ACUTE EFFECTS OF VIGOROUS-INTENSITY AEROBIC EXERCISE ON SPONTANEOUS BRAIN ACTIVITY IN HEALTHY ADULTS: AN EXPLORATIVE RESTING-STATE FMRI STUDY

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TIIVISTELMÄ

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Johdanto: Fyysinen aktiivisuus parantaa kaikenikäisten kognitiivisia kykyjä. Aerobinen liikunta lisää lyhytaikaisesti kognitiivista suorituskykyä. Ilmiötä kutsutaan ”aerobiseksi herkistämiseksi”. Herkistäminen näkyy lisääntyneenä aivokuoren aktivaationa välittömästi liikunnan jälkeen. Toiminnallinen magneettikuvantaminen (fMRI) on tehokas tapa mitata aivojen aktivaatiota. Tutkimuksen tarkoituksena on hyödyntää lepotilan fMRI:ta mittaamaan spontaania aktivaatiota kovatehoisen liikunnan jälkeen.

Menetelmät: Tutkimuksen koehenkilöille (n=14) tehtiin ennakkotarkastus ja kuntotesti (VO_2peak). Tutkimusohjelmaan kuului 5+25 min 50/70%VO_2peak -pyöräergometriakuormitus ja fMRI ennen ja jälkeen kuormituksen. Liikunnan akuutteja vaikutuksia aivojen spontaaniin aktivaatioon tutkittiin eristämällä matalien taajuuksien amplitudit (ALFF) lepotilan fMRI-kuvista ja vertailtiin lepotilan kuvia 30, 60 ja 90 min kuviin. FMRI-kuvien esikäsittelyssä sekä yksilö- ja ryhmäanalyyseissä käytetiin MATLABin työkaluja DPABIa ja SPM12a. Varianssianalyysejä käytettiin tilastollisten erojen sekä laktaatin selittävän vaikutuksen löytämiseen kuvista. Korrelaatioita kuvien ja koehenkilöiden iän, sukupuolen, painoindeksin, rasvaprosentin, laktaatin ja VO_2peak:n välillä katsottiin usean muuttujan regressioanalyysillä. Tulokset korjattiin yhdistetyllä merkitsevyystasolla (FWE) väärien positiivisten varalta.

Tulokset: Akuutti aerobinen liikuntakuormitus lisäsi tehokkaasti alueellista spontaania aivoaktivaatiota 30 min kuormituksen jälkeen. Kuormituksen jälkeen aktivaatio kohosi otsa-, päälaki- ja takaraivolohkossa sekä pikkuaivoissa. Laktaattikorjauksella otsalohkon kohonnutta aktivaatiota ei havaittu. ALFF korreloi negatiivisesti 30 min kuormituksen jälkeen painoindeksin kanssa otsa- ja takaraivolohkossa, mutta muita korrelaatiota ei löydetty.

Johtopäätökset: Tulokset viittaavat korkeatehoisen aerobisen liikunnan lisäävän spontaania aivoaktivaatiota 30 min kuormituksen jälkeen, mikä tukee ”aerobista herkistämistä”. Osa tuloksista viittaa korkean painoindeksin vaimentavan liikunnan jälkeistä aktivaatiota, mutta näyttö on epäjohdonmukaista. Kuormituksen jälkeiset aluetason muutokset näyttävät riippuvan kuormituksen intensiteetistä, sillä laktaattikorjaus muutti tuloksia. Vahvoja johtopäätöksiä ei voida kuitenkaan aluetasoisista muutoksista tehdä, sillä käytetty monivertailumalli ei ehkä sovellu tutkimukseen. Tulevaisuudessa herkistämistutkimuksissa tulisi käyttää yhtenäistä monivertailumallia, ja niihin kannattaisi yhteensovittaa myös muita aktivaation mittaustapoja.

Avainsanat: aerobinen herkistäminen, spontaani aivoaktivaatio, lepotilan fMRI

ABSTRACT

Neuvonen, J (2022). Acute effects of vigorous-intensity aerobic exercise on spontaneous brain activity in healthy adults: an explorative fMRI study, Department of Biology of Physical Activity, University of Jyväskylä, Master's thesis, 44 pp., 2 appendices.

Introduction: Physical activity improves cognitive function in all age groups. Aerobic exercise enhances short-term cognitive performance. This is known as "aerobic priming" effect. Priming effects is shown as heightened brain cortical activity after exercise. Functional magnetic resonance imaging (fMRI) is a noninvasive and effective tool to measure brain activity. In this study resting-state fMRI is utilized to measure spontaneous brain activity acutely after vigorous-intensity aerobic exercise.

Methods: Study subjects (n=14) were screened, and exercise tested (VO_2peak) beforehand. All subjects underwent the same study protocol, which consisted of 5+25 min 50/70%VO_2peak cycle ergometry exercise and fMRI. Exercise-induced changes to spontaneous brain activity was measured by extracting amplitude of low frequency fluctuations (ALFF) from resting-state fMRI and comparing 30-, 60- and 90-min post-exercise images to baseline. FMRI-toolboxes, DPABI and SPM12 in MATLAB, were used for image preprocessing, first- and second-level analyses. Analyses of variance with and without correcting for lactate nuisance effect were used in pre- and post-exercise fMRIs. Correlations in ALFF and subject age, sex, BMI, fat-%, lactate and VO_2peak were checked with multiple regression analysis. All results were family-wise error (FWE) corrected for false positive findings.

Results: Acute aerobic exercise increased regional spontaneous brain activity 30 min post-exercise in the frontal-, the parietal-, the occipital lobe and cerebellum. Correcting with individual lactate responses to exercise, increased frontal lobe activation were not found. 30-min ALFF negatively correlated with BMI in the frontal and occipital lobes but no other correlations with age, sex, fat-%, lactate and VO_2peak were found.

Conclusions: These results suggest that acute vigorous-exercise aerobic exercise effectively increase spontaneous brain activity 30 min after exercise affirming "aerobic priming" theory. Some findings suggest that high BMI attenuates some activation after exercise, but the evidence is inconsistent. Brain region-specific activation patterns seem exercise intensity specific as lactate correction changed results. No strong conclusions in region-specific changes could be drawn as chosen multiple comparison model may not be suitable for this study. A fixed multiple comparison model should be used for priming studies and other activation methods implemented simultaneously to better study priming-related regional effects in future research.

Key words: aerobic priming, spontaneous brain activity, resting-state fMRI

LIST OF ABBREVIATIONS

%HRR	percentage of heart rate reserve
ALFF	amplitude of low frequency fluctuations
ASL	arterial spin labeling
BDNF	brain-derived neurotrophic factor
BOLD	blood-oxygen-level-dependent (contrast)
BMI	body mass index
CBF	cerebral blood flow
CVR	cerebrovascular reactivity
ECG	electrocardiography
EEG	electroencephalography
ERP	event-related potential
EPI	echo planar imaging
FC	functional connectivity
FDG	fluorodeoxyglucose
fMRI	functional magnetic resonance imaging
GRAPPA	generalized autocalibrating partial parallel acquisition
HIIT	high intensity interval training
HRF	hemodynamic response function
MEG	magnetoencephalography
MPRAGE	magnetization-prepared rapid gradient echo
MREG	magnetic resonance encephalography
MRI	magnetic resonance imaging
NIRS	near-infrared spectroscopy
PET	positron emission tomography
PCO_2	partial pressure of carbon dioxide
PD	Parkinson’s disease
PO_2	partial pressure of oxygen
RF	radiofrequency

rs-fMRI	resting-state functional magnetic resonance imaging
TE	echo time
TR	repetition time
VO_2max	maximal rate of oxygen consumption
VO_2peak	peak rate of oxygen consumption

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1 INTRODUCTION

Physical activity and exercise are beneficial for cognitive function in all age groups. They enhance academic achievements and cognitive function in preadolescence (Bidzan-Bluma & Lipowska, 2018; Donnelly et al., 2017) as well as in adult populations (Sanders et al., 2019; P. J. Smith et al., 2010). Cognitive impairment associated with aging can be attenuated with physical exercise and exercise can even reduce rapid cognitive decline related to neurodegenerative diseases like Alzheimer's and Parkinson's (Jia et al., 2019; Sujkowski et al., 2021).

Positive outcome of exercise is not limited to lifestyle influence and long-term adaptations as a single bout of exercise alone, especially aerobic exercise, can elicit temporary effects to cognition (Chang et al., 2012). This is referred as "exercise priming" and can be utilized as tool to improve cognitive tasks like attention and learning (Moriarty et al., 2019; Stoykov et al., 2017). Exercise priming is demonstrated as improvements to perform cognitive tasks (Chang et al., 2012) but also as heightened brain cortical activity (Büchel et al., 2021; Moriarty et al., 2019) after exercise. Evidence of aerobic priming is incomplete as human brain studies are often limited by isolation. Human brain is enveloped by a thick and dense skull and circulation reach is often restricted by the blood-brain barrier.

In modern neuroimaging there are multiple ways to examine brain function. With functional magnetic resonance imaging (fMRI) brain activity can be measured noninvasively. The purpose of this master's thesis is to shortly review the literature in exercise-induced acute changes cognitive function and to conduct an experimental fMRI study to see aerobic exercise priming effects in the brain in healthy adults. Seemingly, resting-state spontaneous brain activity in acute exercise context has been studied using fMRI only in different patient groups (Kelly et al., 2017; Rajab et al., 2014). This study also aims to define the timeline of priming effects and to find individual characteristics which could explain between-subject differences in post-exercise activation.

2 AEROBIC EXERCISE AND ACUTE COGNITIVE FUNCTION

Cognitive function a term to describe person's overall mental ability to adapt to new situations using prior experiences, processing information and making conclusions. Cognitive function includes skills of perception, attention, memory, learning, decision making, and language abilities (Kiely 2014, 974–978). Physical exercise has gained increasing interest and attention as a non-pharmacological intervention to boost abilities of cognitive function. Aerobic exercise as an intervention yields small improvements to cognitive function (Hedges' g between .123 and .158, $p < .05$) in randomized controlled trials (P. J. Smith et al., 2010). In a more recent systemic review and meta-analysis, small exercise-induced improvements were found in executive function and memory (Cohen's $d = .27$ and .24, $p < .01$) but not overall cognition in healthy older adults. In the same study, results were opposite in population with diagnosed cognitive impairments (Cohen's $d = 0.37$, $p < 0.01$ for overall cognition). (Sanders et al., 2019.) There is also small evidence of physical exercise to improve learning skills in children, but the data is inconsistent and limited due to difficulties in statistical power, controlling for confounding factors and lack of control groups (Donnelly et al., 2017). It is safe to say that aerobic exercise has small to modest cognitive function improving properties, but the exact nature of improvements remains unclear. There are also gaps in knowledge when it comes to dose-response of exercise and cognitive function (Sanders, et al., 2019).

Physical exercise intervention periods do not have to be long to notice improvements in neuronal stimulation and cognitive function. Chang et al. (2012) in a meta-analysis found consistent evidence for a single bout of acute aerobic exercise to induce small but significant positive improvements to cognitive performance during and after exercise. Importantly, they evaluated effect sizes on cognitive performance in different types of exercise. Resistance training and anaerobic exercise induced notable negative effect (Cohen's $d = -.325$ and $-.744$) aerobic exercise alone showed small effect (Cohen's $d = .09$) but combined with other types of exercise the effect was larger (Cohen's $d = .371$) (Chang et al., 2012).

There is a problem in sample size as aerobic exercise is most studied. Sample size for aerobic exercise were hundredfold compared to resistance training and therefore effect sizes may not present the whole picture. However, these results may reflect on the timeline of acute exercise and cognitive function improvements. Chang et al. (2012) concluded that intensity of exercise corresponds to delays in cognitive improvements. Low intensity (aerobic) exercise induces more immediate responses, but higher intensity (anaerobic and muscular resistance) exercise may induce delayed responses after exercise (Chang et al., 2012). When studying acute effects, it may be beneficial to choose low to moderate exercise intensity.

The phenomenon of short-term cognitive function boost as an effect of physical exercise is known as exercise priming. Exercise priming or movement-based priming is an interesting new research topic as its applications may be used to promote cognitive or motor skill-based learning. (Stoykov et al., 2017.) Exercise priming can also be utilized skill or goal oriented but aerobic exercise is thought to induces more global and non-specific neuronal stimulus (Stoykov et al., 2017) and therefore could be used in variety of patient or non-patient groups with different learning goals or therapies.

Moriarty et al. in a (2019) review article listed three potential mechanisms for aerobic exercise priming.

1. Brain blood flow and oxygenation
2. Plasticity and neurotrophic factors
3. Neuroendocrine and myokine function. (Moriarty et al., 2019.)

