Journal of Clinical and Translational Research
Peer review process file 07.202104.008

Is lifelong endurance training associated with maintaining levels of testosterone, IL-10, and body fat in middle-aged males?

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Review timeline:

Received: 6 April, 2021
Editorial decision: 10 May, 2021
Revision received: 15 June, 2021
Editorial decision: 27 June, 2021
Published online: 16 July, 2021

1st Editorial decision
10-May-2021

Ref.: Ms. No. JCTRes-D-21-00051
Is lifelong endurance training associated with maintaining levels of testosterone, IL-10, and body fat in middle-aged males?
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Dear Mrs. Gutierrez,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Jun 09, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: The authors showed that endurance-trained middle-aged men has higher testosterone and interleukin 10 level and lower body fat compared to untrained men. The results are quite expected and the authors need to showcase the novelty of their study. I am also concern with the methods of measuring body fat and the lack of adjustment in correlation study. Other comments:
1. Abstract: add the methods of body composition measure.
2. For sample size calculation, was it performed before or after the study? it is strange to set the target power at 0.7, usually 0.8.
3. The UAM are overweight. Why didn't the authors recruit UAM that did not have weight problem?
4. The correlation study was not adjusted for important confounding factors like age and BMI.
5. Other confounding factors are not disclosed, like duration of training for EMA.
6. Apart from providing citation, I hope the authors can describe the body composition measurement in details because it is an important variable here.

Reviewer #2: General
The topic as such is highly relevant and more knowledge is needed within the area.
However, the present data-set does not add much to the area as two very different groups of subjects (both insufficiently described) are compared, but conclusions are drawn in much broader terms than justified (see specific example under Conclusion).

Major missing points include - age-distribution - matching on other parameters - details/considerations on training history - which sports are they competing in

Recruitment as part of larger/other study - clearly refer to data from this - reasoning for it not being compiled, rather than publishing 'just' IL-10, testosterone and body fat?

Inadequate data to support the comparison between the two groups, as partly described in limitations.

Abstact -
I would spell out Testosterone, rather than using the abbreviation T - throughout man-uscript
Page 1
L 39: underwent to - two
L 39: if details on analyses are given in abstract, please specify kit and assay rather than general terms

Page 2
Relevance for patients - there is discrepancy between engaging in lifelong/chronic endurance training, and implementation of this to reduce and prevent metabolic dis-ease etc (adoption of endurance training as part of a healthy lifestyle). When is it too late to begin? What is life-long? Some consideration on duration as part of life-span. I believe it is never too late, but there is a long way from findings in cross-sectional stud-ies of life-long training vs implementation of training regimens in prevention

1.Introduction
Page 3
L12: - resulting in sarcopenia - the reduction in muscle mass and strength is part of the definition of sarcopenia. Sarcopenia definition should be revisited and the introduction reword accordingly.

2. Methods
Page 4
L 38 - should it not be given which ethics committee?

P5
No selection based on age? Only criteria is above 35
Was any mathing between individuals in the two groups performed (eg regarding age - and other parameters)?

L9 - their modality - which in/exclusion criteria for which sport they were competing in
L 14 - sedentary and otherwise healthy needs definition/selection criteria
L34 - seven skin-fold - why was a dexa scan not performed?

3. Results
It is stated that 'Age did not differ between groups' although the trained group were on average 5 years older and a p-value of 0.056 between groups. Giving the actual age-range or distribution within each group would clarify this a bit.
I acknowledge that this would, if anything, reduce the chance of detecting relevant dif-ferences between the two groups, but I think it is a major confounder and disturbs the basic question of the study.

As nothing is stated on inclusion based on age (only above 35), this is a major concern.

Figure 1.
Instead of just *, give p-value, at least in legend

4. Discussion

P10
L 58: while these were

P11
L 4: Frequent is not enough - these athletes have been competing at international lev-els for +15 years
Measurements of other cytokines would be highly relevant. For example TNFa as dis-cussed. To make a more complete story on the pro/anti-inflammatory status of these subjects

L36: include not measure - rephrase

Limitations are not sufficiently addressed - and why was physical fitness not meas-ured?

5. Conclusion
Two very different groups (both insufficiently described) are compared, but conclusions are drawn in much broader terms than justified: eg Page 12:
Given the global preponderance of chronic disease, more widespread adoption
of endurance training as part of an overall healthy lifestyle could eventually reduce the occurrence of such diseases in the population. Adequate physical training may have clinical applications for attenuating T decline, decreasing body fat, and maintaining an adequate anti-inflammatory
level, especially in middle age.

