Jundishapur Journal of Natural Pharmaceutical Products
Volume 20 · Issue 1 · e158742
OPEN PEER REVIEW

The Effect of Endurance Training and Dill Seed Extract on Insulin Sensitivity and Nicotinamide N-methyltransferase Levels in the Liver and Adipose Tissue of Obese Rats

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SUBMITTED	REVISED	ACCEPTED	DOI
13 Dec 2024	27 Jan 2025	13 Feb 2025	10.5812/jjnpp-158742

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1 Original submission

2 comments · 29 Dec 2024 – 5 Jan 2025

Referee 1 Reviewer

MAJOR REVISION 29 Dec 2024

The manuscript presents an interesting investigation into the effects of endurance training (ET) and dill seed extract (DSE) on insulin sensitivity and NNMT levels in obese rats. However, there are several methodological and reporting limitations that should be addressed to improve the overall quality and clarity of the study.
1. The methods for measuring total phenols, flavonoids, and flavonols of the DSE are not adequately described. Provide a more detailed explanation of the methods used for determining phenol, flavonoid, and flavonol content. Include details on the assay methods, standards, and instruments for these measurements. This is essential for reproducibility and validation of the results.
2. The manuscript does not mention the herbarium code or any detailed authentication process for the dill seed used in the study. Provide information on the botanical identification, including the herbarium code and the expert who authenticated the plant.
3. Ensure figures and tables are well-labeled with clear legends that include statistical details (e.g., p-values, group comparisons). The figures lack sufficient annotations and statistical markings for key comparisons. Include clear legends with detailed descriptions of the comparisons and significance levels (e.g., p-values). Label all axes with appropriate units.
4. One notable issue with the manuscript is the excessive use of abbreviations. While abbreviations help simplify technical terms, overusing them can make the text difficult to read and follow. In figures, tables, and their captions, avoid using abbreviations alone. Instead, provide a brief explanation of each abbreviation used to ensure that readers can understand the information without referring back to the text.
5. The study lacks histopathological analysis of liver and adipose tissues. Including such data would have provided valuable insights into tissue-level changes. Histological analysis of liver and adipose tissues could have strengthened the interpretation of NNMT and triglyceride findings.
6. Include more structured descriptions of findings. For example, mention specific percentages for key results instead of relying on vague descriptions (e.g., “significant improvement”).

Associate Editor

MAJOR REVISION 5 Jan 2025

Thank you for submitting your manuscript to JJNPP.
Please revise the manuscript and submit a point-by-point response to the reviewers’ comments and concerns.

I would also greatly appreciate the authors' contribution and time spent reviewing manuscripts for this journal. Without the dedication of reviewers like you, it would be impossible to manage an efficient peer review process and maintain the high standards necessary for a successful journal.

Best regards

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor

We sincerely would like to thank you for constructive comments about our paper entitled "**The Effect of Endurance Training and Dill Seed Extract on Insulin Sensitivity and Nicotinamide N-Methyltransferase Levels in the Liver and Adipose Tissue of Obese Rats**".

As you recommended, we tried to response to the reviewers comments with more details. We hope these modifications meet all expectations of you and reviewers and help publishing of our paper in the "**Jundishapur Journal of Natural Pharmaceutical Products**".

Sincerely,

Answers to reviewer #1's comments

The authors would like to thank the anonymous reviewer the careful reviews and insightful comments. We tried to response to the following comments with more details. We hope these modifications will meet all expectations of reviewer .

1. The methods for measuring total phenols, flavonoids, and flavonols of the DSE are not adequately described. Provide a more detailed explanation of the methods used for determining phenol, flavonoid, and flavonol content. Include details on the assay methods, standards, and instruments for these measurements. This is essential for reproducibility and validation of the results.

Answer:

Thanks for this comment. One of the limitations of the present study was the lack of measurement the content of total phenols, flavonoids, and flavonols of the DSE. But these cases have been measured in articles whose extraction method and dosage are completely similar to the present article (33), which were reported in the new version of the present article.

This issue was also stated in the limitations and suggestions for future studies section.

2. The manuscript does not mention the herbarium code or any detailed authentication process for the dill seed used in the study. Provide information on the botanical identification, including the herbarium code and the expert who authenticated the plant.

Answer:

The following sentences were added to the manuscript.

"After purchasing dried dill seed from the Birjand local market (South Khorasan province, Iran) and its confirmation by a botanist, an electric grinder was used to powder the dried dill seeds."