Brain blood flow and oxygenation and their relation to exercise are reviewed in chapter 3.

Neuroplasticity refers to ability of neuronal networks to change their structure and function through reorganizing and growth. Neuroplasticity is linked to upregulation of neurotrophins, neurotrophic factors and neurogenesis (Thoenen, 1995). There is a variety of known neurotrophic factors but most notable, at least in terms of exercise research, is brain-derived

neurotrophic factor (BDNF). Exercise BDNF responses are mostly researched in animals. Wheel running in mice seems to consistently increase BDNF expression especially in the hippocampus and prefrontal cortex (Baranowski & MacPherson, 2018; Bos et al., 2012; Pervaiz & Hoffman-Goetz 2012.; Venezia et al., 2017) in which BDNF is most active in preserving memory, learning and higher cognitive processes.

In human studies exercise seem to increase serum BDNF regardless of intensity, as proximity to maximal rate of oxygen consumption (VO_2max) or duration (Schmolesky et al., 2013). However, the type of exercise may matter as all-out sprint exercise induced larger elevations in BDNF than set-intensity steady-state or high intensity interval training (HIIT) (Hashimoto et al., 2021; Weaver et al., 2021). These BDNF studies are made from venous blood and some might argue that they do not reflect within-brain conditions. Hashimoto et al. (2018) used paired arterial-jugular venous difference after HIIT exercise neurohumoral responses. They found decreased BDNF differences suggesting increased synthesis and secretion within the brain. Interestingly, they did not find differences in noradrenaline, dopamine or serotonin. This might be due to BDNFs ability to cross the blood-brain barrier whereas others cannot. They also hypothesised that lactate may drive BDNF response as they were correlated in the study. (Hashimoto et al., 2018.) This is consistent with Weaver et al. (2021) results.

Neuroendocrine responses to exercise may promote priming effect. Physical exercise is always a stressor to the neuroendocrine system and exercise induces profound effect to sympathetic nervous system and hypothalamic-pituitary-peripheral gland axes (Hackney, 2006). Neuroendocrine system covers nearly all familiar hormones to exercise scientists but most relevant, in case of exercise priming, seems to be cortisol. Exercise-induced cortisol response is shown to improve learning and memory, but effects are opposite when stress is psychological (Moriarty et al., 2019). This systemic “neuroendocrine hypothesis” to exercise priming can be supplemented with within-brain catecholamines and neurotransmitters. This “catecholamines hypothesis” reviewed by McMorris (2016) describes how even a light exercise induces a noradrenaline response through a brain inner feedback system which in turn excitants the reticular system resulting in increased arousal and vigilance. Brain noradrenaline response is

separate from the peripheral exercise noradrenaline response and therefore effects on cognitive skills can be detected after shorter and lighter exercise bouts that otherwise would not alter plasma noradrenaline concentration. Dopamine response is similar and highest in short moderate-intensity exercise in the prefrontal cortex. (McMorris, 2016.) Dopamine response could partly explain how moderate-intensity exercise elicits most positive effect on executive function, described in the meta-analysis of Chang et al. (2012). Noradrenaline and dopamine do not affect neuronal activation alone but also inhibit non-essential activity and promote signal to "noise" ratio, meaning they amplify neuronal information transmission relative to other activations. Higher intensity exercise induces a higher catecholamine response. When reaching a certain intensity threshold increased catecholamine response begins to damper cognitive skills due to overactivation. High intensity exercise therefore worsens signal to "noise" ratio (McMorris, 2016). This literature is based on animal studies and knowledge in humans is limited. However, the catecholamine hypothesis implies that exercise dose-response to cognitive function follows an inverted U-curve. This is consistent with human studies discussed earlier and the timeline proposed by Chang et al. (2012).

Myokines refer to cytokines secreted from the muscle tissue. They are protein hormones which can exert autocrine, paracrine and long-distance endocrine effects. Some myokines are part of skeletal muscle-brain crosstalk and can acutely affect the nervous system. (Delezie & Handschin, 2018.) Of myokines most notably irisin and cathepsin B have been somewhat linked to exercise priming effect (Delezie & Handschin, 2018; Moriarty et al., 2019) but the research is limited in number of studies. In addition, skeletal muscle derived lactate may indirectly facilitate BDNF response as mentioned earlier.

To summarize, exercise priming is a sum of multiple exercise-induced physiological mechanisms that affect cognitive skills like short-term memory and learning. Effects may be dependent on the intensity and type of exercise favouring moderate-intensity and -duration aerobic exercise. However, evidence from single mechanistic factors may not agree with this model.

3 BRAIN HAEMODYNAMICS AND OXYGENATION IN AEROBIC EXERCISE

Hemodynamics in definition refers to the physical study of blood flow and the structures which it flows through, known as the circulatory system. Blood "flow" is its velocity in the vessel which has length and time components. Flow rate on the other hand also takes blood volume into the equation. These terms are often mixed. (Secomb, 2016.) In recent literature term blood flow is often replaced with "blood velocity". Also, flow rate is often replaced with "blood flow". These more recent terms are used in this thesis.

Blood flow is controlled by physical laws such as blood pressure and viscosity and vessel geometrical properties (Secomb, 2016). Blood flow is also regulated through multiple biological mechanisms controlling central and peripheral blood flow allocating different tissues at different times. Blood pressure can change rapidly for example during physical exercise. Organs need to ensure adequate blood flow, and some have an innate property to do so. Despite changes in arterial perfusion pressure, they can adapt by changing their peripheral resistance to pressure. This is known as autoregulation, and it is most apparent in heart, kidneys and brain. (Johnson, 1986.)

3.1 Mechanisms for Autoregulation

According to Armstead (2016) at least four mechanisms are proposed for autoregulation: myogenic, neurogenic, metabolic and endothelial regulation. These mechanisms and their relevance to acute exercise cerebral autoregulation are briefly discussed.

1. Myogenic regulation

Vascular smooth muscle responds to stretching of the vessel wall pressure (blood pressure) by contracting or relaxing (Bayliss, 1902) keeping blood flow constant. This response to stretch is known as myogenic response or Bayliss effect named according to the original author.

2. Neurogenic regulation

Cerebral arterioles or capillaries respond directly or indirectly to neuronal stimulus. In need of energy substates, neurons chemically stimulate smooth muscle cells. This chemical signal is thus turned into mechanical signal, which alters blood flow. Neurons also stimulate surrounding glial cells that amplify the regulatory effect by also releasing vasoactive agents. However, the exact mechanism for neuron, glial cell and vascular cell responsiveness is still unknown. (Huneau et al., 2015.) This functional change in local cerebral blood flow (CBF) following changes in neuronal activity is known as neurovascular coupling.

3. Metabolic regulation

Changes in arterial gasses regulate cerebral microvasculature diameter (K. J. Smith & Ainslie, 2017). Arterial gasses' partial pressure changes are prevalent in physical exercise and are further discussed below.

4. Endothelial regulation

In addition to neurons and glial cells, endothelial cells have a role in vascular tone. Endothelium-synthesized factors like nitric oxide can also stimulate smooth muscle cells (Ashby & Mack, 2021). Endothelial effects are longer in duration and may therefore not be relevant in exercise CBF response. Endothelial effects are present in long term conditions like cerebral small vessel disease (Ashby & Mack, 2021).

3.2 Cerebral Blood Flow During Physical Exercise

Brain exhibits a high degree of autoregulation to always meet its high metabolic demand and paradoxically has very limited energy reserves. Conserving CBF is essential to brain function especially during physical exercise when systemic blood pressure may fluctuate following

skeletal muscle activity. The onset of exercise directly elevates heart rate and venous return *via* multiple reflexes which increase cardiac output and systemic blood flow to meet the metabolic demand of working skeletal muscle tissue. During exercise cardiac output increases 300-600% but top CBF only about 20% (K. J. Smith & Ainslie, 2017). This small increase in blood flow is important and enough to cancel out exercise-induced changes in blood flow distribution and to maintain brain oxygen and nutrient intake and waste clearance.

But what causes this small but significant increase in CBF during exercise? One could assume that exercise-induced increases in cardiac output is enough to promote CBF, but this can be debunked with exercise intensity comparison. In lower working loads CBF follows the intensity-curve. This pattern is however flipped in higher intensities and as working load increases CBF return towards baseline, despite the linear rise in cardiac output. (Smith & Ainslie, 2017.) Smirl et al. (2014) study also found similarities in middle cerebral artery blood velocity responses to progressive exercise in heart transplant recipients and controls despite transplant recipients having impaired heart function (Smirl et al., 2014). While cardiac output alone does not explain a small rise in CBF during exercise multiple other exercise variants have separately shown to elicit some autoregulatory responses (K. J. Smith & Ainslie, 2017).

Arterial gasses. When exercise intensity rises more and more energy is produced by glycolytic routes. As a by-product carbon dioxide is formed and systemic partial pressure of carbon dioxide (PCO_2) rises in circulation. PCO_2 can has shown to trigger some cerebral vasoactive responses. The PCO_2 dependent theory is an early finding linking inverse parabolic relationship of arterial PCO_2 during exercise and CBF. (K. J. Smith & Ainslie, 2017.) The relationship curve peaks just before ventilation threshold after which blood PCO_2 would rise significantly if workload would be increased. Partial pressure of oxygen (PO_2) may also play a part in autoregulatory responses and is thought to co-operate with PCO_2 in metabolic autoregulation. Smith et al. (2016) followed CBF in both steady and changing arterial PCO_2 in multiple submaximal exercise models in both normoxic and hyperoxic conditions. They also controlled subject breathing. They found differences exercise-induced changes in CBF as a result of both PCO_2 and PO_2. Interestingly, they also found the act of breathing alone to elicit these responses

to CBF during exercise (Smith et al., 2016) demonstrating the complexity of CBF metabolic autoregulation. It is also speculated that both PO_2 and PCO_2 respond to changes in pH and metabolic demands during exercise, but exact mechanisms of metabolic autoregulation are yet to be determined (K. J. Smith & Ainslie, 2017)

Neural control. Autonomic nervous system control has been studied in the context of acute exercise. Purkayastha et al. (2013) found α1-antagonist to attenuate the CBF response during submaximal exercise. This could mean that sympathetic nervous system controls CBF during exercise (Purkayastha et al. 2013). Drastic attenuation of CBF response after sympathetic nervous system blockade could imply a more top-to-bottom regulation of CBF, instead of local autoregulation. However, neural control studies are tricky as it is impossible to target substances to specific organs and therefore it is difficult to draw conclusions on autonomic nervous system control over CBF.

3.2.1 Aerobic Exercise Intensity-Cerebral Blood Flow Curve

Overall, exercise seems to increase CBF, cerebral blood velocity and perfusion most efficiently in moderate (50-80%) workloads (Figure 1 ((K. J. Smith & Ainslie, 2017)). Percentage of maximal workload does not directly reflect percentage of VO_2max as individual differences occur, and often exercise power output does not stop at maximal oxygen consumption. In the review of Moriarty et al. (2020) aerobic exercise at 50-60% workloads were thought to elicit most priming effects. When fixed to oxygen consumption maximal CBF, responses are detected near the ventilatory threshold around 60-70%VO_2max intensities at steady-state aerobic exercise. This model may be dependent on exercise type as HIIT exercise temporary exceeding ventilatory threshold is shown to elicit similar arterial velocity increases as moderate-intensity steady-state exercise (Weaver et al., 2021).