Reviewer #3: This manuscript looks at the effect of lifelong training on testosterone, IL-10 and %body fat by using a population of endurance master athletes. The study is well thought and one of the few using master athletes to look at the effects of lifelong exercise in preventing ageing and chronic diseases. In the methodology it would be important to characterize the groups according to their physical fitness and in the steps that have been taken to make sure the blood sample taken represents a rested baseline state. If possible the inclusion of pro-inflammatory markers like TNF-alpha or IL-6 would improve the study. The discussion could be enhanced by a more detailed discussion. Also in discussion, the phrase "EMA are a successful model of adequate nutrition, stress management and consistent training for decades..." maybe a bit optimistic since "adequate nutrition and stress management" can not be generalized to all endurance athletes.

I suggest correcting the phrases: line 24 "The hypothesis was that EMA has higher IL-10 and..." change has for "have". Line 36, page 10 "not measure the nutritional status" change to "not measuring ..."

Reviewer #4: I suggest to accept the article. Well amended.

Authors' response

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June 9, 2021

Dr. Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Ref. Revised version of the manuscript (id: **JCTRes-D-21-00051**): *"Is lifelong endurance training associated with maintaining levels of testosterone, IL-10, and body fat in middle-aged males?"*

Dear Dr. Heger,

First of all, we appreciate the reviewers' valuable comments on the manuscript. We accepted all suggestions and are now resubmitting this revised version to the **Journal of Clinical and Translational Research**. The changes in the manuscript are highlighted in red and this response letter answers each point of the comments of the reviewers. We hope that these changes together with our replies to the reviewers are acceptable, and we look forward to your final decision on the paper. Also, we want to include a co-author in our article, who participated in the analyses, but

we had forgotten to insert her name. We added her name in the co-authors and also here in the review letter along with her email: Lysleine Alves de Deus (lys.deus@gmail.com).

Sincerely,

Sara Duarte Gutierrez, Ms.

REVIEWER 1

Comments to the Author: The authors showed that endurance-trained middle-aged men has higher testosterone and interleukin 10 level and lower body fat compared to untrained men. The results are quite expected and the authors need to showcase the novelty of their study.

Reply: We agree with the Reviewer's observation. This was our initial hypothesis when we started our research. However, our simultaneous analysis of the associations between testosterone, IL-10 and body fat within a group of master endurance athletes had not yet been conducted to our knowledge. In addition, the results of our study are very promising and relevant to the larger scientific community. Based on our results, we demonstrate the importance of many years of exercise training for the prevention of ever-increasing global obesity, physical inactivity, and related chronic diseases that are exacerbated by unhealthy inflammatory patterns. Our results suggest that focusing on preventing these comorbidities should start as early in life as possible. Based on this concept, we seek to show the relevance of this healthy progressive pattern over the years with endurance master athletes. Although athletes are not representative of the general population, they represent the highest point of the physical exercise continuum and, therefore, provide information about the upper limits of exercise-induced adaptations on physiological characteristics, without confusing the effects of disuse, disease, and typical chronic conditions in a "normal" aged population. Thus, the lifestyle practiced over the decades of sport is an incentive and example to be followed to achieve healthy aging that, within the parameters evaluated in our article, includes mitigating the decline of testosterone, improving the anti-inflammatory pattern, and decreasing body fat levels. Therefore, we want to encourage the general population, through scientific publications, to practice continuous physical exercise throughout life and to raise awareness about the impact of being sedentary.

Specific comments: I am also concern with the methods of measuring body fat and the lack of adjustment in correlation study.

Reply: We adjusted the measurement of body fat, and we also recalculated the correlation adjusted for age, BMI and age together, and BMI (see the table below). We decided to use the BMI and age data together, we rewrote the statistical methods on adjusting the correlations for

these two parameters. We also adjusted Figure 2 according to the correlation and significance values of the adjusted correlation for BMI and age together.

Measuring body fat:

[…] "Body mass index (BMI) was calculated from weight in kilograms divided by height in meters squared (kg/m2). The relative body fat was estimated using the seven skinfolds protocol proposed by Jackson and Pollock, obtaining the values of the pectoral, axillary, triceps, subscapular, medial thigh, suprailliac, and abdominal folds[27]. A single researcher measured all skinfolds with a Lange® caliper (Cambridge Scientific Instruments, MA, USA). Body density was then calculated following the equation: Body density (g/cm^3) = 1.112-0,00043499 * (sum of 7 skinfolds) + 0.00000055 * (sum of 7 skinfolds) 2 - 0.00028826 * (Age), and converted into a percentage of body fat, using a formula: Fat% = [(4.95/DENS) - 4.50] x 100[28]." […]

Correlation-adjusted for Age, Age and BMI together, and BMI:

