Author’s response to reviews

Title: Novel causes and consequences of overtraining syndrome: the EROS-DISRUPTORS study

Authors:

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Author’s response to reviews:

The point-by-point response to reviewers, which shows the new table and figure, and differentiate between reviewers comments, authors responses, and included or modified words and sentences are attached as supplemental material. The text of the responses follows below:

"Point-by-point response to reviewers

“Novel causes and consequences of overtraining syndrome: the EROS-DISRUPTORS study”

Flavio Adsuara Cadegiani, MD, MSc, PhD; Claudio E. Kater, MD, PhD

Legend:

- Italic: authors responses
- Bold: words and sentences included or modified in the manuscript

General responses:

We are truly thankful for the review performed by both researchers. We know that the subject of the manuscript may be challenging to evaluate, due to its complexity and lack of clear characteristics. Both reviewers, regardless of their opinions, really helped us raise the quality of this manuscript.

The highly distinct evaluations between the two reviewers shows the heterogeneity regarding the conceptions on OTS among different experts on the field.
Our responses to reviewers are in italic.

Julien Louis (Reviewer 1):

General response from authors to reviewer 1:

Reviewer 1 was extremely important to highlight the missing information of the present manuscript. We fully addressed the points raised by him and included the information that he noticed to lack. Indeed, the revised version of the present manuscript has become way more complete. The description of the 38 parameters evaluated in an illustrated table, the methodology containing the key data and the baseline characteristics will be of great help and clarification for readers

However, many of the points raised are accessible within the manuscript.

Also, the reviewer claims that this is a report of an existing literature, which it is not. All information in the present study is novel, not previously published, and results from a deep and thorough statistical analysis which was allowed by the large number of different sorts of parameters in a relatively large number of participants (compared to previous papers on the same subject).

Additionally, reviewer 1 questioned about training patterns. We have now included the baseline training characteristics, which were similar in all aspects between affected and healthy athletes. This means that training patterns were not a trigger for OTS in the studied population of athletes.

- This article aimed to better inform on the causes and consequences of the overtraining syndrome (OTS). Although the question is sound and important for the field, the article falls far short of its goal.

R. “Falls far short of its goal”, when the answer seems clear, sounds a bit emotional, as the reviewer fails to point the reasons why it falls far short, while we clearly pointed the novel causes (eating patterns) and consequences (reduced late hormonal responses and the testosterone-to-estradiol ratio, worsened mood, and affected the immunology panel).

- Many inaccuracies and lacks in the different parts of the article make it difficult to understand.

R. “Many inaccuracies” – we would really appreciate that each of these multiple inaccuracies could be pointed out, as constructive/productive critics should focus on the improvement of the quality of the object of the critics.
For instance:

- more information is required on the methods utilised and specifically on the papers selected for analysis. The authors chose to focus their analysis on nutritional variables and sleep, but we do not know what the inclusion criteria were. I also assume that the number of OTS studies reporting dietary and sleep patterns of athletes is rather limited. As such the readers need to understand the sources of information. Therefore all the references selected for analysis should be presented in the article.

R. The reviewer is absolutely right regarding the lack of specification of inclusion criteria. Hence, we decided to include the inclusion criteria, as well as the specification of each of the studies included in this manuscript.

The inclusion criteria help to clarify how true/actual OTS-affected athletes were selected.

Of more than 110 parameters, we analyzed all the most important characteristics and habits that could be correlated with OTS. Among these, nutritional and sleeping patterns were shown to be keys for the comprehension of how OTS occurs.

Compared to previous studies, we consider that we performed a broad and comprehensive analysis, encompassing multiple aspects. If further asked, we may bring ALL previous published papers on OTS, that do not address more than one to two different aspects that could influence OTS. Once this as a complex and multifactorial disorder, we considered that the lack of previous understanding of OTS may have been due to the lack of evaluation of multiple aspects.

A substantial change on how to assess OTS in studies was needed, and this is what we suggest. Among these changes, the evaluation of eating and sleeping patterns, which still lacks in studies on OTS, as considered by the reviewer admits (“I also assume that the number of OTS studies reporting dietary and sleep patterns of athletes is rather limited”). With the introduction of the specification of the material and methods, the sources of information are now clear for readers.

