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Weight loss and improved mood after aerobic exercise training are linked to lower plasma anandamide in healthy people

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ABSTRACT

Anandamide, a major endocannabinoid, participates in energy metabolism homeostasis and neurobehavioral processes. In a secondary analysis of an open-label, randomized controlled trial, we investigated the long-term effect of aerobic exercise on resting plasma anandamide, and explored its relationship with changes in body weight, cardiorespiratory fitness, and mood status in healthy, physically inactive individuals. Participants recruited between March 2013 to August 2015 at the UNIFESP's Neurology/Psychobiology Department were randomly allocated into a 12-weeks supervised moderate exercise program, or into waitlist, control condition. Thirty-four participants (age = 38 ± 11.5, BMI = 26.6 ± 3.6) were intention to treat-analysed (Exercise: $n = 17$; Control: n = 17). After intervention, there were significant decreases in plasma anandamide ($p < .01$), anger, anxiety, and body weight (all $p < .05$), whereas cardiorespiratory fitness increased ($p < .05$) in the exercise group. There were no significant changes in any variable for the control group. In the whole cohort, adjusted R^2 of multiple linear regressions showed that 12.2% of change body weight was explained by changes in anandamide ($\beta = 0.391$, $p = .033$), while 27% of change in mood disturbance ($\beta = 0.546$, $p = .003$), and 13.1% of change in anger ($\beta = 0.404$, $p = .03$) was explained by changes in anandamide. Our data suggest that the weight loss and mood improvement through regular moderate exercise may involve changes in anandamide metabolism/signaling. Trials registration: #NCT01972607.

1. Introduction

Regular moderate aerobic exercise recognizably promotes physical [1,2] and mental health [3]. Growing data suggest a critical role of endocannabinoids on these health-promoting effects of regular aerobic exercise [4–7]. Endocannabinoids are lipid-derived compounds, which affect several neurobiological processes ranging from behavioral regulation at specific brain circuitries [8–11] to energy metabolism homeostasis at peripheral tissues [12,13]. These molecules play a critical role in the pathomechanisms of metabolic diseases and mood disorders [10,13].

N-Arachidonoyl-ethanolamine, or anandamide (AEA), is the most studied endocannabinoid, and exerts complex, pleiotropic, and multiphasic biological actions through cannabinoid type-1 (CB1) and type-2 (CB2) receptors, as well as by binding to others non-cannabinoid receptors, such as transient receptor potential vanilloid type-1 receptors (TRPV1) and peroxisome proliferator-activated receptors (PPARs) [10,13].

At the behavioral level, AEA increases in the plasma of humans under acute mental [14] or physical stress, such as moderate or intense aerobic exercise [15–19]. This response is akin with behavioral preclinical data showing that increased AEA-CB1 signaling mediates antidepressant, anxiolytic, and stress-buffering effects at the neurohormonal axis [8–11]. Agreeably, plasma AEA concentration is positively correlated with plasma cortisol levels at the end of intense exercise and in the recovery period [16], as well as it positively correlates with increased positive mood domains (improved mood) after acute exercise [15,17].

However, less is known regarding the long-term effects of regular aerobic exercise on the resting, steady-state plasma AEA concentration and whether there would be a correlation with exercise training-induced mood-enhancing effects. At peripheral tissues, AEA seems to

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exert opposite effects on health outcomes. AEA impairs insulin signaling and mitochondrial function/biogenesis via CB1 receptors at adipocytes, liver, and musculoskeletal tissues, reducing peripheral oxidative metabolism pathways, therefore, it has been implicated in obesitogenic and diabetogenic processes [12,13]. Conversely, CB1 receptors blockage (thus presumably blocking AEA-CB1 signaling) increases glucose uptake, resting oxygen uptake and energy expenditure in rodents and humans [20,21].