Correlation adjusted	Parameters	Correlation coefficient	
		Body Fat	Testosterone
Age	IL-10	*r*: -0.693 (*p*:<0.0001)	*r*: 0.*559* (*p*: 0.005)
	Testosterone	*r*: -0.*517* (*p*: 0.01)	
Age and BMI	IL-10	*r*: -0.*633* (*p*: 0.001)	*r*: 0.509 (*p*: 0.013)
	Testosterone	*r*: -0.517 (*p*: 0.01)	
BMI	IL-10	*r*: -0.600 (*p*: 0.002)	*r*: 0.507 (*p*: 0.011)
	Testosterone	*r*: -0.475 (*p*: 0.019)	

Statistical analysis methods (on page 8):

[…] "Pearson's correlation coefficients were calculated to determine the relationships among testosterone, IL-10, and body fat, adjusted for BMI and age together." […]

Subtitle Figure 2 (on page 11):

[…] "Relationship between IL-10 and testosterone (A), testosterone and body fat (B), and IL-10 and body fat (C) of resistance-trained (EMA) and untrained middle-aged (UAM) middle-aged individuals adjusted for BMI and age together. IL-10 = interleukin 10." […]

1. Abstract: add the methods of body composition measure.

Reply: Dear Reviewer, we added the requested sentence to the abstract methods (on page 2).

[...] "Both groups underwent body composition measurements (evaluated by a skinfold protocol)" [...]

2. For sample size calculation, was it performed before or after the study? it is strange to set the target power at 0.7, usually 0.8.

Reply: The calculation of the sample power was done after (post hoc) the study. We recalculated the power as 0.8, as requested (on page 8). See below:

[…] The total sample size in this study conferred statistical power of 86% (*post hoc*) with a significance level of α =0.05 and a large effect size of d =0.8." […]

3. The UAM are overweight. Why didn't the authors recruit UAM that did not have weight problem?

Reply: Yes, the UAM group is overweight. We chose to use a convenience sample for our comparison group that is representative of society today, which is overweight, and falls within a similar age range. Because the main inclusion criterion for the control group is to be sedentary, it is difficult for an individual to be of normal weight when compared to EMA.

4. The correlation study was not adjusted for important confounding factors like age and BMI.

Reply: As noted above, we recalculated the correlations adjusted for age, age and BMI, and BMI as suggested. Although the results have not changed and the difference in correlations remains, we agree with the Reviewer and adjusted the manuscript in the statistical analysis of the methods and in the caption in Figure 2 with the suggested statistics. See below:

Correlation-adjusted for Age, Age and BMI together, and BMI:

Correlation adjusted	Parameters	Correlation coefficient	
		Body Fat	Testosterone
Age	IL-10	*r*: -0.693 (*p*:<0.0001)	*r*: 0.*559* (*p*: 0.005)
	Testosterone	*r*: -0.*517* (*p*: 0.01)	
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5. Other confounding factors are not disclosed, like duration of training for EMA.

Reply: The duration of the athlete's training time is a difficult variable to measure in isolation. In our study, the minimum continuous training time was one of the inclusion criteria for athletes. In addition, we added the training time in years, the training minutes in daily sessions, the number of days of aerobic training, and strength of the athletes in our results. See below:

Methodology (on page 6):

[...] "(1) training continuously for at least 15 years" [...]

Results (on page 8):

"The sample consisted of 25 EMA (51.48 ± 9.49 years of age; 21.71 ± 10.19 years of training; 102.08 ± 39 minutes of daily training; 4.67 ± 1.4 days of weekly aerobic training; and 2.23 ± 1.22 days of weekly strength training)" [...]

6. Apart from providing citation, I hope the authors can describe the body composition measurement in details because it is an important variable here.

Reply: We agree that the method of determining body composition needs to be more detailed. We adjusted this description both in the abstract and in the methods.

Abstract (on page 2):
"Participants were EMA (n= 25; 51.48 ± 9.49 years) and UAM (n= 23; 46.0 ± 9.37 years). Both groups underwent body composition measurements (evaluate by a skinfolds protocol)" […]

Article Methods (on page 7):
[…] "Body mass index (BMI) was calculated from weight in kilograms divided by height in meters squared (kg/m2). The relative body fat was estimated using the seven skinfolds protocol proposed by Jackson and Pollock, obtaining the values of the pectoral, axillary, triceps, subscapular, medial thigh, suprailliac, and abdominal folds[27]. A single researcher measured all skinfolds with a Lange® caliper (Cambridge Scientific Instruments, MA, USA). Body density was then calculated following the equation: Body density (g/cm^3) = 1.112-0,00043499 * (sum of 7 skinfolds) + 0.00000055 * (sum of 7 skinfolds) 2 - 0.00028826 * (Age), and converted into a percentage of body fat, using a formula: Fat% = [(4.95/DENS) - 4.50] x 100[28]." […]

REVIEWER 2

General

1. The topic as such is highly relevant and more knowledge is needed within the area. However, the present data-set does not add much to the area as two very different groups of subjects (both insufficiently described) are compared, but conclusions are drawn in much broader terms than justified (see specific example under Conclusion).

