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Aerobic Fitness is Associated with Cerebral mu-Opioid Receptor Activation in Healthy Humans

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ABSTRACT

Introduction: Central μ-opioid receptors (MORs) modulate affective responses to physical exercise. Individuals with higher aerobic fitness report greater exercise-induced mood improvements than those with lower fitness, but the link between cardiorespiratory fitness and the MOR system remains unresolved. Here we tested whether maximal oxygen uptake (VO_{2peak}) and physical activity level are associated with cerebral MOR availability and whether these phenotypes predict endogenous opioid release following a session of exercise. **Methods:** We studied 64 healthy lean men who performed a maximal incremental cycling test for VO_{2peak} determination, completed a questionnaire assessing moderate-to-vigorous physical activity (MVPA, min/week), and underwent positron emission tomography (PET) with [^{11}C]carfentanil, a specific radioligand for MOR. A subset of 24 subjects underwent additional PET scan also after a one-hour session of moderate-intensity exercise and 12 of them also after a bout of high-intensity interval training (HIIT). **Results:** Higher self-reported MVPA level predicted greater opioid release after HIIT, and both VO_{2peak} and MVPA level were associated with a larger decrease in cerebral MOR binding after aerobic exercise in the ventral striatum, orbitofrontal cortex, and insula. That is, more trained individuals showed greater opioid release acutely following exercise in brain regions especially relevant for reward and cognitive processing. Fitness was not associated with MOR availability. **Conclusions:** We conclude that regular exercise training and higher aerobic fitness may induce neuroadaptation within the MOR system, which might contribute to improved emotional and behavioural responses associated with long-term exercise.

Key Words: OPIOID SYSTEM, POSITRON EMISSION TOMOGRAPHY (PET), BRAIN IMAGING, FITNESS, PHYSICAL ACTIVITY LEVEL

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Habitual physical activity and cardiorespiratory fitness (CRF) are well-established modifiable lifestyle factors that promote brain health throughout the lifespan. Higher fitness and greater amounts of physical activity are linked with better cognitive functioning (1), lower levels of anxiety and depression (2), and reduced risk for neurodegenerative disease (3). These biological and psychological benefits of exercise are paralleled in brain structure and function. Better fitness and higher physical activity levels are associated with higher grey (4,5) and white matter volume (4,6). In addition, several intervention studies have demonstrated that improved fitness positively affects brain volumes in older adults, especially in frontotemporal regions that are important for cognition and memory functions, and most susceptible to age-related brain atrophy (7,8). Moreover, higher aerobic fitness promotes efficient functional connectivity of multiple brain networks supporting cognitive control and memory functions (9,10).

Physical exercise also acutely affects the functioning of the brain's neuromodulatory systems, particularly the endogenous opioid system (11). Endogenous opioid system and especially μ-opioid receptors (MORs) are closely involved in processing reward, motivation, and emotions (12). They also have a central role in several physiological functions, such as pain processing (13) and stress regulation (14), and recent evidence links opioid system dysregulation with depressive and anxious symptoms (15). Therefore, the opioid system could potentially mediate the psychological benefits of regular exercise, such as anxiolysis and improved mood.

Animal studies investigating the effects of regular exercise training on the opioid system have found elevated β-endorphin and met-enkephalin levels in the periaqueductal grey area and rostral ventromedial medulla after five weeks of treadmill running (16) and shown that chronic

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exercise, in comparison with short-term exercise or no exercise, decreases MOR expression (17) and overall MOR availability in rat brain (18). Exercising rats also show reduced sensitivity to antinociceptive effects of exogenous opioid agonists such as morphine, which may indicate downregulation of MORs resulting from increased endogenous opioid concentrations elevated by regular exercise training (19,20). Human neuroimaging studies have demonstrated that a single bout of moderate-to-vigorous exercise stimulates endogenous opioid release in the brain, which is associated with affective responses induced by exercise (21-23). Taken together, converging evidence from animal and human studies suggest that regular exercise training might induce neuroadaptation within the central MOR system, subsequently contributing to improvements in mood and stress regulation. Yet, *in vivo* evidence from humans is currently lacking.

Here we investigated whether individual differences in baseline MOR availability are associated with cardiorespiratory fitness and habitual physical activity levels in healthy young men. We used *in vivo* PET imaging with the highly selective MOR agonist ligand [^{11}C]carfentanil. We coupled MOR data with measurement of VO_{2peak}, an objective and direct measure of CRF, and with self-reported physical activity questionnaires. To test whether higher fitness and physical activity levels influence the capacity of acute exercise to activate the MOR system, we also studied a subset of participants with [^{11}C]carfentanil PET after a session of high-intensity interval training (HIIT) and after a one-hour session of aerobic exercise. Based on previous human and animal research, we hypothesized that higher fitness and physical activity levels would be negatively associated with cerebral MOR availability in the brain's reward circuits and positively associated with GM volume. In addition, we predicted that VO_{2peak} and self-reported physical activity would be associated with exercise-induced changes in MOR availability.

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METHODS

Subjects

The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol, and the study was conducted in accordance with the Declaration of Helsinki. The sample size was determined by a priori power analysis based on our prior neuroreceptor PET studies (24,25), which suggested that with expected effect size of r = 0.45 a sample size of 45 would be sufficient for establishing the predicted effects at power of 0.95. Sixty-four male adults with a variable exercise background were enrolled in the study (Table 1). All subjects signed ethics-committee-approved informed consent forms. They were recruited via Internet discussion forums, traditional bulletin boards, university-hosted email lists, and newspaper advertisements. The exclusion criteria were poor compliance, a history of or current neurological or psychiatric disease, use of tobacco products or medication affecting the central nervous system, current or past excessive alcohol or substance abuse, as well as standard PET and MRI exclusion criteria. Laboratory tests, urinalysis, and an ECG were obtained to assess health and the absence of psychoactive drugs. These data have originally been collected in clinical trials EXEBRAIN (NCT02615756; n=24) and PROSPECT (NCT03106688; n=40).