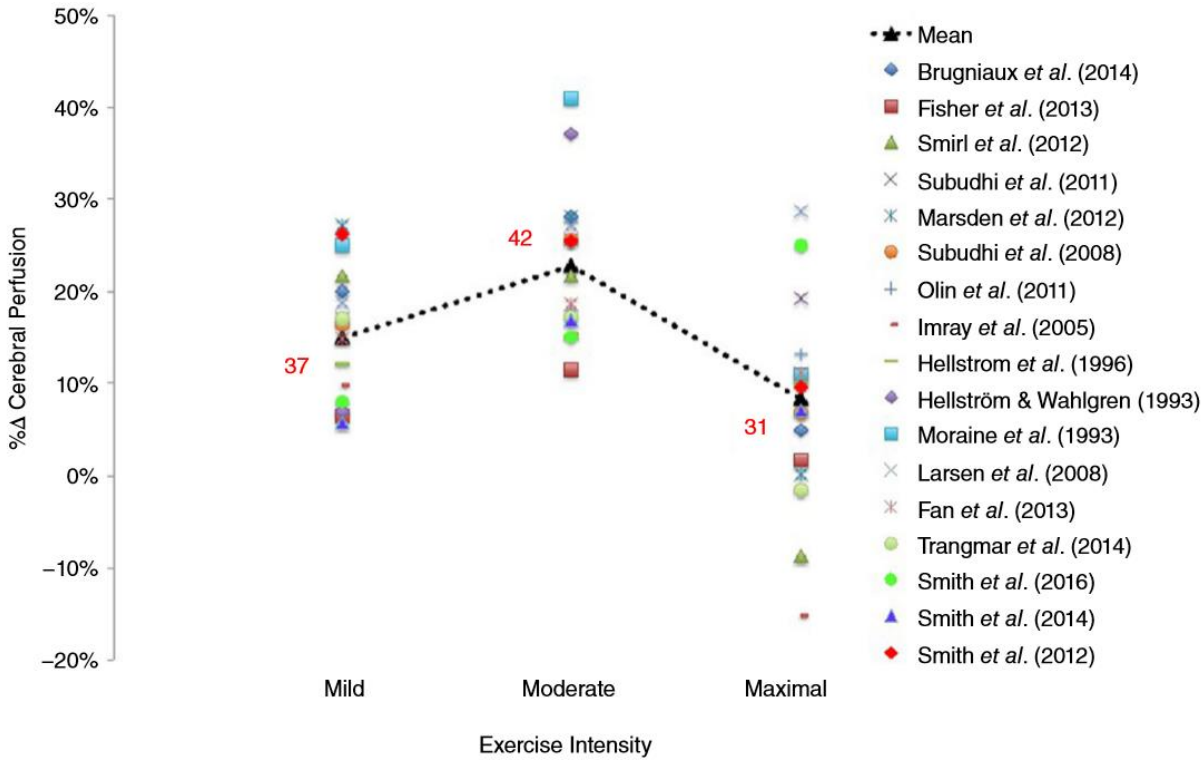

FIGURE 1. Percentage changes in cerebral perfusion from rest, during incremental exercise from mild (20-40%) maximal workload (Wmax) moderate (50-80%Wmax) and maximal (90-100%Wmax) intensities in aerobic exercise studies (K. J. Smith & Ainslie, 2017).

It is important to notice that estimates of CBF are in many cases conducted from measurements of medial cerebral artery velocities using transcranial Doppler sonography (K. J. Smith & Ainslie, 2017). Transcranial Doppler can detect changes in blood flow velocity in arteries but does not take in count arterial diameter. Therefore, direct measurements of blood flow and cerebral perfusion cannot be done using transcranial Doppler. Arterial blood velocities seem to correlate with blood flow and perfusion as transcranial Doppler is shown to match well with perfusion MRIs (Sorond et al., 2010). Still a certain level of precaution should be taken when interpreting these results.

Sorond et al. (2010) used two functional MRI techniques to validate Doppler method: contrast-enhanced perfusion MRI and arterial spin labeling (ASL). ASL is often used to measure whole-

brain CBF. It is a noninvasive magnetic resonance method that uses radiofrequency (RF) pulses at neck level to magnetically "tag" inflowing arterial blood. It compares images before and after magnetization to trace arterial blood movement (MacIntosh et al., 2014; Williams et al., 1993). ASL has an advantage over transcranial Doppler as it measures blood flow throughout whole brain area, not just arterial blood velocities. Macintosh et al. (2014) found 20 minutes cycle ergometry exercise at 70%VO_2max to acutely decrease global gray matter blood flow but increase global white matter blood flow (MacIntosh et al., 2014). This would imply regional differences in blood distribution after exercise that would not show in transcranial Doppler.

Physical fitness seems to be correlated with CBF and cerebrovascular reactivity (CVR). CVR refers to vessel ability to dilate in response to autoregulatory stimuli, like increased PCO_2. In a study by Foster et al. (2020), they used ASL to measure correlations between peak rate of oxygen consumption (VO_2peak) and CBF. They found positive correlations in grey matter CVR, but negative regional correlations in CBF, and fitness level (VO_2peak). No associations between fitness and cognitive performance were found. These results suggest that fitness level may alter baseline cerebral hemodynamics. (Foster et al., 2020.) Whether hemodynamic features are a result of training or congenital remains unknown. Body mass index (BMI) also seems to negatively affect baseline grey matter CBF even more so than aging (Knight et al., 2021). Obesity related diseases, like type 2 diabetes, are also known to attenuate acute exercise increases in CBF and oxygenation due to impaired cardiac output and CVR which could lead to earlier perceived exertion (Kim et al., 2015). Disease state data cannot directly be used to describe healthy population baseline CBF or responses to exercise, but some references to fitness and BMI effects could be made. Higher body mass could increase CBF at baseline, but limit increases during exercise.

3.3 Cerebral Blood Oxygenation During Physical Exercise

Blood oxygenation often refers to oxygen saturation, which is the fraction of oxygen-saturated hemoglobin to total hemoglobin. Cerebral oxygenation is often measured with near-infrared spectroscopy (NIRS). NIRS uses near-infrared (700–1000 nanometers) light to penetrate tissue and to be absorbed or scattered by proteins such as oxy- or deoxyhemoglobin. Detecting returning light at different wavelengths, oxygen saturation or total oxy- and deoxyhemoglobin concentrations can be measured. (Ferrari et al., 2004.) In a systematic review of Rooks et al. (2010), they found prefrontal cortex oxygenation to increase during exercise relative to intensity, peak at near-maximal intensities but drop at maximal exercise (Rooks et al., 2010). This pattern is quite similar to cerebral arterial velocity inverted U-curve presented in Figure 1 but oxygenation seems to increase further approaching VO_2max. NIRS can detect oxygenation at cortical brain levels like prefrontal cortex but cannot reach deeper brain structures.

In brain function research, oxygenation and neuronal activation are almost treated as synonyms. This originates from the field basing its theory over functional magnetic resonance (fMRI) research. Local neuronal activation evokes neurovascular response through neurovascular coupling which can be measured. Most notable fMRI method is blood oxygen-level-depended fMRI (BOLD-fMRI) which as the name suggests, measures local oxygenation. In chapter 4 fMRI and other brain activation measuring methods are reviewed.

4 METHODS FOR MEASURING BRAIN ACTIVITY IN HUMANS

Exercise priming can be defined as behavioral change generated by physical exercise (Stoykov et al., 2017). To study this, tasks or tests would be performed. Exercise priming is also seen as heightened activity in brain regions controlling these cognitive processes (Moriarty et al., 2019) which can be studied using functional brain imaging.

Brain activity research requires understanding on imaging methodology. Functional imaging is different from conventional imaging in that it does not mainly gather information on brain anatomical structures but rather evaluates its physiological function like neuronal activity. Function can also refer to blood flow or perfusion which is the outcome of ASL as discussed earlier. There are direct and indirect methods for functional imaging. Either neuronal (electrical) activity or physiological events related to activity, like hemodynamics or metabolic changes, are measured. Basic methods of functional brain imaging and activity research are reviewed. *Postmortem* and animal studies methodology is excluded from this review.

4.1 Measuring Brain Electrical Activity

Brain activity can be detected as electric or magnetic activity with electroencephalography (EEG) and magnetoencephalography (MEG). EEG uses electrodes on the subject's scalp. Electrical activity can be read through the skull with electrodes. Changes in potential between two electrodes are caused by neurons' electrical net effect, and activity is graphed as patterns over time. EEG is most often evaluated using frequencies. Frequency waveforms or "waves" include infra slow oscillations (<0.5Hz), delta (0.5-4Hz), theta (4-7Hz), alpha (8-12Hz) sigma (12-16Hz), beta (13 to 30Hz) and high-frequency oscillations (>30Hz) and all these waveforms have different physiological importance. (Nayak & Anilkumar 2021.) Büchel et al. (2021) studied aerobic exercise at different intensities in different resting-state network EEGs. They found EEG power to increase equivalently at alpha waveform in all intensity levels consistent with earlier research. They also found EEG efficiency to drop at maximal exercise (Büchel et

al., 2021) which could be explained with McMorris (2016) reduction of signal to noise -theory. There is also an experimental EEG model named event-related potentials (ERPs) in which electrophysiological responses to a stimulus are measured with EEG equipment. This thesis however focuses on “resting state” conditions of brain activity where no external stimuli are given.

EEG/ERP methods are totally noninvasive and harmless to the subject. EEG methods are also relatively inexpensive but hold difficulties in spatial resolution. Incoming signal is difficult to precisely locate to a specific region. EEG/ERP is also limited to cortical levels of the brain and therefore activity in deeper levels is underestimated or go undetected (Nunez, 1988).

MEG detects same neuronal activation net effects as EEG. MEG detects changes in magnetic field caused by electrical activity. Both MEG and EEG have good temporal resolution of a few milliseconds, but MEG has better spatial resolution (Hämäläinen et al., 1993). MEG is however far more expensive and cannot be transported easily. MEG is rarely used to record spontaneous brain activity after exercise, but some studies have been conducted using ERP model of activation. Akatsuka et al. (2015) found moderate-intensity aerobic exercise to increase MEG signal amplitude of an inhibitory system, sensitive to cognitive performance, during cognitive task execution (Akatsuka et al., 2015). Authors concluded this type of exercise to effectively increase short-term cognitive performance through this network, again affirming the aerobic priming theory.

4.2 Measuring Local Events Related to Activity

As mentioned earlier neuronal activation evokes increases in local blood flow known as neurovascular coupling. Simultaneously, energy substrates, like glucose, uptake increases in areas of activity. This is known as neurometabolic coupling. Consequently, increased energy metabolism increases local oxygen consumption. (Huneau et al., 2015.) Therefore, tracking oxygen or glucose would indicate areas of activity. This can be done with positron emission tomography (PET). Radioactive labeling in form of fluor-18-labelled fluorodeoxyglucose (18F-

FDG) or oxygen-15-labelled water (15O-water or “radiowater”) can measure effects related to metabolic rate of glucose and oxygen uptake, respectively (Verger & Guedj, 2018). This functional form of PET imaging is not as popular in activity research as functional MRI, but there is potential for application as it has multiple advantages over fMRI like resolution and signal to noise ratio (Boecker & Drzezga, 2016). Some activity studies using PET have been conducted. Christensen et al. (2000) used PET to affirm primary motor cortex, supplementary motor area and cerebellum involvement during rhythmic cycling exercise in humans (Christensen et al., 2000). While being an attractive functional neuroimaging method notable disadvantages of PET are a dose of ionizing radiation to the subject and imaging expenses.

There is some PET imaging evidence of increased sensimotor activity and decreased prefrontal activity during exercise and potential for more exercise priming research. Still, most PET studies seem to focus on reward system (endorphins and dopamine) effects of exercise as they are almost exclusively studied using PET. (Boecker & Drzezga, 2016.) High intensity exercise may depress glucose and increase lactate uptake in the brain (Kemppainen et al., 2005), which could negatively affect functional imaging, if 18-FDG were used. In this context, 15O-water would be more efficient, especially in higher exercise intensities. In the study of Christensen et al. (2000) 15O-water was used. 15O-water has a short half-life (about two minutes) which has to be taken into consideration when designing post-exercise activity studies. These limitations have made it so activity research is based more on fMRI rather than PET.

4.3 BOLD-FMRI

Blood-oxygen-level-dependent (BOLD) imaging is a fMRI technique using blood hemoglobin as contrast agent. Earlier NIRS and its application as an activity marker were discussed. BOLD-fMRI similarly uses hemoglobin to detect changes in neuronal activation but in a different way. Hemoglobin has different magnetic properties depending on how many oxygen molecules it carries. Therefore, if hemoglobin releases an oxygen molecule, it can be detected with an MRI scanner (Ogawa et al., 1990). An increase in BOLD signal intensity in a region of interest would imply changes in hemodynamics in that area.

Physiological origin of BOLD signal. BOLD signal application assumes that detected hemodynamic response is caused by neurovascular coupling. Hemodynamic response or hemodynamic response function (HRF) is an acute increase in local blood flow to meet metabolic needs, which can be detected with an MRI scanner. Figure 2 describes how activity is detected by MRI through local changes in hemodynamics and neurovascular coupling. As Figure 2 shows there are multiple variables that can change imaging outcome: biological and technical. Basic MRI technical properties include magnetic field strength, repetition time (TR), echo time (TE) and sequence type as mentioned in Figure 2.