Reply: We agree that the UAM and EMA groups are very different. Although master athletes may not be representative of the general population, they represent the highest point of the

physical exercise continuum and therefore provide information on the upper limits of exercise-induced adaptations in physiological characteristics, without confusing effects of disuse, illness, and typical chronic conditions in a "normal" aged population. Therefore, the lifestyle experienced by endurance master athletes can be an example to be followed to achieve healthy aging that, within the parameters evaluated in our article, includes mitigating testosterone decline, a better anti-inflammatory immune pattern, and decreasing body fat. Thus, with our results, we also seek to encourage the general population to practice physical exercise to raise awareness about the relationships among chronic-metabolic diseases, morbidity and mortality of such diseases, and a sedentary lifestyle. In addition, we have included a clearer and more detailed description of the inclusion criteria of these two groups (on page 6) to better characterize our sample, in addition to the characteristics shown in Table 1 (on page 8 and 9) and in Figures 1 (on page 10) and 2 (on page 11). See below:

[...] “The inclusion criteria for EMA were: (1) training continuously for at least 15 years; (2) continue to compete in national and international endurance running events at the time of data collection; (3) not having a diagnosis of recent infection or chronic metabolic diseases; and (4) not taking hormone replacement or any type of pharmaceutical stimulant or depressant of the immune system. The inclusion criteria for UAM were: (1) sedentary; (2) not having a diagnosis of recent infection or chronic metabolic diseases; and (3) not taking hormone replacement or any type of pharmaceutical stimulant or depressant of the immune system."

2. Major missing points include - age-distribution - matching on other parameters - details/considerations on training history - which sports are they competing in

Reply: The age distribution is described in our results and in Table 1. Other parameters for characterizing the sample are described in the "Participants" part of the methods in our article. The volunteer athletes in our study train and compete in endurance running sports, as also described in the "Participants" part of the methods in our article. As for the training history, training continuously for at least 15 years was one of our inclusion criteria, described in the "Participants" part of the methods, and we added the training time in years, the training minutes in the day, the number of days of aerobic training, and the strength of the athletes in our results. See below:

Methodology (on page 6):

[...] "(1) training continuously for at least 15 years" [...]

Results (on page 8):

"The sample consisted of 25 EMA (51.48 ± 9.49 years of age; 21.71 ± 10.19 years of training; 102.08 ± 39 minutes of daily training; 4.67 ± 1.4 days of weekly aerobic training; and 2.23 ± 1.22 days of weekly strength training)"[…]

3. Recruitment as part of larger/other study - clearly refer to data from this - reasoning for it not being compiled, rather than publishing 'just' IL-10, testosterone and body fat?

Reply: This is a preliminary study in which we chose the most relevant triad of parameters when analyzing the sample, with IL-10 being one of the main anti-inflammatory cytokines and testosterone as one of the main male hormones. Our group is evaluating other immunological and hormonal parameters of athletes, which will be complementary to the findings presented in this article. In addition, the initial results we obtained with IL-10, testosterone, and body fat seem relevant enough to be explored in an article. Thus, we decided to publish our preliminary data, both to avoid wasting publication time and to present them to the scientific community before the new analyses were completed.

4. Inadequate data to support the comparison between the two groups, as partly described in limitations.

Reply: We agree that the UAM and EMA groups are different. However, although master athletes are not representative of the general population, they represent the highest point of the physical exercise continuum and, therefore, provide information on the upper limits of exercise-induced adaptations in physiological characteristics, without confusing the effects of typical diseases and chronic conditions in a "normal" elderly population. Therefore, the lifestyle experienced by endurance master athletes can be an incentive and example to be followed to achieve healthy aging that, within the parameters evaluated in our article, includes mitigating the decline of testosterone, improving anti-inflammatory parameters, and decreasing the body fat index. Thus, with our results, we also seek to encourage the general population to practice physical exercise throughout life on a continuous basis to make people aware of the impact of being sedentary to hopefully avoid chronic diseases and increased morbidity and mortality.

Abstract:

1. I would spell out Testosterone, rather than using the abbreviation T - throughout manuscript.

Reply: We replaced "T" with "testosterone" throughout the manuscript.