To clarify, we included an additional table (Table 1) in which we describe all 117 markers, and those that were excluded (and why) and those that were included. We also included an additional figure (Figure 1) to illustrate the parameters included in the present analysis.

Please find the paragraphs and the table included in the manuscript below (sentences included are highlighted in bold):

“For the present analysis, we performed a comprehensive joint statistical analysis of data from five of the arms of the EROS study, including four of primary findings: the EROS-HPA axis, in which we evaluated the hypothalamic-pituitary-adrenal axis hormonal responses in athletes (4);
The EROS-STRESS, in which we evaluated the prolactin and growth hormone (GH) responses to an exercise-independent stimulation test – the insulin tolerance test (ITT) – as well as the glucose behavior during this test (5); the EROS-PROFILE, in which we evaluated eating, psychological, sleeping, and social patterns (6); the EROS-BASAL, in which we evaluated basal hormones, inflammatory, immune, and muscular levels (7), and the additional EROS-HIFT arm, in which we evaluated specific characteristics of healthy and OTS-affected HIFT (including CrossFit) athletes (8).

Full descriptions of the materials and methods (i.e., the selection of participants and study procedures), results of the statistical analyses of data, and their respective discussions are available in these five of the arms of the present study (4-8), as well as in a depository (4-8, https://osf.io/bhpq9), which also has the raw data of the results of each participant.

Subject and parameter selection

We recruited participants through calls for participation in social media and group messages, and invitations to sports coaches. Prior to interview, each candidate self-reported sex, age, body mass index (BMI), and whether he intended to participate as a healthy athlete, clinically suspected for OTS, or healthy sedentary. Aiming homogenous groups, we specific criteria for all groups, of OTS-affected athletes (OTS group), healthy athletes (ATL group), and non-physically active controls (NPAC group) including sex (male), age (18-50 years old), BMI (20–29.9 kg/m2 for sedentary and 20–32.9 kg/m2 for athletes), absence of known hormonal, metabolic, inflammatory, or psychiatric disorders, non-current or recent use of drugs or hormones. In order to avoid false athletes, for the two groups of athletes we required a minimum amount of training per week (> 300 min and > four times a week), intensity of training (at least moderate-intensity, according to their sport coaches), time since started non-stop training (> six months). To avoid misdiagnosis of OTS, for athletes suspected of OTS, we required a sports-coach certified reduction of at least 10% of previous performance, or a loss of > 20% in time to fatigue, increased sense of effort for a same training intensity and volume, persistent fatigue that lasted > two weeks, unresponse to resting, and lack of use of confounding drugs or hormones, and presence of confounding diseases. For all candidates that fulfilled criteria for any of the three groups, we performed hormones and basic biochemical profile and avoided those who presented alterations in any of the tested parameters.

Identification of independent triggers and consequences of OTS

In the present study, we performed a joint multivariate and logistic regression analyses for the identification of independent triggers and consequences of OTS, among those parameters that were suitable for the diagnosis or as characteristic of OTS, and significantly different between
OTS and ATL. From the 117 evaluated parameters in the EROS study (4-8), 32 were non-diagnostic, not useful, or unsubstantiated; nine were qualitative (yes vs no), three had missing data in > 5% of participants, and 27 had similar levels (Table 1). Hence, we elected 43 hormonal responses to stimulation tests, basal and accumulated hormonal levels, social and psychological aspects, specific eating patterns, and body metabolism and composition parameters, among which 38 were variables dependent of five modifiable variables (eating, sleeping, and social patterns) plus the presence of OTS as an additional variable, in a total of 44 variables (38 dependent and 6 independent variables) with two groups of athletes: the OTS and ATL groups (N = 39) (Figure 1). Despite additional analyses of the remaining data were also performed, they were not included in the present analysis, as they were not eligible. The variability of the biochemical markers measured in all arms of the EROS study were as low as 3.5% and 3% for inter- and intra-assay coefficients, respectively.

Table 1. Markers included in the present analysis, among those evaluated by the EROS study.