Physical activity and aerobic fitness measurements

Self-reported physical activity was assessed with a questionnaire where participants rated the frequency (days/week) and duration (hours and minutes/week) of moderate to vigorous physical activity (MVPA) and other physical activity during the last three months. CRF was assessed as peak oxygen consumption (VO_{2peak}), which was determined in a maximal exercise test performed on a cycle ergometer starting at 40-50 W and followed by an increase of 30 W in every 2 minutes

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until volitional exhaustion. Ventilation and gas exchange were measured (Jaeger Oxycon Pro; VIASYS Healthcare) and reported as the mean value per minute. The highest 1-min mean value of oxygen consumption was expressed as the VO_{2peak}.

PET data acquisition

We measured MOR availability with the agonist radioligand [^{11}C]carfentanil that has a high affinity for MORs. Radioligand syntheses for the EXEBRAIN and PROSPECT trials have been described previously (22,26). Subjects refrained from exercise for at least 24 hours and fasted for at least 2 h before scanning. Data were acquired with the 3T Philips Ingenuity TF PET/MR (PhilipsHealthcare, Cleveland, OH, USA) scanner or PET/CT (GE Discovery VCT PET/CT, GE Healthcare (General Electric Medical Systems, Milwaukee, WI, USA) at Turku PET Centre. Data acquisition started concomitantly with the intravenous radioligand bolus injection (M = 250 MBq, SD = 13 MBq), and cerebral radioactivity was measured for 51 min. Data were corrected for dead-time, decay, and measured photon attenuation.

PET challenge paradigm for exercise-induced opioid release

A subset of participants (n = 24; Table S1, Supplemental Digital Content, SDC 1) underwent an additional PET scan after a one-hour session of moderate-intensity cycling exercise on a separate day, and twelve of these participants underwent a PET scan also after a session of HIIT; the exercise protocols and opioid release data have been reported previously (22). The order of the exercise/rest PET studies was randomized and counterbalanced for these participants. Emotional reactions to physical exercise were measured with Positive Affect and Negative Affect Schedule (27).

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MRI acquisition

Anatomical MR images were acquired for VBM as well as for preprocessing the PET images with the 3T Philips Ingenuity TF PET/MR scanner using a T1-weighted sequence with 1 mm^3 resolution (TR 8.1 ms, TE 3.7 ms, flip angle 7°, scan time 263 s). Complementary voxel-based morphometric analyses on the association between aerobic fitness, physical activity, and cerebral density are described in Supplemental Digital Content, SDC 1.

PET data preprocessing and analysis

PET data were processed with the automated Magia pipeline (28) (https://github.com/tkkarjal/magia). Processing began with motion-correction of the PET data followed by coregistration of the PET and MR images. Magia uses FreeSurfer (http://surfer.nmr.mgh.harvard.edu/) to define the regions of interest (ROIs) as well as the reference regions. The ROI-wise kinetic modeling was based on the extraction of ROI-wise time-activity curves. The PET images were slightly smoothed using Gaussian kernel (2 mm full width at half maximum, FWHM) to increase signal-to-noise ratio before model fitting Parametric images were spatially normalized to MNI-space and finally smoothed using a Gaussian kernel (FWHM = 6 mm). [^{11}C]carfentanil binding was quantified by binding potential (BP_{ND}), which is the ratio of specific binding to non-displaceable binding in the tissue (29). The occipital cortex was used as the reference region (30). Because of technical problems with the PET scanner, the PET data following one aerobic exercise scan and one HIIT scan were subsequently found invalid and were excluded from the analysis.

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Statistical analysis

The effects of VO_{2peak} and self-reported physical activity on i) MOR availability, ii) MOR activity following physical exercise, and iii) on GM densities were assessed in SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) using a linear regression model with BMI and PET scanner (for baseline PET data) as a covariate. The statistical threshold was set at $p < 0.05$, FDR-corrected at cluster level. Atlas-based ROIs were generated in the MOR-rich regions in the brain (31) (amygdala, hippocampus, ventral striatum, dorsal caudate, thalamus, insula, orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), middle cingulate cortex (MCC), and posterior cingulate cortex (PCC) using AAL (32) and Anatomy (33) toolboxes. Mean regional [^{11}C]carfentanil BP_{ND} were extracted for each region, and the averaged ROI data were analysed with R statistical software (https://cran.r-project.org) (34). Complementary hierarchical Bayesian analyses are described in Supplemental Digital Content, SDC 1.

RESULTS

VO_{2peak} is associated with physical activity level, age, and BMI

VO_{2peak} was positively associated with self-reported physical activity ($r = 0.45$, $p < 0.01$) and negatively associated with age ($r = -0.37$, $p < 0.01$; Fig. 1A) and BMI ($r = -0.46$, $p < 0.01$; Fig. 1B). Age was positively associated with BMI ($r = 0.38$, $p < 0.01$; Fig. 1C).

Higher MVPA level predicts a larger decrease in MOR availability after high-intensity interval training

We recently showed that HIIT significantly decreased MOR binding in human brain, indicative of endogenous opioid release (22). Here we tested whether exercise-induced changes in BP_{ND} would

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be associated with self-reported physical activity or VO_{2peak}, indicative of exercise habit-dependent MOR activation. We found a negative association between MVPA level and changes in BP_{ND} after HIIT (Fig. 2), such that higher MVPA level was associated with a larger decrease in BP_{ND} after HIIT. This effect was observed in the ventral and dorsal striatum, hippocampus, left amygdala, thalamus, cingulate cortex, insular cortex, somatosensory cortex, temporal areas, and orbitofrontal cortex. VO_{2peak} and total minutes of self-reported physical activity were not associated with HIIT-induced BP_{ND} changes in this small sample of 11 participants.

Higher VO_{2peak} and MVPA level predict a larger decrease in MOR availability after aerobic exercise

MOR availability shows notable variation between individuals following aerobic exercise, such that [^{11}C]carfentanil BP_{ND} decreases in some individuals and increases in others (22). Here we found that both VO_{2peak} (Fig. 3A) and self-reported MVPA level (Fig. 3B) were negatively associated with exercise-induced change in BP_{ND}, such that higher VO_{2peak} and higher MVPA level predicted a larger decrease in BP_{ND} after exercise. This effect was observed in the ventral and dorsal striatum, left hippocampus, left thalamus, insular cortex, somatosensory cortex, temporal areas, and orbitofrontal cortex. No associations were found between total self-reported physical activity and exercise-induced change in BP_{ND}.