2. Page 1 L 39: underwent to – two.

Reply: We agree that this sentence has not been clarified and we have adjusted the text (on page 2) as follows:

"Participants were EMA (n= 25; 51.48 ± 9.49 years) and UAM (n= 23; 46.0 ± 9.37 years). Both groups underwent body composition measurements (evaluate by a skinfolds protocol)" […]

3. Page 1 L 39: if details on analyses are given in abstract, please specify kit and assay rather than general terms.

Reply: We agree that the methodology has been poorly described with regard to the kits used in the dosages. We specify both in the abstract methods and in the General Procedures part of the methods. See below:

Abstract methods (on page 2):
[…] "blood sampling for IL-10 (assessed through ELISA® kit) and testosterone (assessed with Roche Diagnostics® kit, Mannheim, Germany, by chemiluminescence technique in a third-party laboratory)." […]

General Procedures part of the article methodology (on page 6 and 7):
[…] "Serum IL-10 was analyzed in triplicate by ELISA® according to the manufacturer's instructions (R & D Systems, Minneapolis, MN, USA). The detectable limit for IL-10 was 1.0 pg/mL. The overall inter-assay coefficient of variation for IL-10 was 8%. The total testosterone fraction was analyzed in a third-party reference laboratory, with the Atellica® - Siemens® automatic immunoassay equipment, using the chemiluminescence technique[29]. A Roche commercial kit (Roche Diagnostics®, Mannheim, Germany) was applied to the Modular E170

automated platform, which uses biotinylated anti-testosterone antibody and a testosterone derivative labeled with a ruthenium complex. The separation was performed with microparticles covered with streptavidin, captured by magnetic action and subjected to washing. After this procedure, the reading was performed by applying a voltage that induced the emission of light by the ruthenium complex (electrochemiluminescent test). Standard solutions were prepared for all procedures, one containing only the reagent and a control solution containing the reagent plus a known solution, in duplicate."

Relevance for patients: Page 2

There is discrepancy between engaging in lifelong/chronic endurance training, and implementation of this to reduce and prevent metabolic disease etc (adoption of endurance training as part of a healthy lifestyle). When is it too late to begin? What is life-long? Some consideration on duration as part of life-span. I believe it is never too late, but there is a long way from findings in cross-sectional stud-ies of life-long training vs implementation of training regimens in prevention.

Reply: Actually, physical exercise goes beyond just prevention, as it is also beneficial for people who already have a disease or people who decide to start practicing it late. Thus, we agree with you that it is not too late to start and, yes, much still needs to be investigated about training for decades versus cross-sectional findings. Therefore, we adjusted the relevance for patient's part in the abstract.

Relevance for patients (on page 3):

"The adoption of endurance training as part of a healthy lifestyle may contribute to decreasing age-related testosterone reduction, besides reducing markers of inflammaging, preventing the occurrence of chronic age-related diseases, and thus contributing to healthy aging. For people who already have chronic diseases, physical exercise can shift the immune system toward a more anti-inflammatory profile and, thus, improve their pathological condition. In both cases, physical exercise can help attenuate the decline in testosterone, decrease body fat, and increase anti-inflammatory levels."

Introduction:

1. Page 3 L12: resulting in sarcopenia - the reduction in muscle mass and strength is part of the definition of sarcopenia. Sarcopenia definition should be revisited and the introduction reword

accordingly.

Reply: We agree with your consideration of the concept of sarcopenia and have rewritten the initial part of the first paragraph to make the understanding clearer (on page 4). See below:

"The aging process is associated with the clinical condition of sarcopenia[1], whose pathophysiology is associated with progressive degeneration of muscle mass and strength, and consequently, a reduction of musculoskeletal function[2]. Sarcopenia is considered one of the major geriatric syndromes that favor the increase in morbidity and mortality of affected individuals[3]." […]

Methods:

1. P4 L 38: should it not be given which ethics committee?

Reply: We have specified the ethics committee in our methods (on page 5). See below:

"This study was approved by the Ethics and Research Committee of the Catholic University of Brasilia (protocol: 3,779,535), and it was conducted according to the Helsinki declaration." [...]

2. P5 - No selection based on age? Only criteria is above 35.

Reply: The age criterion was chosen according to the beginning of the master athlete categories which, according to the World Masters Athletics (WMA), to be considered a master running athlete and participate in master running athletics events, it is necessary to be over 35 years of age. It is worth mentioning that this age group is an important milestone to visualize the beginning of the effects of hormonal and inflammatory changes, which will be progressively visualized with aging. In addition, other selection criteria were used and you can check them in our methods (on page 6). See below:

[…] "The inclusion criteria for EMA were: (1) training continuously for at least 15 years; (2) continue to compete in national and international endurance running events at the time of data collection; (3) not having a diagnosis of recent infection or chronic metabolic diseases; and (4) not taking hormone replacement or any type of pharmaceutical stimulant or depressant of the

immune system. The inclusion criteria for UAM were: (1) sedentary; (2) not having a diagnosis of recent infection or chronic metabolic diseases; and (3) not taking hormone replacement or any type of pharmaceutical stimulant or depressant of the immune system."