<table>
<thead>
<tr>
<th>Study/Tests</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of markers: 117</td>
<td></td>
</tr>
<tr>
<td>(Included:45)</td>
<td>Whether included or excluded (and if excluded, why)</td>
</tr>
<tr>
<td>EROS-HPA axis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total number of markers: 20 Included markers: 7</td>
</tr>
</tbody>
</table>

Basal ACTH and cortisol and their response to an insulin tolerance test (ITT)

1. Basal cortisol (µg/dL)            INCLUDED
2. Cortisol during hypoglycemia (µg/dL) INCLUDED
3. Cortisol 30 min after hypoglycemia (µg/dL) INCLUDED
4. Cortisol increase during ITT (µg/dL) Similar levels between OTS and ATL
5. Basal ACTH (pg/mL)                Similar levels between OTS and ATL
6. ACTH during hypoglycemia (pg/mL)  INCLUDED
7. ACTH 30 min after hypoglycemia (pg/mL) INCLUDED

8. ACTH increase during ITT (pg/mL) Unsubstantiated marker

9. Basal ACTH/cortisol ratio Unsubstantiated marker

10. ACTH/cortisol ratio during hypoglycemia Similar levels between OTS and ATL

11. ACTH/cortisol ratio 30 min after hypoglycemia Unsubstantiated marker (although different between OTS and ATL)

Cortisol response to a cosyntropin stimulation test (CST)

12. Cortisol at 30 min after synthetic ACTH shot (µg/dL)

13. Cortisol at 60 min after synthetic ACTH shot (µg/dL)

14. Difference between basal cortisol on day 1 (CST) and day 3 (ITT) (%) Similar levels between OTS and ATL

Similar levels between OTS and ATL

Not diagnostic or helpful

Salivary cortisol rhythm (SCR)

15. Salivary cortisol at awakening (ng/dL) INCLUDED

16. Salivary cortisol 30 min after wakening (ng/dL) INCLUDED

17. Salivary cortisol at 4PM (ng/dL) Similar levels between OTS and ATL

18. Salivary cortisol at 11PM (ng/dL) Similar levels between OTS and ATL
19. Cortisol awakening response (CAR) (%) Not diagnostic or helpful

20. Difference between 8AM and 4PM salivary cortisol Similar levels between OTS and ATL

EROS-STRESS  Total number of evaluated markers: 12

    Included markers: 7

GH and Prolactin response to an ITT

1. Basal (GH) (µg/L) INCLUDED

2. GH during hypoglycaemia (µg/L) INCLUDED

3. GH 30 min after hypoglycaemia (µg/L) INCLUDED

4. Basal prolactin (ng/mL) INCLUDED

5. Prolactin during hypoglycaemia (ng/mL) INCLUDED

6. Prolactin 30 min after hypoglycaemia INCLUDED (ng/mL)

7. Prolactin increase during ITT (ng/mL) INCLUDED

8. Basal serum glucose (mg/dL) Not diagnostic or helpful

9. Serum glucose during hypoglycemia Not diagnostic or helpful (mg/dL)

10. Capillary glucose during hypoglycemia Not diagnostic or helpful (although different between OTS and ATL)

11. Adrenergic symptoms during Not diagnostic or helpful
hypoglicemia (0-10)

12. Neuroglycopenic symptoms during hypoglicemia (0-10)

EROS-BASAL (total number of evaluated markers: 32)

Included markers: 9

Hormonal markers

1. Total testosterone (ng/dL) INCLUDED
2. Estradiol (pg/mL) INCLUDED
3. IGF-1 (pg/mL) Similar levels between OTS and ATL
4. TSH (µUI/mL) Similar levels between OTS and ATL
5. Free T3 (pg/mL) Similar levels between OTS and ATL
6. Total catecholamines (µg/12h) INCLUDED
7. Total metanephrines (µg/12h) Similar levels between OTS and ATL
8. Noradrenaline (µg/12h) Similar levels between OTS and ATL
9. Epinephrine (µg/12h) Similar levels between OTS and ATL
10. Dopamine (µg/12h) INCLUDED
11. Metanephrine (µg/12h) Similar levels between OTS and ATL
12. Normetanephrine (µg/12h) Similar levels between OTS and ATL
13. Catecholamine-to-metanephrine ratio Similar levels between OTS and ATL