There is a growing interest on the long-term effects of aerobic exercise on peripheral endocannabinoids, as data point to the potential participation of these molecules in energy metabolism, with implications on weight loss in rodents and humans [5–7]. Two clinical studies have investigated the long-term effect of aerobic exercise on peripheral endocannabinoids concentrations and its influence on weight loss [22,23]. Reductions in salivary AEA levels [23] and plasma 2-Arachidonoyl glycerol levels (another endocannabinoid) [22] were correlated with reductions in anthropometric outcomes and metabolic improvement. Yet, these studies have also focused on diet intervention, thus preventing one to draw conclusions on the effects of exercise alone [22,23]. Furthermore, no study so far has investigated the correlations between changes in cardiorespiratory fitness and AEA after exercise training. AEA affects mitochondrial function and oxidative metabolism pathways [12,13], thus, one could predict associations between changes in AEA and cardiorespiratory fitness (e.g., peak oxygen uptake).

With this background, the purpose of this study was a) to prospectively investigate whether regular aerobic exercise changes the resting, steady-state plasma AEA concentration in previously physically inactive, apparently healthy subjects following a standardized aerobic exercise program, and b) to explore its relationship with changes in body weight, mood profile, and cardiorespiratory fitness. Our alternative hypotheses were a) regular aerobic exercise would reduce the circulating levels of AEA, and b) there would be an association between changes in plasma AEA and changes in body weight, mood scores, and cardiorespiratory fitness. Due to dual actions of AEA on the variables selected, we were prevented from establishing the direction of the hypothesized changes.

2. Material and methods

2.1. Study's design

The data in this study consist of a secondary, post hoc analysis of an open-label, randomized controlled clinical trial aiming to assess clinical outcomes in migraine patients (paper under review). Here, we analyzed only the healthy participants, and the new primary outcome variable was resting plasma AEA concentration. Secondary outcome variables were changes in body weight, mood profile, and cardiorespiratory fitness. The study protocol was approved by the Research Ethics Committee of the Sao Paulo Federal University, and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave their informed consent prior to their inclusion in the study. The trial has been registered in the National Institute of Health (www.ClinicalTrials.gov) under #NCT01972607. The data in this study are reported in agreement with the CONSORT's Statement for non-pharmacological trials [24].

2.2. Participants

Participants were recruited through print and electronic media advertisements and refereed to the Neurology and Psychobiology Departments of the Sao Paulo Federal University for screening and inclusion in the study. The inclusion criteria were: subjects of both sexes, between 18 and 65 years, physically inactive the previous 12 months (defined as ≤1 day/week of leisure-time physical activity). Exclusion criteria were: use of tobacco, alcohol or abuse drugs; taking any prescribed medication, dietary supplements or oral contraceptives, or practicing mind-body activities (e.g., yoga, tai chi, etc.); pregnancy; clinical history of cardiovascular, pulmonary, metabolic, rheumatic, musculoskeletal, psychiatric, or neurological disease. All participants had a neurological and cardiological examination before inclusion in the study.

2.3. Procedures

Participants were randomly assigned to receive aerobic exercise training (EXE group), or enter a waiting list (CT group). Researcher ABO performed simple randomization, with 1:1 allocation ratio, at the psychometric assessment visit. Random numbers were generated by an online software, and even and odds numbers were previously attributed to EXE or CT, respectively.

At experimental visit 1, participants had a neurological examination, and psychological interview for mood assessment, followed by blood collection for AEA quantification. At experimental visit 2, participants were assessed for cardiorespiratory fitness, conducted by a cardiologist and exercise physiologist around 7 days after visit 1. Measurements at both baseline and post-intervention visits were scheduled in the same order and were conducted between 9:00 AM and 11:00 AM. For the EXE group, all measurements at post-intervention visits were carried out within 2–5 days after the last exercise session. All participants were instructed to keep with their usual physical activity and dietary habits throughout the study.

2.3.1. Mood assessment

Questionnaires were filled in at the Psychobiology Department of Sao Paulo Federal University prior to blood collection. We used the Profile of Mood State (POMS) questionnaire [25]. POMS is amply used in exercise and sports field, and it comprises 65 adjectives, each one rated on a 4-point intensity scale (0 = “not at all”, 1 = “a little”, 2 = “moderately”, 3 = “quite a bit”, and 4 = “extremely”), addressing 6 identifiable affective domains: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, Confusion-Bewilderment. Total mood disturbance (TMD) is obtained by summing all negative affective domains scores then subtracting the positive affective domain vigor score [25].