BOLD-fMRI parameters. In fMRI acquisition, TR refers to time to capture whole brain coverage once. Selecting TR is an important trade-off between spatial and temporal resolution. Longer TR produces higher quality images, but brain activity may be time sensitive. Often TR is settled between 2-3 seconds depending on study purpose (Soares et al., 2016). TE is time between RF pulse and peak echo signal returning to coils. To maximize BOLD contrast TE must be designed according to target tissue type and magnetic fields strength. BOLD contrast and resolution favor higher magnetic field strengths going from typical clinical-purpose 1.5 Teslas to 3 Teslas and even higher as high-performance equipment gains popularity (Duyn, 2012). TE is typically around 30 milliseconds in BOLD-fMRI (Soares et al., 2016). Sequence type means how the RF pulses are given and echoes are received. BOLD-fMRI typically uses echo planar imaging (EPI) technique to imaging speed and BOLD contras (Soares et al., 2016). EPI utilizes gradients (secondary magnetic fields) to induce multiple echoes within a single TR. This allows image acquisition with reduced number of TRs. Selecting EPI type (spin-echo or gradient echo) is based on imaging goals more often favoring gradient echo (Soares et al., 2016).

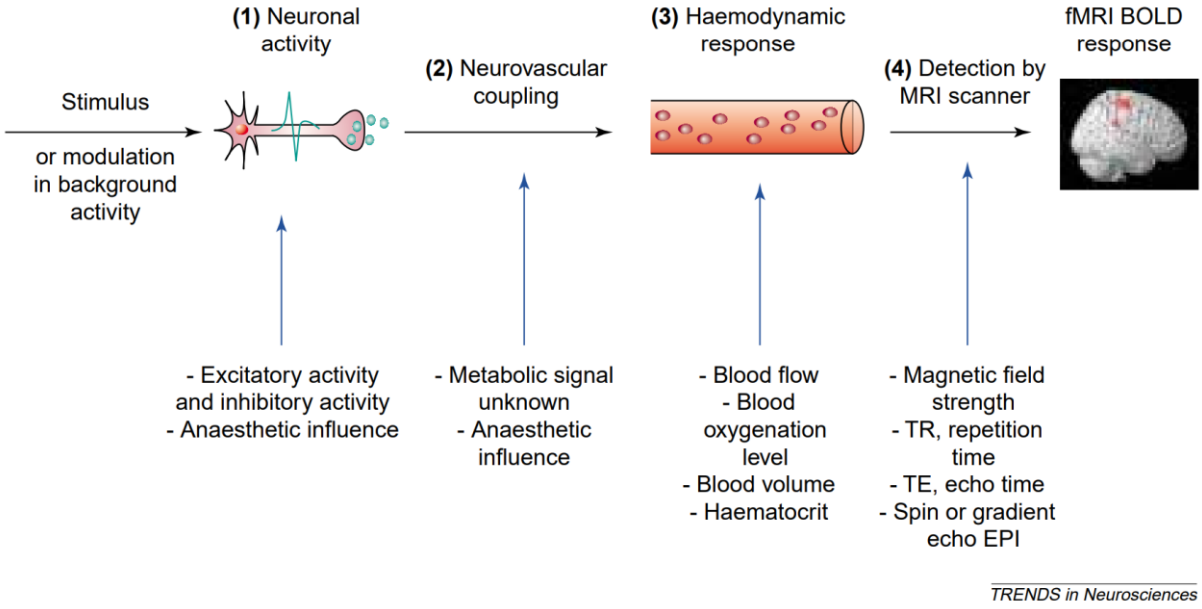

FIGURE 2. Physiological origin of BOLD signal (Arthurs & Boniface, 2002).

BOLD-fMRI study design. Most fMRI studies are task-based in which stimuli are applied in different timepoints followed with periods of rest. When designing fMRI studies, it is important to note that timelines of HRFs are delayed after neuronal activation, and peak BOLD signal is detected *circa* 5-6 seconds after stimulus and slowly declines in following seconds (Arthurs & Boniface, 2002; Soares et al., 2016). Multiple stimuli too close may have overlapping effects. To compare activity to nonactivity HRFs can be extracted from total BOLD signal in the simplest manner by subtracting signal from nonactivity periods to periods where a stimulus is given. There are also other models in stimulus presentation and comparison strategies for task-based fMRI (Amaro & Barker, 2006).

Resting-state fMRI. When imaging spontaneous brain activity, the study design cannot be task-based. Spontaneous brain activity can be imaged using resting-state fMRI (rs-fMRI). In rs-fMRI, no external stimuli are given so study design is totally different from block- or event-related designs. Although being more straightforward in image acquisition design, rs-fMRI does not include periods on activity to nonactivity so signal to noise separation in more difficult.

Regional brain activity can be measured by investigating BOLD signal time course. Biswal and colleagues (1995) originally found fluctuations of BOLD signal to by synchronized between right and left motor cortices in rest and their functional connectivity (FC), or in other words their simultaneous activity patterns, to be similar as during bilateral finger tapping task (Biswal et al., 1995). This is one of the earliest works validating rs-fMRI application and their results indicate low frequency patterns of resting-state BOLD to have physiological origin. FC is often used in rs-fMRI research to identify synchronized and functionally connected resting brain networks. In addition to FC, magnitude of local activity can be measured by extracting amplitude of low frequency fluctuations (ALFF) from BOLD signal. ALFF is a quantifiable outcome of rs-fMRI. ALFF is defined as "the total power within the frequency range between 0.01Hz and 0.1Hz" and most often used as power spectral density within these frequencies (Zuo et al., 2010).

ALFF were first used in clinical setting by Zang et al. (2007) to identify spontaneous brain activity differences in ADHD children, and later has been used to evaluate resting activity patterns in multiple different patient groups (Wang et al., 2019; Wei et al., 2021; Zang et al., 2007). Exercise research using rs-fMRI techniques is rare. Weng et al. (2016) utilized FC and found acute moderate-intensity aerobic exercise to selectively increase synchroneity in brain regions important to cognitive function in young and old people. Increased FC were linked to networks that functionally decline over ageing process (Weng et al., 2016). These findings support the aerobic priming hypothesis acutely improving cognitive function.

Only two exercise rs-fMRI studies using ALFF as a marker for spontaneous brain activity were found in the literature. Huang et al. (2021) found 8-week aerobic exercise intervention to reshape spontaneous brain activity in both subthreshold depressive disorder patient group and healthy controls. Exercise intervention "normalized" ALFF patterns observed in the patient group in baseline. Interestingly, aerobic exercise intervention was able to reshape spontaneous brain activity patterns also in healthy controls. Increased activity was observed in areas related to motor learning. (Huang et al., 2021.) This could imply that aerobic training could have long-term priming effects in form of increased spontaneous activity.

Acute exercise effects on ALFF were studied in Kelly et al. (2017) study with exercise-trained Parkinson's disease (PD) patients (n=17). They found acute exercise to increase ALFF in regions linked to PD progression. In this study, exercise model were a combination of resistance training and aerobic training, and individual responses to exercise were poorly recorded. This study did not have a healthy control group and therefore authors could not make distinctions between PD patients and other population (Kelly et al., 2017). Although being a pilot study with limitations, they found physical exercise to acutely increase spontaneous brain activity in disease specific brain regions. They found ALFF to be useful is recording spontaneous brain activity acutely post-exercise.

5 RESEARCH QUESTIONS AND HYPOTHESES

Question 1: Does vigorous-intensity steady state aerobic exercise acutely change brain spontaneous activity?

Hypothesis: Yes

Rationale: Moderate- and vigorous-intensity aerobic exercise has been shown to elicit multiple "priming" effects of increased CBF and activity (Moriarty et al., 2019) In clinical setting, acute exercise has been shown to reshape spontaneous brain activity patterns in different populations (Kelly et al., 2017; Weng et al., 2016).

*Question 2***:** Are acute changes in spontaneous brain activity after aerobic exercise related to subject characteristics (age, sex, BMI, fat-% and VO_2peak)?

Hypothesis: Yes.

Rationale: Evidence from Kim et al. (2015), Knight et al. (2020) and Foster et al. (2020) suggests that BMI and age negatively and fitness level positively effects CBF responses to exercise, which would be shown as attenuated rise in spontaneous activity after exercise. Caveat here is that noticeable effects could be limited to older, obese or diseased population.

Question 3: Are acute changes in spontaneous brain activity after aerobic exercise related to subject level of exertion (lactate)?

Hypothesis: Yes.

Rationale: Both direct evidence of increased activity (Büchel et al., 2021; Rooks et al., 2010) and evidence from underlying mechanisms (Hashimoto et al., 2018; Kemppainen et al., 2005;

McMorris, 2016; Schmolesky et al., 2013; K. J. Smith & Ainslie, 2017) suggest that spontaneous brain activity is highly intensity-depended. If selected exercise model would elicit different lactate or heart rate responses, different responses in spontaneous brain activity effect size and regional orientation likely occur.

6 METHODS

This study is a part of GLYMREG collaboration research project in Turku PET centre, and Turku University Hospital Neurocenter, in Turku, Finland. GLYMREG studies aerobic exercise effects on the glymphatic system and CBF and perfusion using fMRI modalities (ClinicalTrials.gov identifier: NCT04255758).

6.1 Study Subjects

Study target population was healthy young age-matched men and women aged 18-45 without any underlying diseases like central nervous system, cardiovascular or musculoskeletal diseases or disorders. Subjects also did not have any regular ongoing medication throughout the study period. There were no limits or guidelines on subject physical activity level or physical fitness. The study subjects were recruited through personal contacts and social media (appendix 1: study advertisement poster).

Subject Inclusion and Exclusion Criteria. Study subjects had to fit into following list of criteria as subject safety precaution and to ensure subject suitability to perform maximal fitness testing and exercise and imaging protocol.

Inclusion criteria:

- Age 18-45
- BMI 18-30
- Resting blood pressure < 140/90 mmHg

Exclusion criteria:

- History of cardiac events
- Insulin or medically treated diabetes
- Any chronic disease or condition that could create a hazard to the subject safety, endanger the study procedures or interfere with the interpretation of study results
- Presence of ferromagnetic objects that would make MRI contraindicated
- Claustrophobia
- Abundant use of alcohol
- Use of narcotics
- Smoking of tobacco or consuming snuff tobacco
- Diagnosed depressive or bipolar disorder
- Abnormalities in resting electrocardiogram (revised by the study physician)
- Pregnancy (confirmed by pregnancy test)
- Acute upper respiratory tract infection symptoms, flu-like symptoms, fever, or symptoms in the gastrointestinal tract as a precaution for COVID-19
- Travel abroad 14 days before testing as a precaution for COVID-19

Number of subjects. A total of 20 subjects was recruited, 10 men and 10 women. The number of subjects was estimated using study primary outcome measurement. Study primary outcome measure was sample entropy derived from magnetic resonance encephalography (MREG) imaging, which has shown to represents the glymphatic clearance/flow. To detect a statistically significant change before and after exercise, 14 subjects in total was calculated to be needed ($\alpha = 0.05$, $1 - \beta = 0.9$) to show significant increase in sample entropy from 2.10 to 2.35. (Rajna et al., 2019.) To allow possible dropouts and technical problems in the measurements as well as suitable group size for correlation analyses, it was estimated that 20 subjects, 10 men and 10 women (allowing for sex difference comparison), would be needed for the study. Rs-fMRI results were obtained from 14 subjects, 6 males and 8 females. Five subjects' results were discarded due to technical difficulties in image storage. One subject was unable to finish the intended exercise duration and ended up in exhaustion. Subject characteristics from study

screening protocol are combined in Table 1. Screening protocol is described in detail below. All but one subjects were right-handed, one subject used both hands in daily activities.

TABLE 1. Subject characteristics and designed cycle ergometry power outputs for the aerobic exercise protocol.

	Subjects (n = 14)
Age (y)	31.4 ± 8
Height (cm)	17.9 ± 9.2
Body mass (kg)	71.3 ±10.9
Body mass index	24.9 ± 2.2
Bodyfat percentage (%)	22.6 ± 6.6
VO_2peak (ml/kg/min)	42.3 ± 5.0
Power output at 50%VO_2peak (W)	99 ± 32
Power output at 70%VO_2peak (W)	166 ± 42

Ethics. Prior to participation, all subjects were informed about the potential risks of the study and the possible discomfort associated with high intensity aerobic exercise, blood sampling and MRI. All subjects gave their written informed consent to participate and filled out a Physical Activity Readiness Questionnaire (PAR-Q+), to ensure readiness for strenuous physical activity. All subjects were also examined by a hospital physician. All procedures were approved by the Ethics Committee of the Hospital District of Southwest Finland and the study was carried out according to the Declaration of Helsinki.

6.2 Screening Protocol

Subject screening protocol started with protocol familiarization, informed consent and health and physical activity questionnaires. Resting seated blood pressure was measured twice and lying position electrocardiography (ECG) was recorded. ECG is checked by the study physician who also carried out a thorough physical examination. Once cleared by the physician and stated as applicable to the study and checked for inclusion and exclusion criteria maximal exercise testing could be performed.