Biochemical markers

Not diagnostic or helpful (although diferente between OTS and ATL)
14. Erythrocyte sedimentation rate (ESR, mm/h)  
   Similar levels between OTS and ATL

15. Hematocrit (%)  
   Similar levels between OTS and ATL

16. C-reactive protein (CRP, mg/dL)  
   Similar levels between OTS and ATL

17. Lactate (nMol/L)  
   INCLUDED

18. Vitamin B12 (pg/mL)  
   Similar levels between OTS and ATL

19. Ferritin (ng/mL)  
   Similar levels between OTS and ATL

20. Neutrophils (*mm3)  
   INCLUDED

21. Lymphocyte (*mm3)  
   Similar levels between OTS and ATL

22. Eosinophils (*mm3)  
   Similar levels between OTS and ATL

23. Creatine kinase (CK, U/L)  
   INCLUDED

24. Medium corpuscular volume (MCV)  
   Not diagnostic or helpful

25. Platelets (103/mm)  
   Not diagnostic or helpful

26. Low density lipoprotein cholesterol (LDLc) (mg/dL)  
   Data missed in > 5% of participants

27. High density lipoprotein cholesterol (HDLc) (mg/dL)  
   Data missed in > 5% of participants

28. Tryglicerides (mg/dL)  
   Data missed in > 5% of participants
Ratios

29. Testosterone-to-oestradiol ratio  INCLUDED
30. Testosterone-to-cortisol ratio  Similar levels between OTS and ATL
31. Neutrophil-to-lymphocyte ratio  INCLUDED
32. Platelet-to-lymphocyte ratios  Similar levels between OTS and ATL

EROS-PROFILE  (total number of evaluated markers: 53)
  Included markers: 21

Nutritional patterns

1. Calorie intake (kcal/kg/day)  INCLUDED (as a modifiable habit*)
2. Carbohydrate intake (g/kg/day)  INCLUDED (as a modifiable habit*)
3. % calories from carbohydrate (%)  Intrinsically linked to other parameters
4. Protein intake (g/kg/day)  INCLUDED (as a modifiable habit*)
5. % calories from protein (%)  Intrinsically linked to other parameters
6. Fat intake (g/kg/day)  Similar levels between OTS and ATL
7. % calories from fat (%)  Intrinsically linked to other parameters
8. Carbohydrate intake > 3g/kg/day (Y/N)  Qualitative marker
9. Daily whey protein consumption (Y/N)  Qualitative marker
10. Followed a diet plan (Y/N)  Qualitative marker
11. Post-workout carbohydrate intake > 0.5g/kg (Y/N)  Qualitative marker

Psychological patterns
12. Profile of Mood State (POMS) questionnaire (total score: -32 to +120) INCLUDED

13. Anger subscale (0 to 48) INCLUDED

14. Confusion subscale (0 to 28) INCLUDED

15. Depression subscale (0 to 60) INCLUDED

16. Vigour subscale (0 to 32) INCLUDED

17. Fatigue subscale (0 to 28) INCLUDED

18. Tension subscale (0 to 36) INCLUDED

19. How do you feel today? (0-10) Not diagnostic or helpful (although different between OTS and ATL)

20. Have you been sick in the last two weeks? (Y/N) ? Not diagnostic or helpful

21. How was your last training session compared to the projected goals? (Extremely easy to extremely hard) Not diagnostic or helpful

22. How do your muscles feel? (Nothing at all to extremely painful) Not diagnostic or helpful (although different between OTS and ATL)

23. How friendly do you feel today? (0-6) Not diagnostic or helpful

24. How worthless do you feel today? (0-6) Not diagnostic or helpful

25. How miserable do you feel today? (0-6) Not diagnostic or helpful

26. How helpful do you feel today? (0-6) Not diagnostic or helpful (although different between OTS and ATL)

27. How bad-tempered do you feel today? (0-6) Not diagnostic or helpful

28. How unworthy do you feel today? (0-6) Not diagnostic or helpful

29. How peeved do you feel today? (0-6) Not diagnostic or helpful
30. How cheerful do you feel today? (0-6) Not diagnostic or helpful (although
different between OTS and ATL)

