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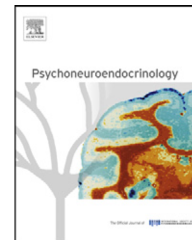

Intense exercise increases circulating endocannabinoid and BDNF levels in humans— Possible implications for reward and depression

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Summary The endocannabinoid system is known to have positive effects on depression partly through its actions on neurotrophins, such as Brain-Derived Neurotrophic Factor (BDNF). As BDNF is also considered the major candidate molecule for exercise-induced brain plasticity, we hypothesized that the endocannabinoid system represents a crucial signaling system mediating the beneficial antidepressant effects of exercise. Here we investigated, in 11 healthy trained male cyclists, the effects of an intense exercise (60 min at 55% followed by 30 min at 75% W_{max}) on plasma levels of endocannabinoids (anandamide, AEA and 2-arachidonoylglycerol, 2-AG) and their possible link with serum BDNF. AEA levels increased during exercise and the 15 min recovery ($P < 0.001$), whereas 2-AG concentrations remained stable. BDNF levels increased significantly during exercise and then decreased during the 15 min of recovery ($P < 0.01$). Noteworthy, AEA and BDNF concentrations were positively correlated at the end of exercise and after the 15 min recovery ($r > 0.66$, $P < 0.05$), suggesting that AEA increment during exercise might be one of the factors involved in exercise-induced increase in peripheral BDNF levels and that AEA high levels during recovery might delay the return of BDNF to basal levels. AEA production during exercise might be triggered by cortisol since we found positive correlations between these two compounds and because corticosteroids are known to stimulate endocannabinoid biosynthesis. These findings provide evidence in humans that acute exercise represents a physiological stressor able to increase peripheral levels of AEA and that BDNF might be a mechanism by which AEA influences the neuroplastic and antidepressant effects of exercise.
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1. Introduction

Chronic physical exercise has beneficial effects on the ''depressive-like'' phenotype and involves changes in adult neurogenesis with possible impact on reward and cognitive behavior (Ernst et al., 2006; Dishman et al., 2006; Brene et al., 2007). Up to date, Brain-Derived Neurotrophic Factor (BDNF), a member of the neurotrophin family promoting neuronal survival and proliferation (Castren and Rantamaki, 2010), has been described as one of the best potential candidate molecules playing a role in exercise-induced antidepressant effects (Duman et al., 2008; Li et al., 2008), particularly through promotion of neurogenesis (Lafenetre et al., 2010; Erickson et al., 2011). Recent data underline the putative role of the endocannabinoid system in the etiology of depression. Thus, the two most studied endocannabinoids, *N*-arachidonoylglycerol (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), which are synthesized on demand in various central and peripheral tissues, have the capacity, through their agonist effects on the cannabinoid CB1 receptor, to alter cognitive and emotional behaviors, neurogenesis, and the levels of neurotrophins, such as BDNF (Gorzalka and Hill, 2010). These behaviors and molecules are also influenced by physical exercise, and one could speculate that the endocannabinoid system represents a crucial signaling system mediating the beneficial antidepressant effects of exercise. In addition to its putative antidepressant effects, the endocannabinoid system may acutely influence mood through its effects on pain perception (Pertwee, 2001) and its facilitation of dopamine release in the nucleus accumbens (Maldonado et al., 2006; Cheer et al., 2007). Endocannabinoids have thus been hypothesized to be linked with the so-called ''runners high'', an intense but transient positive emotion during exercise (Dietrich and McDaniel, 2004; Keeney et al., 2008; Fuss and Gass, 2010; Garland et al., 2011).

To date, only one human study has investigated the specific effects of exercise on plasma endocannabinoid levels in humans, although without dealing with correlates of cognitive or emotional function (Sparling et al., 2003). The authors observed a significant increase of plasma AEA but not 2-AG in trained subjects following a 45-min acute exercise (Sparling et al., 2003). Unfortunately, the exercise intensity was not individualized for each subject and the exercise was performed at different times from the last non-standardized meal, between 14.00 and 17.00 h. This lack of method standardization might have influenced the results since the variability of exercise intensity is reflected into the level of stress (Urhausen et al., 1995) and the differences of quantity and quality of food and of the time-lag from the last meal might influence the endocannabinoid levels (Di Marzo and Matias, 2005).