3. P5 - Was any mathing between individuals in the two groups performed (eg regarding age - and other parameters)?

Reply: The relationship between the UAM and EMA groups is that both are male, over the age of 35, have not been diagnosed with recent infection or chronic metabolic diseases, such as diabetes mellitus, systemic arterial hypertension, cardiovascular diseases, dyslipidemia, and are not under hormonal replacement therapy or any type of pharmaceutical stimulant or immune system depressant. The inclusion criteria for UAM were: not having a history of regular sports practice for more than a year in the last two years. The inclusion criteria for EMA were: to train continuously for at least 15 years and to continue competing in national and international events in an endurance race mode at the time of data collection. You can find this information in the Participants part of the methods (on page 6). See below:

[…] "The inclusion criteria for EMA were: (1) training continuously for at least 15 years; (2) continue to compete in national and international endurance running events at the time of data collection; (3) not having a diagnosis of recent infection or chronic metabolic diseases; and (4) not taking hormone replacement or any type of pharmaceutical stimulant or depressant of the immune system. The inclusion criteria for UAM were: (1) sedentary; (2) not having a diagnosis of recent infection or chronic metabolic diseases; and (3) not taking hormone replacement or any type of pharmaceutical stimulant or depressant of the immune system."

4. L9 - their modality - which in/exclusion criteria for which sport they were competing in.

Reply: The athletes in our study were competing exclusively in endurance running events. We rewrote the part of the methods that includes the inclusion criteria for EMA as suggested (on page 6). See below:

[…] "(2) continue to compete in national and international endurance running events at the time of data collection;" […]

5. L 14 - sedentary and otherwise healthy needs definition/selection criteria

Reply: For the selection of UAM, we define "sedentary" as an individual who does not have a history of regular sports practice for a period greater than one year in the last two years. We define "healthy" as an individual who does not continually use medications, has not had a recent infection, and does not have medical diagnoses of chronic-metabolic diseases, such as diabetes mellitus, systemic arterial hypertension, cardiovascular diseases, and dyslipidemia. For a better understanding of our UAM inclusion criteria, we rewrote the sentence (on page 6). See below:

[…] "The inclusion criteria for UAM were: (1) sedentary; (2) not having a diagnosis of recent infection or chronic metabolic diseases; and (3) not taking hormone replacement or any type of pharmaceutical stimulant or depressant of the immune system."

6. L34 - seven skin-fold - why was a dexa scan not performed?

Reply: DEXA scans were not performed due to the cost and the difficulty of moving this device for measurements on national and international trips in athletics competitions.

Results:

1. It is stated that 'Age did not differ between groups' although the trained group were on average 5 years older and a p-value of 0.056 between groups. Giving the actual age-range or distribution within each group would clarify this a bit. I acknowledge that this would, if anything, reduce the chance of detecting relevant differences between the two groups, but I think it is a major confounder and disturbs the basic question of the study. As nothing is stated on inclusion based on age (only above 35), this is a major concern.

Reply: We appreciate your suggestion. The distribution of each group was calculated according to age, where the minimum and maximum for UAM were 36 and 65 years, respectively. For EMA, they were 38 and 71 years old, respectively. Even making the suggested adjustment, the correlation remained (ANOVA: p=0.146) non-significant. In addition, we have readjusted the sentence for better clarification. See below:

Table Descriptive:

Group	N	Mean	Std. Derivation	Minimum	Maximum
UAM	23	48.22	8.135	36	65
EMA	25	52.21	8.959	38	71
TOTAL	48	50.50	8.744	36	71

Sentence (on page 8):

[...] " Age differed slightly between groups (p =0.056). The age range of EMA was 38-71 years (52.21 ± 8.959 years). The age range of UAM was 36-65 years (48.22 ± 8.135 years). The age range of the two groups together was 36-71 years (50.50 ± 8.744 years)." […]

Figure 1:

1. Instead of just *, give p-value, at least in legend.

Reply: Dear reviewer, we added the p-value in the legend of Figure 1 (on page 10). See below:

"Testosterone, IL-10, and body fat of endurance-trained middle-aged (EMA) and untrained age-matched individuals (UAM). IL-10 = interleukin 10; * statistical difference; p =0.001."

