Author’s response to reviews


Authors:

Flavio Cadegiani (f.cadegiani@gmail.com)
Claudio Kater (claudio.kater@gmail.com)

Version: 2 Date: 21 Aug 2019

Author’s response to reviews:


Overall response:

We truly appreciate the thorough reviews, which were helpful to raise the quality of our paper.

Legends:

1. Changes in the manuscript are highlighted in bold
2. Point-to point responses to reviewers are highlighted in italic, right after each point.

Specific (point-by-point) responses to reviewers:

Mauro Lombardo (Reviewer 1):

The response of the hypothalamic-pituitary-adrenal (HPA) axis to ITT is exacerbated in healthy athletes compared to healthy sedentary subjects. ITT can be a tool to assess whether the athlete is well conditioned and to predict performance once the exacerbation of HPA axis responses can play an important role in the progressive improvement of sports performance.
In this cross-sectional study, healthy athletes (ATL) and nonphysically active healthy controls (NPAC) have been selected to describe the theory of the novel hormonal conditioning mechanism using the findings from another study.

I think the selection criteria for patients should be reviewed. The two small groups have a large numerical difference: 25 healthy athletes (ATL) versus 12 non-physically active healthy controls (NPAC), the age range is very wide: 18-50 years old, and the BMI range (20-30 kg/m2) should be restricted. These criteria means to include young and old people; healthy and overweight patients, these differences could effect HPA as demonstrated in other papers.

The suggestion is to change the inclusion criteria: restrict the subjects' BMI range and limit age differences. It would also be useful to reduce the difference in the number of subjects between the two groups. The criterion of selection of subjects considered "athletes" should also be better investigated.

R.

Indeed, the ranges for age and BMI are apparently wide, which could compromise the results. Despite these potential differences within and between groups, all the groups had similar age and BMI with low variability.

Our search for sedentary yet healthy participants was challenging, as most of them were excluded within the selection criteria, for both higher age and BMI. Expectedly, those who fulfilled the criteria and participated in the study were the youngest and with lower BMI (but within the normal range), and quite similar to the characteristics of athletes.

For further studies, discussions regarding the appropriate ranges for age and BMI are valid, although a general agreement is unlikely, as the appropriate ranges depend on the parameters to be evaluated and differ among them. Meanwhile, in the absence of the demonstration of significant differences when within the proposed ranges, thi

However, we want to bring some arguments for the ranges proposed by the study:

1. Age:
   a. Despite the wide range, when between 18 and 50 years old in males, differences in hormones do occur, but do not tend to be as meaningful to the point that invalidate a study. The most remarkable changes are a yearly decrease of 1% of testosterone and a slight progressive reduction of IGF-1 levels. In addition, studies demonstrated a high variability of hormonal levels, even when adjusted for age. Despite having the strongest evidence of the decrease throughout life, normal range for testosterone levels are not generally according to age, while normal ranges for IGF-1 has great differences between essay kits.
b. In regards to the HPA axis, we were unable to find substantiation in the literature for the differences in the HPA responsiveness according to the age, at least when below 50 y/o, despite the widely spread alleged differences. In case one has original literature (not books or reviews) for this, we would be happy to receive them and improve our methods.

c. Studies in the endocrinology field rarely cluster their findings according to age intervals, unless the comparison between different ages is the main objective of the study, or when they are performed in both young adults and those above 60-65 y/o.

2. Body Mass Index (BMI):

a. Similarly to what occurred with the age range, despite a large BMI range allowed in the inclusion criteria, there is not sufficient scientific demonstration of differences in hormonal responsiveness to stimulations, particularly to the insulin tolerance test (ITT), at least when within the proposed BMI range.

b. The inclusion of BMI up to 32.0 kg/m² took into account the reportedly increased BMI of some of the athletes that practice sports that include strength exercises. The BMI criteria for sedentary was up to 29.9 kg/m².

Just to reinforce, both BMI and age were similar between groups and also within groups, with no substantial differences between any of the participants.

