Sports Medicine and Health Science 2 (2020) 89–94

Contents lists available at ScienceDirect

Sports Medicine and Health Science

journal homepage: www.keaipublishing.com/en/journals/sports-medicine-and-health-science/

Original Research

Structural brain differences between ultra-endurance athletes and sedentary persons

T. Paruk [a], L. Rauch [a], M. Jankiewicz [b], K. Van Breda [a], D.J. Stein [c], M. King [a,*]

[a] Department of Exercise Science and Sports Medicine, University of Cape Town, South Africa
[b] Division of Biomedical Engineering, Department of Human Biology, University of Cape Town, South Africa
[c] SAMRC Unit on Risk & Resilience in Mental Disorders, Dept of Psychiatry & Neuroscience Institute, University of Cape Town, South Africa

ARTICLE INFO

Keywords:
Ironman athletes
Ultra-racing
Ultra-training
MRI
Ultra endurance

ABSTRACT

Participation in ultra-endurance events has increased in recent years and requires extreme levels of moderate to vigorous physical activity (MVPA). Moderate levels of MVPA have been associated with increased brain volume but the effects of extreme levels of MVPA on brain volume is unknown. As a result, we sought to compare the brains of those who engage in extremely high levels of MVPA with those who are sedentary using magnetic resonance imaging. We performed whole brain volumetric analyses and voxel-based morphometry on 12 ultra-endurance athletes (1078.75 ± 407.86 min of MVPA/week) and 9 sedentary persons (18.0 ± 56.9 min of MVPA/week). Whole-brain analyses revealed that those who participate in ultra-endurance training have increased grey ($p < 0.0001$), white ($p = 0.031$), and total matter volume ($p < 0.0001$), while regional analyses revealed that ultra-endurance athletes have smaller regional grey matter volume in the right primary sensory and motor cortex, inferior and middle frontal gyrus, and left thalamus. Future research is warranted to determine why ultra-endurance athletes have lower regional volumes in these areas despite having overall increased grey and white matter volumes.

Introduction

One hundred and 50 min of moderate to vigorous levels of physical activity (MVPA) is recommended per week and it is estimated that 80% of Americans meet or fall below this criteria.[1] However, ultra-endurance or ironman athletes habitually engage in levels of physical activity far greater than the recommended amount. These persons typically perform more than five times the recommended levels of MVPA[2] and participation in ultra-endurance events has dramatically increased in recent years.[3] Ultra-endurance events have been associated with pulmonary and autonomic dysfunction,[4] temporary musculoskeletal injuries,[5] cardiac damage,[6] and temporary markers of tissue damage,[7] which is perhaps not surprising given that endurance races can involve exercise for several consecutive days without rest.[4]

While bodily injuries during endurance races have been well documented,[5] the effects of ultra-endurance training on the brain have received less attention.[8] Ultra-endurance training acutely activates the hypothalamic pituitary adrenal (HPA) axis[9] and ultra-endurance athletes have been shown to have chronic high levels of cortisol.[10] Interestingly, individuals with Cushing's disease, which is characterized by high levels of cortisol, show reduced grey matter (GM)[11] volume. On the other hand, increased aerobic fitness has repeatedly been associated with increased GM[12,13] and both rat[14] and human models[15,16] show that exercise leads to increased GM and white matter (WM).[17] Furthermore, mice that are bred to run excessively show increased neurogenesis.[18] However, the effect of extreme levels of habitual exercise on the human brain has not been examined.

The objective of this study was to understand the potential impact of habitual extreme levels of physical activity on the brain. To do this we sought to compare the brains of those who engage in extremely high levels of physical activity with those who are sedentary. We analyzed T1-weighted magnetic resonance images of sedentary and ultra-endurance athletes, which were acquired as part of another study we recently completed that compared the differences in resting state brain activity in these populations.[19] T1-weighted images are generated using a scanning protocol sensitive to fat tissue and are used to reveal detailed structural anatomy. Our hypothesis was that ultra-endurance athletes would have greater GM and WM volumes.[12–18,20] A recent review demonstrated that the majority of the brain is impacted by exercise[21] and thus we do not have a directed regional hypothesis of the areas that will be disproportionally altered as a result of high levels of exercise. In order to test these

* Corresponding author. University of Cape Town, Rondebosch, 7700, South Africa.
E-mail addresses: michael.king@mun.ca, kngmic015@myuct.ac.za (M. King).

https://doi.org/10.1016/j.smhs.2020.05.004
Received 10 March 2020; Received in revised form 14 May 2020; Accepted 16 May 2020
Available online 28 May 2020

List of abbreviations

ANCOVA	analysis of covariance
BMI	body mass index
GM	grey matter
GPAQ	generalized physical activity questionnaire
HPA	hypothalamic pituitary axis
IFG –	inferior frontal gyrus
M1	primary motor cortex
MNI	Montreal Neurological Institute
MRI	magnetic resonance imaging
MVPA	moderate to vigorous levels of physical activity
SD	standard deviations
S1	primary sensory
TE	echo time
thal	Thalamus
TIV	total intracranial volume
TR	repetition time
VBM	voxel based morphometry
WM	white matter

hypotheses, we employed voxel-based morphometry (VBM) to perform whole brain analyses.