31. How sad do you feel today? (0-6) Not diagnostic or helpful

32. Number of hours of activities besides professional training (h/day) INCLUDED (as a modifiable habit*)

Qualitative marker

Social patterns

33. Duration of night sleep (h) Similar levels between OTS and ATL

34. Self-reported sleep quality (0–10) INCLUDED (as a modifiable habit*)

35. Self-reported libido (0–10) INCLUDED

36. Initial insomnia (Y/N) Qualitative marker

37. Terminal insomnia (Y/N) Qualitative marker

38. More than two wake-ups during sleep (Y/N) Qualitative marker

39. Work and/or study (Y/N) Qualitative marker

40. Libido during resting periods / vacations (0-10) Not diagnostic or helpful

Body metabolism analysis

41. Measured-to-predicted basal metabolic rate (BMR, %) INCLUDED

42. Percentage of fat burning compared to total BMR (%) INCLUDED

Body composition
43. Body fat percentage (%)   INCLUDED
44. Visceral fat (cm2)   INCLUDED
45. Muscle mass weight (%)   INCLUDED
46. Body water percentage (BW, %)   INCLUDED
47. Extracellular water compared to total BW (%)   INCLUDED
48. Visceral fat (cm2)   INCLUDED
49. Chest to waist circumference   Not diagnostic or helpful
50. Waist circumference (cm)   Not diagnostic or helpful
51. Chest circumference (cm)   Not diagnostic or helpful
52. Biceps circumference (cm)   Not diagnostic or helpful
53. Hip circumference (cm)   Not diagnostic or helpful

*For statistical purposes, modifiable factors were considered as independent variables, from which the dependent variables were statistically

- It is also not clear whether the authors considered OTS (a rare and extreme stage of overreaching) or overreaching (which is more common and reported in several studies).

R. Although overreaching is indeed much less rare than overt OTS, all athletes were diagnoses with true OTS. In addition we observed that OTS is highly misdiagnoses, as we excluded almost 85% of the athletes initially suspected for OTS. We included a sentence describing the characteristics of OTS in the affected athletes, in which actual OTS can be identified in all them, as follows below:

“All 14 participants selected for the OTS group had true and naturally occurring presence of OTS, not functional or non-functional overreaching, as all athletes had a verified decrement of > 10% of previous sports performance and fatigue that were prolonged (average duration of fatigue and decreased performance = 44.3±23.0 days), and none has fully recovered by the time of the study. Supplementary information regarding the selection process and baseline characteristics have been previously published (4-8).”
- In the methods, the authors indicate that 38 variables were taken into account in the analysis. What are these variables?

R. We included an illustrative figure showing the 38 variables. In Table 1, which is attached above, we describe the selection process for the parameters evaluated in the present analysis. Please find Figure 1 below:

- The abstract is also not clear enough.

R. We improved abstract by giving more details and making it more clear, and almost “self-sufficient” for readers, as below (change are highlighted in bold):

“Background: Hormonal physiology in athletes, dysfunctional paths leading to overtraining syndrome (OTS), and clinical and biochemical behaviors that are independently modified by the presence of OTS remain unclear. Although the Endocrine and Metabolic Responses on Overtraining Syndrome (EROS) study unveiled multiple markers of OTS, the independent influence of OTS on hormones and metabolism were not assessed in that study. Hence, the objective of the present study was to uncover the previously unrecognized independent predictors of OTS and understand how OTS independently modifies the behaviors of clinical and biochemical parameters, using novel data from the EROS study.

Methods: In a total of 39 athletes (OTS = 14 and ATL = 25), we performed two clusters of statistical analyses using the full data of the EROS-HPA axis, EROS-STRESS, EROS-PROFILE, and EROS-BASAL arms of the EROS study, in a total of 117 markers. We first used logistic regression to analyze five modifiable parameters (carbohydrate, protein, and overall caloric intake, sleep quality, and concurrent cognitive effort) as potential additional independent risk factors for OTS, and OTS as the outcome. We then used multivariate linear regression to analyze OTS as the independent variable and 38 dependent variables. Training patterns were found to be similar between OTS and ATL, and therefore excessive training was not a risk, and consequently not a predictor, for OTS.