2.3.2. Endocannabinoid extraction/quantification

After questionnaire filling, participants were conducted to blood collection. Participants breakfasted regularly but were asked to abstain from coffee, teas, and any food containing cocoa derivatives, which are foods known to influence AEA metabolism [26], the previous 24 h from blood collection. All women were scheduled in the early phase of the follicular menstrual period. Blood samples were obtained by venipuncture from the antecubital vein of the forearm, collected in ice-chilled EDTA tubes (BD Vacutainer®, Franking Lakes, NY, USA), and immediately centrifuged (3.400 rpm at 4 °C) for 10 min. The plasma was separated and aliquoted in 2 mL-Eppendorf vials and stored at −80 °C until assay.

For plasma extraction, the 500 μL-vials with plasma were transferred into a 10 mL reaction tube. It was added 100 μL of internal standard (purity > 95%) of the stable isotope-labeled AEA-d8 (Cayman, Ann Arbor, MI, USA). Then 4 mL of methyl tertiary-butyl ether (MTBE, Sigma-Aldrich, San Louis, MO, USA) was added and the mixture was vortexed for 30 s and centrifuged at 12,000 *g* for 6 min. The clear supernatant was transferred into a clean 10 ml glass tube and evaporated under N_2 at 37 °C. Dried organic phases were reconstituted in 100 μL of acetonitrile, vortexed for 30s, centrifuged at 12,000 *g* for 6 min and the clear solution was transferred to a LC/MS-MS system. AEA separation and quantification were carried out by a LC-10 CE system (binary pump and auto sampler; Shimadzu®, Columbia, MD, USA) couple to a triple quadrupole mass spectrometer (Waters® Micromass Quattro Micro,

Milford, MA, USA) equipped with a electrospray ionization (ESI) source operated in negative mode. The mobile phase was performed isocratically (Kinetex ® analytical column; 50 × 2.10 mm, 2.6 μm particle size) with acetonitrile:water/ammonium formate 2 nM (90:10, *v*/v) at a flow rate of 200 μL/min, source temperature of 550 °C, and source gas flow of 50 psi (nitrogen as sprayer and heater gas). The Injection volume was 20 μL. The selected ion monitoring for the AEA precursor and product ions was, respectively, *m/z* 348.2 → 62.1. Pure solutions of AEA (Sigma-Aldrich, San Louis, MO, USA) and blank plasma (Golden West Biologicals Inc. ®, Temecula, CA, USA) were utilized for the calibration curve. Seven points (from 0.05 to 50 ng/mL) of AEA concentrations were tested in duplicate, and this showed to be linear within these calibration ranges ($r = 0.998$). The lower limit of detection of this method (defined as a signal/noise ratio 4:1) was 0.025 ng/mL.

2.3.3. Cardiorespiratory fitness/body weight assessment

Participants underwent a symptom-limited maximal cardiopulmonary exercise test on a treadmill (Centurion 300, MICROMED, Brasília, DF, Brazil) with ramp protocol for determination of peak oxygen uptake (VO_{2Peak}) and the ventilatory threshold (VT) [27]. Patients were asked to abstain from coffee 24 h before the test and to restrain from food 2 h before the test. Respiratory gas exchange measurements were obtained breath-by-breath with use of an open-circuit computerized spirometry system (Quark CPET, COSMED, Rome, Italy) and 30-s averages were calculated for analysis. Detailed criteria for VO_{2Peak} and VT can be found elsewhere [27,28]. The VT for each participant was determined by 2 independent, blinded exercise physiologists with at least 2 years of training in exercise testing and interpretation. Body weight (BW) was measured before cardiorespiratory exercise tests, using a Filizola® scale and recorded to the nearest 100 g, according to standardized procedures.

2.3.4. Aerobic exercise training protocol

The protocol was conducted at the Center for Studies on Psychobiology and Exercise with room temperature set between 20 °C and 22 °C, 55–60% relative humidity, and consisted of 12 weeks of aerobic exercise on a treadmill, 3 times per week, with duration of 40 min/session (including warm up = 5 min. and cool down = 5 min.). The exercise intensity was set at the work rate, heart rate and rate of perceived effort elicited at the VT. Heart rate was recorded by a cardiac monitor (model F5, Polar Electro®, Finland). All sessions had the supervision of an experienced exercise physiologist.