We previously reported enhanced mood responses after aerobic exercise (22). Here, we found a positive association between VO_{2peak} and change in positive affect as measured with Positive Affect and Negative Affect Schedule before and after aerobic exercise ($r = 0.59$, $p < 0.01$; Fig. 1D), indicating that higher VO_{2peak} was associated with greater mood improvement.

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Association of aerobic fitness and physical activity level on MOR availability

We next tested whether baseline differences in aerobic fitness are associated with MOR availability. Full-volume analysis showed a negative association between VO_{2peak} and baseline MOR availability (BP_{ND}) in a large cluster extending to both hemispheres from the frontal lobe to the parieto-occipital sulcus (Fig. 4). Significant associations were also observed in bilateral putamen, thalamus, insula, and temporal cortices. In addition, the ROI analysis revealed significant associations in orbitofrontal and middle cingulate cortices ($p < 0.05$). Comparable analysis where MOR availability was predicted with self-reported physical activity yielded similar effects, but only when BMI was not controlled for in the model (data not shown). Because gross brain atrophy is negligible in healthy subjects under 37 years of age, partial volume effects due to atrophy are unlikely to significantly bias our results. Therefore, we did not correct the data for partial volume effects.

Ageing has regionally specific effects on MOR availability (31). Although our subjects had a relatively narrow age range (M = 25.4, SD = 4.6), we nevertheless wanted to statistically control for potential ageing-dependent effects in the MOR availability. When age was entered in the analysis as a covariate, no associations were observed between MOR availability and VO_{2peak} or self-reported physical activity at the a priori statistical threshold. The unthresholded result files are available in Neurovault (https://neurovault.org/collections/PTWTWOGG/). VO_{2peak} showed a positive association with GM density when controlling for age and BMI (see Figure S1, Supplemental Digital Content, SDC 1). These findings are described in detail in Supplemental Digital Content, SDC 1.

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DISCUSSION

The present findings indicate that aerobic fitness and habitual physical activity level have a previously unrecognized role in brain opioid signaling. Higher self-reported moderate-to-vigorous physical activity (MVPA) level was associated with a larger decrease in cerebral MOR binding after high-intensity interval training (HIIT), and both higher MVPA level and higher cardiorespiratory fitness, as measured by VO_{2peak}, were associated with a larger decrease in cerebral MOR binding after moderate-intensity aerobic exercise. These effects were observed in widespread cortical and subcortical areas, most notably in anterior cingulate cortex, insula, orbitofrontal cortex, and ventral striatum. In other words, more trained individuals showed greater acute opioid release following exercise in brain regions involved in reward and cognitive processing. Fitness and physical activity level were not associated with baseline $BP_{ND.}$ Conversely, VO_{2peak} was positively associated with GM density in several brain regions, including medial and lateral frontal and orbitofrontal cortices, cingulate cortex, and striatum. Taken together, these data suggest that aerobic fitness and exercise training at moderate-to-vigorous intensity may modulate cerebral MOR function, which might be an important pathway regulating exercise habits, and that aerobic fitness and physically active lifestyle are associated with neurobiological markers of brain health (i.e., GM density) not only in older adults but also in early adulthood.

Higher MVPA level and aerobic fitness predict a greater decrease in MOR binding after exercise

Previous research has shown that exercise intensity modulates opioid action in the brain (22,23). High-intensity exercise induces a robust opioid release, whereas moderate-intensity exercise results in decreased MOR availability in some and increased in other individuals (22). Here we

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report, for the first time, that higher MVPA level is associated with higher cerebral opioid release after a bout of HIIT and that both higher VO_{2peak} and MVPA levels are associated with larger decrease in cerebral MOR binding after moderate-intensity aerobic exercise. Together these findings suggest that aerobic fitness and physical activity level may shape opioidergic response following exercise. The dependency on prior aerobic fitness may also explain why moderate-intensity exercise does not result in net opioid release when individuals with different fitness statuses are assessed together (22). The present findings go beyond past reports, which have only examined the association of training status on peripheral opioid concentrations after exercise. Higher circulating β-endorphin levels have been found in well-trained athletes, in comparison with untrained individuals, after a graded exercise test (35) and after a bout of supramaximal exercise (36). In contrast, high-intensity cycling (70% and 80% of VO_{2max}) resulted in a similar increase in plasma β-endorphin concentration in both trained and untrained individuals whereas moderate-intensity cycling (60% of VO_{2max}) showed no effect on plasma β-endorphin concentration (37). While it has been suggested that training-induced adaptation within the opioid system could increase the response capacity to extreme exercise stress (36), peripheral opioid levels probably do not mirror those of the brain (38) and thus limits reasonable comparison of these studies.

We observed greater decreases in MOR binding in more trained individuals after exercise in the ventral and dorsal striatum, left amygdala, hippocampus, thalamus, insular cortex, somatosensory cortex, temporal areas, and orbitofrontal cortex, and across the cingulate. MORs in these regions are closely involved in processing both nociceptive and hedonic signals (12) as well as modulating decision making and cognitive control (39). Spatially more widespread correlation was observed between training variables and the change in MOR binding after HIIT than after

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aerobic exercise, although unequal sample sizes may confound their comparison. While both increased and decreased MOR binding were observed after moderate-intensity aerobic exercise, it should be noted that the within-subject variability in MOR responses after aerobic exercise far exceeds test-retest reproducibility. For example, in the thalamus and anterior cingulate cortex, the test-retest variabilities were 350% and 60% higher, respectively, in the present study in comparison to the prior study examining [^{11}C]carfentanil test-retest reliability (40).

