Aerobic Fitness Is Associated with Cerebral μ-Opioid Receptor Activation in Healthy Humans

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BASIC SCIENCES

ABSTRACT

SAANIJOKI, T., T. KANTONEN, L. PEKKARINEN, K. KALLIOKOSKI, J. HIRVONEN, T. MALÉN, L. TUOMINEN, J. J. TUULARI, E. ARPONEN, P. NUUTILA, and L. NUMMENMAA. Aerobic Fitness Is Associated with Cerebral μ-Opioid Receptor Activation in Healthy Humans. *Med. Sci. Sports Exerc.*, Vol. 54, No. 7, pp. 1076–1084, 2022. **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 ($\dot{V}O_{2peak}$) and physical activity level are associated with cerebral MOR availability and whether these phenotypes predict endogenous opioid release after a session of exercise. **Methods:** We studied 64 healthy lean men who performed a maximal incremental cycling test for $\dot{V}O_{2peak}$ determination, completed a questionnaire assessing moderate-to-vigorous physical activity (MVPA; in minutes per week), and underwent positron emission tomography with [^{11}C]carfentanil, a specific radioligand for MOR. A subset of 24 subjects underwent additional positron emission tomography scan also after a 1-h session of moderate-intensity exercise and 12 of them also after a bout of high-intensity interval training. **Results:** Higher self-reported MVPA level predicted greater opioid release after high-intensity interval training, and both $\dot{V}O_{2peak}$ 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 after 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 behavioral responses associated with long-term exercise. **Key Words:** OPIOID SYSTEM, POSITRON EMISSION TOMOGRAPHY (PET), BRAIN IMAGING, FITNESS, PHYSICAL ACTIVITY LEVEL

Habitual physical activity and cardiorespiratory fitness (CRF) are well-established modifiable lifestyle factors that promote brain health throughout the life span. 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 gray (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

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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 gray area and rostral ventromedial medulla after 5 wk of treadmill running (16) and shown that chronic 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 suggests that regular exercise training might induce neuroadaptation within the central MOR system, subsequently contributing to improvements in mood and stress regulation. However, *in vivo* evidence from humans is currently lacking.

Here we investigated whether individual differences in baseline MOR availability are associated with CRF and habitual physical activity levels in healthy young men. We used *in vivo* positron emission tomography (PET) imaging with the highly selective MOR agonist ligand [^{11}C]carfentanil. We coupled MOR data with measurement of peak oxygen consumption ($\dot{V}O_{2peak}$), 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 1-h 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 gray matter (GM) volume. In addition, we predicted that $\dot{V}O_{2peak}$ and self-reported physical activity would be associated with exercise-induced changes in MOR availability.

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, and standard PET and magnetic resonance imaging (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; $n = 40$).

TABLE 1. Subject characteristics ($n = 64$).

	Mean (SD)	Range
Age (yr)	25.4 (4.6)	20–36
BMI (kg·m^{-2})	24.1 (2.8)	18.5–31.0
Total physical activity (min·wk^{-1})	291 (165)	0–870
MVPA (min·wk^{-1})	160 (113)	0–870
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	44.5 (7.9)	25.8–61.7

Physical activity and aerobic fitness measurements. Self-reported physical activity was assessed with a questionnaire where participants rated the frequency (days per week) and duration (hours and minutes per week) of moderate-to-vigorous physical activity (MVPA) and other physical activity during the last 3 months. CRF was assessed as $\dot{V}O_{2peak}$, 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 min 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 $\dot{V}O_{2peak}$.

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 h and fasted for at least 2 h before scanning. Data were acquired with the 3 T Philips Ingenuity TF PET/MR (PhilipsHealthcare, Cleveland, OH) scanner or PET/CT (GE Discovery VCT PET/CT, GE Healthcare (General Electric Medical Systems, Milwaukee, WI)) 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, http://links.lww.com/MSS/C538) underwent an additional PET scan after a 1-h session of moderate-intensity cycling exercise on a separate day, and 12 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 the Positive Affect and Negative Affect Schedule (27).