2.4. Statistical analysis

All variables were tested for the assumption of normal distribution (Kolmogorov-Smirnov test), and variables with non-normal distribution were analysed by non-parametric test. Between-group differences at baseline were compared by independent *t*-test. Within-group changes from baseline to post-intervention were analysed by Student's paired *t*-test or Wilcoxon signed-rank test. To further explore the relationship between variables, we entered those variables that significantly changed after the intervention period into stepwise multiple linear regression models. Non-parametric variables were standardized by *z*-score transformation. Bisserial point correlation (r) was adopted as a measure of effect size for changes in AEA, while Cohen's f^2 was adopted as the effect size measure in the regression models. Analyses were performed using the SPSS software (IBM, Version 19.0, Chicago, IL). A p-value < .05 was considered statistically significant.

3. Results

Thirty-four participants (M = 12, F = 18; age: 38 ± 11.5, BMI: 26.6 ± 3.6) were included in the intention to treat analysis. Participants' flow in the study is illustrated in Fig. 1. Participant's sociodemographic and anthropometric characteristics are expressed in Table 1. Variables were not statistically different between groups at baseline (Table 2). Participants reported no harm or any adverse effect with the measurements and intervention.

For our primary outcome variable, the EXE group showed a significant decrease of plasma AEA concentration [$t_{(14)} = 3.429$, $p = .004$, $r = 0.67$] after the intervention period, without significant changes in CT group [$t_{(14)} = 1.015$, $p = .32$, $r = 0.26$] (Table 2). For secondary variables, the EXE group showed a significant decrease in anxiety, anger, and BW, and significant increases in VO_{2Peak} and oxygen uptake at the VT (VO_{2VT}) after the intervention period, whereas no secondary variables changed in the CT group (Table 2).

Using the whole cohort, we carried four stepwise multiple linear regression models in order to test possible contributors of changes in body weight (BW) and mood (Table 3). In this models, we used the delta values of those variables that significantly changed after intervention (Fig. 2). Delta AEA values were transformed in percentage values. In the first model, we aimed at identifying possible contributors of changes in body weight (dependent variable). The independent variables chosen to enter the model were AEA, VO_{2Peak}, and VO_{2VT}. Only AEA was a predictor of body weight ($\beta = 0.391$, $p = 0.033$, $f^2 = 0.13$). Adjusted R^2 of multiple regressions showed that 12.2% of change in the body weight was explained by changes in AEA. In the second model, we tested possible contributors of anger (dependent variable) and selected body weight, AEA, VO_{2Peak}, and VO_{2VT} as independent variables. Only AEA was a predictor of anger ($\beta = 0.404$, $p = 0.03$, $f^2 = 0.15$), and the computed adjusted R^2 showed that 13.1% of variance in anger was explained by AEA. In the third model, we tested the contributors of anxiety (dependent variable) and selected the same independent variables tested in the previous model. None of the selected independent variables was predictor of anxiety. Lastly, because there was a marginal significance approaching medium effect size for reduced TMD in the EXE group after intervention period [$t(14) = 1.97$, $p = 0.06$, $r = 0.46$], we tested a model with TMD as dependent variable against the same independent variables of the previous model. This model showed only AEA as a predictor variable ($\beta = 0.546$, $p = 0.003$, $f^2 = 0.36$). The adjusted R^2 revealed that the changes in AEA explained 27% of variance in TMD. In all regression models, tolerance and VIF values ranged from 0.6–0.9 and 1.0–1.2, respectively, implying that multicollinearity was not a concern in these models.

4. Discussion

The main findings of this study are that regular moderate aerobic exercise training reduces plasma AEA levels, and, based on the positive β values of the regression models, this reduction was associated with weight loss and improved mood, in particular with reduced anger.

Regarding the association between AEA and weight loss, our data corroborate previous literature showing reduced AEA concentrations in plasma and saliva of obese people after weight loss through lifestyle changes including caloric restriction and aerobic exercise [22,23], but not after caloric restriction alone [29]. In the study of Matias et al. (2012), salivary AEA levels were positively correlated with body mass index, waist circumference, and fasting insulin levels. These data also align with preclinical studies indicating a negative effects of AEA on peripheral metabolism by impairing insulin signaling and mitochondrial function, which are hold as major molecular pathomechanisms in the etiology of obesity and diabetes [13].

