Author’s response to reviews

Title: The relationship between the leptin/ghrelin ratio and meals with various macronutrient contents in men with different nutritional status: a randomized crossover study.

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Author’s response to reviews:

We wish to thank the Editor and Reviewers for their contributions and comments, and our manuscript has been improved by incorporating their suggestions. The grammatical errors and language style have been corrected, using a professional language editing service recommended by the Nutrition Journal. We have submitted a revised version that addresses the comments, and our responses to each Reviewer are detailed below.

Reviewer reports: This is a follow-up study to a paper published by the same authors in 'Nutrients', where the same cohort was used. The authors do not mention that paper, from which much of the design and statistical approach was obtained (or copied word for word in many instances). Regardless, using the leptin to ghrelin ratio to differentiate obese from normal individuals fed different carbohydrate diets is not a novel finding, nor does it fulfill an unknown in the field. There are also many grammatical errors and awkward sentences throughout the manuscript.

Answer: The description of design and statistical approach has been corrected that it differs from our other, just published paper (lines 146-161), and our previously and recently published papers have been mentioned (lines 114-116). To our knowledge, the studies that investigated leptin/ghrelin ratio are very limited, and we did not find any with the same aims and results, as our experiment. Moreover, the aim of our study was not to investigate the use of leptin/ghrelin ratio to differentiate normal weight from obese men fed various diets, but to investigate the differences in acute postprandial responses dependently on the different meal composition, and to assess the possible differences in postprandial leptin/ghrelin ratio between lean and obese.
Subjects. The grammatical errors and language style have been corrected, using a professional language editing service, one of the recommended by the Nutrition Journal.

Answers for Reviewer 1:

Reviewer’s comment: The authors have conducted a cross over RCT investigating leptin/ghrelin ratio in response to meals with different macronutrient content, in a study population of normal weight, as well as overweight/obese healthy males. It was of interest to note there were marked differences in fasting and postprandial hormone levels in response to macronutrient composition of meals, when comparing results of normal weight to overweight/obese subjects. Findings from this paper may be useful to inform the delivery of targeted advice regarding macronutrient composition of meals to help regulate satiety.

This paper contributes interesting findings to nutrition research, however, will benefit from revising grammatical errors, and adopting a scientific style of writing. For example, references to "people" within the manuscript should be replaced with "subjects" or "participants". Various sentences require re-structuring and/or re-wording, and it is recommended that the manuscript in its entirety be reviewed.

Answer: We thank the Reviewer for these comments. The grammatical errors have been corrected and the style of writing has been improved, and adopted to a scientific style, using a professional language editing service.

Reviewer’s comment: There also appears to be a lack of consistency in spacing between references and text.

Answer: The consistency in spacing between text and references has been checked and corrected. The spaces between references and text have been removed.

Reviewer’s comment: Further comments regarding specific sections of the manuscript are as follows:

Background

The study aim should be stated within the background section, not in the methods section.

Answer: The aim of the study has been stated within the Background Section (lines 105-108).

Reviewer’s comment: Background could benefit from including 1-2 referenced statements describing the cascade of hormonal response to different macronutrients, to acknowledge leptin & ghrelin are not the sole hormones responsible for regulating satiety/hunger.
Answer: The Background has been updated as suggested, and the other hormones and factors involved in satiety/hunger regulation has been mentioned, with references (lines 73-81).

Reviewer’s comment: Stronger justification is needed to highlight the importance of leptin & ghrelin, and why other hormones were not investigated in this study.

Answer: The importance been updated (lines 104-105), and it was explained that leptin and ghrelin were chosen to investigate in our study, because their interaction plays one of the key roles in hormonal regulation of food intake (lines 78-81).

Reviewer’s comment: Does any published literature specify an ideal leptin/ghrelin ratio range?

Answer: To our knowledge there are just few studies, which investigated the leptin/ghrelin or ghrelin/leptin ratio, and the ideal range has not been specified yet.

Reviewer’s comment:

Method

Please specify/reference classification for overweight/obese subjects.

Answer: The classification of overweight/obese subjects has been specified (lines 119-120).

Reviewer’s comment: Need clarity of study duration, i.e. each study phase.

Answer: The study duration was approximately 3 weeks, and this information has been added in text (lines 130-132).

Reviewer’s comment: Was the wash out period 1 or 2 weeks? Lack of consistency with the wash out period can confound results.

Answer: The wash out period was at least 1 week, and maximum 2 weeks, dependently on subject availabilities (lines 131-132), and participants were asked to maintain their usual diet and lifestyle throughout the study, to minimalize the influence of the confounding factors, what was mentioned in lines 132-133.

Reviewer’s comment: Please specify if this study was designed to investigate acute, or long-term, hormonal responses.
Answer: The aim of this study was to investigate an acute hormonal response. It has been clarified in the Background section (lines 105-108).

Reviewer’s comment: Description of meals will also be helpful for the methods section.

Answer: The meals are described in Methods section (lines 121-122, 124-125, 127-129), as well as in Table 2, providing also the manufacturer, energy and macronutrient content.

Reviewer’s comment: Could more in-depth description be provided for the biochemical analysis?

Answer: The detailed procedures according to which the biochemical analysis were conducted are available on the websites of kits producers (provided in text, lines 139-144), therefore we did not want to duplicate information that is generally available.

Reviewer’s comment: Results

Significant results should be discussed within the results section and denoted accordingly in the supporting tables and figures. This is currently missing.

Answer: The results of leptin/ghrelin ratio have been described in Results section, and discussed as recommended in Discussion section. Moreover results are presented in Table 3 and Table 4, as well as in Figure 2 and Figure 3.