Few other studies used rodents to investigate the effects of exercise on endocannabinoid signaling, specifically in the brain. Authors showed that 15 days, 10 days, or eight days of free access to running wheels sensitized the CB1 receptor-mediated responses in the striatum (De Chiara et al., 2010), increased the expression of CB1 receptor mRNA in the hippocampus (Wolf et al., 2010), or increased CB1 receptor binding and intrinsic activity and AEA levels in hippocampus (Hill et al., 2010a), respectively.

Four recent animal studies addressed the question as to whether the exercise-induced modification of endocannabinoid signaling mediates wheel-running-induced effects on depression by correlating with, e.g. neurogenesis (Dubreucq et al., 2010; Hill et al., 2010a; Wolf et al., 2010) or stress-induced anxiety (De Chiara et al., 2010). In three of such studies, it was suggested that modification of endocannabinoid signaling may represent a crucial factor in exercise-induced neurogenesis (Hill et al., 2010a; Wolf et al., 2010) and stress coping (De Chiara et al., 2010). However, caution should be taken when extrapolating to humans the results of these animal studies, in which pharmacological and genetic approaches were used. These approaches have the usual disadvantages of systemic applications and congenital alterations, possibly inducing basal neurohormonal alterations (Steiner and Wotjak, 2008) and not necessarily representative of local physiological changes induced by exercise. Furthermore, although wheel-running is voluntary, the time and frequency spent exercising differ between rodents and humans, since, in humans, voluntary training sessions consist of acute, often single, daily bouts of exercise. Most importantly, free access to wheel running represents not only a voluntary exercise, but also the opportunity of being an enriched environment, two environmental conditions known to act positively on neurogenesis (Will et al., 2004). The observation of a specific role of exercise may be overestimated by the presence of the enriched environment.

Based on this background, the purpose of the current study was to examine, in an homogeneous group of cyclists, the effect of a well-standardized exercise on the plasma levels of the two most studied endocannabinoids (2-AG, AEA), and of the two AEA congeners with activity at peroxysome proliferator-activated receptor-α (PPAR-α) [*N*-oleylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA)], and their possible link with BDNF, the major candidate molecule for exercise-induced brain plasticity. Other factors such as β-endorphins, which might also play a role and synergize with endocannabinoids in reward (Trezza et al., 2011) and cortisol, which stimulates endocannabinoid biosynthesis and is down-regulated by CB1 in the brain (Hill et al., 2010b), were also measured. The exercise protocol and the time intervals of blood sampling used in this study allowed to investigate the effects of two individualized intensities of exercise and the impact of the duration of exercise as well as of the recovery phase.

2. Methods

2.1. Subjects

Eleven young well-trained male cyclists [age 23.3 ± 5.1 (SD) yr, body mass 77.4 ± 8.3 (SD) kg, height 1.83 ± 0.07 (SD) m] participated in the study. All subjects gave written informed consent after receiving information regarding the nature and purpose of the experimental protocol. The study was approved by the ethical committee of the Vrije Universiteit Brussel, Belgium.

2.2. Study design

No exercise practice, alcohol, coffee were permitted in the 24 h before each exercise. All subjects were non-smokers.

2.3. Maximal exercise test

Before starting the experimental exercise protocol, subjects underwent a thorough physical examination and completed an incremental maximal exercise test until exhaustion (80 Watt + 40 Watt/3 min) on an ergometric bicycle to determine maximal power output (W_{max}) (mean: 330.7 ± 19.7 (SD) *W* in the eleven subjects). Results obtained with this test were used to calculate the intensities of 55% and 75% of W_{max} for the experimental trial.

2.4. Experimental exercise protocol

Subjects completed one familiarization trial and then one experimental trial, separated by one week. The purpose of the familiarization trial was to accustom the subjects to the exercise protocol. The procedure during the familiarization trial was identical to the experimental trial in all aspects except for blood sampling.

The day of the experimental trial, subjects entered the laboratory in the morning 90 min after a standardized breakfast. Height and nude body mass were measured. After a thorough examination and a resting electrocardiogram, an indwelling venous canula was introduced into a superficial forearm vein to enable repeated blood samplings at rest, during exercise and during recovery.