Discussion:

1. P10 - L 58: while these were

Reply: We corrected the sentence (on page 13). See below:

"In addition, we showed that UAM have lower IL-10, an anti-inflammatory marker, and decreased testosterone, while these were elevated in EMA. While testosterone levels were positively associated with IL-10, body fat was negatively associated with both testosterone and IL-10." […]

2. P11 - L 4: Frequent is not enough - these athletes have been competing at international levels for +15 years.

Reply: We agree that EMA have an intense training regimen and lifestyle and that the term "frequent" is not appropriate, so we have removed and rephrased the phrase for better understanding (on page 13). See below:

[…] "Besides being the first study to date to demonstrate the relationships among testosterone, IL-10, and body composition in endurance trained middle aged individuals, our findings reinforce that chronic exercise training and the maintenance of low body fat may be pivotal for a healthy anti-inflammatory status and testosterone levels as previously demonstrated[35,39]. Moreover, lifelong exercise training also improves the antioxidant defenses[8,17,15,16], and all of the above mentioned aspects are associated with longevity and healthy aging[13]." […]

3. Measurements of other cytokines would be highly relevant. For example, TNFa as discussed. To make a more complete story on the pro/anti-inflammatory status of these subjects

Reply: We agree that the measurement of other immunological parameters would be highly relevant to complement and elucidate the pro- and anti-inflammatory status of UAM and EMA. This study was a preliminary study, and our group continues to carry out new assessments of other pro- and anti-inflammatory parameters of the groups surveyed. The initial results that we obtained with IL-10, testosterone, and body fat seem to be relevant enough to be already published in an article. For this reason, we decided to publish our initial data to disclose it to the scientific community even before the new analyses were completed.

4. L36: include not measure – rephrase

Reply: We changed the text as suggested (on page 13). See below:

"The possible limitations of this study include not measuring nutritional status and dietary intake and not controlling for the use of dietary supplementation among participants." […]

5. Limitations are not sufficiently addressed - and why was physical fitness not measured?

Reply: We did not measure physical fitness, as our study is cross-sectional. We made international trips to collect the data and materials of athletes in international competitions in countries far from ours. This logistical issue made the analysis of physical fitness unfeasible

because we could not transport the necessary equipment. For UAM, physical fitness was not measured since the inclusion criterion for volunteers was to be sedentary. We had already described the non-measurement of physical fitness in the limitations of the study, but we agree that the reasons were unclear (on page 13 and 14). See below:

"The possible limitations of this study include not measuring nutritional status and dietary intake and not controlling for the use of dietary supplementation among participants. Also, the level of physical fitness of participants was not analyzed because of the short time researchers had with each participant and also because of limitations inherent to international trips for data collection. The initial intent of the study was to form two groups only, athletes and untrained individuals, regardless of their level of physical activity." […]

Conclusion:

1. Two very different groups (both insufficiently described) are compared, but conclusions are drawn in much broader terms than justified: eg Page 12: Given the global preponderance of chronic disease, more widespread adoption of endurance training as part of an overall healthy lifestyle could eventually reduce the occurrence of such diseases in the population. Adequate physical training may have clinical applications for attenuating T decline, decreasing body fat, and maintaining an adequate anti-inflammatory level, especially in middle age.

Reply: We agree that the UAM and EMA groups are different. Although master athletes may not be representative of the general population, they represent the highest point of the physical exercise continuum and therefore provide information on the upper limits of exercise-induced adaptations in physiological characteristics, without confusing effects of disuse, disease, and typical chronic conditions in a "normal" aged population. For this reason, the lifestyle experienced by master endurance athletes can be an example to be followed to achieve healthy aging that, within the parameters evaluated in our article, includes mitigating testosterone decline, a better anti-inflammatory immune pattern, and decreasing body fat. Thus, with our results, we also seek to encourage the general population to practice physical exercise throughout life on a continuous basis to make people aware of the impact of being sedentary to hopefully avoid chronic diseases and increased morbidity and mortality. In addition, we have included a clearer and more detailed description of the inclusion criteria of these two groups to better characterize our sample (on page 6), in addition to the characteristics shown in Table 1 (on page 8 and 9) and in Figures 1 (on page 10) and 2 (on page 11). See below:

[…] “The inclusion criteria for EMA were: (1) training continuously for at least 15 years; (2) continue to compete in national and international endurance running events at the time of data collection; (3) not having a diagnosis of recent infection or chronic metabolic diseases; and (4) not taking hormone replacement or any type of pharmaceutical stimulant or depressant of the immune system. The inclusion criteria for UAM were: (1) sedentary; (2) not having a diagnosis of recent infection or chronic metabolic diseases; and (3) not taking hormone replacement or any type of pharmaceutical stimulant or depressant of the immune system.”

REVIEWER 3

Comments to the Author

1. This manuscript looks at the effect of lifelong training on testosterone, IL-10 and %body fat by using a population of endurance master athletes. The study is well thought and one of the few using master athletes to look at the effects of lifelong exercise in preventing ageing and chronic diseases.

Reply: Thank you for reviewing this manuscript and your kind appraisal of the quality of our work.