Anthropometry. Subject screening included body anthropometric measurements and estimates. Height and weight were measured on the screening day by standard scales in patient care found in Turku PET Centre, Turku, Finland. Body fat percentage was determined using Durning & Womersley (1974) four site skinfold measurement (bicep, tricep subscapular and suprailiac sites). Skinfolds were measured three times by the same tester. Sum of averaged skinfolds and subject sex and age were inserted into logarithmic equations calculated from the original Durning & Womersley (1974) article data to calculate a body fat estimate.

Exercise testing. Maximal exercise testing was outsourced to Paavo Nurmi Center, which is a sports medicine and exercise testing facility with trained personnel in Turku, Finland. Testing included measurements of respiratory gasses, power output and heart rate. Subjects were informed not to exercise and consume alcohol 24 hours before screening and fitness tests. Consumption of large meals and caffeinated products were also prohibited 2 hours prior testing. Cycling power output started at 50 W and 25 W were added incrementally every 2 minutes. Heart rate were measured simultaneously using Polar H10 sensor (Polar Electro Oy, Kempele, Finland). Test were stopped at not being able to keep the intended power output or unwillingness to continue due to fatigue

VO_2peak. Peak rate of oxygen consumption (VO_2peak) was determined in the cycle ergometry maximal exercise test with direct real-time respiratory measurements of oxygen and carbon dioxide. Breath-by-breath method of respiratory gas exchange was used with Vyntus CPX (Vyaire Medical Inc., Chicago, USA). VO_2peak were used as an outcome, instead of VO_2max, because maximal oxygen consumption criteria were not assessed individually in this study.

6.3 Aerobic Exercise and Imaging Protocol

Exercise intensity was determined from previously performed personal maximal exercise test results (VO_2peak). All subjects performed the same relative intensity (%-personal VO_2peak) and same duration exercise protocol using a cycle ergometer. Intensity was be determined and followed with the cycle ergometer power output. Relative intensities were calculated using oxygen consumption at given power output as an indicator rather than power output *per se* and rounded down to previous load in 25 W margin. The exercise protocol continued for total of 30 minutes: including 5 minutes warm-up session at 50%VO_2peak followed by 25 minutes 70%VO_2peak steady-state cycle ergometric work. The exercise protocol was terminated if the subject chooses to or if any subject safety jeopardizing symptoms occur. Inability to keep up the intended workload would also lead to termination.

Measurements were taken before, during and after the exercise protocol at different timepoints: PRE imaging timepoint before exercise and POST 1, POST 2 and POST 3 *circa* 30, 60 and 90 minutes after exercise, respectively. Measurements at these timepoints included include MRI, blood samples, heart rate monitoring and blood pressure measurements. Heart rate were also measured at the start and end of exercise and lactate 2-3 minutes after to evaluate individual exercise intensity. Whole imaging and exercise protocol is shown in Figure 3.

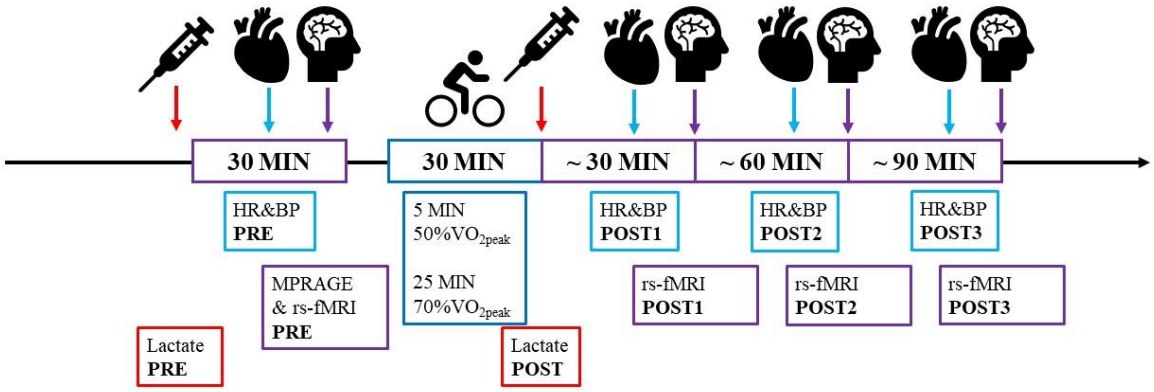

FIGURE 3. Study imaging and aerobic exercise protocol and physiological measurements. HR = heart rate, BP = blood pressure, MPRAGE = T1-weighted structural MRI, rs-fMRI = resting-state fMRI.

Heart Rate and Blood Pressure. Philips Expression MR400 MRI-compatible patient monitor (Philips Healthcare, Andover, USA) was used to measure heart rate and blood pressure. Heart rate was measured with fingertip pulse oximeter compatible to the patient monitor both during scanning and cycle ergometry exercise.

Lactate and heart rate reserve. Before and after aerobic exercise protocol lactate was measured to assess individual responses to exercise protocol. Lactate was measured from blood drawn through intravenous cannula inserted at the start of imaging protocol. Cannula were inserted by hospital personnel and lactate were measured using Lactate Scout 4 (EKF Diagnostics, Cardiff, UK).

Percentage of heart rate reserve (%HRR) during exercise was a way to assess individual responses to exercise protocol and were calculated with following formula:

$$\%HRR = HR_{exercise} - HR_{rest} / HR_{maximal} - HR_{rest},$$

in which $HR_{exercise}$ were peak HR during exercise protocol, HR_{rest} were HR just before starting the protocol and $HR_{maximal}$ were peak HR during maximal exercise testing.

6.4 Magnetic Resonance Imaging

Study scanner. Siemens MAGNETOM Skyra FIT 3.0T MRI system equipped with a standard twenty-channel head coil (Siemens Healthcare GmbH, Erlangen, Germany) was used in this study. Scanner was used in patient care and research in Turku University Hospital, Turku, Finland.

Structural MRIs. For this study, noninvasive magnetic resonance methods were used: a combination of structural MRIs paired with fMRIs. Baseline imaging included anatomical sequences including T1- and T2-weighted images (often referred as T1 and T2). They are basic diagnostic MRIs in which T1 expresses high tissue fluid signal and low tissue fat signal, and T2 vice versa. In images, T1 shows water in high signal intensity, and T2 fat. T2s were not used for study analyses but were checked by the study radiologist for health-related abnormalities.

From T1s, three dimensional (3D) T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) images were gathered. MPRAGEs have high spatial resolution (1.1x1.1x1.2 mm voxel size). Parallel imaging technique called "generalized autocalibrating partial parallel acquisition" (GRAPPA) were also used to speed up imaging acquisition time. MPRAGEs used both short TR = 8.6 milliseconds and TE = 3.69 milliseconds with flip angle 20°. These structural high spatial resolution images are used in fMRI preprocessing as BOLD-fMRI images are fitted into 3D structural head models.

Functional MRIs. Rs-fMRI sequences were scanned with voxel size 3.4x3.4x3.4 mm and using gradient echo EPI design. 198 scans were taken in all timepoints with TR = 3000 milliseconds which add up to ~ 10 minutes per sequence. TE = 30 milliseconds with flip angle 90° were used. To ensure "resting-state" conditions subjects were asked to keep their eyes open look directly to a black cross on a whiteboard in the middle of their field of vision. No music was played during scanning. Scanner head coil limited subject head movement, but additional motion correction was added later as part of image preprocessing. Subjects were asked to stay awake during imaging. Outside of this study the protocol included MREG and ASL scans between rs-fMRIs.

6.5 FMRI Preprocessing and First-Level Statistical Analysis

Rs-fMRI data were preprocessed and first-level analyses were calculated using DPABI toolbox, Data Preprocessing and Analysis of brain imaging (DPABI software version 2.3) (Yan et al., 2016), which is MATLAB (MathWorks, Natick, MA, USA) -based. DPABI calculates original DICOM images into NIfTI format and removed first ten out of 198 timepoints which is equivalent to first 30 seconds of the rs-fMRI sequence. NIfTIs were processed by following steps.

DPABI's advanced preprocessing pipeline (DFARSA) included slice timing correction, spatial realignment, brain extraction tool (BET) (Smith, 2002) to delete non-brain tissue. Functional images were segmented using study MPRAGE images and normalized and smoothed using DARTEL procedure (Ashburner, 2007). Nuisance regression was applied to remove white matter and cerebrospinal fluid noise and to realign head motion using first subject scan as template and correcting motion in three dimensions and their rotations (rigid-body 6 model). Images were spatially normalized into the Montreal Neurological Institute (MNI) template and smoothed with 6 mm full width at half-maximum Gaussian kernel. Quality control was implemented by visually checking MNI normalization in all individual images.

First-level analysis was conducted using the same software. DPABI software uses fast Fourier transform (FFT) to gather power spectrum from filtered time series. The square root of the power spectrum was calculated and averaged across 0.01–0.1 Hz at each voxel. Averaged square root was deemed as ALFF. In further analyses ALFF was used as a raw value and as a Z-standardized value in which mean (across voxels) ALFF was subtracted from each individual voxel and divided by standard deviation of all ALFF values.

6.6 Second-Level Statistical Analysis

Second-level or population level analysis was calculated using SPM12 software (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/), which is also MATLAB-based. SPM12 draws maps using statistical parametric mapping (SPM) t-distribution. These maps are referred as SPM t-maps.

Population level SPM t-maps were created using one sample t-test in all four timepoints with positive and negative contrasts. Contrasts are used in SPM12 for different comparisons, as these statistics are often tested in one direction. To visualize regions with above and below mean (positive and negative) ALFF, images were Z-standardized beforehand. Population images are used only to visualize regional changes in ALFF and not to draw statistical conclusions. Therefore, heavy correction is not needed. There are different guidelines for statistical difference in neuroimaging but for visualization purposes $p < .001$ was thought to be adequate. For group mapping purposes no cluster size requirements were set ($k = 1$ means all clusters above 1 voxel are accepted). Statistically significant SPM t-maps were visualized in slices and overlayed over MNI 152 -template using MRIcroGL software (mricrogl: v1.2.20211006, https://www.nitrc.org/projects/mricrogl/).

To calculate statistically significant changes in ALFF before and after exercise, analysis of variance (ANOVA) was implemented. As the study were constructed as within-subject model, repeated measures ANOVA was used. SPM12 uses a voxel-wise approach to statistical analysis in which each voxel is analysed as their own between all subjects. Statistically significant

clusters were drawn as a SPM t-map. Statistically significant and family-wise error (FWE) corrected SPM t-maps were visualized in 3D also using MRIcroGL software. In 3D images, transverse view is visualized from above, coronal view from the front and sagittal from the right side.

Influences of subject characteristics to ALFF in baseline and post-exercise conditions were investigated using sex, BMI, bodyfat-%, VO_2peak and lactate as covariates in second-level multiple regression analysis in SPM12 in all timepoints. Individual VO_2peak values were used as "fitness level" score and lactate as "exercise intensity" score. Individual lactate levels were also used as covariates in ANCOVA analysis, to correct results with individual changes in exercise exertion: analysis of variance was run again in all four time points, but data were corrected with lactate values. Both multiple regression and ANCOVA results were FWE corrected. Findings on regional activity were reported using MNI coordinates. Coordinates were afterwards checked with DBAPI and regions were located using automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002).

Because fMRI images consist of more than hundred thousand voxels that are compared, type 1 errors are common. If significance level of $p < .05$ were to be used, in 100 000 voxels this would mean 5 000 false positive findings. Simply adjusting p-value may not be efficient to cancel out false positive findings, so other correction models are needed. Family-wise error (FWE) correction was also implemented at cluster-level for results. SPM12 uses random field theory (Nichols & Hayasaka, 2003) for the basis of FWE correction. After FWE correction, $p < .05$ is considered standard significance threshold.

Other statistics. Before and after exercise lactate, heart rate and blood pressure were checked for statistical significance using R (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). Paired two-tailed t-test were used.

7 RESULTS

7.1 Heart rate, Blood Pressure and Lactate Responses to Exercise

Physiological measurements and responses to exercise are shown in table 2. Imaging measurements were measured at supine position inside the scanner. Exercise measurements were taken seated on the cycle ergometry.

TABLE 2. Heart rate, blood pressure and lactate responses to exercise protocol. HR = Heart rate, SBP = systolic blood pressure, DBP = diastolic blood pressure.

	PRE imaging	PRE exercise	POST exercise	POST 1 imaging	POST 2 imaging	POST 3 imaging
HR (bpm)	63 ± 8	74 ± 6	160 ± 16***	74 ± 8***	70 ± 10**	70 ± 8*
SBP (mmHg)	114 ± 7			110 ± 7*	111 ± 8*	112 ± 9
DBP (mmHg)	64 ± 6			60 ± 8**	61 ± 8	63 ± 9
Lactate (mmol/l)	0.9 ± 0.3		3.1 ± 0.8***		1.1 ± 0.4	0.9 ± 0.3

* = significant difference compared to PRE, $p < .05$, ** = $p < .01$, *** = $p < .001$.