After HIIT, higher training level was associated with greater opioid release in the thalamus, insula, left amygdala, and anterior cingulate cortex – brain regions known to process sensory and affective dimensions of pain (41–43). Enhanced MOR activation in these regions may improve exercise tolerance in more trained individuals by reducing pain and discomfort levels and thus, help to sustain the metabolic and mental demands of high-intensity exercise. Higher fitness level was recently also found to be associated with larger pain tolerance after high-intensity but not after low-intensity exercise (44), indicating a relationship between fitness, exercise intensity and opioid modulation. Moreover, the insular cortex is the main brain site responsible for the awareness of subjective feelings from the body (45), and it has been shown to play a central role especially in fatiguing exercise. The magnitude of insular activation varies with the intensity of exercise and associates with subjective ratings of perceived exertion (46). Interestingly, opioid modulation have shown to influence perceived exertion and exercise capacity (47). In addition to insula, also prefrontal areas and anterior cingulate cortex have been proposed to be implicated in the capacity to tolerate high levels of physical exertion and the determination of exercise termination (48). Consequently, greater HIIT-induced opioid release in more trained individuals may modify

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subjective perception of pain and fatigue and may thereby contribute to greater tolerance of higher exercise intensities and improved exercise performance.

The improved capacity of more trained individuals to activate the MOR system in response to exercise may also represent a neurobiological adaptation process induced by regularly repeated exercise bouts. Thus, improved MOR activation capacity could reinforce the adoption and maintenance of new exercise routines and, speculatively, underlie the rapid positive affective adaptation observed already within a few training sessions of both moderate- and high intensity exercise in previously sedentary individuals (49,50). In line with this, we found that participants with higher CRF experienced greater improvements in mood following the aerobic exercise. This accords with previous studies reporting that better fitness level (51) and regular exercise participation are associated with more positive affective responses (52,53) and enhanced anxiety relief (52,54) following a bout of exercise. We propose that greater opioid release could explain enhanced emotional and antinociceptive responses reported by people with higher exercise levels and thus, bear implications in exercise adoption and engagement (55), yet this idea remains to be determined in future studies.

Association of aerobic fitness and physical activity level on MOR availability

We found that VO_{2peak} and self-reported physical activity were negatively correlated with baseline [^{11}C]carfentanil BP_{ND}, suggesting that higher levels of regular exercise promoting better aerobic fitness may induce neuroadaptation within the endogenous opioid system. However, these associations were no longer statistically significant when age was introduced as a covariate in the model between MOR availability and aerobic fitness or self-reported physical activity, despite our

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subjects' narrow age range (20-36 years). Indeed, recent work has shown a prominent effect of age on MORs, especially before the age of 40 (31). Ageing increases MOR availability in frontotemporal areas and decreases it in the thalamus and nucleus accumbens (31). Thus, pure age effects would unlikely explain our findings, for example, in the thalamus. Given that also CRF is markedly influenced by age, physical activity level, and BMI (56,57), it remains inconclusive whether the presently observed effects on MOR availability reflect mere age-specific effects or joint effects between fitness and age. Age-dependent MOR downregulation may, in fact, be propelled by declining physical fitness, yet this speculation warrants further research in more controlled subject cohorts allowing differentiation between the intertwined effects of age, CRF, physical activity level, and BMI on MOR availability.

Prior animal studies have established a relationship between habitual physical activity and MOR expression (17,18). Chronic exercise, in comparison with short-term exercise or no exercise, decreases MOR expression (17) and overall MOR binding in rat brain (18), demonstrating a causal link between exercise and MOR binding. Reduced MOR binding may reflect either down-regulation of MORs, increased opioid tone and following competition between endogenous opioids and the radioligand, or a combination of the two. Animal studies suggest that regular exercise training increases tonic endogenous opioid levels. In rats, exercise training for five to eight weeks increases basal β-endorphin concentration in cerebrospinal fluid (58) and plasma (59) and elevates both β-endorphin and met-enkephalin levels in the periaqueductal grey area and rostral ventromedial medulla (16). Such rise in tonic opioid levels is also associated with altered pain processing, as regular exercise training increases nociceptive threshold in rats (60), reverses measures of pain in animal models of chronic pain (16,61,62), and as these effects can be reversed

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by an opioid receptor antagonist naloxone (16,60–62). Exercise-induced opioidergic pain modulation has also been demonstrated in humans. Six-week aerobic exercise intervention decreased pain intensity and interference in low active patients with chronic low back pain, and participants with the highest number of minutes in the target exercise intensity zone exhibited the greatest increases in endogenous opioid analgesia as indexed by naloxone–placebo condition differences in evoked pain responses (63). This suggests a dose-response effect so that higher intensity and amount of exercise may generate greater adaptation of the MOR system. Chronic exercise has also been demonstrated to result in decreased sensitivity to analgesic effects of exogenous opioid agonists such as morphine both in humans (64) and animals (19,20), which indicates that regular exercise may induce cross-tolerance to exogenously administered opioid agonists due to greater concentrations of endogenous opioid peptides. Altogether these studies indicate a role of habitual physical activity on the MOR system, which may contribute to long-term adaptations of various physiological and behavioural responses associated with regular exercise, such as improved mood and pain and stress regulation.

Exercise-induced affective and antinociceptive responses may also be modulated by other neurotransmitter systems, such as the endocannabinoid system (65). Plasma endocannabinoid levels increase peripherally after acute aerobic exercise in humans (65–68) independent of physical activity level (67), while long-term aerobic exercise decreases circulating endocannabinoid levels (69,70). Given the close interaction of opioid and endocannabinoid systems in the reward and emotion processing brain pathways (71–73), further studies should also examine central endocannabinoid signaling in exercise settings.