(INCLUDE THE SELECTION PROCESS IN A DIFFERENT GRAPH)

The selection of athletes with unclear criteria is another limitation of the study. Mixing strength and endurance exercises and not registering the trainer’s load systematically does not allow to be sure of the degree and type of physical activity.

R. Mixed sports, such as high intensity functional training (HIFT), largely known by one of its brand names (CrossFit), which includes both endurance and strength exercises, are challenging not only due to an apparent lack of standardization methods for the register of mixed exercises are based on composed evaluation of the training level, but also due to its inherent irregularity of the sequence of exercises and training sessions.

For this, standardized tests have been systematically evaluated, in order to homogenize the criteria for competitions and classifications of the athletes according to the performance, particularly in the face of a sort of sports whose competitions are many times performed simultaneously in different places, which reinforces that homogenous criteria is imperative.
From this perspective the athletes that performed HIFT were evaluated for within-group and between-groups comparisons.

Those athletes that did not practice HIFT but practiced both strength and resistance exercises were regularly and closely followed by individual coaches. We excluded those who did not fulfill the criteria for a minimum level of training. Together with the athletes excluded for OTS, more than 50% of the candidates were excluded, due to our rigidness with the proposed criteria.

Anthony C. Hackney (Reviewer 2):
There were no comments from this reviewer.

Karl Neff (Reviewer 3):
Thank you for this interesting study comparing the results of CST and ITT between a group of athletes (ATL) and a sedentary control group (NPAC) matched for BMI and age (but not for fat mass or lean muscle mass).

R. We truly thank the reviewer for the points raised. Indeed, the concerns of the reviewer #3 are consistent, and we were already considering to clarify these points.

With questions and concerns raised by the reviewer #3 brought us the opportunity to raise the quality of our paper, by providing a logical and fully-based manuscript. We tried to perform a beautiful work to make the understanding of the theory and why this is true novelty, decreased the level of certainty of the findings, and illustrated the arguments that support our hypothesis.

As seen below, we consider that we fully addressed the points of the reviewer #3.

The results show that there are differential responses within multiple pituitary axes. These data are clear and well-presented. However, they are not novel. The effect of exercise on pituitary/hypothalamic function is well documented, and while the authors data contribute to consolidation of this evidence base, they do not really offer any new insights. I appreciate that the use of paired CST and IST allows for some distinction between adrenal and pituitary function in the ATL group, and this is something that could be developed to enhance our understanding of the HPA axis in the ATL cohort. However, we have known for many years that HPA responsiveness is enhanced by exercise. The element that you can really contribute to the evidence base is that the basis for this effect seems to be pituitary or hypothalamic rather than adrenal. This is not a novel concept, but your data do aid our understanding of this effect. The biggest problem for your paper is that you claim to demonstrate evidence for a hormonal conditioning process. You do not. The data you present show that responses to ITT differ between ATL and NPAC participants at one moment in time.
R.

Before the systematic review that we performed on hormonal responses on overtraining syndrome (OTS), we had assumed that the hypothalamic-pituitary (HP) axes, particularly the adrenal (HPA) axis, were enhanced in athletes, compared to general population, exactly as proposed by the reviewer.

However, we failed to find supportive original data for any independent process of hormonal optimization. Reviews and chapters referred their statements to references that did not actually evaluate differences between paired athletes and sedentary, differently from what the revisor thinks and from what we used to think.

We also found no support for the assumption of optimized HPA axis response to stimulations to be found in athletes, in an independent manner from enhanced musculoskeletal or cardiovascular signaling. For this specific point, we performed a thorough review searching for studies that performed tests that did not have these confounders, such as optimized muscular signaling, and we were not able to identify any manuscript that compared athletes with sedentary that tested hormonal responses independently of performance.

Thus, although widely spread, to us, the assumption of enhanced HPA as well as other axes responses should be disregarded.

This example reminds us of other parameters which were widely employed by studies in sports medicine, such as the testosterone-to-cortisol ratio. Although testosterone and cortisol are initially anabolic and catabolic hormones, respectively, in the long run cortisol become a visceral fat anabolic hormone, which can be observed in patients with Cushing’s syndrome, in which they tend to gain a lot of weight, not only due to water retention. Also, there is not enough arguments from an endocrinology perspective to support a rationale for this ratio.

