Author’s response to reviews

Title: Effects of Aerobic and Resistance Exercises on Circulating Apelin-12 and Apelin-36 Concentrations in Obese Middle-Aged Women: A Randomized Controlled Trial

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Author’s response to reviews:

Response to Reviewers

Thank you for your helpful comments. Please find our responses below.

Reviewer reports:
Mashael K Alshaikh, PhD candidate (Reviewer 1): 1. why the sample were selected to be 8-8-8? Does it correspond to something as as a sample size calculation?

Response: Sample distribution was determined using the distribution of means. To minimize imbalance in sample size between groups, we applied block randomization in the study. Initially, the study was conducted with sample sizes of 9, 8, and 9. Because of participant dropout owing to personal reasons, the final sample size was 8, 8, and 8.

The following text was added to the manuscript (in blue): To balance the number of subjects per group, a random number table was created and the subjects were assigned by block randomization. (Page 8: Line 138-140)

2. why the study period was conducted for 8 weeks? (why previous study was 6 months?)

Response: In a previous study that studied aerobic and anaerobic exercise in diabetes patients for 6 months, the results showed a reduction in apelin only after resistance exercise. In another study of middle-aged obese subjects (Sheibani et al., 2012), reduction in apelin was found after 8 weeks of aerobic exercise at a different intensity with training at 60-70% of HRmax. To demonstrate the effects
of exercise on reduction in apelin among middle-aged obese women, our study aimed to investigate changes in apelin levels according to exercise type (aerobic versus anaerobic), which could not be clearly proven in the two previous studies.

Accordingly, we set the study period to 8 weeks to match the previous study that showed reduction in apelin from aerobic exercise among middle-aged subjects with no disease history. This was done to validate the results from the previous study and also to identify the effects of anaerobic exercise on apelin.

This is covered in the Background section of the manuscript: Studies have shown a decrease in apelin after 8 weeks of aerobic training at 60–70% maximum heart rate (HRmax) in middle-aged obese individuals [26] and after 6 months of resistance training at 60–80% 1 repetition maximum (1RM) in type 2 diabetes patients [27]. (Page 6: Line 104-108)

3. Patient sample free or not of chronic conditions? (Any exclusion criteria?)

Response: The following people were excluded from the study: those taking medication for chronic disease (such as diabetes, rheumatism, or arthritis); those who have been diagnosed or currently have angina pectoris, myocardial infarction, or brain disease; and those who cannot participate in the experiment due to physical, mental, or physiological impairment.

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4. What was the dietary requirements prior to blood samples collection? (Did the study subjects had to regulate certain food groups?)

Response: Subjects were instructed to abstain from food and drink for at least 12 hours prior to blood sample collection. Alcohol intake was strictly forbidden from the day before the pre-test to after the post-test. Besides alcohol, there were no other dietary restrictions, allowing the subjects to maintain their normal diet.

The following text was added to the manuscript (in blue): Subjects abstained from food and drink for at least 12 hours prior to blood sample collection. Alcohol intake was strictly forbidden from the day before the pre-test to after the post-test. Besides alcohol, there were no other dietary restrictions, allowing the subjects to maintain their normal diet. (Page 8: Line 143-147)

5. social demographic characteristics of the study samples (Married? Occupation? Income?)

Response: We selected middle-class middle-aged married women (50-61 years of age) who were homemakers with no other occupation and had no experience of participating in systematic exercise within 6 months.

The following text was added to the manuscript (in blue): In this study, the subjects were middle-class,
middle-aged women aged 50–61 years who were homemakers with no other occupation and residents of Seodaemun District in Seoul where the exercise experiment was conducted. (Page 7: Line 127-129)

6. how was the randomisation process conducted?
Response: For ethical consideration and prevent skewed results, the experiment used random allocation design. To minimize imbalance in sample size between the groups, we constructed a random number table for block randomization.

As noted above, the following text was added to the manuscript (in blue): To balance the number of subjects per group, a random number table was created and the subjects were assigned by block randomization. (Page 8: Line 138-140)

7. How was the sample recruited? ( Same city/area/part of the country?)
Response: To encourage continued participation and minimize energy being spent in participating in the experiment, such as due to travel, we recruited residents of Seodaemun District, where the experiment was being conducted.

Zuzanna Goluch, PhD., D.Sc., Eng. (Reviewer 2): Comments:
TITLE: correct, corresponds to the work content.
ABSTRACTS: correct.
KEYWORDS: correct.
BACKGROUND:

* line 76 diabetes type 1 or 2?
Response: Type 2 diabetes

We have clarified this in the manuscript (shown in blue).

* line 97 explain abbreviation ACSM.
Response: American College of Sports Medicine

We have clarified this in the manuscript (shown in blue).

* line 102 explain abbreviation HR max.
Response: HRmax: maximum heart rate

We have clarified this in the manuscript (shown in blue).

* line 103 explain abbreviation 1 RM.
Response: 1RM: 1 Repetition Maximum
We have clarified this in the manuscript (shown in blue).

OBJECTIVES: they were properly established and were fulfilled.

MATERIALS AND METHODS
* Materials as well as statistical methods applied are described correctly.
* The authors did not explain in what conditions the anthropometric measurements, necessary for body composition estimates with the use of bioelectrical impedance analysis (BIA), were made.