Approximately 135 min after their breakfast, subjects entered the climatic chamber (18 °C, relative humidity between 50% and 60%) and rested in a seating position for 15 min. Then the long duration exercise protocol was started, involving 60 min of pedaling at 55% of their W_{max} on an ergometric bicycle. This enabled to assess the effects of a constant exercise of moderate load on endocannabinoids. This moderate exercise was then immediately followed by a time trial protocol, an intense endurance performance test with a known endpoint (i.e. a certain target amount of work). Subjects were requested to complete a predetermined amount of work equal to 30 min at 75% of W_{max} as quickly as possible (Jeukendrup et al., 1996) but they did not get any feedback on the time elapsed. The time trial was used to test if an increase in exercise intensity would have an additional influence on the plasma levels of endocannabinoids. Following the end of the time trial, subjects sat down to recover during 15 min. During the whole exercise protocol, subjects received water *ad libitum*.

Ratings of perceived exertion (RPE) levels (Borg, 1982) were registered every 15 min during the 60 min exercise, and every 10 min during the time trial.

2.5. Blood collection and biochemical analysis

Venous blood samples were taken at four different time points: at rest (after 15 min quietly sitting), after the continuous exercise of 60 min at 55% of W_{max}, at the end of the time trial, and after 15 min of recovery. Samples were drawn directly into pre-cooled 5-mL K_3EDTA tubes and 7-mL tubes with silica clot activator. A 0.5 mL aliquot of whole blood was extracted and used for the determination of hematocrit. EDTA blood was immediately centrifuged (less than 5 min after sampling) and plasma was removed and frozen (−80 °C) pending analysis, in duplicate, of endocannabinoids (2-AG, AEA), AEA congeners (PEA and OEA) (as detailed below), and β-endorphin (Radioimmunoassay Kit, Nichols Institute Diagnostics, DA, USA). Clot activator containers were left to clot for 1 h at room temperature before centrifugation. The resulting serum was stored at −80 °C pending analysis in duplicate of BDNF (ELISA, Chemicon, Temecula, CA, USA) and cortisol (RIA, Diasorin Stillwater, USA).

2.5.1. Extraction, purification and quantification of plasma endocannabinoids and AEA congeners

Extraction, purification and quantification of AEA, 2-AG, PEA and OEA from blood require several steps as described previously (Cote et al., 2007). First, blood samples were dounce-homogenized and extracted with chloroform/methanol/Tris—HCl 50 mM pH 7.5 (2:1:1, v/v) containing internal deuterated standards for AEA, 2-AG, PEA and OEA quantification by isotope dilution ($[^2H]_8$AEA, $[^2H]_5$2AG, $[^2H]_4$PEA, and $[^2H]_4$OEA (Cayman Chemicals, MI, USA)). The lipid-containing organic phase was dried down, weighed and pre-purified by open bed chromatography on silica gel. Fractions were obtained by eluting the column with 99:1, 90:10 and 50:50 (v/v) chloroform/methanol. The 90:10 fraction was used for AEA, 2-AG, PEA and OEA quantification by liquid chromatography—atmospheric pressure chemical ionization-mass spectrometry (LC—APCI-MS), as previously described and using selected ion monitoring at [M+1] values for the four compounds and their deuterated homologues (Di Marzo et al., 2001).

2.6. Statistical analysis

Statistics were computed using Statistica 6.0 software. Normality was tested using Kolmogorov—Smirnov tests. Changes in hematocrit levels, plasma β-endorphins and endocannabinoids, serum BDNF and cortisol, were analysed by one-way ANOVAs (time effect) with repeated measures. If a significant main effect was observed, Duncan's multirange post hoc tests were applied to examine specific pairwise differences. Rates of perceived exertion (non-parametric) were analysed using Friedman ANOVAs and Wilcoxon Matched Pairs tests. Pearson (or Spearman for non-parametric data) rank order correlation coefficients were used to detect correlations between variables. $P < 0.05$ was considered statistically significant. Data are reported as means ± standard deviation (SD).

3. Results

Hematocrit levels increased significantly during the intense exercise (time trial) ($P < 0.005$) and decreased significantly during recovery ($P < 0.001$) (45.2 ± 1.8% at rest, 45.7 ± 2.1% after 60 min of moderate exercise, 46.7 ± 2.2% after time trial, 44.9 ± 1.5% after 15 min recovery) (ANOVA time effect, $P < 0.001$). Therefore, blood metabolite and hormone concentrations have been corrected to account for plasma volume changes (Van Beaumont, 1972).