We consider that hormonal aspects, physiology, and adaptive changes in response to exercise should be deeply revisited, from a standardized perspective, in association with endocrinologists or endocrinology societies.

In regards to claim for the possibility of the existence of a novel intrinsic, and independent hormone conditioning process in athletes,

1. The actual lack of existence of a true, intrinsic, and independent enhancement hormonal responsiveness to stimulations that do not require signaling from any other organ or tissue.

2. The current optimization of hormonal responses may be secondary to increased muscular or cardiovascular stimulations, as these stimulations were dependent on exercise.

3. The conditioning processes found by the study remain present after overestimated adjustments for body fat, muscle mass, and water content, although how these adjustments should be employed is unknown, as well as if these factors really influence the hormonal responses
4. The differences found between athletes and sedentary are not due to performance, body composition, presence of dysfunctions, and are not secondary to differences in any other tissue. Hence, the most plausible data is indeed the hormonal conditioning proposed by the study.

However, as we highlighted, this should be confirmed longitudinally within the same participants during a conditioning program, from a sedentary state until the level of an athlete, which will also help to identify when and in which sequence these conditioning processes occur.

It is important to note that differences in responses in performance-dependent tests are vastly described. But they do not allow the conclusion regarding the level of the optimization of the hormonal response.

Although apparently largely spread in the sports medicine field, the concepts of hormonal optimization responses to physical activity, independently from other systems, are unfamiliar to endocrinologists.

In a constructive manner, in case the reviewer has literature to support any of the points he brought, and that we were unable to find, we would highly appreciate to receive them, and them to rewrite our paper. But we would like actual studies, not books or empty references.

An additional figure comparing the previous studies with the current findings to highlight the AN ADDITIONAL FIGURE COMPARING THE PREVIOUS WITH THE KNOWLEDGE PROPOSED BY THE PRESENT PAPER WILL BE PROVIDED, showing the human body and the axes.

This could be due to differences in any number of confounding variables such as muscle mass (fat mass is clearly different between groups despite comparable weights and BMIs, inferring significant differences in muscle mass), diet or sleep.

R.

The production of myokines and adipokines by the muscle and fat tissue, respectively, likely influences the level of the hypothalamic-pituitary responsiveness, as well as other factors, although unlikely to an extent that could even the differences between athletes and sedentary.

We need to consider that unlike exercise-dependent stimulations, such as maximal exercise or two-bout exercise protocol, the hypothalamic-pituitary responses to the ITT and CST are entirely independent from any signaling from other tissues or organs, which help prevent from the influences of body composition.
Indeed, with the exception of the GH response to a central stimulation test, which has been demonstrated to be influenced by body fat, (and which we adjusted), we were unable to find literature that supports the existence of differences of hormonal responsiveness to central stimulations (i.e., stimulations that do not depend on musculoskeletal, cardiovascular, or other external signaling to respond) according to the level body fat or muscle mass.

We were also unable to identify studies in the past that aimed to evaluate differences in hormonal responsiveness according to muscle, fat, or water content, in healthy athletes or healthy sedentary.

Despite the arguments above, we decided to perform fat- and muscle-adjusted results using an overestimation of the influences of these factors, although this sorts of adjustments are not present in studies, even on those in which participants with both normal and high BMI are present. Differences remained significant, substantial, and with a narrow confidence interval, which reinforces our findings. As a standardized calculation for these adjustments, and even until which extent these factors really influences the hormonal responses to a direct, independent stimulation test, are unknown.

We included the following paragraph:

In regards with differences as being secondary to muscle of fat mass, there is no sufficient literature to support their influences, except for the negative influence of body fat on GH release. Despite the lack of supporting literature for these influences, we employed overestimated adjustments for muscle and fat mass, and differences between healthy athletes and sedentary remained significant.