3. Ensure figures and tables are well-labeled with clear legends that include statistical details (e.g., p-values, group comparisons). The figures lack sufficient annotations and statistical markings for key comparisons. Include clear legends with detailed descriptions of the comparisons and significance levels (e.g., P-values). Label all axes with appropriate units.

Answer:

In the new version, all the items requested by the respected reviewer have been reviewed and corrected.

4. One notable issue with the manuscript is the excessive use of abbreviations. While abbreviations help simplify technical terms, overusing them can make the text difficult to read and follow. In figures, tables, and their captions, avoid using abbreviations alone. Instead, provide a brief explanation of each abbreviation used to ensure that readers can understand the information without referring back to the text.

Answer:

In the new version, abbreviations were reduced as much as possible in the text. In figures, tables, and their captions were added brief explanation of each abbreviation.

5. The study lacks histopathological analysis of liver and adipose tissues. Including such data would have provided valuable insights into tissue-level changes. Histological analysis of liver and adipose tissues could have strengthened the interpretation of NNMT and triglyceride findings.

Answer:

Thank you for your valuable feedback. We agree that histopathological analysis of liver and adipose tissues would have provided deeper insights into tissue-level changes. However, our study was designed to assess molecular-level changes, focusing on insulin sensitivity and NNMT levels, rather than tissue-level alterations. Unfortunately, due to the completion of experiments and resource limitations, we are unable to include histopathological analysis at this stage. We acknowledge this limitation and have explicitly mentioned it in the revised manuscript. We hope our findings on molecular-level improvements in insulin sensitivity contribute meaningfully to the field, and we plan to incorporate tissue-level analyses in future studies.

6. Include more structured descriptions of findings. For example, mention specific percentages for key results instead of relying on vague descriptions (e.g., "significant improvement").

Answer:

In the new version, we tried to rewrite this item and added the percentage of changes:

4.1. Body Weight

The repeated measures ANOVA revealed significant effects for time ($F [1, 35] = 196.03$, $P = 0.01$), group ($F [4, 35] = 14.46$, $P = 0.01$), and time × group interaction ($F [4, 35] = 10.40$, $P = 0.01$). The results showed that body weight in the ET, DSE, ET+DSE, and OC groups was significantly higher than in the NC group before the 10-week intervention (with percentage changes of approximately 42.65%, 50.13%, 44.58%, and 49.70%, respectively; $P < 0.001$ for all comparisons). No significant differences were observed between other paired comparisons ($P \geq 0.05$). After the intervention period, body weight was significantly lower in the ET+DSE (15.52%, $P = 0.003$) and ET (14.91%, $P = 0.005$) groups compared to the OC group. Additionally, body weight in the ET+DSE (11.56%, $P = 0.03$) and ET (10.91%, $P = 0.04$) groups was significantly lower than in the DSE group. Higher body weight after the intervention was observed in the DSE (21.76%, $P \leq 0.001$) and OC (27.47%, $P \leq 0.001$) groups compared to the NC group (Figure 2). No significant differences were observed between other paired comparisons ($P \geq 0.05$).

And:

4.2. Glycemic Indices

A significant difference in blood glucose across the groups was observed using one-way ANOVA ($F [4, 35] = 8.04$, $P = 0.001$). Tukey's post hoc test showed that blood glucose was significantly lower in the DSE (10.63%, $P = 0.001$), ET (15.89%, $P = 0.001$), ET+DSE (22.09%, $P = 0.001$), and NC (25.30%, $P = 0.001$) groups compared to the OC group (Figure 3A). The Kruskal-Wallis test revealed significant differences in insulin levels ($\chi^2 = 15.80$, $P = 0.003$) and the QUICKI index ($\chi^2 = 21.24$, $P = 0.001$) between the groups. The Mann-Whitney U test showed significantly lower insulin levels and higher QUICKI indices in the DSE (57.30%, $P = 0.01$ and 11.87%, $P = 0.003$, respectively), ET (48.07%, $P = 0.02$ and 10.66%, $P = 0.01$, respectively), ET+DSE (63.88%, $P = 0.002$ and 16.82%, $P = 0.001$, respectively), and NC (59.00%, $P = 0.007$ and 15.18%, $P = 0.001$, respectively) groups compared to the OC group

(Figure 3B-C). No significant differences were observed in blood glucose, insulin levels, or QUICKI index between the DSE, ET, and ET+DSE groups ($P \geq 0.05$).

Also, for the information of the respected referee, we reported the percentage of changes in other indicators here, but they were not reported in the article due to word limit.