The cycling exercise protocol induced changes in lactate, heart rate and blood pressure. Exercise protocol effectively elevated blood lactate 2-3 minutes after exercise, but effects no more found at ~60-minute post-exercise (POST 2) timepoint. Intensity at the end of exercise were equivalent to %HRR = 74±10. Heart rate remained elevated in all post-exercise timepoints through ~90-minute recovery period after exercise. Both systolic and diastolic blood pressure was elevated at ~30-minute post-exercise (POST 1) timepoint and therefore postexercise hypotension effect was found.

7.2 ALFF Group Maps

Figure 4 shows Z- standardized group SPM t-maps in baseline (PRE), before exercise. Figures 5 to 7 shows same results in 30, 60 and 90 minutes (POST 1, POST 2 and POST 3), after exercise, respectively. Both positive and negative ALFF are visualized.

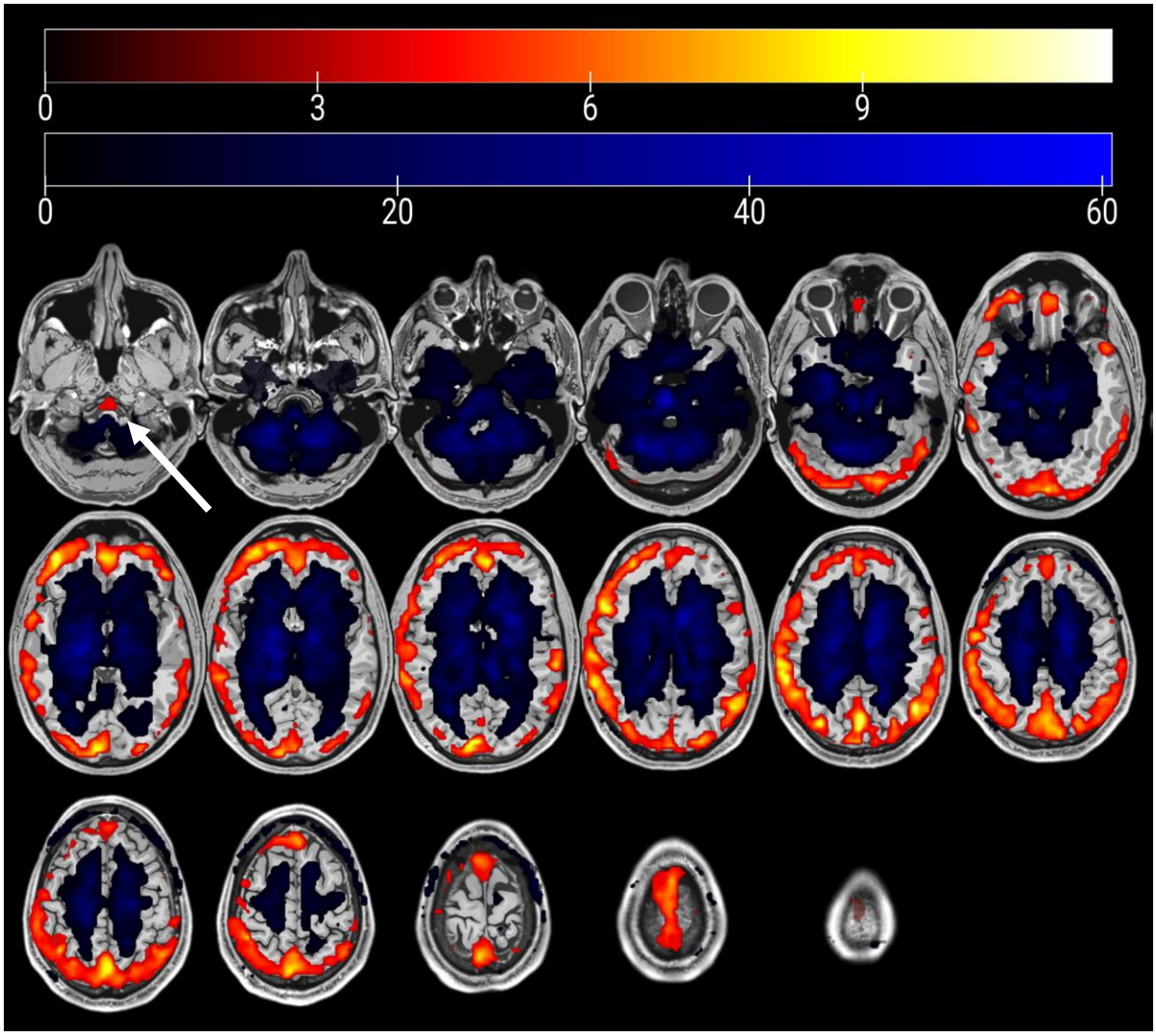

FIGURE 4. Baseline (PRE) group level ALFF results. SPM t-map thresholded at $p < .001$ uncorrected, $k = 1$. Warm color scale shows regions with above mean and blue color scale shows below mean ALFF. White arrow shows the brainstem region.

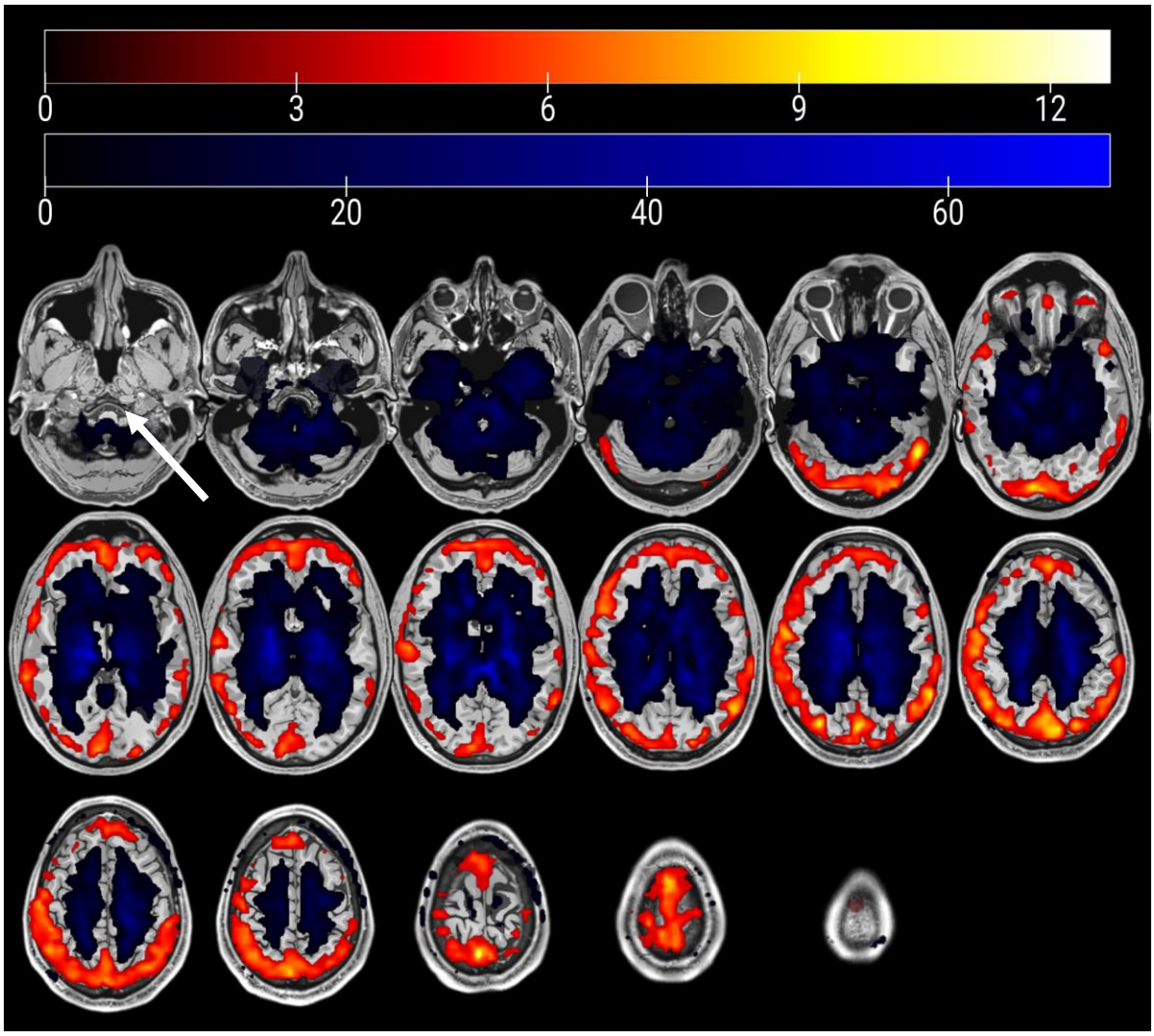

FIGURE 5. ~30 minutes post-exercise (POST 1) group level ALFF results. SPM t-map thresholded at $p < .001$ uncorrected, $k = 1$. Warm color scale shows regions with above mean and blue color scale below mean ALFF. White arrow shows the brainstem region.

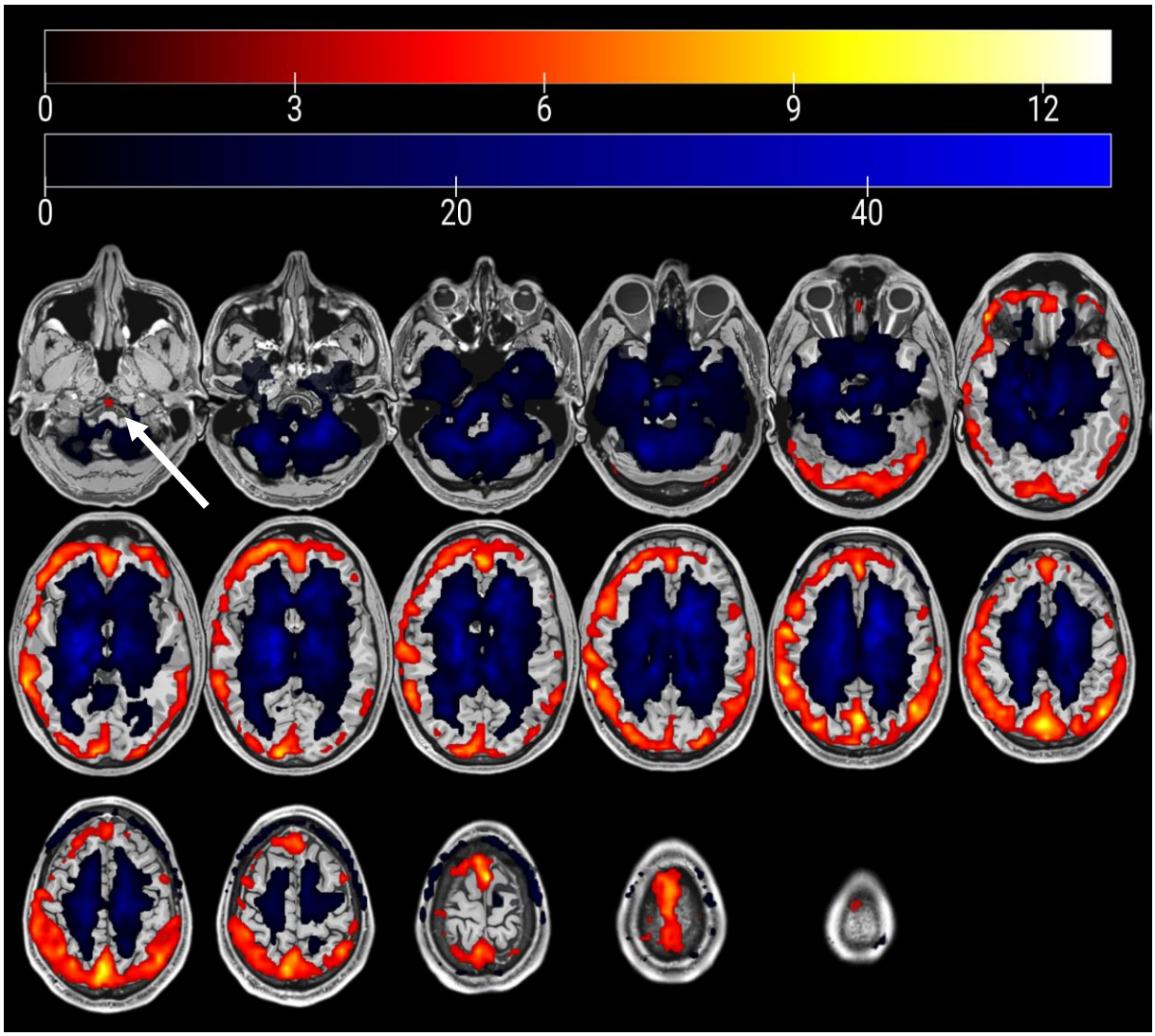

FIGURE 6. ~60 minutes post-exercise (POST 2) group level ALFF results. SPM t-map thresholded at $p < .001$ uncorrected, $k = 1$. Warm color scale shows regions with above mean and blue color scale below mean ALFF. White arrow shows the brainstem region.