The ATL group may eat or sleep very differently to the NPAC group. How can we say it it the exercise or training of the ATL group that produce the proposed pituitary effect, and not simply changes in diet or sleep hygiene?

R.

For both selection criteria and as parameters of the EROS study, we evaluated specific dietary patterns using a thorough and specific 7-day record of the diet, and specific characteristics of sleep, mostly self-reported, but employed in a validated and standardized way. This was published in the EROS-PROFILE study. Besides, tests were performed during fasting state, which precluded from differences in macronutrient intake or even glycogen storage.

We included the following paragraphs to the manuscript:

“In addition, a 7-day dietary record with specific calorie and macronutrient account, and self-reported sleeping patterns, social and psychological characteristics, basal muscular, inflammatory, immunologic and hormonal parameters, and body composition and metabolism were evaluated in all selected participants.”
"These findings were not related to differences in sleeping or sleeping patterns, as groups had similar sleeping quality, duration, and hygiene, and tests were conducted after a period of fasting, respectively."

So while we can say there is a difference between groups, we cannot say for sure why this difference occurs.

R.

Taking into account that:

a. The ITT directly tests the integrity of the hypothalamic-pituitary axes, without any interference of other systems
b. The CST, which directly stimulates the cortisol release using a synthetic ACTH, which was shown to be normal
c. Differences are not justified by differences in age, BMI, and even after adjusted for fat or muscle

There seems to be no explanation other than a “hyperresponsiveness” of the hypothalamic-pituitary axes, which occurred in a diffuse manner, as optimized responses were not selective.

To us, this is the first study to:

a. Compare age-, sex-, and BMI-matched healthy athletes and healthy non-physically active subjects
b. Exclude for a massive number of confounding factors
c. Did not have any bias in terms differences in hormonal responsiveness, from the perspective of actual literature
d. Performed tests that isolated the endocrine from cardiovascular, musculoskeletal, autonomic, and other systems, to avoid interferences from external signaling
e. The criteria and methods utilized fully satisfy the strict endocrinological criteria for a standardized test

Taking into account the arguments above, we did find sufficient data to propose the existence of an independent process of hormonal conditioning in athletes, despite the low number of athletes, due to the considerable differences with results almost clustered in their respective groups, with very few overlapping responses between athletes and sedentary.

To highlight the support why our findings should be considered, we included the following:

“Considering that:
d. Different from other tests, the ITT directly tests the integrity of the hypothalamic-pituitary axes, without any interference from other systems, including cardiovascular and musculoskeletal;

e. Unlike previous studies that employed exercise-dependent tests, in which differences in responses could be attributed to differences in performance, the ITT does not have any influence from the physical capacity or performance, which evens athletes and non-athletes;

f. Differences between athletes and sedentary are not justified by differences in age, sex, or BMI, since groups had similar baseline characteristics, and differences remained after further adjustments;

g. A large number of clinical and biochemical confounding factors, including the presence of clinical conditions, biochemical abnormalities, and influences from eating and sleeping patterns, were excluded;

h. There are not any potential biases from what the existing literature has already demonstrated regarding the tests performed;

i. The criteria and hormonal functional tests employed in the present study are highly standardized, and fully satisfy the strict endocrinological criteria for an appropriate test;

j. Results were highly distinct, with important differences in the mean of median, and narrow standard deviations and confidence intervals, respectively, and almost absence of overlapping results; and

k. There seems to be no plausible explanation other than the existence of a “hyperresponsiveness” of the hypothalamic-pituitary axes to justify the present findings in athletes,”

Even if we assume there is a difference that is due to training or exercise, to prove that there is pituitary adaptation, a persistent effect would need to be demonstrated.

R.

We should an optimization of an acute response of pituitary, for at least 60 minutes after the stimulation. For studies in the endocrinology field, this is enough to demonstrate an interference in the HPA and lactotrophic (prolactin) axes. For the GH axis (somatotrophic axis), when GH response is below 3 µg/L, ITT should be repeated. However, sedentary athletes did respond with GH levels above 3 µg/L, which does not require repeated tests. Hence, at least from an endocrinology perspective, there are no need to evaluate a persistent effect in this case.