Variables	NC	%	OC	DSE	%	ET	%	DSE+ET	%
Liver NNMT	0.86±0.34	8.86	0.79±0.12	0.86±0.19	8.86	0.86±0.51	8.86	0.87±0.17	10.13
Adipose NNMT	3.87±0.74	3.20	3.75±1.82	3.30±1.24	12.-00	3.30±1.20	12.-00	4.14±1.31	17.60
Liver TG	10.72±3.80	11.-26	12.08±1.83	11.26±3.61	6.7-9	9.33±1.33	22.-76	10.00±3.42	17.-22

%: Percentage change compared to obese control group

Answers to reviewer #2's comments

The authors would like to thank the anonymous reviewer the careful reviews and insightful comments. We tried to response to the following comments with more details. We hope these modifications will meet all expectations of reviewer .

1. The article's title is long and vague. Please refer to the journal guidelines for help.

Answer:

Thanks. In the new version, this item has been rewritten and modified.

"The effect of endurance training and dill seed extract on insulin sensitivity and nicotinamide N-methyltransferase levels in liver and adipose tissue of obese rats"

2. The abstract is not well organized and lacks sufficient coherence. For example, the background could be written better and more precisely so that the reader has a better understanding of the topic. In comparison, the majority of the content in the abstract should be devoted to the results.

Answer:

In the new version, we tried to rewrite this item.

"Background: Evidence suggests that exercise training and dill extract can influence obesity and related metabolic conditions, such as type 2 diabetes.

Results: After 10 weeks of intervention, blood glucose levels were significantly lower in the DSE (10.63%, P = 0.001), ET (15.89%, P = 0.001), ET+DSE (22.09%, P = 0.001), and NC (25.30%, P = 0.001) groups compared to the OC group. Similarly, insulin levels were significantly reduced in the DSE (57.30%, P = 0.01), ET (48.07%, P = 0.02), ET+DSE (63.88%, P = 0.002), and NC (59.00%, P = 0.007) groups compared to the OC group. The QUICKI index was significantly higher in the DSE (11.87%, P = 0.003), ET (10.66%, P = 0.01), ET+DSE (16.82%, P = 0.001), and NC (15.18%, P = 0.001) groups compared to the OC group. However, no significant differences were observed in NNMT levels in the liver and adipose tissue or in liver triglyceride levels between the groups ($P \geq 0.05$)."

3. The authors could expand the introduction by more closely reviewing past studies on the effect of NNMT on metabolic regulation and present the research hypotheses more clearly.

Answer:

The new version examines the effect of NNMT on metabolic regulation in more detail and attempts to express the research hypotheses more clearly.

We added:

Prolonged metabolic stress, such as obesity, leads to adipose tissue dysfunction and altered adipokine secretion, which increases lipid flux to the liver and contributes to an altered metabolic state characterized by insulin resistance (2, 3).

And:

NNMT localizes primarily in adipose tissue and liver (5, 8) and based on evidence NNMT expression is increased in the liver and white adipose tissue of diabetic and obese mice. Also, reducing NNMT expression can reduce insulin resistance and diet-induced obesity (9-11). The preventive effects of NNMT blockade at early stages of adipogenesis have been

highlighted, inhibiting the differentiation of preadipocytes into adipocytes in response to glucocorticoids (12). Moreover, knockout of the NNMT gene resulted in reduced adipogenesis (11).
And:
However, there is no clear scientific evidence that dill extract can regulate the level of some enzymes effective in metabolic diseases such as NNMT, especially in the liver and adipose tissue, which play an effective role in controlling energy homeostasis and occurrence of these diseases.

4. Why was no significant change in NNMT levels in adipose tissue and liver despite improved insulin sensitivity? Discuss this further.
Answer:
Thanks for valuable comment. We have discussed this issue in detail in the discussion section:
“However, a key point in the present study is that despite improvements in glycemic indices following ET and DSE, no significant changes were observed in NNMT levels in the liver and adipose tissue of obese rats. It has been reported that NNMT levels and activity in different organs, particularly the liver and adipose tissue, as well as circulating MNA concentrations, vary under different physiological conditions, such as disease, exercise, and HFD (6, 22). One study showed that NNMT levels in adipose tissue decreased significantly after 12 weeks of ET in diabetic patients with high BMI, although the underlying mechanisms remain unclear (22). The role of NNMT in aerobic and anaerobic energy metabolism also differs (22, 41, 42). For instance, anaerobic exercise has been shown to enhance NNMT expression in skeletal muscles more than aerobic exercise, especially when glucose is the primary energy source (41). Additionally, inhibiting NNMT activity with MNA impaired the performance of rats during anaerobic endurance training (42). The endurance nature of the exercise training used in the present study may explain the lack of significant changes in NNMT levels in the liver and adipose tissue.”
And:

“Another possible reason for the lack of change in NNMT levels in adipose tissue and liver, despite improved insulin sensitivity, is the small sample size and large standard deviation of NNMT data. Larger sample sizes reduce the standard deviation of the distribution of sample means, enhancing confidence in the results and increasing the likelihood of detecting statistical significance (46).”