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Limitations

The interpretation of [^{11}C]carfentanil PET studies is challenging. High-intensity exercise led to significant decrease in MOR binding, whereas moderate-intensity exercise resulted in both decreased and increased MOR binding. According to the competition principle, reduced [^{11}C]carfentanil BP_{ND} is typically interpreted as evidence of increased endogenous opioid release. Similarly, increased BP_{ND} has been suggested to mirror MOR "deactivation" as an acute decrease in synaptic endogenous opioids (42,74). However, as BP_{ND} is a composite measure that does not differentiate between receptor density and affinity, radioligand binding can also be affected by receptor trafficking and changes in binding affinity. While we cannot directly specify which interpretation is most appropriate, our findings nevertheless are consistent with the modulatory role of aerobic fitness and physical activity level in MOR responses following both high-intensity and moderate-intensity exercise. Next, our data were sampled from two distinct projects using two different PET scanners. Although the outcome measure BP_{ND} theoretically controls for minor differences in scanner signal-to-noise ratios (75), comparable PET scanning protocols may yield different BP_{ND} estimates across scanners. Our recent study comparing BP_{ND}s of various PET ligands across PET scanners, however, suggest that the BP_{ND}s obtained from PET/CT and PET/MRI are sufficiently comparable for [^{11}C]carfentanil (76). Even though we corrected for potential scanner-related biases in the analyses, possible confounding effect of different scanners remain in the analyses. Furthermore, as females and males may have differential neurochemical responses to exercise (77) and differences in brain MOR availability (31), the findings may not be generalisable to females. Finally, due to underpowered sample, this first demonstration of the role of fitness and training measures on exercise-induced opioid release should be considered as preliminary evidence.

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CONCLUSIONS

We conclude that higher training status is associated with greater reductions in MOR availability after a bout of high-intensity exercise, suggesting greater exercise-induced opioid release in more trained individuals. Higher MVPA level and higher aerobic fitness also predict exercise-induced changes in MOR binding after a bout of aerobic exercise, indicating a role of habitual physical activity on MOR modulation also at moderate exercise intensities. Aerobic fitness was positively associated with grey matter density but not associated with baseline MOR availability. Altogether our findings suggest that improving aerobic fitness by regular physical activity of moderate to high intensity may induce neuroadaptation within the MOR system by improving exercise-induced opioid functioning, which may further modulate physiological and behavioural responses governed by the opioid system.

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Conflict of interest

The authors declare no conflict of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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FIGURE LEGENDS

Figure 1. VO_{2peak} correlated negatively with age (A) and BMI (B). Age correlated positively with BMI (C). VO_{2peak} was positively associated with change in positive affect after aerobic exercise (D).

Figure 2. Negative association between self-reported moderate-to-vigorous physical activity (MVPA) level and changes in BP_{ND} after high-intensity interval training session in a subset of 11 participants. The data are thresholded at $p < 0.05$, FDR-corrected at the cluster level. Scatterplot shows the corresponding association (LS-regression line with 95% CI) in the orbitofrontal cortex.

Figure 3. Higher fitness level predicted higher exercise-induced opioid release after moderate-intensity exercise, as indicated by a negative association between changes in BP_{ND} after one-hour session of aerobic exercise and VO_{2peak} (A) and self-reported moderate-to-vigorous physical activity (MVPA) level (B). The data are thresholded at $p < 0.05$, FDR-corrected at the cluster level. Scatterplots show the corresponding association (LS-regression line with 95% CI) in the putamen.

Figure 4. Negative association between VO_{2peak} and baseline [^{11}C]carfentanil BP_{ND}. The data are thresholded at $p < 0.05$, FDR-corrected at the cluster level. Scatterplots show the corresponding association (LS-regression line with 95% CI) in representative anatomical regions of interest.

SUPPLEMENTAL DIGITAL CONTENT (SDC)

