs)”. While a Letter to the Editor may

undoubtedly contribute a debate on such a relevant issue, unfortunately the submitted Letter has been shaped in quite an aggressive tone, as it is clearly indicated by sentences like: “It is unbecoming to judge the effect of other cytokines in the SP sample on MSCs without excluding the influence of androgens”. Indeed, to this end, Alessio et al. are well aware of the issue of sex hormones controlling MSC fate: “We selected only male since estrogen fluctuation may introduce further complexity to data analysis. Indeed, estrogen may influence MSC osteo-adipo commitment [24,25]”. Thus, the Authors likely decided to avoid confounding bias associated with the well-known effect of sex hormones on MSC differentiating/paracrine potential. Overall, the main value of the study of Alessio et al. is the novel finding that SP priming of MSCs affected adipocyte cell commitment and reduced spontaneous adipogenesis, showing striking differences between differentiation in SP-primed samples, compared with the ones that had been primed with serum harvested from normal people (NP). The Authors are aware of the preliminary nature of their cytokine assay in NP and SP sera: “we performed a preliminary analysis of SP cytokine content to identify possible molecules that may play a role in the physio-pathology of adipogenesis and related phenomena”. Nevertheless, the performed assessment was a wide-ranging cytokine profiling: cytokine content of NP and SP sera was evaluated by array analysis. “The relative levels of 62 cytokines were analyzed in NP and SP groups by the Human Obesity Antibody Array C1 (RayBiotech, United States)”. In actual fact, the same Authors “focused on four factors (ACRP30, ANGPT1, IGFBP1, and RANTES) that could be related to the physiological status of SP: Eating an excess of calories without weight gain”. In other words, they decided to focus on a number of factors whose differential expression in SP sera may account for the observed MSC responses, as shown in Figure 6. Overall, the reported findings may have molecular underpinning in the pattern of observed differential cytokine expression. Of course, considering other additional factors potentially contributing to the observed phenomena is something deserving further

investigation and it may well be a subject for a Letter to the Editor. Nevertheless, this Letter cannot be shaped with an excessive opinionated tone, largely neglecting the reliability of the study itself ("In such a small sample size, statistical analysis and conclusion inference are prone to bias, and too small sample size will significantly reduce the credibility of the conclusion"). Besides, the submitted Letter presents flaws in both grammar and sentence construction.

Response:

Thank you for your patience in reading our article. We humbly accept your comments on us. After reading your comments in detail, we agree that our article does have the above problems. We have rewritten the statistical methods section as detailed below

Statistical method

The authors of this study collected serum from a total of 12 adult males and divided them into two groups, NP and SP, with 6 samples in each group. We used PS (Power and Sample size calculation) software (HyLown Consulting LLC • Atlanta, GA) for sample size estimation. With this method, we estimated the sample size of Triglycerides according to a type II error β of 0.2, that is, a power of 0.8 ($1-\beta$) and a first type error (α) of 5%. According to the data in table 1 normal and skinny patient parameters, the mean values of triglycerides in the NP and SP groups were 79.2 mmol/L and 98.1 mmol/L, respectively, sample ratio is 1, standard deviation(σ) is 22, after calculation, the theoretical conservative estimate of the sample size of each group should be at least 22 cases, while the sample size of each group in this experiment is only 6 cases, so we believe that the statistical power of this experiment is low, and it is recommended that the author supplement the samples of each group content.

We do not deny the author's experimental results, we emphasize that sex selection cannot completely circumvent the effect of sex hormones on stem cell differentiation, and we suggest that the authors take into account our proposal that sex hormones may have some effect on stem cell differentiation.

We have polished and revised the language to make up for our lack of expression. We changed the statement pointed out by the reviewer from “It is unbecoming to judge the effect of other cytokines in the SP sample on MSCs without excluding the influence of androgens”. to “Therefore, we suggest that the interference of androgen itself on cell differentiation and commitment should be excluded as much as possible when studying cytokine stimulators of MSCs in adult male serum.”