5.In the discussion, mention the possible role of confounding factors such as fatty acid composition or timing of intervention that could affect NNMT.
Answer:
We have discussed these factors in the discussion:
“It has been reported that feeding mice with HFD selectively increases NNMT activity in adipose tissue while leaving liver activity unchanged (5). Interestingly, we observed no changes in NNMT levels in the liver or adipose tissue under HFD (no difference between the NC and OC groups), which may explain why this enzyme remained unaffected by training and extract consumption. Some studies suggest that the composition of dietary fat and the use of different fatty acids can produce varying metabolic responses, particularly in fat oxidation rates across tissues (43, 44). Diets with a higher proportion of polyunsaturated and monounsaturated

fatty acids (e.g., oleic acid) compared to saturated fatty acids increase fat oxidation in adipose tissue and reduce fat deposition in other tissues, such as the liver, which is critical for preventing HFD-induced metabolic dysfunction. In the present study, the higher ratio of polyunsaturated and monounsaturated fatty acids to saturated fatty acids in the HFD may have increased beta-oxidation of fats, thereby reducing the impact of HFD on NNMT and TG levels. The presence of compounds like oleic acid in HFD can also enhance beta-cell activity and insulin sensitivity (44). Thus, not only the quantity of dietary fats but also their composition plays a fundamental role in metabolic changes, including fat accumulation and lipid metabolism. The duration of HFD exposure may also influence metabolic responses. For example, fat mass increases continuously over 6, 10, and 20 weeks of HFD consumption. However, the expression of adipogenesis regulatory genes (e.g., PPAR, adiponectin, and leptin) peaks early in HFD exposure and gradually declines over time (45). In the current study, prolonged HFD exposure may explain the lack of significant effects on NNMT levels in the liver and adipose tissue, and improvements in insulin sensitivity and glycemic indices may have occurred through mechanisms other than NNMT. Definitive conclusions in this area should be made cautiously, and further research is needed with varying durations of HFD and different fatty acid compositions. Another possible reason for the lack of change in NNMT levels in adipose tissue and liver, despite improved insulin sensitivity, is the small sample size and large standard deviation of NNMT data. Larger sample sizes reduce the standard deviation of the distribution of sample means, enhancing confidence in the results and increasing the likelihood of detecting statistical significance (46)."

However, these sentences have also been incorporated into the revised version:
"Furthermore, NNMT knockdown has been shown to significantly reduce body weight, fat mass, and insulin levels in female mice fed a Western diet (47% kcal from fat and 34% kcal from carbohydrates). However, no changes were observed in body weight, fat mass, fasting blood glucose, insulin levels, or glucose tolerance in HFD-fed NNMT knockdown male mice (60% kcal from fat and 20% kcal from carbohydrates). Gender differences have been proposed as a potential explanation for this discrepancy (47). The exclusive use of male rats in the present study may explain the lack of HFD effects on NNMT. Including both male and female rats in future studies could provide a more comprehensive understanding of this phenomenon."

6.Could the method of extracting the dill or how it was consumed have affected the results? How do you suggest this issue be investigated in future research?
Answer:
Thanks for this comment. In the new version of the article, we have included these sentences in the Limitations:
"Another limitation is the method of dill extraction and administration, which may have influenced the results related to insulin sensitivity and NNMT levels in the liver and adipose tissue. Techniques such as ultrasonic-assisted extraction can enhance the efficiency of extracting beneficial compounds, while hydrodistillation provides an alternative method to isolate essential oils with distinct chemical compositions. In this study, hydrodistillation was used, which is a practical and common extraction method. Future studies should compare different extraction methods (e.g., steam distillation versus solvent extraction) and their effects on insulin sensitivity and NNMT activity in both animal models and humans."