To do this, you would need to recruit a sedentary group, put them on an exercise programme for a period of at least a few weeks, and then allow them to become sedentary again, while testing them with ITT and CST at each phase.
This is the next step, which we are currently working on. Actually, besides working in a longitudinal study (we termed “from the sedentary to the athlete”), we also divided into 4 groups: program of a. endurance training, b. strength training, c. “stop-and-go” (explosive) training (ball sports, at a professional level), and d. mixed sports (“CrossFit”, HIFT). Then we will also be able to determine whether some of these optimized responses are dependent on the sort of exercise performed.

We are controlling for eating, sleeping, and concurrent cognitive patterns, in order to avoid these important sources of interferences. By the way, we claim studies in the sports medicine field to improve the level of control of these external factors.

By design, the ATL group exercise frequently. How can we be sure that the ITT differences seen are not due to recent exercise (rather than a chronic adaptation)?

Athletes did not perform any exercise for 120 hours (05 days) before the test, which completely prevents from any interference from recent exercise. Thus, this is indeed a chronic adaptation.

We included this important information in the manuscript, correctly raised by the reviewer: “Subjects underwent the ITT 48 hours after the CST, following an 8-hour fasting and a minimum of 120 hours without exercising.”

There are no longitudinal data in your paper. We cannot say based on the data presented that there is a persistent pituitary response to exercise. Therefore the proposal that there is evidence for a pituitary’adaptation' is fundamentally flawed.

The first step of the demonstration of a novel mechanism as a result from an intervention is to compare two groups in which the fundamental difference is the intervention – in this case, physical activity at an intense and regular level.

Once the presence of this novel mechanism is demonstrated, then longitudinal studies should be perform, because they will show:

1. Whether the conditioning process occur within the same participants, which reinforces the existence of the proposed mechanism, and

2. When during the longitudinal process, and in which sequence it occurs.
However, taking into account that groups were similar, and the thorough and strict multistep selection process that was employed, and the availability of the full raw data, the present study does have sufficient data to demonstrate the existence of this mechanism.

Similar demonstrations with weaker arguments and control for variabilities, and lower number of participants, have been extensively published in the sports medicine and in the endocrinology literature. We are open to send as many examples as required.

Hence, we respectfully disagree that we flawed, at least from an endocrinology point of view, as we extensively demonstrated the reasons why we found the evidence for a hypothalamic-pituitary adaptation (we cannot conclude this is a pituitary adaptation, because none of the tests differentiated them), and why the arguments against are mostly based in assumptions, rather than scientific data that belie our findings.

I think you have some good data, but the conclusions drawn are not supported by the data presented. Therefore, I cannot recommend publication of this paper in its current form.

R. We have decreased the strength of words we used in the conclusion in order to demonstrate what we found as a possibility, not a confirmation.

Also, we included the additional figure (Figure 4) to demonstrate why there is sufficient data to propose the existence of “hormonal conditioning”:

I would recommend redrafting your paper to focus on the evidence that the hormonal changes seen are due to a pituitary or hypothalamic effect, rather than a primary adrenal effect.

R. We have now highlighted this in the manuscript, as below:

“The exacerbated response observed in the hypothalamic-pituitary-adrenal (HPA) axis of the athletes was not found when adrenals were directly stimulated through the CST, showing that the conditioning process of the HPA axis does not occur in the adrenal glands, but centrally instead.”

Again, this is expected, but your paper does offer some confirmatory data that may be suitable for publication.

R. We decreased the level of strength of the findings, but at the same time questioned what could have occurred.
I hope the above is useful. Thank you again for your paper.

R. Was very useful and consistent! Thank you.

Final comments to reviewer #3:

Despite the rich arguments brought by this great reviewer, which are, indeed, a general perception among the best scientists in the field, some are based on assumptions, not actual scientific data, as demonstrated in this point-by-point response. Our aim is also to clarify which statements spread in the sports medicine field are actually supported and which require further confirmatory data to be stated.