Plasma AEA, OEA and PEA concentrations increased significantly during exercise and/or recovery (ANOVA time effects, $P < 0.001$ for the three compounds), whereas plasma 2-AG concentrations remained stable during exercise and recovery (Fig. 1). Noteworthy, the increase in AEA, when expressed per time unit, was higher during the 15 min recovery (0.035 ± 0.04 pmol/mL/min) compared to the moderate

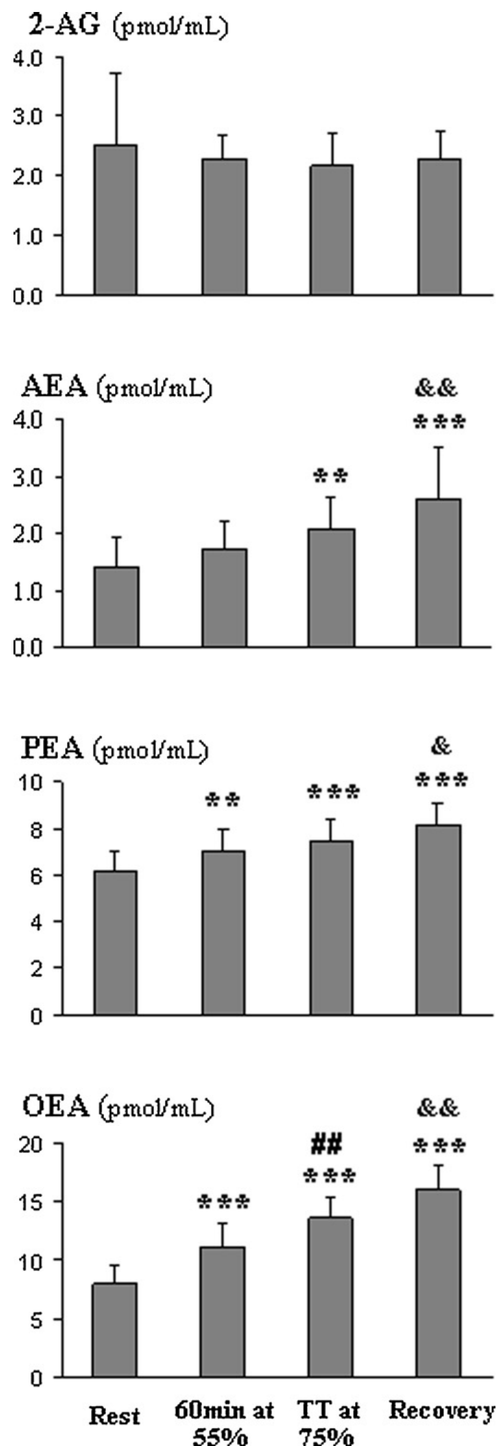

Figure 1 Plasma concentrations of endocannabinoids and non-endocannabinoid *N*-acylethanolamines during exercise and recovery. Values are presented as mean ± SD. Blood samples taken at rest, at the end of the 60-min exercise at 55% W_{max} (60 min at 55%), at the end of the time trial (TT at 75%) and after 15 min of recovery (recovery). Comparable results were obtained when concentrations were expressed in pmol/mg of lipid extract. Significant difference from rest: $^{**}P < 0.01$, $^{***}P < 0.001$. Significant difference from 60 min at 55%: $^{\#\#}P < 0.01$. Significant difference from TT at 75%: $^{\&}P < 0.05$, $^{\&\&}P < 0.01$.

exercise phase (0.006 ± 0.006 pmol/mL/min) or to the intense exercise phase (0.010 ± 0.018 pmol/mL/min) ($P < 0.05$).

Serum cortisol levels increased significantly during the intense exercise and continued to increase during recovery (ANOVA time effect, $P < 0.001$) (Fig. 2).

The increase in AEA concentrations during the 15 min recovery (+27.5 ± 29.9%) was positively correlated to the increase in serum cortisol during the same time (+12.8 ± 15.1%) ($r = 0.60$, $P < 0.05$). In addition, a positive correlation was detected between AEA ($r = 0.61$, $P < 0.05$) and cortisol levels after 15 min of recovery.