In a recent study, aerobic exercise training promoted reductions of AEA accumulation in the skeletal muscle of rodents under high-fat diet, an effect accompanied by improved glycemia [30]. In humans, although acute aerobic exercise has been consistently shown to increase circulating AEA [5–7], Gasperi et al. (2013) [31] found increased up-regulation and activity of resting fatty acid amide hydrolase (FAAH) - a major enzyme responsible for AEA breakdown - in the lymphocytes of physically active compared to sedentary young men, suggesting higher peripheral AEA metabolism with increased exercise levels. In a more

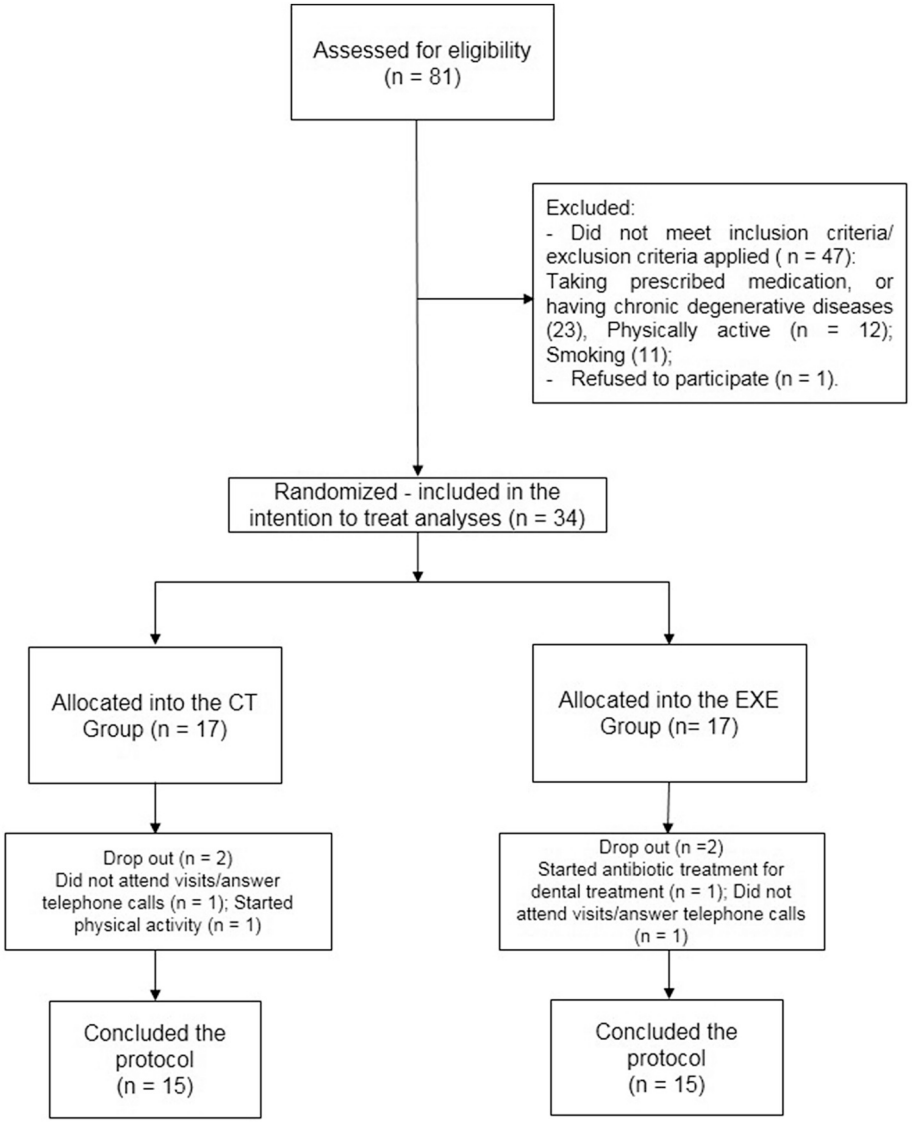

Fig. 1. Participants´ flow in the study.