Experimental procedure

Ethical approval

Participants of the study provided written informed consent. This study was approved by the human research ethics committee of the University of Cape Town, South Africa (Ref:336:2009) and was carried out in accordance with the Declaration of Helsinki on the use of human participants in experiments.

Participants

We performed secondary analyses on 22 healthy right-handed participants without any history of cardiovascular, metabolic, neurological, or psychiatric disease. These participants were originally recruited for another study,[19] which examined resting state connectivity in these two populations. As part of the resting state connectivity study, T1-weighted images were collected and this manuscript examines those images.

Participants had no recent history of drug, nicotine, or alcohol use and were excluded if in the past month they had used psychoactive medication (determined by structured interview, see Familiarization section); regardless of whether that was substance of abuse (determined by saliva sample, Detect a Drug, South Africa) or prescription. All of our participants had at least one year of tertiary education. Participants were telephonically screened as being either sedentary or an ultra-endurance athlete who has been training for at least 1 year. Our ultra-endurance athlete participants were recruited from the ironman athlete community in Cape Town, South Africa using social media, sports clubs, through posters, and by word of mouth. The term athlete in this study is defined as a person who aims to improve his/her performance, is actively participating in competition, formally registers in sport, and to have sport training and competition as his or her focus for an amount of time that exceeds the time allocated for other types of professional or leisure activities.[22] Groups were defined by the amount of physical activity they completed in a typical week using the generalized physical activity questionnaire (GPAQ[23]). The GPAQ includes physical training and MVPA occurring outside training, such as active transportation. Physical activity was defined by the amount the minutes of MVPA. Moderate activity was defined to participants as moderate physical effort causing small increases in breathing or heart rate, while vigorous activity was defined as hard physical effort causing large increases in breathing or heart rate.

We excluded one of the participants in the sedentary population with a body mass index (BMI) of 59.1, which was 3.65 standard deviations (SD) from the BMI mean of the study population. As a result, the dataset we analyzed included 21 participants, which was split into two groups; the ultra-endurance group were highly active participants (n= 12, 1078.75 ± 407.86 MVPA in minutes) while the sedentary group were highly inactive (n = 9, 18 ± 56.9 MVPA in minutes, Table 1). It should be noted that the sedentary population were also overweight (BMI of 29.59 ± 5.71) but were otherwise healthy.

Familiarization

Participants underwent a structured diagnostic interview (Mini International Neuropsychiatric Interview) by a psychologist in order to assess the presence of any psychiatric disorders.[24] Height and weight were measured to obtain BMI and two participants in each group were excluded as outliers. We defined an outlier by a BMI z-score of greater than 2.68. All participants were included as part of another investigation examining brain activity during a fatiguing handgrip task and were familiarized with procedures relevant to those investigations at this time.[25–27]

MRI image acquisition, processing and analyses

All participants were scanned on a 3 T S Skyra whole-body MRI scanner (Erlangen, Germany) at the Cape Universities Body Imaging Centre in South Africa with a 32 channel head coil. For each participant a structural T1-weighted volume was acquired using a multiecho magnetization prepared rapid gradient echo sequence (TR (repetition time) = 2530 ms, TE (echo time) = 1.59 ms, 7° flip angle, voxel size = 1.14 × 1.14 × 1.14 mm, field of view 256 × 256 × 192 mm^3, 128 slices). Images were analyzed using VBM 8 statistical package within SPM 8 (Wellcome Dept of Cognitive Neurology, London, UK) and Matlab 2017a (The MathWorks Inc. Natick, MA, USA) according to default preprocessing steps. All images were inspected for image quality, manually centered to the anterior/posterior commissure, and segmented into GM and WM using SPM 8 prior to pre-processing. All images were then normalised to the MNI (Montreal Neurological Institute) standard space and smoothed using an 8 mm full-width half-maximum Gaussian kernel, which was done to reduce noise and reduce the effect of small potential errors in registration.

Statistical analysis for demographic data was conducted using IBM Statistical Software package version 21 (SPSS, IBM Corporation, NY, USA) and results were graphed using Prism (GraphPad Prism 5.0 software, La Jolla, CA, USA). We performed t-tests to compare age, BMI, and MVPA. We performed Fisher's exact test to compare gender ratio between groups. We defined significant findings as $p < 0.05$.