Reviewer #5:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: I would like to congratulate the authors for this article. It can bring new perspective on the development of the referred study. I have some comments: The referred study by Alessio et al. is interesting and I believe it has been reviewed by the reviewers accordingly. Since the referred study is already done, the suggested study which involve sex hormones (estrogen and androgens) on the differentiation and commitment of MSCs would be a great idea and interesting for another research or project development. Regarding the statistical method used and sample size, it would be more informative if the authors can give suggestion about the appropriate method and sample size to avoid bias, in detail.

Response:

Thanks for your recognition of our article, we have updated the description of statistical methods as follows.

The authors of this study collected serum from a total of 12 adult males and divided them into two groups, NP and SP, with 6 samples in each group. We used PS (Power and Sample size calculation) software (HyLown Consulting

LLC • Atlanta, GA) for sample size estimation. With this method, we estimated the sample size of Triglycerides according to a type II error β of 0.2, that is, a power of 0.8 ($1-\beta$) and a first type error (α) of 5%. According to the data in table 1 normal and skinny patient parameters, the mean values of triglycerides in the NP and SP groups were 79.2 mmol/L and 98.1 mmol/L, respectively, sample ratio is 1, standard deviation(σ) is 22, after calculation, the theoretical conservative estimate of the sample size of each group should be at least 22 cases, while the sample size of each group in this experiment is only 6 cases, so we believe that the statistical power of this experiment is low, and it is recommended that the author supplement the samples of each group content.

Science editor:

The submitted Letter to the Editor, aims at providing a critical reading the work of Alessio et al (2019) published in the World Journal of Stem Cells about mesenchymal stem cells (MSCs) differentiation in fat cells influenced by circulating factors present in the sera of naturally skinny people. A Letter to the Editor is interesting to contribute to a debate on such a relevant topic. However, this Letter seems more like a critique of previous work than an opening for debate because the authors of the letter to the Editor point out several important weaknesses of the published paper and hypothesizes an important role of sexual hormones in the results found by Alessio N. et al. The title and the letter should point explicitly the differentiation of MSC into fat cells. Authors of this Letter refers to MSC as pluripotent cells, and it is widely described than MSC are multipotent cells. In addition, the abstract should be rewritten describing the most important comments within the Letter to the editor. Moreover, the authors should describe better the study design and the results of the published paper of Alessio N. et al. The authors of this letter recommend to Alessio N. et al a study which involve sex hormones (estrogen and androgens) on the differentiation and commitment of MSCs. Of course, considering other additional factors potentially contributing to the observed

phenomena by Alessio N. et al. is something deserving further investigation and it may well be a subject for a Letter to the Editor. But, given that the study referred to in the letter has already been carried out and published, the authors should point out that the study they recommend would be an interesting idea for an additional research project. The authors of the Letter criticize the sample size and the statistical method used Alessio N et al. However, it would be more informative if the authors can give a suggestion about the appropriate method and sample size to avoid bias. In addition, it is not appropriate for the authors to conclude that the sample size and the statistical method used Alessio N et al. will significantly reduce the credibility of the conclusion. Finally, the reference bibliography of the Letter has insufficient references and of those should be replaced with other more recent that can best support the issue being addressed. Besides, the submitted Letter has several flaws in both grammar and sentence construction.

Language Quality: Grade C (A great deal of language polishing)

Scientific Quality: Grade E (Do not publish)

Response:

Thank you for your patience in reading our article. We humbly accept your comments on us. After reading your comments in detail, we agree that our article does have the above problems. We have carefully revised the article and revised the abstract. Re-written, statistical methods re-described, and bibliography updated. Details are as follows,

Abstract

The original study by Alessio N. et al, found that skinny people (SP) serum may promote the formation of brown adipocytes, but not the differentiation of white adipocytes. This finding may explain why SPs do not tend to become obese despite consuming more calories than the body's normal needs. More importantly, they found that circulating factors in SP serum could promote the expression of UCP-1 protein, thereby reducing fat accumulation. In this study, only male serum samples were selected to avoid the interference of sex

hormones in this experiment, but adult males also contain estrogen, which mainly comes from the direct production of testes; at the same time, adult females also secrete androgens, and females have androgens are mainly secreted from the adrenal cortex. We believe that the method of excluding sex hormone interference by sex selection alone may be flawed, so we make some comments on the article and debate the statistical method of the article.