Table 1
Participants' sociodemographic and anthropometric characteristics.

	Groups	
	CT (n = 17)	EXE (n = 17)
Age (years)	37.0 ± 9.6	39.0 ± 13.4
BW (kg)	73.0 ± 13.3	74.1 ± 14.3
Height (m)	1.66 ± 0.1	1.66 ± 0.09
BMI (kg/m^2)	26.6 ± 3.3	26.8 ± 4.0
Sex (n)		
Male	6(40%)	6(40%)
Female	9(60%)	9(60%)
Education (n)		
> 15 years	11(73.3%)	11(73.3%)
8–15 years	4(26.7%)	4(26.7%)
Ethnicity (n)		
Caucasian	9(60%)	7(46.7%)
Hispanic	4(26.7%)	6(40%)
Black	1(6.7%)	1(6.7%)
Asian	1(6.7%)	1(6.7%)

recent study, male runners exhibiting exercise addiction symptoms showed lower plasma AEA levels at rest and after acute exercise than non-addicted mates [32]. Conversely, induced physical inactivity in rodents - through hind limb suspension aiming at inducing skeletal muscle atrophy - promoted upregulation of synthetic enzymes responsible for AEA and others endocannabinoids synthesis, as well as downregulation of FAAH in skeletal muscle, suggesting higher AEA accumulation with physical inactivity [33]. Thus, AEA seems to be relevant for the metabolic flexibility with changes in physical activity levels, akin with the evidences suggesting a critical role in mitochondrial function [12], with implications on body weight control.

Nonetheless, AEA can also target non-CB receptors with opposite results in peripheral tissues energy metabolism [6,13]. It is believed that by binding to TRPV1 and PPARs receptors, AEA downstream signaling improves oxidative metabolism by stimulating the expression of transcriptors of mitochondrial function, such as peroxisome proliferator-activated receptor gamma co-activator 1 (PGA1-α) [34] [35]. Indeed, these effects have been ascribed for the potential beneficial effect of increased AEA levels in the skeletal muscle during acute exercise [5–7]. As such, AEA actions on pro- and anti-oxidative metabolism through either TRPV1/PPARs or CB1 receptors, respectively, might have been balanced by the changes in peripheral AEA observed here, and could partly explain the lack of associations between AEA levels

Table 2
Anandamide, body weight, mood and cardiorespiratory data.

Variables		CT (ITT n = 17)			EXE (ITT *n* = 17)		
		95% CI			95% CI		
		Mean	Lower	Upper	Mean	Lower	Upper
BW (kg)	Baseline	69.9	65.0	75.1	74.1	67.9	81.6
	Post	69.9	65.5	74.7	73.2*	67.5	80.2
VO_{2Peak} (ml.kg.min-1)	Baseline	33.0	28.5	37.2	34.2	31.0	37.7
	Post	33.5	29.7	37.9	37.4*	32.5	42.7
VO_{2VT} (ml.kg.min-1)	Baseline	18.6	16.3	21.3	19.0	16.8	21.6
	Post	18.5	15.5	22.3	21.8*	18.4	25.2
Anxiety	Baseline	8.6	5.6	12.2	10.2	7.5	13.1
	Post	8.5	5.7	11.5	7.6*	5.5	10.1
Depression	Baseline	4.6	1.2	8.9	4.8	1.8	8.4
	Post	4.9	2.2	8.0	3.8	1.6	6.3
Anger	Baseline	4.5	2.0	7.0	5.7	2.7	9.7
	Post	5.5	2.8	8.3	3.3#	1.2	6.2
Vigor	Baseline	16.1	12.5	19.5	19.1	17.0	21.2
	Post	16.6	13.6	19.7	20.0	18.3	21.9
Fatigue	Baseline	5.1	3.0	7.7	6.4	3.9	9.2
	Post	4.7	2.5	7.1	4.6	2.7	7.1
Confusion	Baseline	5.0	3.2	6.7	5.1	3.2	6.9
	Post	4.9	2.8	6.9	4.7	2.6	7.0
TMD	Baseline	11.8	−0.41	25.7	13.2	0.1	28.0
	Post	12.0	1.7	25.1	4.2	−5.8	16.0
AEA (pmol.mL)	Baseline	0.31	0.25	0.39	0.33	0.28	0.41
	Post	0.26	0.22	0.30	0.22**	0.18	0.26