Whole brain tissue analyses and correction for total intracranial volume (TIV)

In order to compare whole brain tissue differences in WM, and GM,

Table 1
Demographic information (mean and SD) of ultra-endurance and sedentary populations. Abbreviations: M – Male, F – Female, BMI – body mass index, MVPA – moderate to vigorous physical activity in minutes. SD – standard deviation.

Variables	Ultra-Endurance Group (n = 12, 10M, 2F)	Sedentary Group (n = 9, 2M, 7F)
Age (years)	27.9 ± 6.44	31.4 ± 13.8
BMI (kg/m^2)	22.67 ± 1.62	29.59 ± 5.71
MVPA (min/week)	1078.75 ± 407.86	18.0 ± 56.9

we used a custom script to extract brain volumes from images after pre-processing was completed. This script also extracted cerebrospinal fluid volume. Males have been shown to have larger TIV than females and this is thought to have an effect on brain matter volume.[28] To address this issue, we corrected each matter type for TIV using a recently developed method by Sanchis-Segura et al. (2019).[29] The adjusted matter volume controlling for TIV was calculated using the following formula with WM used as an example: $WM_{adjusted} = WM_{subject}$ - b (TIV - TIV_{mean}), where b = the slope of the regression line that described the relationship between $WM_{subject}$ and $TIV_{subject}$. We then compared $GM_{adjusted}$ and $WM_{adjusted}$ volumes in separate one-tailed t-tests between groups and assessed effect size (Cohen's *d*, which indicates the number of standard deviation differences between the group mean) using the SPSS. A one-tailed *t*-test was chosen given the extensive research illustrating the effect of exercise on exercise induced total brain volumetric increases in WM and GM.[15,16] Using SPM 8 and VBM, regional brain differences in GM between groups were examined using an analysis of covariance (ANCOVA) model with total matter volume and gender as covariates and post-hoc t-tests to compare groups. We set a cluster threshold to $p < 0.05$ corrected for family-wise error (FWE). A cluster is a set of continuous significant voxels whose significance is determined by various methods such as FWE or false discovery rate.[30] To further account for non-stationary biases associated with cluster significance in VBM analyses, all significant clusters were further corrected using the non-stationary correction toolbox.[31,32]

We interpreted our results using the SPM Anatomy Toolbox 1.7. This toolbox is a probabilistic atlas defined by cytoarchitecture structure[33] and was created to resolve the problematic use of macro-anatomical landmarks, which do not take into account microscopical architectonic organization[34] which is aligned with function.

Results

Demographic results

Our groups did not differ in age ($T(18) = 0.107$, $p = 0.916$), but the sedentary group had a higher BMI ($T(18) = 4.02$, $p = 0.001$, Cohen's $d = 1.86$), had more males ($p = 0.009$), and lower MVPA ($T(18) = 7.68$, $p < 0.0001$, Cohen's $d = 3.56$).

Whole brain results

Analyses comparing GM and WM volumes adjusted for TIV between ultra-endurance and sedentary groups found that the ultra-endurance group has significantly higher GM volume (Fig. 1 A, $T(18) = 7.24$, $p < 0.0001$, Cohen's $d = 3.24$, large effect[35]) and higher WM volume (Fig. 1B, $T(18) = 1.99$, $p = 0.0311$, Cohen's $d = 0.911$, large effect[35]).

Regional results

Our ANCOVA model revealed a main effect of physical activity levels and follow up contrast analyses between ultra-endurance and sedentary groups show that volumes of GM structures in ultra-endurance were smaller as compared to corresponding GM volumes in sedentary groups. Specifically, the right IFG (p. triangularis, Area 45), right middle frontal gyrus, right motor cortex (M1, area 1), Right somatosensory cortex (S1, area 1), left thalamus (thal: prefrontal and temporal), left pallidum (thal: prefrontal) were smaller in ultra-endurance group (Fig. 2, Table 2).

Discussion

Main findings

Our main findings were that (1) the ultra-endurance group had greater overall GM and WM than the sedentary group (Fig. 1) but (2) demonstrated smaller regional volumes in the right S1, M1, inferior frontal gyrus, middle frontal gyrus, and left thalamus.

Whole brain volumetric changes

Our results are consistent with previous findings showing an association between exercise and increased GM and WM volume.[12–18,20] Possible mechanisms for this association include increased vascularization (i.e. angiogenesis) in order to meet the demand of increased activity, which in turn facilitates the development of new neurons (i.e. neurogenesis) and neuron survival. Angiogenesis and neurogenesis are thought to be mediated by exercise-induced increases in angiogenic and neurotrophic factors, such as insulin-like growth factor and brain derived neurotropic factor. Exercise has been shown to influence WM by increasing oligodendrogenesis[36] and myelination.[37] Furthermore, exercise has been shown to induce neuron remodelling by increasing total neuron length, dendritic length, and complexity.[38] It is possible that these mechanisms underlie the observed whole brain differences in this study.