Specific comments:

1. In the methodology it would be important to characterize the groups according to their physical fitness and in the steps that have been taken to make sure the blood sample taken represents a rested baseline state.

Reply: We did not measure physical fitness in our study for logistical reasons, as it was not possible to transport the necessary equipment on international trips to countries far from ours. In addition, as it is a cross-sectional study, in which we met athletes in international athletics events in which they were going to compete, it was not possible to measure physical fitness. We describe this limitation more clearly below. For the UAM group, physical fitness was not measured, since the inclusion criterion for volunteers was to be sedentary. Regarding the stages of venous blood collection from participants, we rewrote our methodology to make it clear that the baseline resting state was followed in our protocol. See below:

Limitations (on page 13 and 14):

"The possible limitations of this study include not measuring nutritional status and dietary intake and not controlling for the use of dietary supplementation among participants. Also, the level of physical fitness of participants was not analyzed because of the short time researchers had with each participant and also because of limitations inherent to international trips for data collection. The initial intent of the study was to form two groups only, athletes and untrained individuals, regardless of their level of physical activity." […]

Methods (on page 6):
"All volunteers arrived at the laboratory in the morning (between 7:00 am and 8:00 am), on days according to the volunteer's convenience and availability, with 8-hour fasting, and abstaining from physical exercise for at least 24 hours prior to procedures. The blood collections of 2 samples of ~4 mL was drawn and deposited each in Vacutainer tubes with and without EDTA." [...]

2. If possible, the inclusion of pro-inflammatory markers like TNF-alpha or IL-6 would improve the study.
The discussion could be enhanced by a more detailed discussion.

Reply: In fact, the inclusion of other immunological markers, such as TNF-alpha and IL-6, would be very relevant to the present study and would leave the discussion more detailed. This study is a preliminary study, in which we chose to only publish our initial results, since the findings appear to be relevant enough to be explored in an article. For this reason, we have already decided to publish them to expose to the scientific community even before the new analyses are completed. However, our research requires funding from the Brazilian government and, as we know, in this current context of crisis arising from the current pandemic, we are experiencing financial difficulties, a fact that delayed some of our analyses and, soon, we will evaluate other parameters.

3. Also in discussion, the phrase "EMA are a successful model of adequate nutrition, stress management and consistent training for decades..." maybe a bit optimistic since "adequate nutrition and stress management" cannot be generalized to all endurance athletes.

Reply: We agree with your comment, however, in our research and in the published scientific literature about master athletes, adequate nutrition and stress management are observed. This

group has been training for more than 15 consecutive years, and they compete in international events, maintaining and even improving their test times, which is consistent with people who maintain aligned dietary, psychological, and conditioning routines. In addition, studies show that endurance athletes have a high level of mental endurance. This concept refers to the ability to produce high levels of subjective and/r objective performance, despite stressful challenges [1,2]. Still, stress management, emotion control, recovery from failure, persistence and resilience are components of their routine, including mental stamina [3]. Therefore, older endurance athletes can be considered to have a high level of mental endurance [4].

[1] Gucciardi DF, Hanton S, Gordon S, Mallett CJ, Temby P. The concept of mental toughness: tests of dimensionality, nomological network, and traitness. J Pers. 2015; 83 (1): 26-44.
[2] Lin Y, Mutz J, Clough PJ, Papageorgiou KA. Mental Toughness and Individual Differences in Learning, Educational and Work Performance, Psychological Well-being, and Personality: A Systematic Review. Front Psychol. 2017; 8: 1345.
[3] Jones G. What Is This Thing Called Mental Toughness? An Investigation of Elite Sport Performers. Journal of Applied Sport Psychology. 2002; 14 (3): 205–218.
[4] Zeiger JS, Zeiger RS. Mental toughness latent profiles in endurance athletes. PLoS One. 2018; 13 (2): e0193071.

4. I suggest correcting the phrases: line 24 "The hypothesis was that EMA has higher IL-10 and..." change has for "have". Line 36, page 10 "not measure the nutritional status" change to "not measuring ..."

Reply: We corrected the sentence as suggested. See below:

Page 5: “The hypothesis was that EMA have higher IL-10.” […]

Page 13: “The possible limitations of this study include not measuring the nutritional status” […]

REVIEWER 4

I suggest to accept the article. Well amended.

Reply: Thank you for reviewing and accepting this manuscript.

2nd Editorial decision
27-Jun-2021

Ref.: Ms. No. JCTRes-D-21-00051R1
Is lifelong endurance training associated with maintaining levels of testosterone, IL-10, and body fat in middle-aged males?
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers:

Reviewer #1: Thank you for addressing my previous comments.

Reviewer #4: The manuscript has been improved in the quality and can be be published.