Reviewer’s comment: It is unclear what "standardised meals" refer to in line 170.

Answer: The “standardized meals” means that the composition of the meals was known, and it was exactly the same in every tested HC-, NC- and HF-meal, and this term is known and commonly being used in other papers.

Reviewer’s comment: There needs to be a more distinct explanation to distinguish the difference between results presented in Tables 3 & 4 to Figures 2 & 3.

Answer: In Tables are presented the comparisons dependent on the meal type, in normal body weight or overweight/obese men. The Figures present the differences between normal body weight and overweight/obese men after the same HC-, NC-, or HF-meal intake. It has been explained in the Tables/Figures descriptions and legends.

Reviewer’s comment: Titles are missing from all figures.
Answer: All of the titles were available in the text, in the Figure Legends section (after the References, lines 378-396).

Reviewer’s comment: Please specify units for BMI in Table 1.

Answer: The units for BMI have been updated in Table 1.

Reviewer’s comment: Discussion:

Paragraphs are needed throughout this section.

Answer: The paragraphs throughout the Discussion section have been added.

Reviewer’s comment: Lines 179 - 182 will benefit from re-structuring to justify the study aim.

Answer: The sentence (after updating-lines 184-187) has been re-structured as suggested, to justify the study aim.

Reviewer’s comment: Lines 191-196 will benefit from providing referenced studies describing suggested mechanisms and/or implications for reported observations.

Answer: The studies describing suggested mechanisms for our observations have been cited (lines 202-207).

Reviewer’s comment: Please provide brief explanation of findings from references cited in lines 210-212.

Answer: In mentioned lines we just cited the findings of other Authors, which support the aim of our study, and the explanations of these findings are widely discussed in the cited articles. In our opinion, providing explanation will extend our article significantly, because there are many pathways and mechanisms, which can not be explained briefly.

Reviewer’s comment: Study limitations have not been addressed.

Answer: The study limitations have been added (lines 238-244).

Reviewer’s comment: References:
Formatting for 1st reference needs to be amended.

Answer: The 1st reference has been removed, since it was not necessary, those information's are available in the 2nd reference (after updating it is the 1st one).

The answers to Reviewer 2

Reviewer’s comment: Title and conclusion: the study concerned only to men

Answer: The title has been updated, and it was mentioned that only men were included.

Reviewer’s comment: Background: the lack of the aim of this study and identify the validity of the subject.

Answer: The aim of the study, and the validity of the subject have been added (lines 104-108).

Reviewer’s comment: Methods: In my opinion, the description of composition of model meals should be more precise - kind of fat and carbohydrate is needed.

Answer: The aim of our study was to compare meals with varying macronutrients content (% of total energy), without taking into account details, since if we would consider and discuss the kinds of fats, the kinds of carbohydrates, the kinds of proteins, then following, it should be compared for example two different HF meals with saturated and unsaturated fats, and next monounsaturated with polyunsaturated, and so on and so on… but it was not the aim of our study, therefore we did not include these information's, which by the way are generally available at website of the manufacturer (that we provided), if needed. Moreover, there are also studies, which show that if meals are matched for energy, macronutrient, and fiber content, then the effects on appetite sensations are similar, independently on the macronutrient sources (and therefore its kinds)(Nielsen LV et. al. Protein from Meat or Vegetable Sources in Meals Matched for Fiber Content has Similar Effects on Subjective Appetite Sensations and Energy Intake—A Randomized Acute Cross-Over Meal Test Study. Nutrients. 2018 Jan 16;10(1). pii: E96. doi: 10.3390/nu10010096.).

Reviewer’s comment: Moreover tested hypotheses were too simple, may be body composition should be added.

Answer: The aim of the study was to compare between normal body weight and overweigh/obese men, and the commonly accepted criteria to identify these groups are based on BMI, therefore participants were divided as described, based on BMI.
Reviewer’s comment: Authors wrote in Background that p.4; 89-90: "Leptin secretion is proportional to total amount of adipose tissue", but it did not include in analysis

Answer: The mentioned sentence is not an observation from our study, but it is a citation of results of other Authors. Please see the linked reference, which was included at the end of the sentence (line 95).

Reviewer’s comment: Figure 1 not presented the study design, it does not take into account visits and examinations

Answer: We can not agree with this comment, since this is a one of the way that studies designs are presented in many other papers.

Reviewer’s comment: Results: In table 3 and 4, please provide explanations of all symbols and statistical tests. I think that the data contained in Figures 2 and 3 can be added to Tables 3 and 4.

Answer: All symbols were already explained in the Figure Legends section, as well as all of the statistical tests were described in Methods section. The Figures and Tables present different comparisons, which were described in the titles and legends. Could we kindly ask the Reviewer to explain in details, or to propose any way to combine the results from Figures and Tables, to keep them still legible to read and not get confused looking at them? In our opinion we could not put all results and comparisons together, because it would be difficult to follow.

Reviewer’s comment: Generally, due to the modest number of results and analyzes, both their description and discussion are simplified and bring nothing new. The final conclusion recommending the avoidance of HC-meals by obese people with frequent insulin resistance, is too obvious.

Answer: We can not agree with this opinion, since the leptin/ghrelin ratio has been investigated only by few studies, and furthermore, with the different aims. Moreover, one of the main conclusion of our study is that “in normal body weight men, a more beneficial leptin/ghrelin ratio was noted after the HC-meal intake, compared to the NC- and HF-meals” what is not so obvious, especially considering the current “fashion” of very low carbohydrate diets.