Regional changes

Despite global matter increases, we observed that ultra-endurance athletes had smaller GM volumes of the right S1, M1, inferior frontal gyrus, middle frontal gyrus, and left thalamus. These regions are highly relevant given their fundamental role in sensorimotor function (S1, M1) and proposed role in mediating exercise fatigue (inferior frontal gyrus,[39] thalamus[40]). Freund et al. (2014)[41] examined the effect of an ultra-endurance race (4487 km in 64 days, ~70 km of running per day) on GM volume and demonstrated focal decreases in the temporal cortex, occipito-parietal cortex and caudate.[41] These volumetric changes were

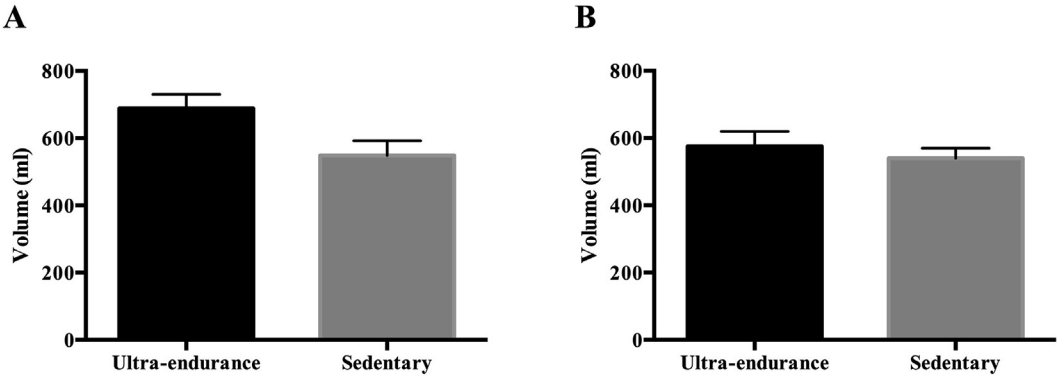

Fig. 1. Mean (±SD) volumetric differences between ultra-endurance and sedentary groups. The ultra-endurance group shows higher volume of GM **(A)** and WM **(B)**. SD – standard deviation, GW – Grey matter, WM – White matter.

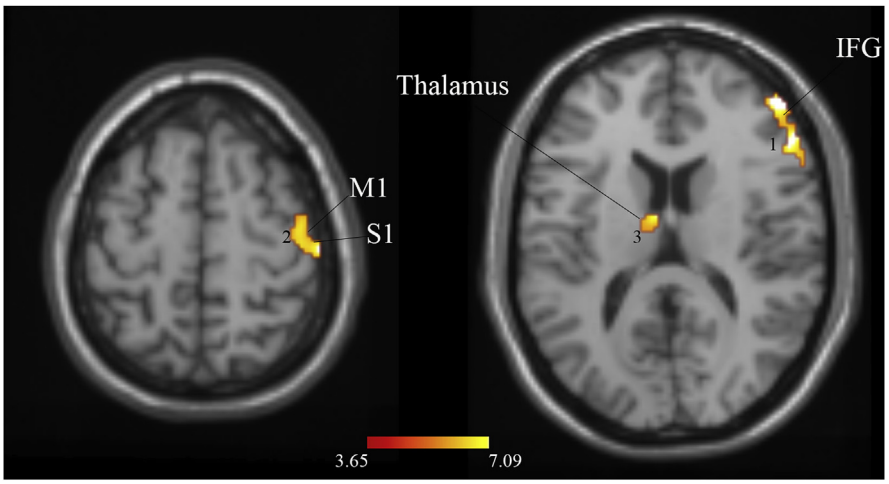

Fig. 2. Sedentary participants show greater volume changes regionally in the R Middle Frontal Gyrus, R IFG, (p. Triangularis), R Precentral Gyrus (M1), R Postcentral Gyrus (S1), L Thalamus, L Pallidum (Table 2). Colour bar on graph indicates T value range. (*Color reproduction for web only*). IFG – inferior frontal gyrus, M1 – primary motor cortex, S1 – primary sensory cortex.