MRI acquisition. Anatomical MR images were acquired for voxel based morphometry and for preprocessing the PET

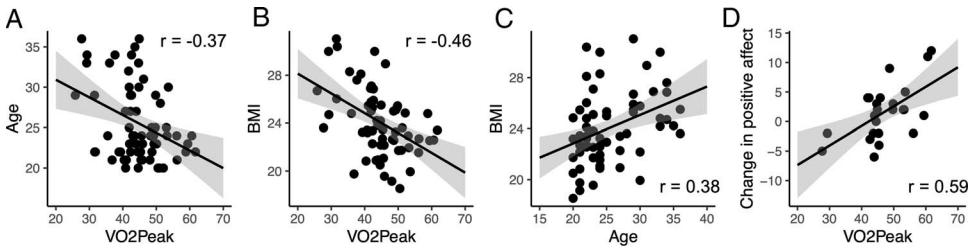

FIGURE 1—$\dot{V}O_{2peak}$ correlated negatively with age (A) and BMI (B). Age correlated positively with BMI (C). $\dot{V}O_{2peak}$ was positively associated with change in positive affect after aerobic exercise (D).

images with the 3 T Philips Ingenuity TF PET/MR scanner using a T1-weighted sequence with 1-mm^3 resolution (repetition time, 8.1 ms; echo time, 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, http://links.lww.com/MSS/C538.

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) to increase the signal-to-noise ratio before model fitting. Parametric images were spatially normalized to Montreal Neurological Institute space and finally smoothed using a Gaussian kernel (full width at half maximum = 6 mm). [^{11}C]carfentanil binding was quantified by binding potential (BP_{ND}), which is the ratio of specific binding to nondisplaceable 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 after one aerobic exercise scan and one HIIT scan were subsequently found invalid and were excluded from the analysis.

Statistical analysis. The effects of $\dot{V}O_{2peak}$ and self-reported physical activity on i) MOR availability, ii) MOR activity after physical exercise, and iii) GM densities were assessed in SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) using a linear regression model with body mass index (BMI) and PET scanner (for baseline PET data) as a covariate. The statistical threshold was set at $P < 0.05$, false discovery rate (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, anterior cingulate cortex, middle cingulate cortex, and posterior cingulate cortex) using AAL (32) and Anatomy (33) toolboxes. Mean regional [^{11}C]carfentanil BP_{ND} was extracted for each region, and the averaged ROI data were analyzed with R statistical software (https://cran.r-project.org) (34). Complementary hierarchical Bayesian analyses are described in Supplemental Digital Content, http://links.lww.com/MSS/C538.

RESULTS

$\dot{V}O_{2peak}$ is associated with physical activity level, age, and BMI. $\dot{V}O_{2peak}$ 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 HIIT. 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 be associated with self-reported physical activity or $\dot{V}O_{2peak}$, 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. $\dot{V}O_{2peak}$ and total minutes of self-reported physical activity

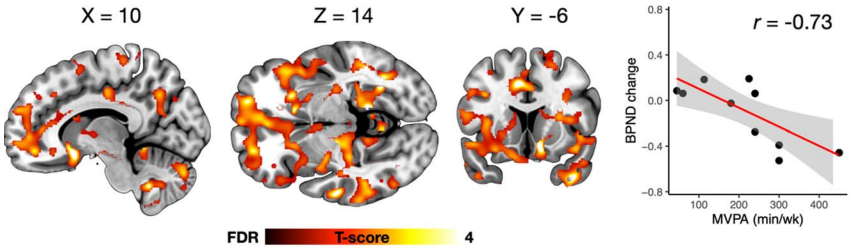

FIGURE 2—Negative association between self-reported MVPA level and changes in BP_{ND} after HIIT session in a subset of 11 participants. The data are thresholded at $P < 0.05$, FDR corrected at the cluster level. Scatterplot shows the corresponding association (least squares regression line with 95% confidence interval) in the orbitofrontal cortex.