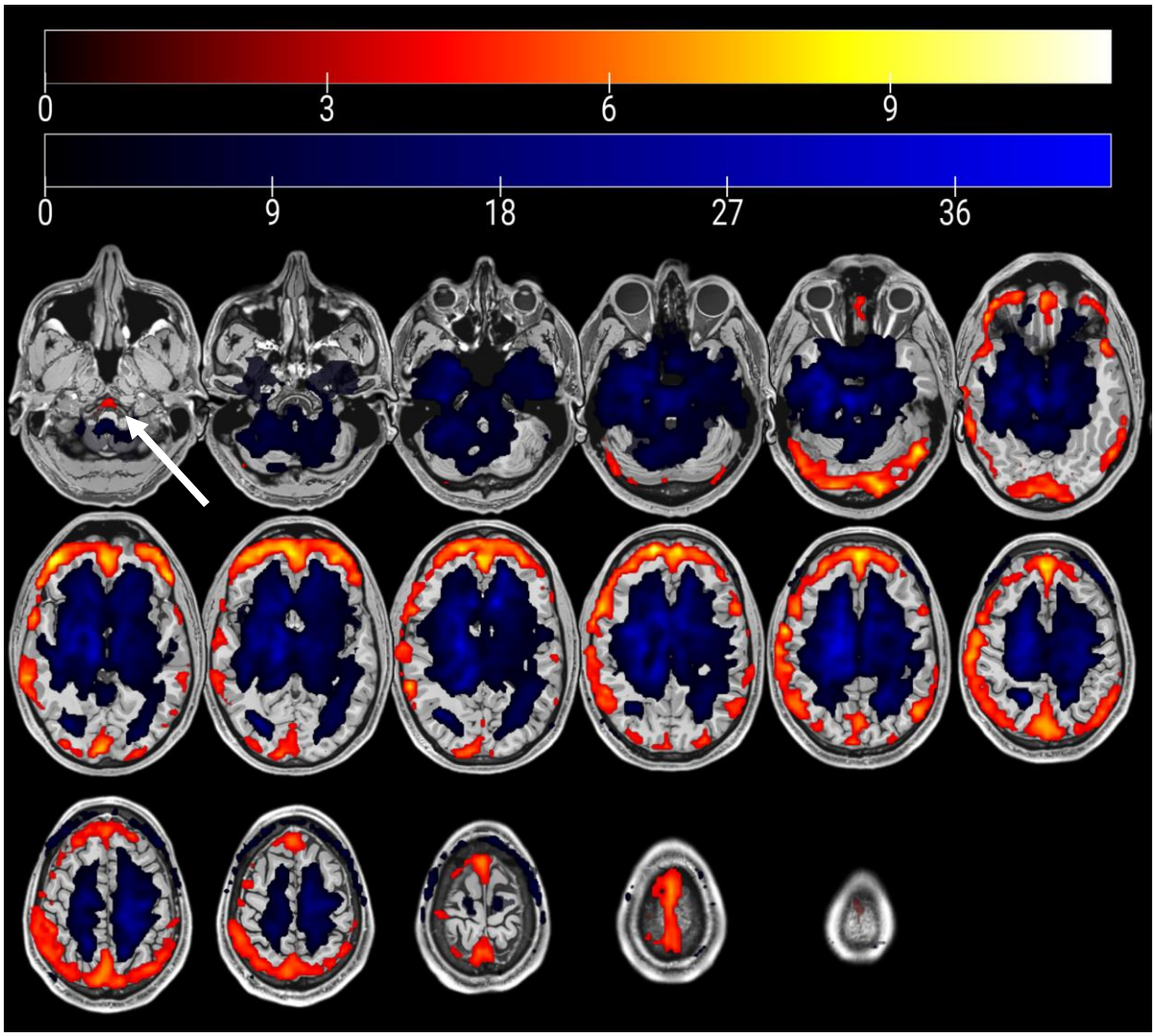

FIGURE 7. ~90 minutes post-exercise (POST 3) group level ALFF results. SPM t-map thresholded at $p < .001$ uncorrected, $k = 1$. Warm color scale shows regions with above mean and blue color scale below mean ALFF. White arrow shows the brainstem region.

In SPM t-maps, above mean ALFF is most prevalent in cortical brain regions and cerebellum. Below mean ALFF is seen in deeper brain structures, although there are some discrepancies like increased ALFF in the brainstem region in PRE, POST 2 and POST 3 timepoints.

7.3 Differences in ALFF Before and After Exercise

After repeated measures ANOVA, only five clusters in ~30 minutes post-exercise SPM t-map (POST 1) and one in ~90 minutes post-exercise SPM t-map (POST 3) survived FWE correction (figures 8 and 9). All clusters found were k > 35 voxels in size. Further inspection showed POST 1 cluster peaks (figure 8) were located at MNI coordinates: (44, 44, −27), (-44, -44, 48), (-3, -78, 0), (20, -54, 61) and (14, -68, 17) and according to AAL atlas, cluster locations matched with:

1. Right middle frontal gyrus in the frontal lobe
2. Left inferior parietal gyrus and left postcentral gyrus in the parietal lobe
3. Left lingual gyrus in the occipital lobe and left cerebellum
4. Right superior parietal lobule and right postcentral gyrus in the parietal lobe
5. Right calcarine sulcus, right superior occipital gyrus and right cuneus in the occipital lobe

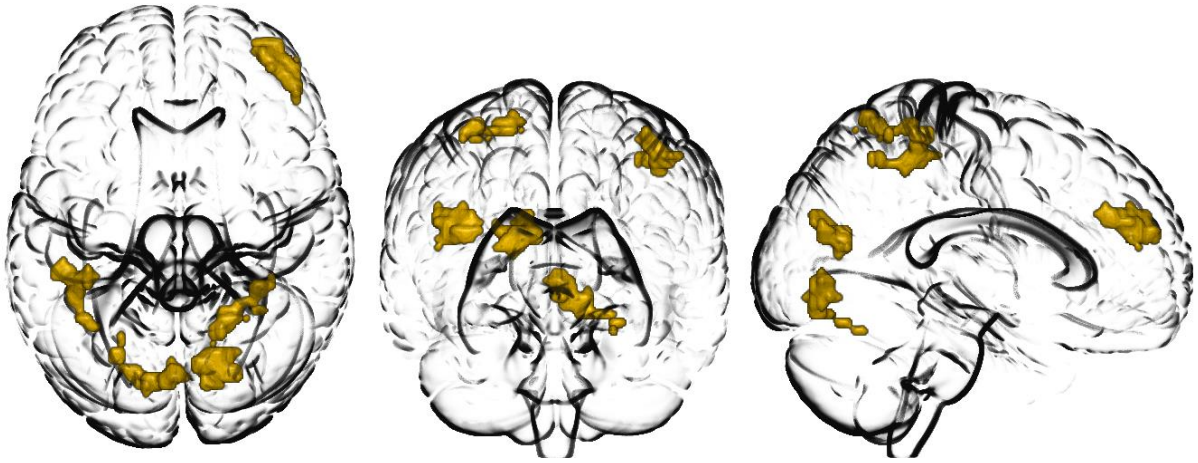

FIGURE 8. Brain regions, where ALFF was increased ~30 minutes post-exercise (POST 1). Results are FWE corrected ($p < .05$, $k = 35$).

POST 3 cluster peak (figure 7) were located at MNI coordinates: (3, −20, −44) which could not be located in AAL atlas.

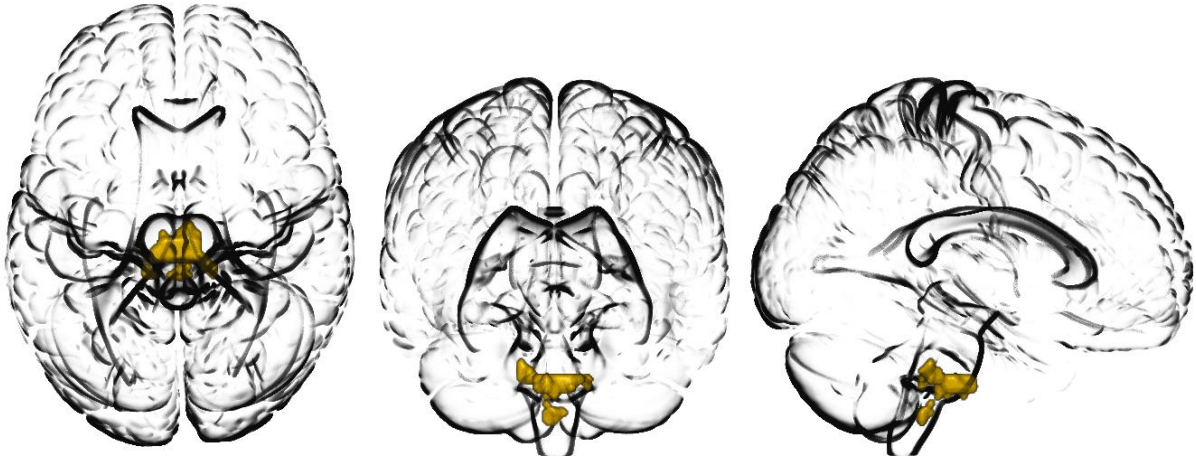

FIGURE 7. Brain region, where ALFF was increased ~90 minutes post-exercise (POST 3). Results are FWE corrected ($p < .05$, $k = 35$).

No significant increases in ALFF were found ~60 minutes post-exercise SPM t-maps. ALFF did not decrease significantly at any timepoint after exercise protocol.

7.4 Differences in ALFF Before and After Exercise Corrected with Lactate

Individual responses to exercise were found (high standard deviation in between-subject lactate in Table 2). After ANCOVA with lactate correction, results changed and only three clusters in ~90 minutes post-exercise SPM t-map (POST 1) survived FWE correction. POST 1 cluster peaks (figure 8) were located at MNI coordinates: (41, -54, -34), (44, -82, -14) and (-37, -31, 58) and according to AAL atlas, cluster locations matched with:

1. Cerebral crus and right cerebellum
2. Left middle occipital gyrus in the occipital lobe
3. Left postcentral gyrus in the parietal lobe

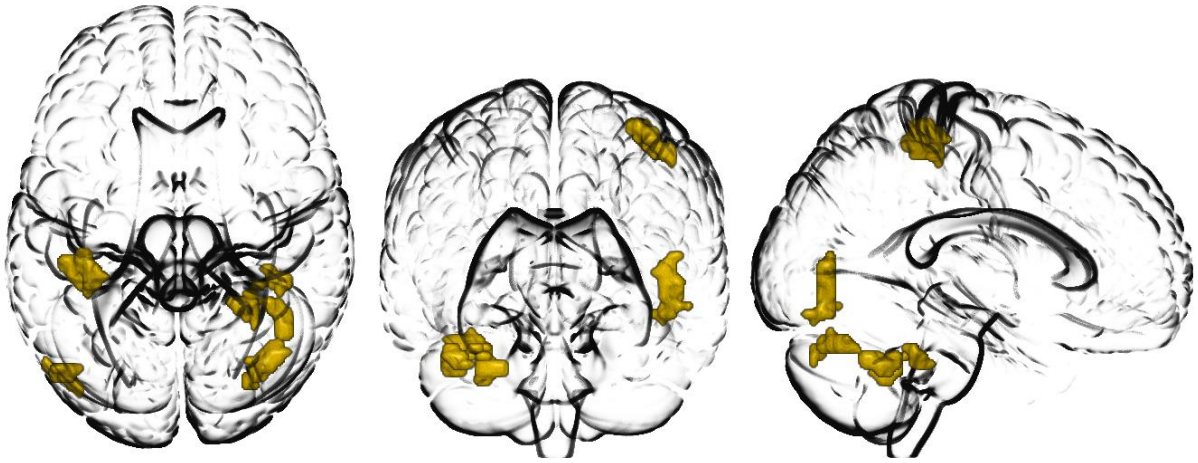

FIGURE 8. Brain regions, where ALFF was increased ~30 minutes post-exercise (POST 1) after lactate correction. Results are FWE corrected ($p < .05$, $k = 35$).