Table 2
The ultra-endurance group demonstrated significantly smaller regional volumes than the sedentary group. The locations of these differences are listed in terms of macroscopic areas (column 3) cytoarchitectonic areas (column 4) as well as MNI brain coordinates (column 6). The clusters listed here correspond to the clusters in Fig. 2 and were located in the R IFG (p. triangularis, Area 45), R middle frontal gyrus, R M1 (Area 1), R S1 (Area 1), L thalamus (prefrontal and temporal), L pallidum (thalamus prefrontal) (Fig. 2). Abbreviations – FWE – family wise error, IFG – inferior frontal gyrus, S1 – somatosensory cortex, M1 – primary motor cortex, thal – thalamus, MNI – Montreal Neurological Institute.

Cluster #	FWE Cluster Significance	SPM Anatomy Macroscopic Label	Cytoarchitectonic Assignment and Probability	Coordinate Peak T - Value	Coordinate (x,y,z)
#1	<0.001	R IFG (p. Triangularis)	Area 45 (31%)	5.66	52, 26, 22
	–	R Middle Frontal Gyrus	–	7.09	48, 22, 42
	–	R Middle Frontal Gyrus	–	6.10	50, 46, 15
#2	0.003	R S1	Area 1 (51%)	5.34	54, −21, 58
	–	R M1	Area 1 (14%)	5.58	45, −24, 64
	–	R M1	Area 4a (11%)	4.76	45, −12, 57
#3	0.034	L Thalamus	Thal (prefrontal) 66%	6.27	−9, −9, 18
		L Thalamus	Thal (temporal) 66%	3.65	−6, −9, 12
		L Pallidum	Thal (prefrontal) 10%	5.79	−9, 0, 0

thought to be independent of dehydration and were restored to baseline levels before the race and thus, it appears that this effect is temporary. Our results are partially consistent with Freund's study[41] but the findings are in different regions. Another study, examining dendritic complexity in high and low trained rats demonstrated that highly trained rats show reduced dendritic complexity in cardiorespiratory and locomotor regions.[42] It was proposed that high volumes of locomotor activity preserve the most critical components related to locomotor processing. We may be observing this pattern of plasticity whereby overall GM volume is increased but specific regions related to exercise are specifically pruned.

On the other hand, it is possible that excessive levels of cortisol observed in endurance athletes may have led to glucocorticoid mediated decreased volume in these regions. Endurance exercise activates the HPA axis[10] and chronic activation of this pathway has been shown to lead to neuronal atrophy in rodent models[43] and adult humans, potentially through BDNF mediated[44] neuronal remodelling.[43] Our voxel based morphometry analyses did not reveal any differences between populations in the hippocampus, a region with high glucocorticoid receptor concentration and that has previously been shown to be altered in response to chronic stress.[43] Furthermore, it is not clear why a glucocorticoid mediated induced decrease in volume would occur selectively in the regions that our analyses revealed since these regions are not selectively high in glucocorticoid receptors. Freund's work[45] demonstrated that ultra-endurance athletes were lesion-free, which might indicate that atrophy might not be the mechanism for decreased volume. Future research is warranted to further investigate the source of the potential volumetric differences.

This study has several limitations. Firstly, because of the secondary nature of our dataset we did not recruit participants to be aged and gender matched controls. The groups differed not only in physical activity levels but also in gender, which is known to be an important factor for brain tissue volume.[46,47] We mitigated this confounding factor in two ways: (i) we corrected for TIV in our whole brain analyses using a recently established method[29] and (ii) we covaried for gender for the regional analyses. Despite this approach our results should be interpreted with caution.

Secondly, we quantified MVPA by self-report using the GPAQ and did not differentiate between aerobic and resistance training, although presumably the large majority of the training was aerobic based given that these athletes were taking part in ultra-endurance racing. It is not clear whether ultra-endurance athletes have inherently different brain morphology or whether the observed differences are due to the extreme

levels of MVPA. Previous research[48] suggests that elite power-based athletes (javelin, sprinting, long jump) show greater GM in the striatum but no difference in M1 or S1 or whole brain volume as compared to non-active age matched controls (defined as persons with MVPA< 120 min/week with no formal athletic training). Furthermore, data from the same study suggested that up-and-coming athletes (defined as training for 9years at least 5 times per week but not elite) did not show GM differences with non-active age matched controls; suggesting that highly skilled power athletes are uniquely different. Comparatively, the participants were not elite ultra-endurance athletes yet demonstrated volumetric differences, presumably from aerobic exercise mediated changes.

Thirdly, the sedentary group was overweight as defined by BMI and the ultra-endurance group was not, which offers a potential confounding factor given that BMI is thought to affect brain morphology.[49] It has previously been found that waist circumference and BMI are *negatively* associated with volume of the motor cortices[50] and IFG,[51] respectively. Importantly, we found the opposite where these regions were larger in the sedentary (higher BMI) group. One might expect that this group would have smaller volumes in these regions but this was not the case. Thus, it is possible that the confounding factor of BMI may not have played a role in these results.