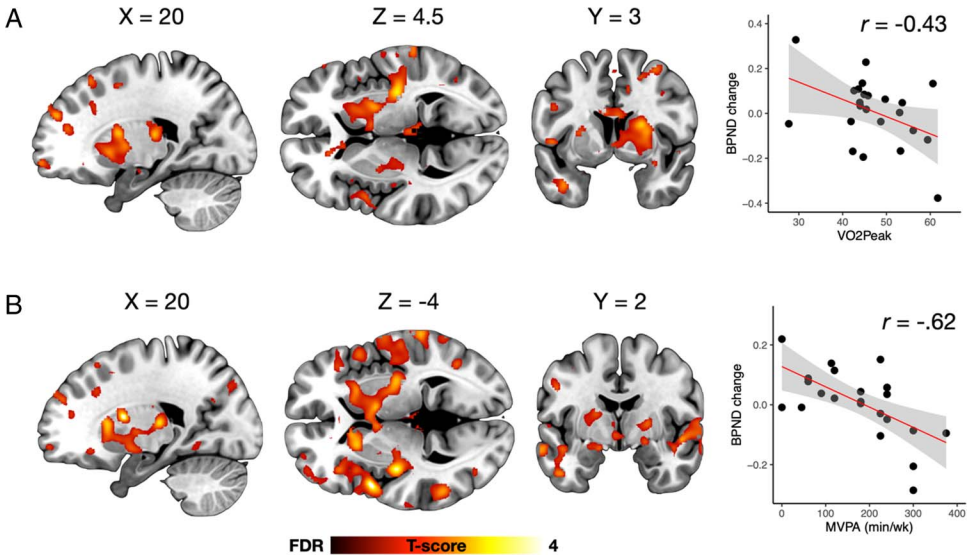

FIGURE 3—Higher fitness level predicted higher exercise-induced opioid release after moderate-intensity exercise, as indicated by a negative association between changes in BP_{ND} after 1-h session of aerobic exercise and $\dot{V}O_{2peak}$ (A) and self-reported MVPA level (B). The data are thresholded at $P < 0.05$, FDR corrected at the cluster level. Scatterplots show the corresponding association (least squares regression line with 95% confidence interval) in the putamen.

were not associated with HIIT-induced BP_{ND} changes in this small sample of 11 participants.

Higher $\dot{V}O_{2peak}$ and MVPA level predict a larger decrease in MOR availability after aerobic exercise. MOR availability shows notable variation between individuals after aerobic exercise, such that [^{11}C]carfentanil BP_{ND} decreases in some individuals and increases in others (22). Here we found that both $\dot{V}O_{2peak}$ (Fig. 3A) and self-reported MVPA level (Fig. 3B) were negatively associated with exercise-induced change in BP_{ND}, such that higher $\dot{V}O_{2peak}$ 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 $\dot{V}O_{2peak}$ and change in positive affect as measured with the Positive Affect and Negative Affect Schedule before and after aerobic exercise ($r = 0.59$, $P < 0.01$; Fig. 1D), indicating that higher $\dot{V}O_{2peak}$ was associated with greater mood improvement.

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 $\dot{V}O_{2peak}$ 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 younger than 37 yr, partial volume effects due to atrophy are unlikely to significantly bias our results. Therefore, we did not correct the data for partial volume effects.

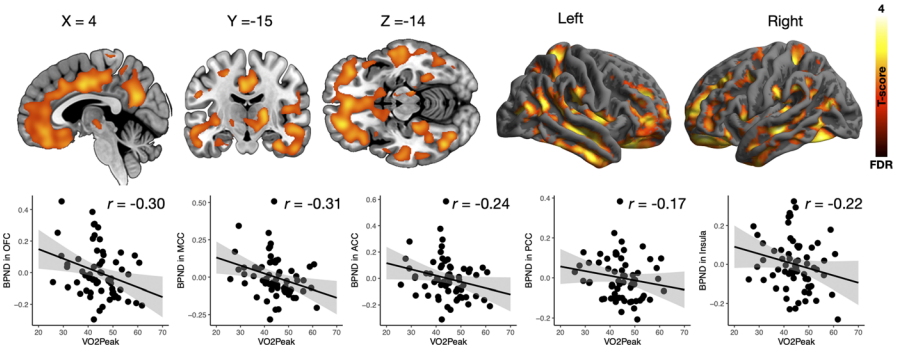

FIGURE 4—Negative association between $\dot{V}O_{2peak}$ and baseline [^{11}C]carfentanil BP_{ND}. The data are thresholded at $P < 0.05$, FDR corrected at the cluster level. Scatterplots show the corresponding association (least squares regression line with 95% confidence interval) in representative anatomical ROIs.