Exercise had also an important effect on plasma BDNF levels, which rose significantly during exercise and then decreased during the 15 min of recovery to return to basal levels (ANOVA time effect, $P < 0.01$) (Fig. 2). Plasma AEA and

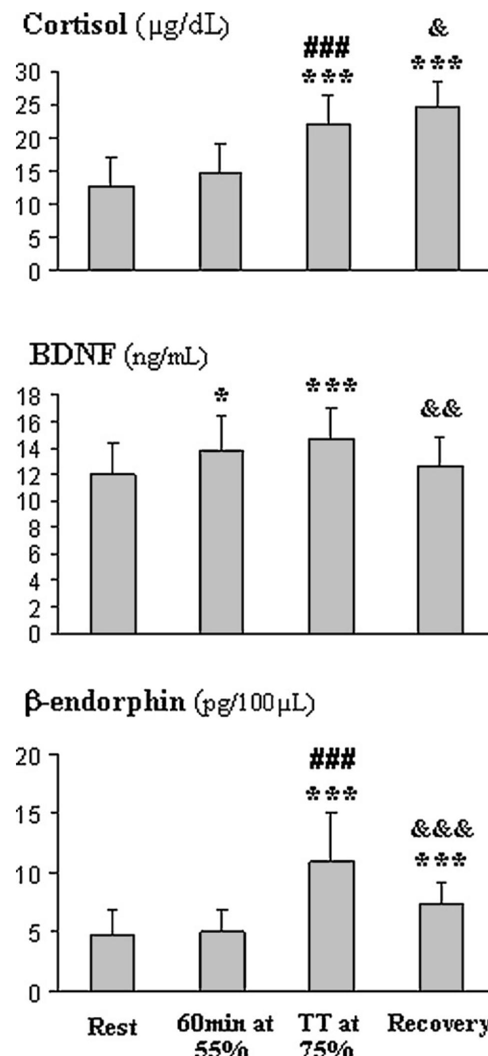

Figure 2 Change in other neurobiological correlates of stress and depression in blood during exercise and recovery. Values are presented as mean ± SD. Blood samples taken at rest, at the end of the 60-min exercise at 55% W_{max} (60 min at 55%), at the end of the time trial (TT at 75%) and after 15 min of recovery (recovery). Significant difference from rest: $^{*}P < 0.05$, $^{***}P < 0.001$. Significant difference from 60 min at 55%: $^{\#\#\#}P < 0.001$. Significant difference from TT at 75%: $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, $^{\&\&\&}P < 0.001$.

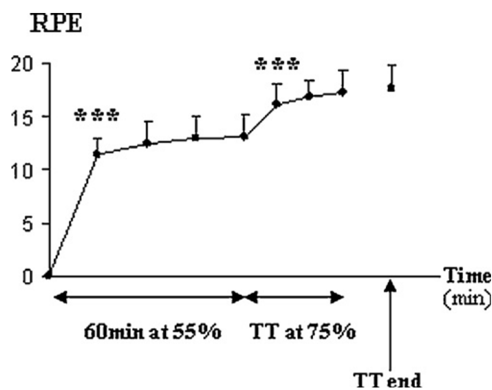

Figure 3 Rates of perceived exertion during moderate and intense exercises. Values are presented as mean ± SD. 60-Min exercise at 55% W_{max}: 60 min at 55%, time trial: TT at 75%. Difference from the preceding time point: ***$P < 0.001$.

serum BDNF concentrations were positively correlated at the end of time trial ($r = 0.71$, $P < 0.05$) and at 15 min recovery ($r = 0.66$, $P < 0.05$). No significant correlations appeared between BDNF and cortisol concentrations or variations.

β-Endorphin concentrations increased significantly only when intensity was high (during the time trial) and decreased significantly during recovery albeit without returning to basal levels (ANOVA time effect, $P < 0.001$) (Fig. 2).

RPE levels during moderate and intense exercises are presented in Fig. 3. No significant correlations appeared between RPE levels and endocannabinoid concentrations. We only detected a positive correlation between the percent change in β-endorphin and the percent change in RPE during the time trial exercise ($r = 0.62$, $P < 0.05$).