Conclusion

Our results indicate that those who participate in ultra-endurance training have overall increased GM and WM, while sedentary individuals have larger regional volume in the right inferior frontal gyrus, middle frontal gyrus, S1, M1, and left thalamus. Previous research by Freund and Schutz[41] showed that participating in an ultra-endurance activities can reduce total GM volume and decrease regional brain volume. On the other hand, our analyses shows that those who habitually exercise at extremely high levels of MVPA have greater baseline levels of GM and WM volumes but also reduced regional GM volumes. Future research is required to confirm the effect of extreme levels of exercise on whole brain and regional changes.

Submission statement

This manuscript was not submitted to another journal while the journal of Sports Medicine and Health Science reviewed and considered it for publication.

Authors' contributions

The contributions of the authors were as follows: Tasneem Paruk: data collection, analysis, figure preparation, and manuscript preparation, Laurie Rauch: manuscript preparation and study design, Marcin Jankiewicz: data collection and manuscript preparation, Keelyn Van Breda: data collection and study design, Dan Stein: manuscript preparation and study design, Michael King: analysis, figure preparation, study design, and manuscript preparation.

Ethics statement

Participants of the study provided written informed consent. This study was approved by the human research ethics committee of the University of Cape Town (Ref:336:2009), South Africa and was carried out in accordance with the Declaration of Helsinki on the use of human participants in experiments. Written informed consent was obtained from all participants before baseline measurements and a copy of the signed consent form was given to each participant.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. I declare that this manuscript is original, has not been published before, is not currently being considered for publication elsewhere.

Acknowledgement

Dan J. Stein was supported by the SAMRC.

References

1. Piercy KL, Troiano RP, Ballard RM, et al. The physical activity guidelines for Americans. *J Am Med Assoc*. 2018;320(19):2020–2028. https://doi.org/10.1001/jama.2018.14854.
2. Knechtle B, Wirth A, Baumann B, Knechtle P, Rosemann T, Oliver S. Differential correlations between anthropometry, training volume, and performance in male and female ironman triathletes. *J Strength Condit Res*. 2010;24(10):2785–2793. https://doi.org/10.1519/JSC.0b013e3181c643b6.
3. Hoffman MD, Ong JC, Wang G. Historical analysis of participation in 161 km ultramarathons in North America. *Int J Hist Sport*. 2010;27(11):1877–1891. https://doi.org/10.1080/09523367.2010.494385.
4. Tiller NB. *Physiological and Pathophysiological Consequences of a 25-Day Ultra-endurance Exercise Challenge*. May 2019:1–8. https://doi.org/10.3389/fphys.2019.00589. fphys-10-00589tex.
5. Vernillo G, Savoldelli A, La Torre A, Skafidas S, Bortolan L, Schena F. Injury and illness rates during ultratrail running. *Int J Sports Med*. 2016;37:565–569. https://doi.org/10.1055/s-0035-1569347, 07.
6. Rao P, Hutter AM, Baggish AL. The limits of cardiac performance: can too much exercise damage the heart? *Am J Med*. 2018;131(11):1279–1284. https://doi.org/10.1016/j.amjmed.2018.05.037.
7. Knechtle B, Nikolaidis PT. Physiology and pathophysiology in ultra-marathon running. *Front Physiol*. 2018;9:634. https://doi.org/10.3389/fphys.2018.00634.