Aging has regionally specific effects on MOR availability (31). Although our subjects had a relatively narrow age range (M = 25.4 yr, SD = 4.6 yr), we nevertheless wanted to statistically control for potential aging-dependent effects in the MOR availability. When age was entered in the analysis as a covariate, no associations were observed between MOR availability and $\dot{V}O_{2peak}$ or self-reported physical activity at the *a priori* statistical threshold. The unthresholded result files are available in Neurovault (https://neurovault.org/collections/PTWTWOGG/). $\dot{V}O_{2peak}$ showed a positive association with GM density when controlling for age and BMI (Fig. S1, Supplemental Digital Content, http://links.lww.com/MSS/C538). These findings are described in detail in Supplemental Digital Content, http://links.lww.com/MSS/C538.

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 MVPA level was associated with a larger decrease in cerebral MOR binding after HIIT, and both higher MVPA level and higher CRF, as measured by $\dot{V}O_{2peak}$, 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 the anterior cingulate cortex, insula, orbitofrontal cortex, and ventral striatum. In other words, more trained individuals showed greater acute opioid release after exercise in brain regions involved in reward and cognitive processing. Fitness and physical activity level were not associated with baseline $BP_{ND.}$ Conversely, $\dot{V}O_{2peak}$ was positively associated with GM density in several brain regions, including the 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 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 $\dot{V}O_{2peak}$ 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 after 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 maximal oxygen uptake ($\dot{V}O_{2max}$)) resulted in a similar increase in plasma β-endorphin concentration in both trained and untrained individuals, whereas moderate-intensity cycling (60% of $\dot{V}O_{2max}$) showed no effect on plasma β-endorphin concentration (37). Although 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 aerobic exercise, although unequal sample sizes may confound their comparison. Although 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 has shown to influence perceived exertion and exercise capacity (47). In addition to insula, 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 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 after 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) after 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 with MOR availability. We found that $\dot{V}O_{2peak}$ 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 subjects' narrow age range (20–36 yr). Indeed, recent work has shown a prominent effect of age on MORs, especially before the age of 40 yr (31). Aging 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 for 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 downregulation of MORs, increased opioid tone, or, after competition between endogenous opioids and the radioligand, a combination of the two. Animal studies suggest that regular exercise training increases tonic endogenous opioid levels. In rats, exercise training for 5 to 8 wk increases basal β-endorphin concentration in cerebrospinal fluid (58) and plasma (59) and elevates both β-endorphin and met-enkephalin levels in the periaqueductal gray 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) and reverses measures of pain in animal models of chronic pain (16,61,62), and as these effects can be reversed 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 because of greater concentrations of endogenous opioid peptides. Altogether, these studies indicate a role of habitual physical activity in the MOR system, which may contribute to long-term adaptations of various physiological and behavioral 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), whereas 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.

Limitations. The interpretation of [^{11}C]carfentanil PET studies is challenging. High-intensity exercise led to a 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, because 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. Although 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 after both high-intensity and moderate-intensity exercises. 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} values of various PET ligands across PET scanners, however, suggests that the BP_{ND} values 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, a possible confounding effect of different scanners remains in the analyses. Furthermore, because females and males may have differential neurochemical responses to exercise (77) and differences in brain MOR availability (31), the findings may not be generalizable to females. Finally, because of underpowered sample, this first demonstration of the role of fitness and training measures on exercise-induced opioid release should be considered as preliminary evidence.

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 in MOR modulation also at moderate exercise intensities. Aerobic fitness was positively associated with GM 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 behavioral responses governed by the opioid system.

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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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