REFERENCE

- 1 Alessio N, Squillaro T, Monda V, Peluso G, Monda M, Melone MA, Galderisi U, Di Bernardo G. Circulating factors present in the sera of naturally skinny people may influence cell commitment and adipocyte differentiation of mesenchymal stromal cells. *World J Stem Cells* 2019; 11: 180-195 [PMID: DOI: 10.4252/wjsc.v11.i3.180]
- 2 Hong L, Sultana H, Paulius K, Zhang G. Steroid regulation of proliferation and osteogenic differentiation of bone marrow stromal cells: A gender difference. *J Steroid Biochem Mol Biol* 2009; 114: 180-185 [PMID: DOI: 10.1016/j.jsbmb.2009.02.001]
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- 4 Herbst KL, Bhasin S. Testosterone action on skeletal muscle. *Curr Opin Clin Nutr Metab Care* 2004; 7: 271-277 [PMID, <https://pubmed.ncbi.nlm.nih.gov/15075918>]
- 5 Nelson LR, Bulun SE. Estrogen production and action. *J Am Acad Dermatol* 2001; 45: S116-S124 [PMID, <https://pubmed.ncbi.nlm.nih.gov/11511861>]
- 6 Rubinow KB. Estrogens and body weight regulation in men. *Adv Exp Med Biol* 2017; 1043: 285-313 [PMID: DOI: 10.1007/978-3-319-70178-3_14]
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8 Chang E, Varghese M, Singer K. Gender and sex differences in adipose tissue. *Current diabetes reports* 2018; 18: 69 [PMID: PMC6525964 DOI: 10.1007/s11892-018-1031-3

9 Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. *Endocr Rev* 2013; 34: 309-338 [PMID: DOI: 10.1210/er.2012-1055