4. Discussion

The present study provides evidence in humans that acute exercise represents a physiological stressor able to increase peripheral levels of AEA and its congeners without changing 2-AG levels. The use of two exercise intensities individually fixed, as well as of strict conditions in terms of food intake prior to exercise, and hour of the day when the experiment was performed, strengthen the impact of our results. Above all, our data suggest that the plasma AEA increment during exercise might be one of the factors involved in the exercise-induced increase in peripheral BDNF, a factor promoting antidepressant effects of exercise.

AEA, OEA, and PEA are all *N*-acylethanolamines synthesized through the hydrolysis of *N*-acylphosphatidylethanolamines formed from the *N*-acylation of phosphatidylethanolamine with arachidonic, oleic, or palmitic acids, respectively, and mostly degraded by a common enzyme, the fatty acid amide hydrolase (FAAH) (Petrosino et al., 2009). In contrast, 2-AG biosynthesis depends from other precursors (diacylglycerols) and enzymes (diacylglycerol lipases) and this endocannabinoid is mainly degraded by monoacylglycerol lipase (Petrosino et al., 2009). Thus, it is not surprising that AEA, OEA and PEA plasma concentrations, by sharing similar metabolic pathways, present similar patterns of changes following exercise in our study, completely different from the lack of changes of 2-AG. AEA, OEA and PEA derive from different biosynthetic precursors, but, apart from FAAH, they can be degraded in humans by two other enzymes, FAAH-2 and *N*-acylethanolamine acid amidase, which, however, have very low affinity for AEA vs. OEA and PEA, respectively (Di Marzo, 2008). Therefore, if the common regulation of the plasma concentrations of these compounds is exerted at the level of their inactivation, it is more likely that FAAH operates such regulation. It should be noted that PEA and OEA do not bind the CB1 but they act on PPAR-α nuclear receptors, which are involved in lipolysis. Hence, unlike the elevation of plasma AEA levels, which is likely to stimulate CB1 receptors with anti-lipolytic activity (Di Marzo, 2008), the increases in OEA and PEA levels might produce the opposite effect via PPAR-α. However, these compounds might also prolong and enhance AEA biological activity by competing with AEA for FAAH-mediated degradation (Petrosino et al., 2009). Further studies are required to understand the cellular and tissue origin of plasma endocannabinoids and *N*-acylethanolamines in order to fully interpret the meaning of the exercise-induced changes described here.

In fact, many peripheral tissues involved in exercise adaptation, among which the adipose tissue, the liver (Matias et al., 2006) and also the skeletal muscle (Newman et al., 2007), possess the ability to synthesize and release endocannabinoids and *N*-acylethanolamines into the blood. In addition, due to their high lipophilic properties, endocannabinoids readily cross the blood—brain barrier. Hence, the peripheral increase in circulating levels of AEA in our study might either be accompanied by, or reflect, a central increase. Accordingly, in an animal study, eight days of free access to running wheels elevated the total content of AEA in hippocampus, without any change in FAAH maximal hydrolytic activity, reflecting a potential increase in AEA biosynthesis (Hill et al., 2010a). On the contrary, and similar to plasma 2-AG levels in our study, hippocampal 2-AG levels were not significantly changed by exercise (Hill et al., 2010a).

The mechanisms by which exercise might alter specifically AEA, PEA and OEA levels, either centrally or in the periphery, remain to be explored. Endocannabinoid signaling is known to be recruited by stress and glucocorticoid hormones, particularly cortisol (Hill and McEwen, 2010). Hill et al. (2010c) demonstrated that systemic administration of the glucocorticoid corticosterone resulted in an increase in the tissue content of AEA within several brain structures (amygdala, hippocampus, hypothalamus) in rats, while 2-AG appeared less responsive (as it increased only in hypothalamus). The rapidity of AEA increase (10 min after corticosterone administration) suggests that glucocorticoids act trough a non-genomic pathway (Hill et al., 2010c), compatible with acute adaptations to a physiological stressful condition such as intensive physical exercise. Indeed, in agreement with the literature (Urhausen et al., 1995), we observed a great increase in serum cortisol levels after intense exercise, which even continued during recovery. We therefore hypothesize that this increase in cortisol secretion, triggered by long intense exercise, might have stimulated AEA production. This possibility is corroborated by the significant positive correlation between serum cortisol and plasma AEA levels in our study. Nevertheless, before generalizing our findings to the whole population, supplementary data in sedentary subjects should be obtained.