SDC 1: Supplemental_Digital_Content.docx – Appendix

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Figure 1

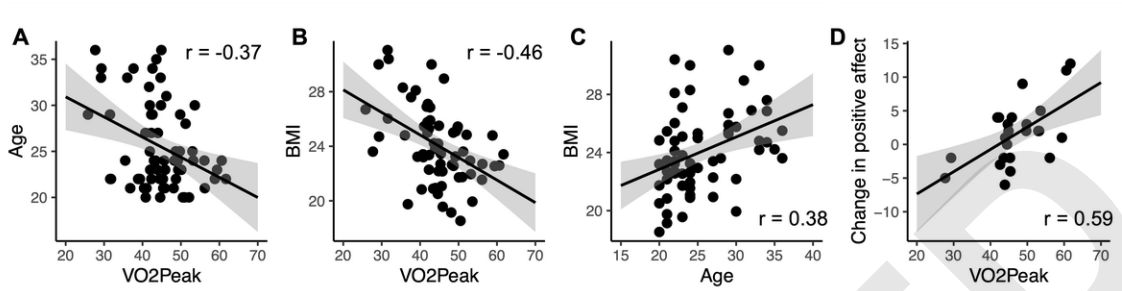

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Figure 2

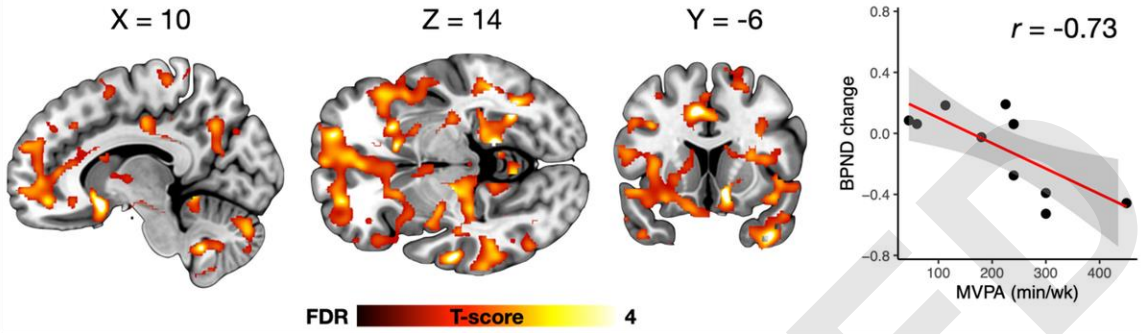

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Figure 3

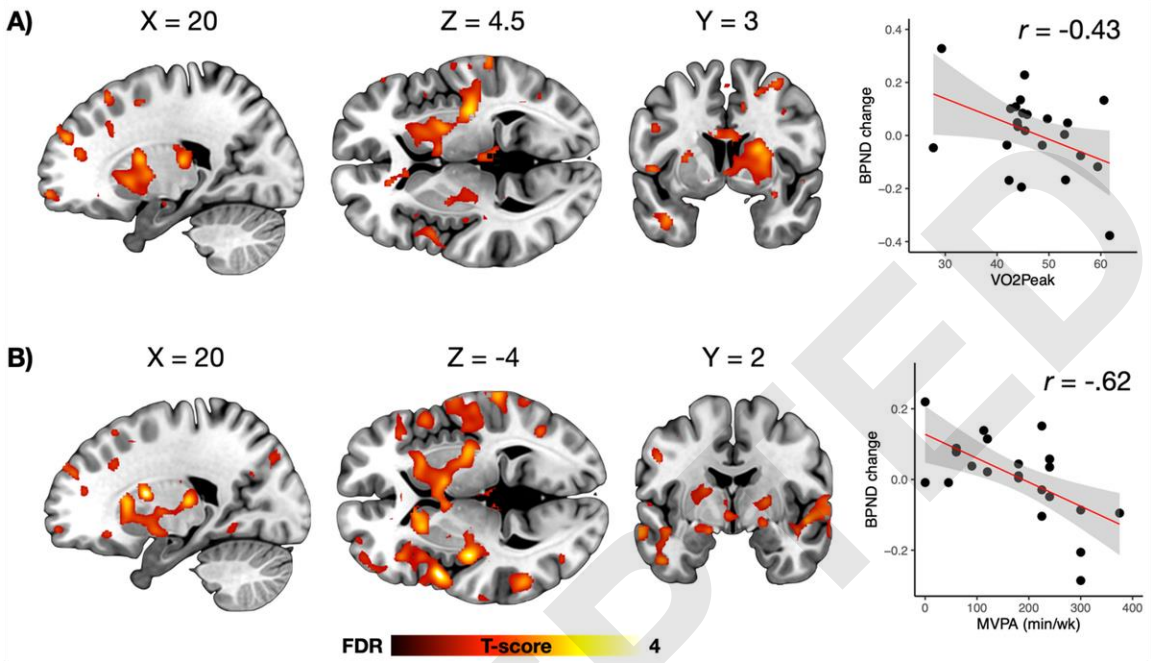

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Figure 4

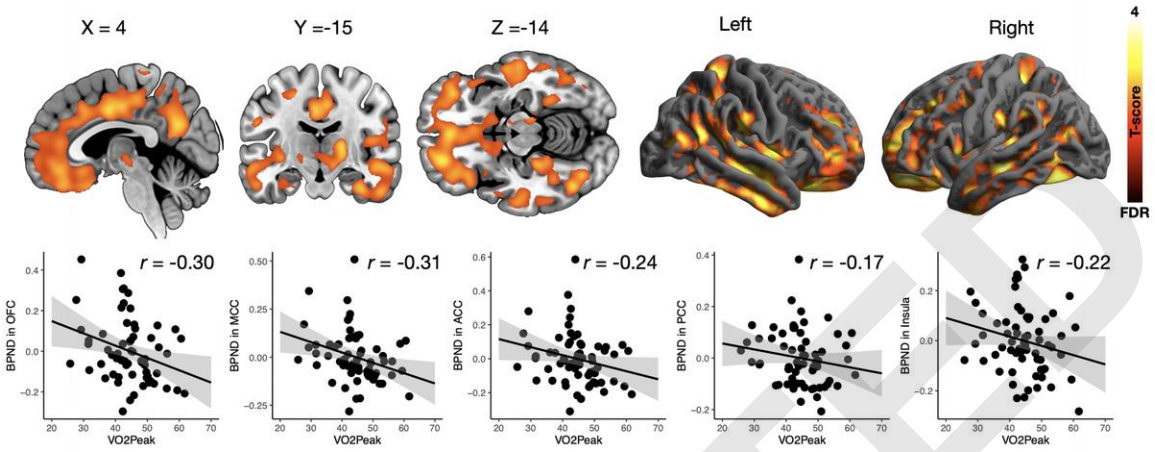

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Table 1. Subject characteristics (n=64).

	Mean (SD)	Range
Age (years)	25.4 (4.6)	20−36
BMI	24.1 (2.8)	18.5−31.0
Total physical activity (min/week)	291 (165)	0−870
MVPA (min/week)	160 (113)	0−870
VO_{2max} (ml/kg/min)	44.5 (7.9)	25.8−61.7

BMI, body mass index; MVPA, moderate-to-vigorous physical activity; $VO2_{max}$, maximal oxygen uptake

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Supplemental Digital Content 1

Aerobic fitness is associated with cerebral mu-opioid receptor activation in healthy humans

Tiina Saanijoki[1,2], Tatu Kantonen[1,3], Laura Pekkarinen[1,4], Kari Kalliokoski[1], Jussi Hirvonen[1,5], Tuulia Malén[1], Lauri Tuominen[1,6], Jetro J. Tuulari[1,7], Eveliina Arponen[1], Pirjo Nuutila[1,4], Lauri Nummenmaa[1,8]

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Table S-1. Characteristics of the subset of participants who were also examined for exercise induced opioid release after aerobic exercise and after high-intensity interval training (HIIT).

	Aerobic exercise (n=24)		**HIIT (n=12)**	
	Mean (SD)	Range	Mean (SD)	Range
Age (years)	27.1 (5.3)	21−36	26.9 (5.4)	21−36
Body Mass Index (kg/m^2)	23.5 (1.7)	19.9−26.9	23.9 (1.9)	19.9−26.9
Total physical activity (min/week)	255 (146)	0−533	291 (141)	105−533
MVPA (min/week)	188 (115)	0−450	209 (115)	45−450
Peak oxygen consumption (VO_{2peak}; ml/kg/min)	47.2 (8.3)	27.7−61.7	48.8 (7.0)	41.9−61.7

HIIT, high-intensity interval training; BMI, body mass index; MVPA, moderate-to-vigorous physical activity; $VO2_{max}$, maximal oxygen uptake

Supplementary methods

Voxel-based morphometry

Voxel-based morphometry was done with SPM12 (Wellcome Trust Center for Imaging, London, UK, http://www.fil.ion.ucl.ac.uk/spm), which enables automated spatial normalization, tissue classification and radio-frequency bias correction to be combined with the segmentation step. Cut-off of spatial normalization was 25 mm and medium affine regularization 0.01 was used. Following normalization and segmentation into grey matter (GM) and white matter (WM), a modulation step was incorporated to take into account volume changes caused by spatial normalization. Importantly, the modulation step corrects for the differences in total brain size across subjects. Finally, the segmented, normalized, and modulated GM images were smoothed using a Gaussian kernel of 8 mm FWHM.