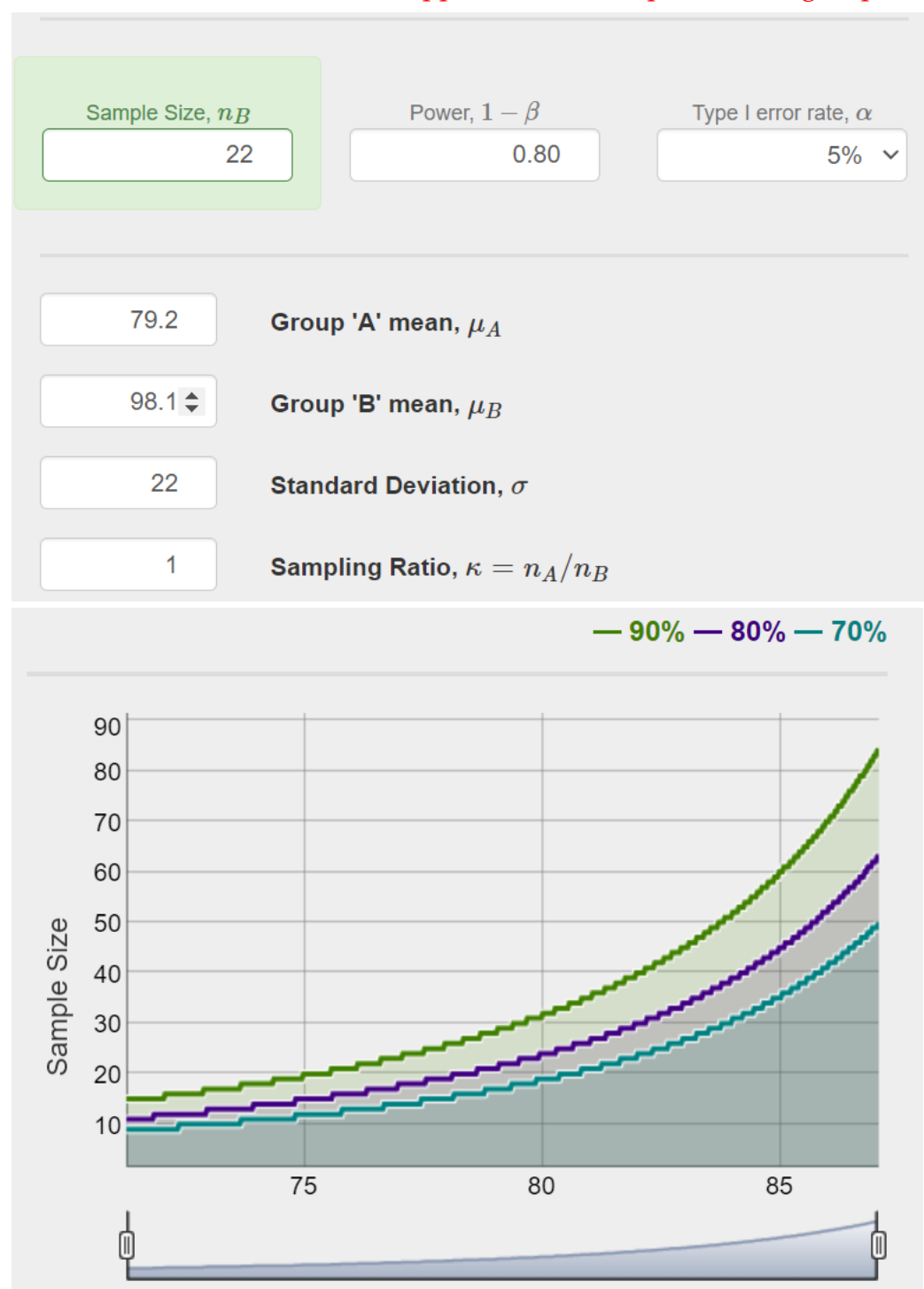
10 Takao K, Iizuka K, Liu Y, Sakurai T, Kubota S, Kubota-Okamoto S, Imaizumi T, Takahashi Y, Rakhat Y, Komori S, Hirose T, Nonomura K, Kato T, Mizuno M, Suwa T, Horikawa Y, Sone M, Yabe D. Effects of chrebp deficiency on adrenal lipogenesis and steroidogenesis. *The Journal of endocrinology* 2021; 248: 317-324 [PMID: DOI: 10.1530/joe-20-0442

11 Schnabl K, Westermeier J, Li Y, Klingenspor M. Opposing actions of adrenocorticotrophic hormone and glucocorticoids on ucp1-mediated respiration in brown adipocytes. *Frontiers in physiology* 2018; 9: 1931 [PMID: PMC6344423 DOI: 10.3389/fphys.2018.01931

Statistical method

The authors of this study collected serum from a total of 12 adult males and divided them into two groups, NP and SP, with 6 samples in each group. We used PS (Power and Sample size calculation) software (HyLown Consulting LLC • Atlanta, GA) for sample size estimation. With this method, we estimated the sample size of Triglycerides according to a type II error β of 0.2, that is, a power of 0.8 ($1-\beta$) and a first type error (α) of 5%. According to the data in table 1 normal and skinny patient parameters, the mean values of triglycerides in the NP and SP groups were 79.2 mmol/L and 98.1 mmol/L, respectively, sample ratio is 1, standard deviation(σ) is 22, after calculation, the theoretical conservative estimate of the sample size of each group should be at least 22 cases, while the sample size of each group in this experiment is only 6 cases, so we believe that the statistical power of this experiment is low, and it is

recommended that the author supplement the samples of each group content.



Company editor-in-chief:

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Stem Cells, and the manuscript is

conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. However, the quality of the English language of the manuscript does not meet the requirements of the journal. Before final acceptance, the author(s) must provide the English Language Certificate issued by a professional English language editing company. Please visit the following website for the professional English language editing companies we recommend: <https://www.wjgnet.com/bpg/gerinfo/240>. Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file.

Response:

We have reviewed and edited the language of the article, optimized the English language, and the provided English Language Certificate issued has been uploaded to the attachment. The chart file is also uploaded to the attachment.