7.Many cited sources are old and need to be updated with new ones.
Answer:

In the new version of the article, we tried to use more recent articles to review the research background and analyze the results. For example, the following references were added to the introduction and discussion of the article:

8.Wei X, Tan Y, Huang J, Dong X, Feng W, Liu T, et al. N1-methylnicotinamide impairs gestational glucose tolerance in mice. *Journal of Molecular Endocrinology*. 2024;**72**(2).

9. Sun W-D, Zhu X-J, Li J-J, Mei Y-Z, Li W-S, Li J-H. Nicotinamide N-methyltransferase (NNMT): a novel therapeutic target for metabolic syndrome. *Frontiers in Pharmacology*. 2024;**15**:1410479.

11. Dong H, Guo W, Yue R, Sun X, Zhou Z. Nuclear nicotinamide adenine dinucleotide deficiency by Nmnat1 deletion impaired hepatic insulin signaling, mitochondrial function, and hepatokine expression in mice fed a high-fat diet. *Laboratory Investigation*. 2024;**104**(3):100329.

17.Babula JJ, Bui D, Stevenson HL, Watowich SJ, Neelakantan H. Nicotinamide N-methyltransferase inhibition mitigates obesity-related metabolic dysfunction. *Diabetes, Obesity and Metabolism*. 2024;**26**(11):5272-82.

8.The manuscript text should undergo a major revision in terms of writing.

Answer:

Thank you for your valuable feedback regarding the language and writing quality of our manuscript. We have carefully revised the text to improve grammar, diction, and overall clarity. A thorough language editing process has been conducted to ensure the manuscript meets high standards of academic writing. We believe these revisions have significantly enhanced the readability and presentation of our work. Thank you again for your constructive suggestion, which has helped us improve the quality of our manuscript.

Answers to reviewer #3's comments

The authors would like to thank the anonymous reviewer the careful reviews and insightful comments. We tried to response to the following comments with more details. We hope these modifications will meet all expectations of reviewer .

1.Please check the whole manuscript grammatically.

Answer:

Thank you for your valuable feedback regarding the language and writing quality of our manuscript. We have carefully revised the text to improve grammar, diction, and overall clarity. A thorough language editing process has been conducted to ensure the manuscript meets high standards of academic writing. We believe these revisions have significantly enhanced the readability and presentation of our work. Thank you again for your constructive suggestion, which has helped us improve the quality of our manuscript. We marked all corrections in blue (second reviewer's opinion).

2. Please mention the details about the importance of this study and why this study was done (novelty) in the background.

Answer:

Thanks for this comment In the new version, we tried to rewrite this item.

We rewrite:

"However, there is limited scientific evidence on whether dill extract can regulate the levels of enzymes involved in metabolic diseases, such as NNMT, particularly in the liver and adipose tissue, which play a critical role in controlling energy homeostasis and the development of these diseases. In summary, understanding the effects of exercise training and dill extract on metabolic responses in the liver and adipose tissue could serve as a therapeutic strategy for reducing metabolic disorders caused by obesity and type 2 diabetes."

3.Please statistically compare the results of each DSE, ET, and ET+DSE together. Which one is a more significant decrease.

Answer:

In the new version, we tried to rewrite this item.

We added this section in new version:

4.1. Body Weight

The repeated measures ANOVA revealed significant effects for time ($F [1, 35] = 196.03$, $P = 0.01$), group ($F [4, 35] = 14.46$, $P = 0.01$), and time × group interaction ($F [4, 35] = 10.40$, $P = 0.01$). The results showed that body weight in the ET, DSE, ET+DSE, and OC groups was significantly higher than in the NC group before the 10-week intervention (with percentage changes of approximately 42.65%, 50.13%, 44.58%, and 49.70%, respectively; $P < 0.001$ for all comparisons). No significant differences were observed between other paired comparisons ($P \geq 0.05$). After the intervention period, body weight was significantly lower in the ET+DSE (15.52%, $P = 0.003$) and ET (14.91%, $P = 0.005$) groups compared to the OC group. Additionally, body weight in the ET+DSE (11.56%, $P = 0.03$) and ET (10.91%, $P = 0.04$) groups was significantly lower than in the DSE group. Higher body weight after the intervention was observed in the DSE (21.76%, $P \leq 0.001$) and OC (27.47%, $P \leq 0.001$) groups compared to the NC group (Figure 2). No significant differences were observed between other paired comparisons ($P \geq 0.05$).