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Bayesian hierarchical regression analyses

Full-volume analysis showed a negative association between VO_{2peak} and baseline MOR availability (BP_{ND}), however including age as a covariate in the model, these associations were no longer statistically significant. VO_{2peak}, moderate-to vigorous physical activity (MVPA) level, age, and BMI are highly correlated with each other, making it difficult to differentiate their effects on the BP_{ND} variation (1). Hence, we supplemented the statistical analysis of the age, VO_{2peak}, MVPA, and BMI effects on BP_{ND} applying hierarchical Bayesian modeling using brms package (2,3) in R (4).

The data are hierarchical in the sense that i) they include repeated measures from the subjects (one measure from each ROI) and ii) data come from two different scanners. We ran models 1-5 including the following predictors (fixed factors): 1) age, VO_{2peak}, MVPA level, and BMI; 2) age; 3) VO_{2peak}; 4) MVPA level; and 5) BMI. Otherwise, the models were comparable. In each model, we used log-transformed binding potential as a dependent variable and allowed the overall binding level to vary by subject. We adjusted for the scanner effect and allowed it to vary by ROI. Such model structure was implemented by including both the scanner and ROI, as well as their interaction, as random (i.e. grouping) factors (5). As a first prior, we set the regression coefficients to follow normal distribution with zero-mean and unit variance (https://rdrr.io/cran/brms/man/set_prior.html). As a second prior, we applied normal distribution (0,1) for standard deviations of random (group-level) effects (https://rdrr.io/cran/brms/man/set_prior.html). We facilitated the model sampling by setting max treedepth as 20 and adapth delta close to one (varied between the models but always in the range 0.9999-0.99999).

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Differentiating the effects of two or more colinear predictors is difficult in both Bayesian and Fisherian frameworks. The Bayesian model estimates how much each predictor explains the variation in BP_{ND}, when the rest of the predictors are known (1). This may lead to estimates where none of the correlated predictors are associated with the dependent variable, as all of them may have zero explanatory value once the model has ruled out the variance induced by the other predictors (1). Thus, although we are aware of the information loss in leaving predictors out, we also analyzed the single-predictor models to gain more in-depth understanding of the individual effect of each predictor.

Supplementary Results

Higher VO_{2peak} and physical activity level are positively associated with GM density

VBM analyses revealed a positive correlation between VO_{2peak} and GM density when controlling for age and BMI (Figure S1). These associations were found in prefrontal and orbitofrontal cortex, nucleus accumbens, amygdala, primary motor cortex, parietal regions (precuneus, supramarginal gyrus, somatosensory cortex, temporal lobes (superior, middle, and inferior temporal gyri, fusiform gyri, hippocampi), occipital cortex and cerebellum. Self-reported physical activity also correlated positively with GM density in the same regions but only when age was not used as a covariate (data not shown). These effects were most salient in prefrontal regions governing emotional processing and executive functions, limbic regions governing emotional functions, parietal and temporal areas sub-serving long-term memory as well as somatosensory, auditory, speech, and vision processing, and occipital visual cortices, and cerebellum. These regional findings align with those observed in previous studies examining a link between aerobic fitness and cortical structure in older age groups (6) and complement the present evidence in younger

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subjects (7–11). Our data suggest that aerobic fitness might be an important neuroprotective factor already in the early adulthood.

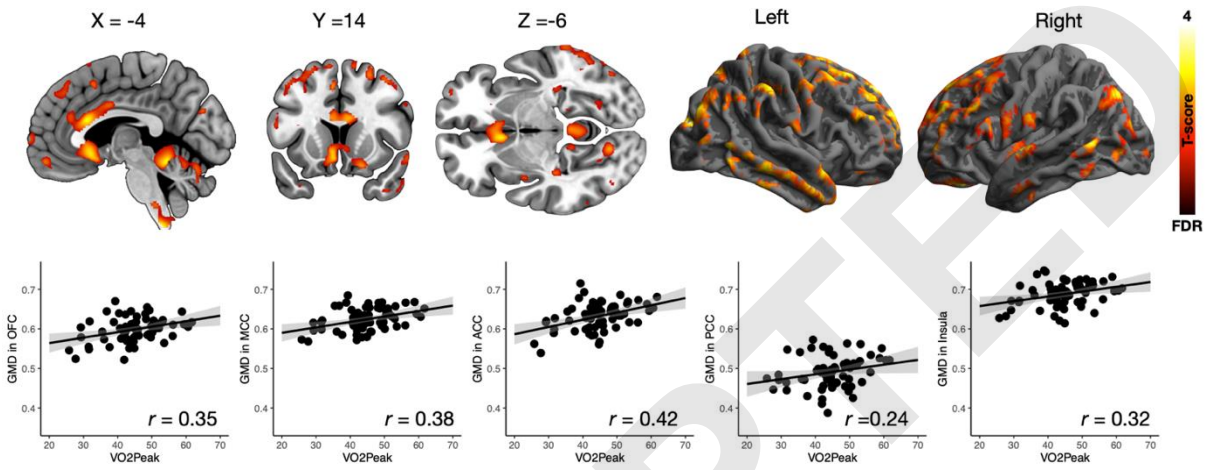

Figure S1. *Positive association between VO_{2peak} and grey matter density. The data are thresholded at $p < 0.05$, FDR-corrected at the cluster level. Scatterplots show the corresponding association (LS-regression line with 95% CI) in representative anatomical regions of interest (GMD; grey matter density).*

Regional effects of age, VO_{2peak}, MVPA level, and BMI on BP_{ND}

The Bayesian hierarchical multi-predictor (age, VO_{2peak}, MVPA level, and BMI) model revealed positive association between age and BP_{ND} in the majority of the ROIs (putamen, posterior cingulate cortex, orbitofrontal cortex (OFC), midcingulate cortex (MCC), insula, dorsal caudate, nucleus accumbens (Figure 2SA), while it suggested no clear effects of the rest of the predictors (Figure S2 B-D). In the single-predictor modeling, age was again positively associated with BP_{ND} in the same set of ROIs (Figure 3SA), as was BMI in OFC and MCC (Figure 3SD), while VO_{2peak}

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was negatively associated with BP_{ND} in MCC, OFC and anterior cingulate cortex (Figure 3SB). Modeling showed no clear associations of MVPA and BP_{ND} (Figure 3SC).