Results: Each of the three dietary patterns (daily carbohydrate, daily protein, and daily overall calorie intake) were found to be the independent triggers of OTS, while sleeping, social, and training characteristics depended on other factors to induce OTS. Once triggered, OTS independently induced multiple changes, including reductions of cortisol, late growth hormone
and adrenocorticotropic hormone responses to stimulations, testosterone-to-estradiol ratio, neutrophils, neutrophil-to-lymphocyte ratio, vigor levels, hydration status, and muscle mass, while increase of tension levels and visceral fat.

Conclusions: The EROS-DISRUPTOR study unveiled that OTS can be independently triggered by eating patterns, regardless of training patterns, while the occurrence of OTS reduced late hormonal responses and the testosterone-to-estradiol ratio, worsened mood, and affected the immunology panel. These novel findings may explain underperformance, which is the key characteristic of OTS. "

-The figure is incomplete as physical activity must be present to induce OTS and not just sleep and food intake. There is also no mention of a reduction in physical performance as a consequence of OTS.

R. The presence of reduced performance is a criteria for the diagnosis of OTS, not a disruption caused by OTS. After revising, we improved the figure by providing a more detailed explanation of triggers (1st column), characteristics (2nd column) and consequences (3rd column) of OTS, as below:

Current figure:

Previous figure:

- The results section includes some elements of discussion which is quite confusing.

R. The reviewer was right both points. We transferred sentences that should had been included in the discussion section, and clarified the results section.

Transferred from results to discussion: “In addition, it is important to mention that a very high OR is likely to be a statistical overestimation of an association of different variables when one variable is the sole predictor of an outcome (in this case, OTS) without controlling for other variables.”

Improvement of the results section:

“When analyzed together, at least one factor between low carbohydrate, low protein intake, low overall caloric intake, and poor sleep quality was present in 100% of the study’s cases.”

“Carbohydrate intake was found to be an independent trigger of OTS when it was analyzed together with sleep and social patterns, with an odds ratio (OR) = 1.61, [confidence limits (CL) =
1.03–2.50] for the risk of developing OTS, while its ability to induce OTS was lost without the concurrent analysis of sleep quality.

Conversely, protein intake was shown to independently induce OTS without the concurrent analysis of any of the other possible triggers in all scenarios. Likewise, overall caloric intake independently induced OTS, irrespective of the proportions of macronutrients, indicating that if caloric intake (but no carbohydrate or protein intake), work hours, and sleep quality had been analyzed together as the three modifiable habits, caloric intake would have been the only independent trigger \((p = 0.004; \ OR = 1.13 \ [CL = 1.04-1.23])\) between these three variables. In contrast, excessive work and poor sleep quality, each failed to induce OTS independently, regardless of the combinations of predictors.”

“With respect to the basal hormones, OTS reduced the testosterone-to-estradiol (T:E) ratio by 43, while it did not modulate total testosterone, estradiol, or any of the other hormones. Conversely, the basic immunology panel, including neutrophils, lymphocytes, and the neutrophil-to-lymphocyte ratio were influenced by the occurrence of OTS, although at lower degrees of association, and only when combined with other triggers.

OTS also affected tension, fatigue, and vigor levels when evaluated through the Profile of Mood States (POMS) questionnaire, accounting for 43%, 84%, and 86% of their levels, respectively. While OTS did not show to affect any aspect of body metabolism, it independently led to reductions in muscle mass and body water content to 34% and 51%, respectively, and an increase in visceral fat to 38%. While visceral fat was increased in OTS, overall body fat was unchanged by the presence of OTS.”

At this stage, considering the existing literature on OTS, it is recommended to focus on experimental projects designed to identify the mechanisms of OTS rather than summaries/analyses of the literature.

R. In the EROS study we strongly focused on projects designed for the identification of the mechanisms and characteristics of OTS. The present paper is a highly valuable paper bringing PREVIOUSLY UNPUBLISHED results of a more comprehensive and deeper statistical analysis of the multiple parameters evaluated by the EROS study.