Response: Waist circumference was measured not only by direct measurement, but also by BIA. The subjects were prohibited from wearing any metallic accessories that may interfere with electrical impedance during BIA and the analysis was performed with all subjects barefooted and wearing the same light clothing provided.

The following text was added to the manuscript (in blue): WC was measured using a tape measure (Seca200, Germany), with the measurement made at the approximate midpoint between the lower margin of the last rib and the top of the iliac crest after exhaling while in upright position, as indicated in the WHO guidelines for Asia and Western Pacific region [29]. (Page 9; Line 159-163).

* Were anthropometric measurements and body composition estimates were done in the morning?

Response: All pre-test and post-test measurements were taken early in the morning before the subjects started any other activities, within 1 hours from waking up after at least 8 hours of sleep.

The following text was added to the manuscript (in blue): Moreover, all pre-test and post-test measurements were taken early in the morning before they started any other activities, within 1 hours from waking up after at least 8 hours of sleep. To meet the measurement conditions, the subjects were provided with light clothing for the measurement. (Page 10; Line 171-175).

* There was no information given in what places were the measures of waist circumference taken? Was it half distance between the inferior margin of costal arch and upper crest of iliac bone or was it another.

Response: WC was measured using a tape measure, with the measurement made at the approximate midpoint between the lower margin of the last rib and the top of the iliac crest after exhaling while in upright position, as indicated in the WHO guidelines for Asia and Western Pacific region (WHO, 1997) World Health Organization. Obesity: preventing and managing the global epidemic: report of a WHO Consultation on Obesity. Geneva, 1997.

The following text was added to the manuscript (in blue): WC was measured using a tape measure (Seca200, Germany), with the measurement made at the approximate midpoint between the lower margin of the last rib and the top of the iliac crest after exhaling while in upright position, as indicated in the WHO guidelines for Asia and Western Pacific region [29]. (Page 9; Line 159-163).
There was no information given as to which reference values (literary source) was the source of classifying the measurements of waist circumference and BMI. BSM330 (Biospace, KOREA) was used and BMI was calculated using the formula weight (kg) ÷ \{height (m)}^2. For waist circumference, a tape measure (Seca200, Seca, Germany) was used.

Response: This was clarified in the manuscript: Body weight, body fat, and BMI (calculated using the formula weight (kg) ÷ \{height (m)}^2) were measured by bioelectrical impedance analysis using an Inbody720 (Bio-space, Korea), a tetrapolar 8-point tactile electrode system.

* Were the patients prepared for measurements (on an empty stomach, after urination and defecation)? Did they do any stretching exercises? Were they administered any diuretic medicine or/and liquids including caffeine 24 hours before the body composition estimates? This information has to be included so that the reader does not doubt the reliability of the performed measurements and the results of the body composition estimates.

Response: To minimize errors that may occur during measurement, the subjects were restricted from exercising, starting from 24 hours prior to the measurement, while they were also instructed not to eat or drink for at least 12 hours prior to the test. The subjects were also instructed to use the restroom to urinate or defecate between 30 minutes and immediately before the measurement and the tests were conducted after the subjects performed simple stretching and warm-up exercise.

Alcohol intake was strictly prohibited during the study period and the subjects were not artificially administered with caffeine, diuretics, or fluids.

The following text was added to the manuscript (in blue): Subjects abstained from food and drink for at least 12 hours prior to blood sample collection. Alcohol intake was strictly forbidden from the day before the pre-test to after the post-test. Besides alcohol, there were no other dietary restrictions, allowing the subjects to maintain their normal diet. Subjects were restricted from exercising, starting from 24 hours prior to measurements, while they were also instructed not to eat or drink for at least 12 hours prior to the test. The subjects were also instructed to use the restroom to urinate or defecate between 30 minutes and immediately before the measurement was taken. (Page:8; Line 143-150)

* Moreover, please add information that the body composition estimate with bioelectrical impedance analysis (BIA) was made on Inbody720 apparatus in a tetrapolar setting.

Response: We added an explanation in the manuscript that Inbody720 used in the experiment is a tetrapolar 8-point tactile electrode system.

RESULTS
* Properly and readably written.

* Internationally accepted rules and the international system of units (SI) should be used in Table

Response: The system of unit was changed from ng/ml to ng/μmL.

DISCUSSION:
* Factual discussion, but the Authors should more broadly refer to the results (value) of other researchers who have done similar experiments.

Response: Recently, there are active studies on apelin as an adipokine, but it was difficult to find studies from broad fields. Accordingly, we conducted the study based on our belief that there is a need. In the future, we also plan to conduct additional studies on the relationship between improvement in apelin concentration and insulin resistance.

CONCLUSION:
* lines the 283-309 are not specific conclusions but summary. The only conclusions a reader might find are suggested in the abstract.

Response: Thank you. The content in the abstract has been supplemented and the content in the conclusion sections has been revised.

REFERENCES:
* 93.6% of literature comes from the last decade.
  Response: After apelin was initially identified as one of the G protein-coupled receptor in 1993 (O’Dowd et al., 1993), it was also identified as an adipokine that helps regulation of insulin secretion and lipolysis in 2008 (Kunduzova et al., 2008). As a result, active studies have been conducted within the last 10 years. Accordingly, we also felt the need to study this topic.

* According to publements identifier DOI should be given in the reference list.

Response: Thank you. References, DOI list //DOI (digital content identifiers) have been added.