The supplementary Bayesian modeling suggests that ageing has a more profound effects on BP_{ND} than VO_{2peak}, MVPA level, and BMI. However, it is noteworthy that the single-predictor models miss the information of the other predictors. Our analysis may well signal that one effect is mediating the other(s). While the correlation structure of age, VO_{2peak}, MVPA level, and BMI remain complex for statistical analysis, we need settings where one or two of these predictors can be controlled (e.g. subjects from precise age and BMI cohort). This would allow us to differentiate the apparently intertwined effects of age, VO_{2peak}, MVPA level, and BMI on BP_{ND}.

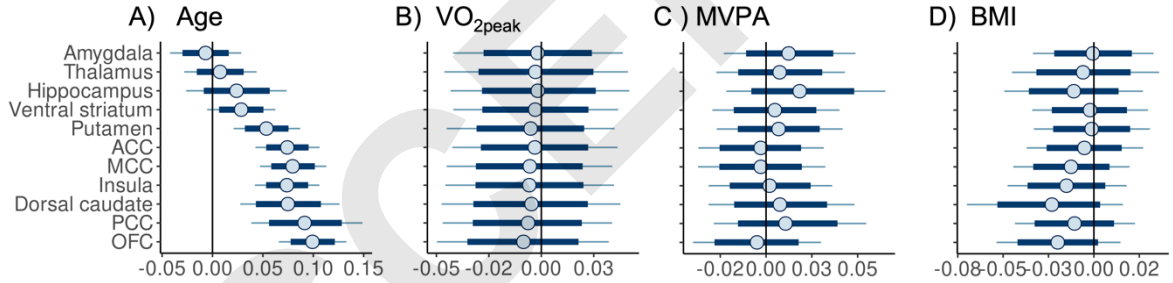

Figure 2S. *The Bayesian hierarchical multi-predictor (age, VO_{2peak}, MVPA level, BMI) model showed positive association between age and BP_{ND} in the majority of the ROIs (A), while it suggested no clear effects for the rest of the predictors (B-D). The plots show medians (circles), 80% (thick line) and 95% (thin line) posterior intervals of the regression coefficients on a logarithmic scale.*

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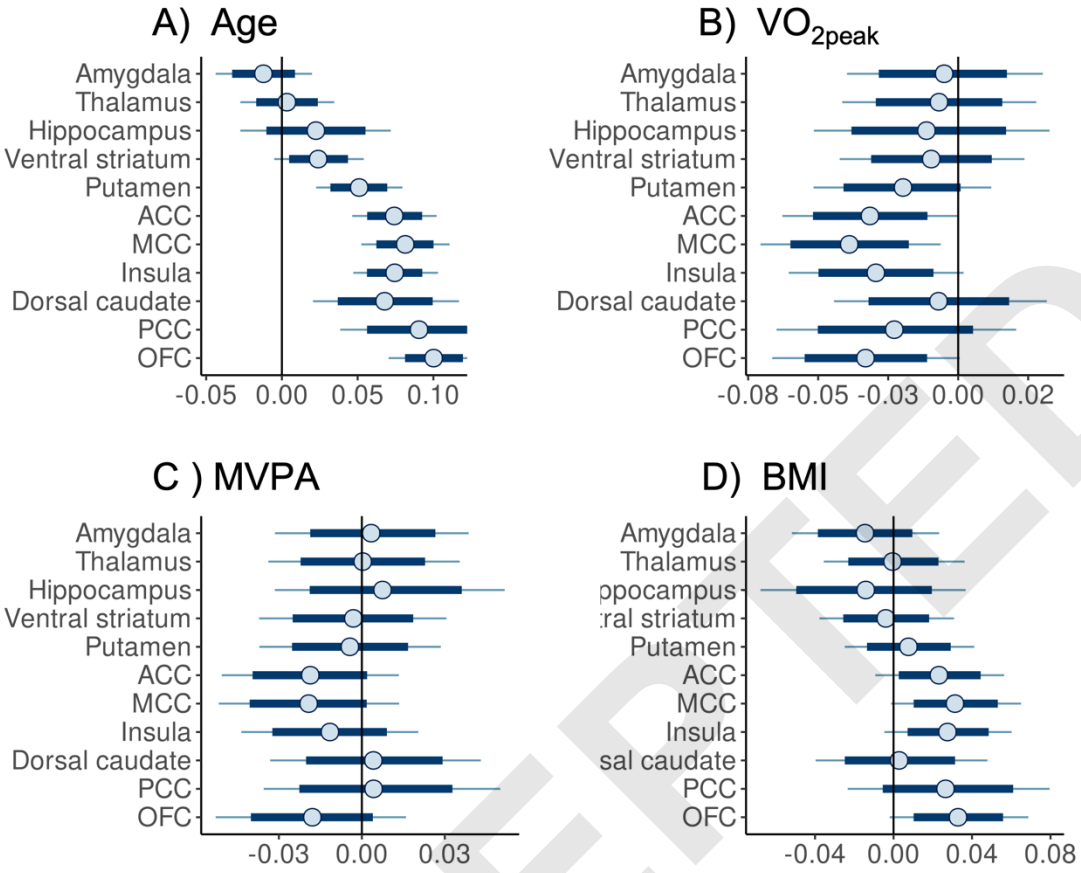

Figure 3S. *In the single-predictor modeling, age (A) and BMI (D) were positively associated with* BP_{ND} *in several ROIs, while* VO_{2peak} *(B) was negatively associated with* BP_{ND} *in the middle cingulate, orbitofrontal, and anterior cingulate cortices. MVPA level (C) was not associated with* BP_{ND}*. The plots show medians (circles), 80% (thick line) and 95% (thin line) posterior intervals of the regression coefficients on a logarithmic scale.*

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