8. Perrey S, Mandrick K. Evidence from neuroimaging to explore brain plasticity in humans during an ultra-endurance burden. *BMC Med*. 2012;10(1):171–173. https://doi.org/10.1186/1741-7015-10-171.
9. Howe CCF, Pummell E, Pang S, Spendiff O, Moir HJ. Emotional intelligence and mood states impact on the stress response to a treadmill ultramarathon. *J Sci Med Sport*. 2019;22(7):763–768. https://doi.org/10.1016/j.jsams.2019.02.008.
10. Skoluda N, Dettenborn L, Stalder T, Kirschbaum C. Elevated hair cortisol concentrations in endurance athletes. *Psychoneuroendocrinology*. 2012;37(5):611–617. https://doi.org/10.1016/j.psyneuen.2011.09.001.
11. Simmons NE, Do HM, Lipper MH, Laws ER. Cerebral atrophy in Cushing's disease. *Surg Neurol*. 2000;53(1):72–76. https://doi.org/10.1016/S0090-3019(99)00197-4.
12. Erickson KI, Prakash RS, Voss MW, et al. Aerobic fitness is associated with hippocampal volume in elderly humans. *Hippocampus*. 2009;19(10):1030–1039. https://doi.org/10.1002/hipo.20547.
13. Chaddock L, Erickson KI, Prakash RS, et al. A neuroimaging investigation of the association between aerobic fitness, hippocampal volume, and memory performance in preadolescent children. *Brain Res*. 2010;1358(C):172–183. https://doi.org/10.1016/j.brainres.2010.08.049.
14. Swain RA, Harris AB, Wiener EC, et al. Prolonged exercise induces angiogenesis and increases cerebral blood volume in primary motor cortex of the rat. *Neuroscience*. 2003;117(4):1037–1046. https://doi.org/10.1016/S0306-4522(02)00664-4.
15. Erickson KI, Voss MW, Prakash RS, et al. *Exercise training increases size of hippocampus and improves memory*. January 2011:1–6. https://doi.org/10.1073/pnas.1015950108/-/DCSupplemental/pnas.201015950SI.pdf.
16. Hoffman MD, Krishnan E. Health and exercise-related medical issues among 1,212 ultramarathon runners: baseline findings from the ultrarunners longitudinal TRAcking (ULTRA) studyLucia A, ed. *PloS One*. 2014;9(1). https://doi.org/10.1371/journal.pone.0083867. e83867-e83868.
17. Braskie MN, Boyle CP, Rajagopalan P, et al. Physical activity, inflammation, and volume of the aging brain. *Neuroscience*. 2014;273:199–209. https://doi.org/10.1016/j.neuroscience.2014.05.005.
18. Rhodes JS, van Praag H, Jeffrey S, et al. Exercise increases hippocampal neurogenesis to high levels but does not improve spatial learning in mice bred for increased voluntary wheel running. *Behav Neurosci*. 2003;117(5):1006–1016. https://doi.org/10.1037/0735-7044.117.5.1006.
19. van Breda K. *The Influence of Methylphenidate on Heart Rate and Brain Connectivity*. PhD Thesis. South Africa: University of Cape Town; 2018. Available from: http://hdl.handle.net/11427/27818.
20. Colcombe SJ, Erickson KI, Scalf PE, et al. Aerobic exercise training increases brain volume in aging humans. *J. Gerontol. Biol. Med. Sci.* 2006;61(11):1166–1170.
21. Batouli SAH, Saba V. At least eighty percent of brain grey matter is modifiable by physical activity. A review study. *Behav Brain Res*. 2017;332:204–217. https://doi.org/10.1016/j.bbr.2017.06.002.
22. Araújo CGS, Scharhag J. Athlete: a working definition for medical and health sciences research. *Scand J Med Sci Sports*. 2016;26(1):4–7. https://doi.org/10.1111/sms.12632.
23. Bull FC, Maslin TS, Armstrong T. Global physical activity questionnaire (GPAQ): nine country reliability and validity study. *J Phys Activ Health*. 2009;6(6):790–804.
24. Amorim P, Lecrubier Y, Weiller E, Hergueta T, Sheehan D. DSM-IH-R psychotic disorders: procedural validity of the mini international neuropsychiatric interview