ITT: Intention to treat; CT: Control; EXE: Exercise training; BW: Body weight; VO_{2Peak}: Peak oxygen uptake; VO_{2VT}: Oxygen uptake at the ventilatory threshold; TMD: Total mood disturbance; AEA: Anandamide. *: $p < .05$, $r > 0.5$ vs baseline; **: $p < .01$, $r > 0.5$ vs baseline; Paired t-test #: $p < .05$, $r = -0.43$ vs baseline; Wilcoxon Signed-Rank test.

Table 3
Stepwise multiple regression models showing significant predictors of change in weight loss and mood.

Model Set I: Predictors of BW change (AEA, $VO_{2Peak,}$ and VO_{2VT})						
Adjusted R^2		B	SE	*β*	t	*p*
AEA	0.12	0.029	0.013	0.39	2.24	0.03
Model Set II: Predictors of Anger change (BW, AEA, $VO_{2Peak,}$ and VO_{2VT})						
AEA	0.13	26.2	11.6	0.40	2.25	0.033
Model Set IV: Predictors of TMD change (BW, AEA, $VO_{2Peak,}$ and VO_{2VT})						
AEA	0.27	128.7	39.5	0.54	3.25	0.003

BW: Body weight; AEA: Anandamide; VO_{2Peak}: Peak oxygen uptake; VO_{2VT}: Oxygen uptake at the ventilatory threshold; TMD: Total mood disturbance.

and oxygen uptake after intervention.

The observed lower circulating AEA levels associated with improved mood, in particular reduced anger, merits a special appraisal, as this finding seems to contradict the current understanding of the relationship between exercise-related mood enhancement and endocannabinoids [15–19]. Several studies indicate a stress-buffering, anxiolytic, and antidepressant effects of endocannabinoids via CB1 receptors [8–11]. AEA fine tunes the activation and the feedback inhibition of the neuroendocrine response to stress, and is thus believed to participate in the mood improvement following acute exercise in both healthy people [15–17] and people with stress-related disorders [36]. In this context, increased rather than lower AEA would be associated with positive mood outcomes. Although these studies describe the acute AEA response to exercise (i.e., after a single exercise session), it is also believed to be the case as for chronic response (i.e., exercise training). Accordingly, in the study of Antunes et al. (2016), which showed reduced resting plasma AEA in exercise-addicted runners [32], the observed lower AEA was accompanied by higher negative mood scores (higher depression, fatigue, confusion, and anger). The authors suggest, therefore, an endocannabinoid dysfunction underlying exercise addiction mood symptoms, including higher anger [32]. It is worth mentioning that the discrepancies found among these studies with regard to the mood and endocannabinoid relationship may lie in the distinct effects of acute versus chronic exercise, measures undertaken during exercise versus resting condition, or heterogeneous populations (i.e., physically inactive, active, or excessively active). Thus, the endocannabinoid responses to acute and chronic exercise in healthy people and under pathological conditions merit further investigation.

Alternatively, our data may be explained by the dual action of AEA on emotionality, depending on the receptor type it binds [10,37]. For example, Moreira et al. (2012) have shown that AEA exerts a dose-dependent dual effect on anxiety-related behavior in translational studies [38]. At low doses, and via CB1 receptors, AEA elicits anxiolytic behavior, while at higher doses AEA facilitate anxiety-like behavior through the activation of TRPV1 channels [38]. However, it is unknown whether AEA-TRPV1 signaling participates specifically in aggressiveness-related behavior (e.g., “anger” in humans). The seminal researches conducted by Dr. Mechoulam's lab suggest the participation of AEA and other cannabinoids in aggressive behavior [39], but this research line has never been further explored. Therefore, one alternative explanation for our data would be that the reduction in the AEA-TRPV1 signaling pathways through regular exercise would mediate mood improvement. This assumption is still conjectural, but merits further research.