And:

4.2. Glycemic Indices

A significant difference in blood glucose across the groups was observed using one-way ANOVA ($F [4, 35] = 8.04$, $P = 0.001$). Tukey's post hoc test showed that blood glucose was significantly lower in the DSE (10.63%, $P = 0.001$), ET (15.89%, $P = 0.001$), ET+DSE (22.09%, $P = 0.001$), and NC (25.30%, $P = 0.001$) groups compared to the OC group (Figure 3A). The Kruskal-Wallis test revealed significant differences in insulin levels ($\chi^2 = 15.80$, $P = 0.003$) and the QUICKI index ($\chi^2 = 21.24$, $P = 0.001$) between the groups. The Mann-Whitney U test showed significantly lower insulin levels and higher QUICKI indices in the DSE (57.30%, $P = 0.01$ and 11.87%, $P = 0.003$, respectively), ET (48.07%, $P = 0.02$ and 10.66%, $P = 0.01$, respectively), ET+DSE (63.88%, $P = 0.002$ and 16.82%, $P = 0.001$, respectively), and NC (59.00%, $P = 0.007$ and 15.18%, $P = 0.001$, respectively) groups compared to the OC group (Figure 3B-C). No significant differences were observed in blood glucose, insulin levels, or QUICKI index between the DSE, ET, and ET+DSE groups ($P \geq 0.05$).

4. "Obesity is an epidemic in many developed countries with increasing prevalence in developing countries, which increases the risk of metabolic disorders. "Developing countries' word was repeated two times; please revise this sentence.

Answer:

Thanks. It was corrected.

5. The place of this sentence is not suited."Prolonged metabolic stress such as obesity leads to adipose tissue dysfunction and adipokine secretion that results in increased lipid flux to the liver and an altered metabolic state with insulin resistance" Please put them earlier in the introduction.

Answer:

Thanks for this comment. Based on the reviewer 's opinion, we revised and rewrote this section.

"Prolonged metabolic stress, such as obesity, leads to adipose tissue dysfunction and altered adipokine secretion, which increases lipid flux to the liver and contributes to an altered metabolic state characterized by insulin resistance (2, 3)."

6. The title of this is too long and contains the result. Please revise it:" Assessing the effect of endurance training and dill seed extract on insulin sensitivity and nicotinamide N-methyltransferase levels in liver and adipose tissues in Obese rat."

Answer:

Thanks.We revised it.

7. How can authors define the effective dose (300mg/kg) to receive in the rats? Please add references for it.

Answer:

In the new version, we explained the reason for choosing this dose.

"Previous studies have reported that 300 mg/kg is an effective dose for metabolic properties in animal models (34, 35)."

8. Please add subtitles for figures in the manuscript.

Answer:

We added this section.

9. In the result and body weight section, what does the number in the bracket mean?

Answer:

The numbers in brackets are the values of the df for time, group, and the interaction of time and group. For example: The time (F [1 (df of time), 35 (df of Error(time))] = 196.03, P = 0.01), group (F [4 (df of group), 35 (df of Error(time))] = 14.46, P = 0.01), and time × group interaction (F [4 (df of time * group), 35 (df of Error)] = 10.40, P = 0.01) all produced significant effects according to the repeated measures ANOVA.

10. According to Figure 2, is the body weight statistically significant difference between OC and DSE, ET+DSE, and DSE? Please add the statistical analysis between these two groups in the manuscript.

Answer:

Thanks for these comments. We reported all significant differences between groups in the results:

4.1. Body Weight

The repeated measures ANOVA revealed significant effects for time (F [1, 35] = 196.03, P = 0.01), group (F [4, 35] = 14.46, P = 0.01), and time × group interaction (F [4, 35] = 10.40, P = 0.01). The results showed that body weight in the ET, DSE, ET+DSE, and OC groups was significantly higher than in the NC group before the 10-week intervention (with percentage changes of approximately 42.65%, 50.13%, 44.58%, and 49.70%, respectively; $P < 0.001$ for all comparisons). No significant differences were observed between other paired comparisons ($P \geq 0.05$). After the intervention period, body weight was significantly lower in the ET+DSE (15.52%, P = 0.003) and ET (14.91%, P = 0.005) groups compared to the OC group. Additionally, body weight in the ET+DSE (11.56%, P = 0.03) and ET (10.91%, P = 0.04) groups was significantly lower than in the DSE group. Higher body weight after the intervention was observed in the DSE (21.76%, $P \leq 0.001$) and OC (27.47%, $P \leq 0.001$) groups compared to the NC group (Figure 2). No significant differences were observed between other paired comparisons ($P \geq 0.05$).