Considering the putative role of the endocannabinoid system in the etiology of depression (Gorzalka and Hill, 2010), it seems reasonable to speculate that the intense exercise-induced increase in circulating AEA levels observed in the present study might promote antidepressant-like effects. For instance, with respect to the effect of endocannabinoid signaling on analgesia (Pertwee, 2001) and reward (Cheer et al., 2007; Maldonado et al., 2006) and to the anti-inflammatory and antinociceptive effects of PEA (LoVerme et al., 2006), we hypothesized that the increment in circulating AEA and PEA levels during intense acute exercise might contribute to a possible immediate feeling of well-being and explain why the repetition of such exercises might drive people to a kind of exercise addiction (Kanarek et al., 2009). However, we did not find any correlations between RPE and AEA or PEA concentrations in the present study, possibly because RPE remains a subjective emotional feeling, the evolution of which may sometimes differ from that of pleasure—displeasure rating during and after exercise (Backhouse et al., 2007). It should be emphasized that traditionally the molecule considered to mediate the fleeting sense of euphoria and calm experienced after prolonged exercise is β-endorphin (Morgan, 1985). However, the major limitation of the β-endorphin hypothesis is that endorphins in the systemic circulation cannot be taken as indicative of central effects, since endorphins do not cross the blood—brain barrier. Nevertheless, the role of β-endorphins in exercise-derived pleasure merits further investigation since we detected a positive correlation between increase in circulating β-endorphin concentrations and RPE only during the time trial, the intensity of which is compatible with effects on pain perception (Hoffman et al., 2004).

Another well-known mechanism underlying the antidepressant effect of exercise is the increase in neurogenesis and neuronal plasticity (Brene et al., 2007). The recent study by Hill et al. (2010a) in rats raised the possibility that AEA/CB1 receptor signaling would be an important mediator of the exercise-induced plasticity in the hippocampus. In the present investigation in humans, serum BDNF was measured because it represents an indicator of brain BDNF production during exercise (Rasmussen et al., 2009) and because an increase in peripheral BDNF is known to increase neurogenesis in hippocampus (Erickson et al., 2011), hence to contribute to the antidepressant effects of exercise (Duman et al., 2008; Li et al., 2008). A major result of our study is the observation of significant positive correlations between plasma AEA and serum BDNF concentrations both at the end of time trial and after recovery. It is then tempting to suggest that the AEA increase following high intensity exercise contributes to BDNF production and that high levels of AEA during recovery delay the return of BDNF to basal levels. In line with our interpretation, an acute peripheral administration of a CB1 receptor agonist was shown to induce an increase in serum BDNF levels in humans, hypothetically through activation of the ERK signaling pathway (D'Souza et al., 2009). Accordingly, in specific rat brain areas, notably those involved in reward and addiction, a BDNF up-regulation was observed after one-week of peripheral exposure to a CB1 agonist (Butovsky et al., 2005). Moreover, BDNF was shown to be a key mediator of endocannabinoid-induced neuroprotection against other types of acute stress, such as glutamate excitotoxicity (Khaspekov et al., 2004). Thus, the positive effect of intense exercise on AEA plasma levels and its putative repercussion on BDNF secretion merit further investigation, both in healthy and depressive populations.

Finally, considering that the endocannabinoid system exerts also important roles in skeletal muscle metabolism (Eckardt et al., 2009; Watt, 2009) and tension (Newman et al., 2007; Huerta et al., 2009), future research on the effects of acute and chronic exercise on endocannabinoid signaling in patients with metabolic disorders, and on the potential role of this system in skeletal muscle regeneration following intense and prolonged contraction, is also to be fostered.

5. Conclusion

This study demonstrates, in humans, that acute exercise, all the more if intense, induces a large increase in circulating levels of AEA, which persists during recovery, possibly through mechanisms involving cortisol. Our findings also shed new light on the fact that BDNF may be a mechanism by which AEA/CB1 signaling influences the neuroplastic and antidepressant effects of exercise. However, since our data are still of a correlative nature, mechanistic studies will have to be performed to confirm the cause—effect relationships between cortisol and AEA and between AEA and BDNF, in humans during acute exercise.

Role of funding source

Nobody forced us in collecting and processing data.

Conflicts of interest

All authors report no biomedical financial interests or potential conflicts of interest.

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