One should be cautious while interpreting our results regarding the mood outcomes. We did not provide data here on the source of the circulating AEA measured; therefore, we cannot assure that the circulating AEA levels would reflect its metabolism in the central nervous system. Likewise, we also cannot assert which tissue compartment the measured circulating AEA level would reflect most (e.g., adipose tissue, skeletal muscle, blood born cells, etc.). Therefore, our association data could represent spurious correlations.

The limitations of this study are as follow: this is a secondary, post hoc analysis, therefore the primary variable of interest was changed, and data were analysed after randomization. Yet, as the most important concern would be related to blinding, the change in the primary outcome variable is acceptable in this case, as this is an open-label trial (i.e., one cannot blind exercise intervention) [40]. Our sample size was small, which reflected the small effect sizes observed in the regression models. In addition, our sample was heterogeneous regarding age, ethnicity, and sex, and we did not control covariates that could affect circulating AEA levels, such as dietary composition [41]. Future powered studies should test the a priori alternative hypothesis assuming significant effects of changes in AEA levels on body weight and mood outcomes.

5. Conclusions

In summary, the data of this study suggest a reduction in the circulating levels of AEA through moderate aerobic exercise training, which was associated with weight loss and improved mood. Further studies are necessary to confirm these responses. The understanding of endocannabinoids regulation by acute and chronic exercise constitute an important research area with implications on weight control and mental health.

Conflict of interest

The authors declare no conflict of interest.

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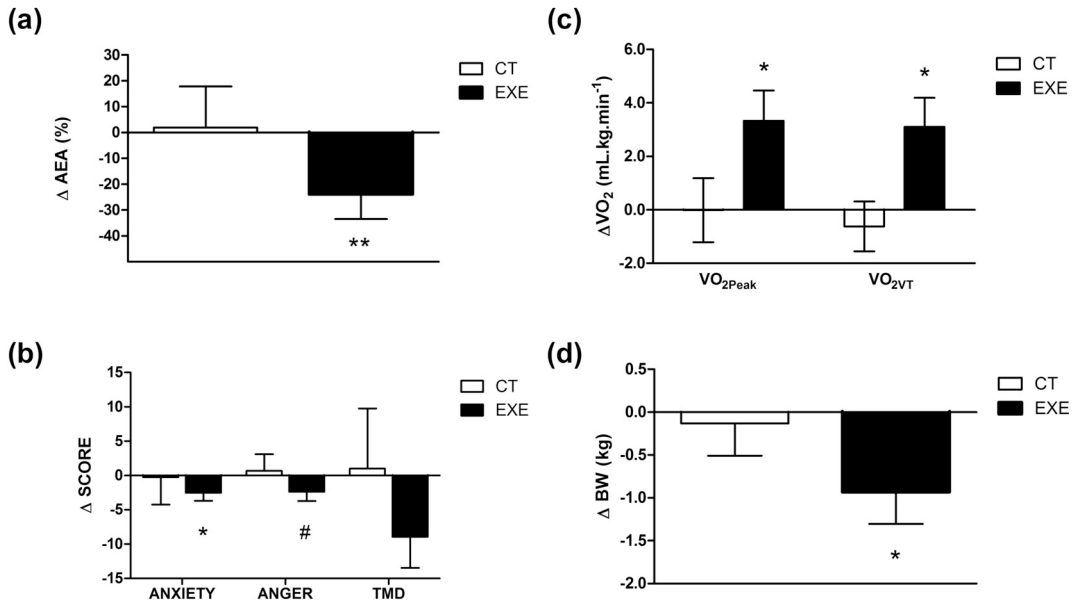

Fig. 2. Delta values of AEA (a), mood scores (b), cardiorespiratory fitness (c), and BW (d) variables after intervention that entered regression models. Variables are expressed as mean ± SEM.
CT: Control; EXE: Exercise training; AEA: Anandamide; VO_{2Peak}: Peak oxygen uptake; VO_{2VT}: Oxygen uptake at the ventilatory threshold; TMD: Total mood disturbance. BW: Body weight. *: $p < 0.05$; **: $p < 0.01$, paired *t*-test. #: $p < 0.05$, Wilcoxon signed-rank test.

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