11. Please add statistical analysis between DSE, ET, and DSE+ET in the manuscript.

Answer:

In the new version, we rewrited this item.

12. According to Figure 4, significant differences could be seen, especially for Figure 4. B. how authors did not see significant differences? Also, please add a statistical analysis of each OC, DSE, ET, and DSE+ET.

Answer:

All reported results are derived from data analysis and statistical testing. The statistical analysis revealed no significant differences between the groups in the NNMT levels in liver and adipose tissue and liver TG. A potential explanation for the concerns raised by the esteemed referee may be the small sample size of the groups, as well as the dispersion of the data, both of which are acknowledged as limitations of this study.

13. Why use "Contrastingly" word in this sentence, as a result, is compatible with the result

of the previous study "Contrastingly, it has been reported that exercise training is a therapeutic intervention and preventive method for insulin resistance "

Answer:

Thanks. It was corrected.

"Consistent with these findings, our results showed that HFD affected glycemic indices (as evidenced by the differences between the NC and OC groups). Additionally, exercise training has been recognized as a therapeutic and preventive intervention for insulin resistance."

14. This sentence should be revised. "No significant change was observed in the NNMT levels of the liver and adipose tissue of obese rats." As I see in Figure 4, a significant reduction of NNMT level was observed in the DSE and ET groups in adipose tissue. Interestingly, in the DSE+ET group, the NNMT level going up. Why?

Answer:

Thanks for this comment. All reported results are derived from data analysis and statistical testing. The statistical analysis revealed no significant differences between the groups in the NNMT levels in liver and adipose tissue and liver TG. A potential explanation for the concerns raised by the esteemed referee may be the small sample size of the groups, as well as the dispersion of the data, both of which are acknowledged as limitations of this study.

15. The word "or" is not suited in this sentence" The role of NNMT in endurance or aerobic and anaerobic energy metabolism is different" Please revise it.

Answer:

Thanks. We rewrote and corrected this sentence in the new version.

16. Why the HFD feeding could increase NNMT in adipose tissue but not in the liver? Please mention the reason. Also, according to the current study, the NNMT level in the ET+DSE group was higher than in the OC group. Why? Why The OC group did not have a higher NNMT level according to this sentence "It was reported that feeding mice with HFD leads to a selective increase in the adipose tissue NNMT activity while the activity in the liver remains unchanged "

Answer:

We would like to express our gratitude for your thorough review, esteemed reviewer. As you noted, this report examines the influence of HFD on NNMT in adipose tissue "*It was reported that feeding mice with HFD leads to a selective increase in the adipose tissue NNMT activity while the activity in the liver remains unchanged*".

And it is stated that due to limitations in the literature research, the precise mechanisms by which a high-fat diet affects NNMT across different tissues remain unclear. Various factors, including diet composition and the duration of the intervention, can significantly influence the relationship between HFD and metabolic variables.

In our article, we have comprehensively addressed these concerns in the discussion section, where we outline potential reasons for the observed lack of effect of HFD on the NNMT levels in liver and adipose tissue. Furthermore, regarding the non-significantly elevated NNMT levels in adipose tissue observed in the ET+DSE group, we propose that these findings may be attributed to the small sample size and data variability. This version maintains the original meaning while enhancing clarity and formality.

17. The fatty acid diet affects the HFD-inducing, beta cell activity and insulin sensitivity. Why did the authors use this composition for this study?

Answer:

Thank you for your insightful comment regarding the high-fat diet (HFD) composition and its effects on beta cell activity and insulin sensitivity. We chose this specific HFD composition because it is a well-established model for inducing obesity and insulin resistance in rodents, closely mimicking the metabolic dysregulation observed in human obesity. This model allowed us to investigate the effects of endurance training (ET) and dill seed extract (DSE) on insulin sensitivity and NNMT levels in a controlled setting of diet-induced metabolic impairment.
While we acknowledge that HFD can influence beta cell activity and insulin sensitivity, our primary focus was to evaluate the therapeutic potential of ET and DSE in improving metabolic parameters in an obese state. The HFD model provided a robust baseline for assessing these interventions. We have clarified this rationale in the revised manuscript to better justify our experimental design. Thank you for raising this important point, which has allowed us to provide a clearer explanation of our methodology.

18. The authors mentioned that the time of feeding rats with an HFD diet could affect the liver's NNMT level. Why authors use 8 weeks for feeding and why the author did not use a period to follow the NNMT level?