(MINI). Concordance and causes for discordance with the CIDI. *Eur Psychiatr*. 1998; 13(1):26–34. https://doi.org/10.1016/S0924-9338(97)86748-X.
25. King M, van Breda K, Rauch LH, Brooks SJ, Stein DJ, Ipser J. Methylphenidate alters brain connectivity after enhanced physical performance. *Brain Res*. 2018;1679: 26–32. https://doi.org/10.1016/j.brainres.2017.10.026.
26. King M, Rauch LHG, Brooks SJ, Stein DJ, Lutz K. Methylphenidate enhances grip force and alters brain connectivity. *Med Sci Sports Exerc*. 2017;49(7):1443–1451. https://doi.org/10.1249/MSS.0000000000001252.
27. King M. Predicting the ergogenic response to methylphenidate. *Eur J Appl Physiol*. 2018. https://doi.org/10.1007/s00421-018-3800-8, 0(0):0-0.
28. Leonard CM, Towler S, Welcome S, et al. Size matters: cerebral volume influences sex differences in neuroanatomy. *Cerebr Cortex*. 2008;18(12):2920–2931. https://doi.org/10.1093/cercor/bhn052.
29. Sanchis-Segura C, Ibañez-Gual MV, Adrián-Ventura J, et al. *Sex differences in gray matter volume: how many and how large are they really?*. June 2019:1–19. https://doi.org/10.1186/s13293-019-0245-7.
30. Poldrack RA, Fletcher PC, Henson RN, Worsley KJ, Brett M, Nichols TE. Guidelines for reporting an fMRI study. *Neuroimage*. 2008;40(2):409–414. https://doi.org/10.1016/j.neuroimage.2007.11.048.
31. Ashburner J, Friston KJ. Voxel-based morphometry—the methods. *Neuroimage*. 2000;11(6):805–821. https://doi.org/10.1006/nimg.2000.0582.
32. Hayasaka S, Phan KL, Liberzon I, Worsley KJ, Nichols TE. Nonstationary cluster-size inference with random field and permutation methods. *Neuroimage*. 2004;22(2): 676–687. https://doi.org/10.1016/j.neuroimage.2004.01.041.
33. Eickhoff SB, Stephan KE, Mohlberg H, et al. A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage*. 2005; 25(4):1325–1335. https://doi.org/10.1016/j.neuroimage.2004.12.034.
34. Amunts K, Zilles K. Advances in cytoarchitectonic mapping of the human cerebral cortex. *Neuroimaging Clin*. 2001;11(2):151–169 (vii).
35. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. second ed. Hillsdale, NJ: Erlbaum; 1988.
36. Alvarez-Saavedra M, De Repentigny Y, Yang D, et al. Voluntary running triggers VGF-mediated oligodendrogenesis to prolong the lifespan of Snf2h-null ataxic mice. *Cell Rep*. 2016;17(3):862–875. https://doi.org/10.1016/j.celrep.2016.09.030.
37. Jensen SK, Yong VW. Activity-dependent and experience-driven myelination provide new directions for the management of multiple sclerosis. *Trends Neurosci*. 2016; 39(6):356–365. https://doi.org/10.1016/j.tins.2016.04.003.
38. Eadie BD, Redila Van A, Christie BR. Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density. *J Comp Neurol*. 2005;486(1):39–47. https://doi.org/10.1002/cne.20493.
39. King M, Rauch LHG, Brooks SJ, Stein DJ, Lutz K. Methylphenidate enhances grip force and alters brain connectivity. *Med Sci Sports Exerc*. 2017;49(7):1443–1451. https://doi.org/10.1249/MSS.0000000000001252.
40. Hilty L, Jäncke L, Luechinger R, Boutellier U, Lutz K. Limitation of physical performance in a muscle fatiguing handgrip exercise is mediated by thalamo-insular activity. *Hum Brain Mapp*. 2011;32(12):2151–2160. https://doi.org/10.1002/hbm.21177.
41. Freund W, Faust S, Gaser C, et al. Regionally accentuated reversible brain grey matter reduction in ultra marathon runners detected by voxel-based morphometry. *BMC Sports Sci Med Rehabil*. 2014;6(1):4. https://doi.org/10.1186/2052-1847-6-4.
42. Nelson AJ, Juraska JM, Musch TI, Iwamoto GA. Neuroplastic adaptations to exercise: neuronal remodeling in cardiorespiratory and locomotor areas. *J Appl Physiol*. 2005; 99(6):2312–2322. https://doi.org/10.1152/japplphysiol.00693.2005.
43. Magariños AM, Verdugo JM, McEwen BS. Chronic stress alters synaptic terminal structure in hippocampus. *Proc Natl Acad Sci Unit States Am*. 1997;94(25): 14002–14008. https://doi.org/10.1073/pnas.94.25.14002.
44. Kuipers SD, Trentani A, Boer Den JA, Horst Ter GJ. Molecular correlates of impaired prefrontal plasticity in response to chronic stress. *J Neurochem*. 2003;85(5): 1312–1323.
45. Freund W, Faust S, Birklein F, et al. Substantial and reversible brain gray matter reduction but no acute brain lesions in ultramarathon runners: experience from the TransEurope-FootRace Project. *BMC Med*. 2012;10(1):170. https://doi.org/10.1186/1741-7015-10-170.
46. Peters M. Sex differences in human brain size and the general meaning of differences in brain size. *Can J Psychol*. 1991;45(4):507–522.
47. Kruggel F. MRI-based volumetry of head compartments: normative values of healthy adults. *Neuroimage*. 2006;30(1):1–11. https://doi.org/10.1016/j.neuroimage.2005.09.063.
48. Taubert M, Wenzel U, Draganski B, et al. Investigating neuroanatomical features in top athletes at the single subject levelKotz S, ed. *PloS One*. 2015;10(6). https://doi.org/10.1371/journal.pone.0129508. e0129508-e0129515.
49. García-García I, Michaud A, Dadar M, et al. Neuroanatomical differences in obesity: meta-analytic findings and their validation in an independent dataset. *Int J Obes*. 2019;43(5):943–951. https://doi.org/10.1038/s41366-018-0164-4.
50. Janowitz D, Wittfeld K, Terock J, et al. Association between waist circumference and gray matter volume in 2344 individuals from two adult community-based samples. *Neuroimage*. 2015;122:149–157. https://doi.org/10.1016/j.neuroimage.2015.07.086.
51. Taki Y, Kinomura S, Sato K, et al. Relationship between body mass index and gray matter volume in 1,428 healthy individuals. *Obesity*. 2008;16(1):119–124. https://doi.org/10.1038/oby.2007.4.