Ian Walshe (Reviewer 2)

General response from authors to reviewer 2
Reviewer 2 performed a thorough analysis and provided useful suggestions for the improvement of the manuscript. We considered the review performed by reviewer 2 highly valuable for our work.

- The study aimed to use logistic regression to analyze five modifiable parameters to predict OTS as an outcome. In addition, Multivariate linear regression was used to analyze the impact of OTS on hormonal and immunological parameters as well as body composition and subjective feelings.

This is a very interesting article and the authors should be congratulated on working with a population of this nature in such depth. I do, of course, have some comments.

Introduction

- Only 3 reviews have been cited in the introduction (excluding self-citation). You should include more literature from other groups in this section.

R. As properly recommended, we included 06 additional references to reinforce the introduction and background for the objective of the study.

- Line 71: Maybe add in …Despite the name "overtraining syndrome," referring to excessive exercise training, other modifiable factors may trigger OTS.

R. We added accordingly, which helped to improve the comprehension of the sentence.

Methods

- It would be useful to have very brief description of the study procedures, diagnosis of OTS etc. rather than referring your previous work; some articles may require subscription.

R. We have not described the selection process for healthy athletes and for the actual diagnosis of OTS, and markers tested were described in the new table that we designed and included (Table 1).

- Line 105: N=39, is this both OTS and ATL? If so, would it be possible to report the n of each group?

R. We included accordingly (OTS = 12 and ATL = 25).
- Line 106: Should this be selected rather than elected?

R. Corrected accordingly.

- Line 125-127: Can you clarify what you mean by this? It would seem that the study is under powered.

R. We’re sorry, we made ourselves misunderstood. We corrected the sentence as below:

“Given the context of the present study and its main objective, the number of participants in the present study was found to be sufficient for the number of variables and outcomes for the present logistic regression analyses”

Results

- Without seeing the full methods, it is hard to interpret these results, making it hard for the reader. e.g. line 141-143:

R. With the methods now included, results are now easy to interpret.

- "Carbohydrate intake was found to be an independent trigger of OTS when it was analyzed together with sleep and social patterns with an odds ratio (OR) = 1.61, [confidence limits (CL) = 1.03-2.50] for the risk of developing OTS…”

Were the variables measured before diagnosis of OTS? If so, this would indeed indicate the risk of developing OTS. However, if the variables were assessed after diagnosis of OTS, this could be interpreted as OTS leading to a loss of appetite (reduced CHO intake), impaired sleep and change in other cognitive activity.

R. All athletes strictly followed their diet plans, regardless of their appetite (we also question this type of dietary approach to athletes). The carbohydrate intake was measured using a 7-day diet record, prior to the diagnosis of OTS, indicating this as a risk of developing OTS. In the methods section, more specifically in the new Table 1, we detail this.

- Line 166: I think this should refer to body composition rather than body metabolism.

R. This refers to body metabolism. We clarified as below:
“any aspect of body metabolism (ratio between measured and expected basal metabolic rate (BMR) and percentage of fat oxidation)”

Discussion

- You mention that each athlete with OTS exhibited a unique combination of altered markers. While it is important to assess multiple markers, it is also important to note that the thresholds for working and studying activity will differ between athletes. Equally, sleep requirements and dietary requirements will also differ between athletes. It is important to acknowledge that the change in these activities are more important than absolute values.

R. The reviewer is absolutely right. We included highlights of the points brought by the reviewer, as below: “Noteworthy, OTS is more likely to occur after changes in eating, sleeping and/or social patterns. In clinical practice, dietary characteristics should be assessed prior to other triggers, and whenever they do not indicate the presence of OTS, sleep and social patterns should be investigated. However, there is not a specific threshold for each activity or habit, as each of them will highly depend on the combination with other potential triggers of OTS.”

- Line 212-216: You mention changes in behaviors. The methods and results do not reflect that you have assessed changes in behavior.

R. OTS was shown to independently change tension, vigor, and enhance fatigue levels (as a sort of vicious cycle), as we made explicit:

“supported the conclusion that these changes in behaviors were inherently due to the presence of OTS, as the occurrence of OTS was shown to independently increase tension levels and blunt vigor levels, while may independently enhance fatigue, as a sort of a vicious cycle, since fatigue is also one of the features of OTS.”