Answer:

Thanks for valuable comment.
We stated in the paper “The duration of HFD exposure may also influence metabolic responses. For example, fat mass increases continuously over 6, 10, and 20 weeks of HFD consumption. However, the expression of adipogenesis regulatory genes (e.g., PPAR, adiponectin, and leptin) peaks early in HFD exposure and gradually declines over time (45). In the current study, prolonged HFD exposure may explain the lack of significant effects on NNMT levels in the liver and adipose tissue, and improvements in insulin sensitivity and glycemic indices may have occurred through mechanisms other than NNMT. Definitive conclusions in this area should be made cautiously, and further research is needed with varying durations of HFD and different fatty acid compositions.”

19. As the HFD diet could not able to affect the NNMT level why authors use this diet to perform obese rats? Why authors did not measure intermediate compounds of this pathway addition to NNMT?

Answer:

Thanks. HFD can influence the levels of NNMT; however, in the present study, we observed no significant effect of the HFD on NNMT levels. This finding is particularly noteworthy given that the HFD was effective in enhancing insulin sensitivity. Additionally, the research team faced limitations in measuring further indicators due to the high costs associated with research kits, especially in Iran, as discussed in the limitations section of our study. This revision maintains the original meaning while enhancing clarity and formality.

Answers to Reviewer Assistant Comments

The authors would like to thank the anonymous reviewer the careful reviews and insightful comments. We tried to response to the following comments with more details. We hope these modifications will meet all expectations of reviewer .

Peer Review Using STROBE-Cohort Checklist

1. **Title and Abstract**
    - **Question:** Indicate the study's design with a commonly used term in the title or the abstract.
    - **Peer Review:** The abstract does not explicitly mention that it is a cohort study, which is a minor omission.
2. **Abstract**
    - **Question:** Provide in the abstract an informative and balanced summary of what was done and what was found.
    - **Peer Review:** The abstract provides a clear summary of the objectives, methods, results, and conclusions.
3. **Introduction**
    - **Question:** Explain the scientific background and rationale for the investigation being reported.
    - **Peer Review:** The background mentions the significance of NNMT in obesity and type 2 diabetes, providing a rationale for the study.
4. **Objectives**
    - **Question:** State specific objectives including any prespecified hypotheses.
    - **Peer Review:** The objectives are clearly stated, focusing on the impact of endurance training and dill seed extract on insulin sensitivity and NNMT levels.
5. **Methods**
    - **Question:** Present key elements of study design early in the paper.
    - **Peer Review:** The methods section describes the division of rats into groups and the interventions applied, which are key elements of the study design.
6. **Setting**
    - **Question:** Describe the setting, locations, and relevant dates including periods of recruitment, exposure, follow-up, and data collection.
    - **Peer Review:** The study describes the intervention period (10 weeks) but does not specify the setting or exact dates.
7. **Eligibility Criteria**
    - **Question:** Give the eligibility criteria and the sources and methods of selection of participants. Describe methods of follow-up.
    - **Peer Review:** The eligibility criteria for the rats are not explicitly mentioned, which is a minor omission.
8. **Variables**
    - **Question:** Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria if applicable.
    - **Peer Review:** Outcomes such as insulin sensitivity and NNMT levels are defined, but potential confounders are not discussed.

9. **Data Sources/Measurement**
    - **Question:** For each variable of interest, give sources of data and details of methods of assessment (measurement).
    - **Peer Review:** The methods of assessing insulin sensitivity and NNMT levels are not detailed in the abstract.
10. **Bias**
    - **Question:** Describe any efforts to address potential sources of bias.
    - **Peer Review:** The abstract does not mention efforts to address bias, which is a minor omission.
11. **Study Size**
    - **Question:** Explain how the study size was arrived at.
    - **Peer Review:** The study size is mentioned (40 rats), but the rationale for this size is not provided.
12. **Statistical Methods**
    - **Question:** Describe all statistical methods including those used to control for confounding.
    - **Peer Review:** The abstract mentions statistical significance ($P < 0.0001$) but does not detail the statistical methods used.

Best regards,

Corresponding author

2 Revision 1

1 comment · 11 Feb 2025

Referee 1 Reviewer

ACCEPT 11 Feb 2025

Dear Authors,
Thank you for your thorough revisions and detailed responses to the comments.
While you have cited previous studies that measured these compounds using a similar extraction method, it would strengthen your study if you included direct measurements of these bioactive components in your extract. The absence of this data remains a limitation.
The addition of details regarding the sourcing and authentication of dill seeds is noted. However, providing an official herbarium code would enhance the credibility of botanical identification.