After lactate correction, ~90 minutes post-exercise images (POST 3) remained almost the same. POST 3 cluster peak (figure 9) were also located at MNI coordinates: (3, −20, −44) which could also not be located using AAL atlas.

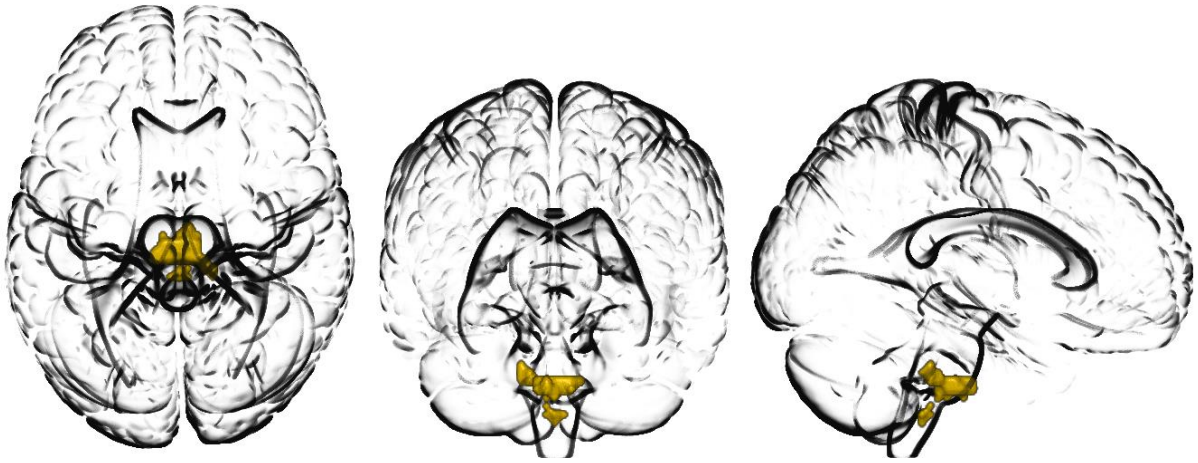

FIGURE 9. Brain region, where ALFF was increased ~90 minutes post-exercise (POST 1) after lactate correction. Results are FWE corrected ($p < .05$, $k = 35$).

No significant increases in ALFF were found ~60 minutes post-exercise SPM t-maps after lactate correction. Again, ALFF did not decrease significantly at any timepoint after exercise protocol.

7.5 Correlations to Subject Characteristics and Responses to Exercise

BMI were negatively correlated with ~30 minutes post-exercise (POST 1) ALFF in two separate clusters. Cluster peaks were located at MNI coordinates: (7, -58, 27), (-14, 37, 37) and according to AAL atlas cluster locations matched with right precuneus and cuneus in the occipital lobe and left superior and medial frontal gyri in the frontal lobe. Clusters are found in figure 10. No positive correlations were found regarding ALFF and BMI.

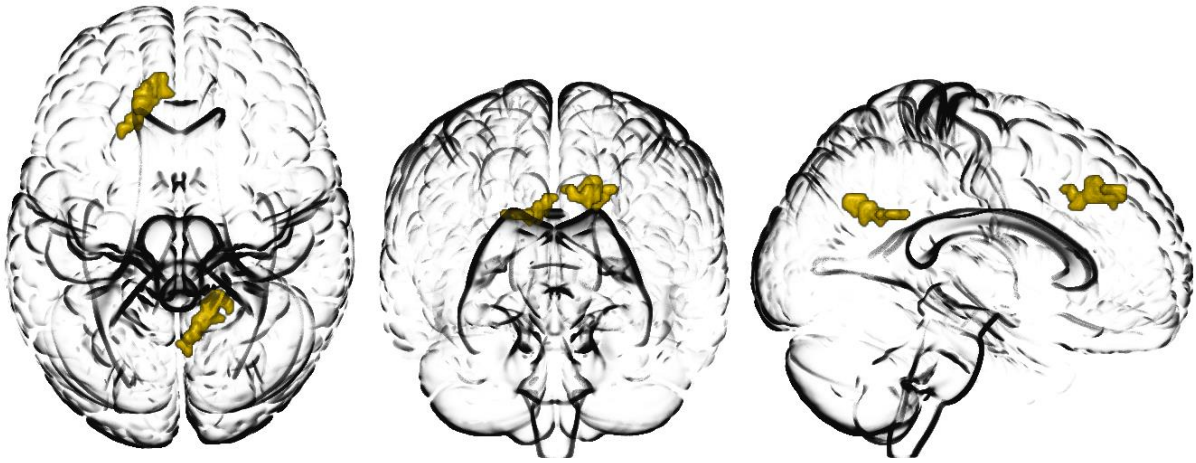

FIGURE 10. Regions where ~30 minutes post-exercise (POST 1) ALFF and BMI were negatively correlated. Results are FWE corrected ($p < .05$, $k = 35$).

Other subject characteristics including sex, age, VO_2peak, lactate and bodyfat-% were not positively nor negatively correlated with ALFF at any time point.

8 DISCUSSION

The study main finding was that moderate-intensity aerobic exercise acutely increased spontaneous brain activity in healthy young adults. Five distinct clusters were found in which ALFF signal were significantly higher 30 minutes after exercise. It is safe to say that this type of vigorous intensity steady-state aerobic exercise elicits some excitatory responses to spontaneous brain activity when measured with BOLD-fMRI. These five clusters are located in brain regions that have different functions. For purposes of this study, a detailed speculative review of individual regional functions is not relevant but some regions' involvement in theoretical aerobic priming effect are discussed.

Timeline of effects were also studied. It seems clear that ~30 minutes after exercise, excitatory effects are found, but not ~60 minutes after. This pattern of increased activation timeline is however disrupted at ~90 minutes timepoint in which a cluster was found in pons/medulla region. At first glance, this cluster was thought to be in fMRI imaging lower border and emitting false BOLD signal. Further inspection in imaging parameters found that not to be the case. Brainstem takes part in multiple neuronal processes like autonomic regulation, sensory and motor pathways, but it is known to be a source of false BOLD signal. BOLD from the brainstem is usually physiological noise, not direct activity (Wei et al., 2020). Brainstem ALFF signal in group maps disappeared ~30 minutes after exercise, where respiratory and cardiac activity would be highest. Increased BOLD noise could hide spontaneous brain activity. As ALFF is considered sensitive to background physiological noise, fractional ALFF (ratio of power spectrum of low frequencies to that of the entire frequency range) could be utilized in further research to improve signal to noise ratio (Zuo et al. 2010).

Within these five clusters found ~30 minutes after exercise, regions of the parietal lobe could be related to improved sensory response and attention (Shomstein, 2012). Increased activity in these regions were also found by Huang et al. (2021) after aerobic exercise intervention. Intervention induced increased spontaneous activity in the parietal lobe, but only for the subthreshold depression group, not in healthy controls (Huang et al., 2021). In this study

depressive symptoms were not screened beforehand, but it seems an unlikely explanation. This could reflect exercise effects in prevention of depression (Hu et al., 2020), but evidence is far too limited to find causal relationships, and more research is needed to explain direct neural mechanisms of aerobic exercise and depression.

Even though exercise intensity were set at power in which individual 70%VO_2peak were found, there were differences individual lactate responses. Further analysis was conducted to figure out individual responses to exercise relations to ALFF. Adding lactate as covariate in analysis of variance, changed the results signioficantly. Frontal lobe cluster found in ANOVA ~30 minutes after exercise, were not present when corrected with lactate levels. This would imply that frontal lobe ALFF is more susceptible to higher intensity exercise. This finding is consistent with NIRS findings in studies reviewed by Rooks et al. (2010). Approaching near-maximal intensities, prefrontal cortex activity could also cause a decline in cognitive task performance due to overactivation (McMorris, 2016). Vigorous-intensity exercise effects on cognitive performance remain unknown as they were not tested in the study protocol. For prospects, cognitive tasks should be implemented to test out aerobic priming effects at standardized exercise intensities and durations. Kelly et al. (2017) found increased activity in frontal lobe after high-intensity mixed-model acute exercise. In their study in PD patients, exercise protocol used included a mixture of resistance training and aerobic training components. Lactate were not measured Kelly et al. (2017) study, but one could assume lactic responses in this type of exercise protocol. Even though lactate was able to change results, lactate was not correlated with ~30 minutes after exercise ALFF in multiple regression analysis. These two analyses are different in design, but if lactate would have been a major driver of prefrontal activation, some correlation would have been expected. Hashimoto et al. (2021) recently proposed lactate to be a major component in exercise-induced cognitive function and brain health benefits, but in this study, lactate alone did not account for changes in spontaneous brain activity.

Another important finding was that cerebellar activity increased, even more so when adjusted for exercise intensity, but not motor cortex activity. This contradicts prior stimulation studies where aerobic exercise was shown to “prime” cerebellar-motor cortex cooperation (Mang et al.,

2016). Although, no motor cortex activity was found, increased ALFF post-exercise in left postcentral gyrus was present in lactate-corrected ANCOVA results. Primary somatosensory cortex is located in the postcentral gyrus. All but one subjects were fully right-handed so finding in the left side is logical. Rajab et al. (2014) had similar findings after acute moderate-intensity exercise. They found increased FC in somatosensory networks, indicating aerobic priming effects to the sensory system (Rajab et al., 2014). ALFF and FC are different. ALFF measures magnitude of local effects and FC synchroneity of spatially different regions. Still, origin of signal remains the same, and there is some valuable cross-over in these techniques.

While other subject characteristics did not correlate with baseline or after exercise ALFF, BMI did so negatively on two clusters. Exercise-induced changes in precuneus/cuneus and superior and medial frontal gyri could reflect on decreased priming effects in these regions like working memory (Nee et al., 2013). Most likely explanation could be that BMI negatively effects post-exercise CBF (Knight et al., 2021). This is so far speculative as none of the subjects were obese (BMI > 30), and therefore a wider rage of BMI could produce significant results in more than two clusters. BMI effects over ALFF are also contradicted with study finding that fat-% did not correlate with ALFF in any way. Skin caliper fat-% estimate could be inaccurate but still, similar findings as BMI could have been expected. Additionally, VO_2peak did also not correlate with ALFF contradicting Foster et al. (2020) results of relationship fitness level and hemodynamics. A more heterogenous source population in terms of body composition and fitness could be needed for higher correlations.

Overall, no direct comparisons to prior studies cannot be drawn regarding regional spontaneous activity, because of dissimilarities in methods or source population. There are small incidences of similar effects regarding lobe-level effects, but small region-level similarities seem incidental. It is important to know that research in this topic is very limited. Difficulties in region-level analysis might be partly due to FWE correction. FWE correction can be considered be too conservative for a whole-brain analysis and other forms of multiple comparison could be used to gain more applicable results (Lindquist & Mejia, 2015). Heavy correction strategies could “hide” results, but too light might result in false positives. From this study, uncorrected

results show increased activity at nearly all cortical regions after exercise (appendix 2) even when significance set at < .001, but these results cannot be used due to the lack of multiple comparison. It is widely accepted that some form of multiple comparison is needed in fMRI research. All exercise rs-fMRI studies discussed in this work used different multiple comparison models (Huang et al., 2021; Kelly et al., 2017; Rajab et al., 2014) and for future research, a standard method of multiple comparison and level of significance should be universally set.

Interpreting the study results, one definitive question should be considered: are the effects found just underlying hemodynamics rather than real spontaneous brain activity? Explained in a review of Arthurs & Boniface (2002), BOLD signal has multiple origins, neuronal and non-neuronal. Increases in CBF and oxygenation alone could increase BOLD signal. But does increase in CBF or oxygenation increase neuronal function, or vice versa? This is "the chicken or the egg" type of dilemma in which no one seems to have definite answers. As of now, exercise effects to cognitive function (Chang et al., 2012) and EEG (Büchel et al., 2021) is enough evidence to accept that aerobic exercise has regional brain activity altering effects, despite BOLD-fMRI shortcomings. In this research project ASL were used alongside rs-fMRI and combining these two methods, spontaneous activity, CBF and cerebral perfusion relationship and timeline could be studied further.

In conclusion, vigorous-intensity aerobic exercise was able to increase spontaneous brain activity ~30 minutes after exercise in some brain regions. Other findings suggest that these changes activity are highly intensity-dependent and for future research exercise models inducing more homogenous lactate responses should be used. Definite relationship between studied ALFF and spontaneous brain activity in physiologically unstable environment remains debatable, but future implementation of cognitive tasks and other fMRI techniques or EEG/MEG could clarify intricacies of aerobic priming phenomenon.

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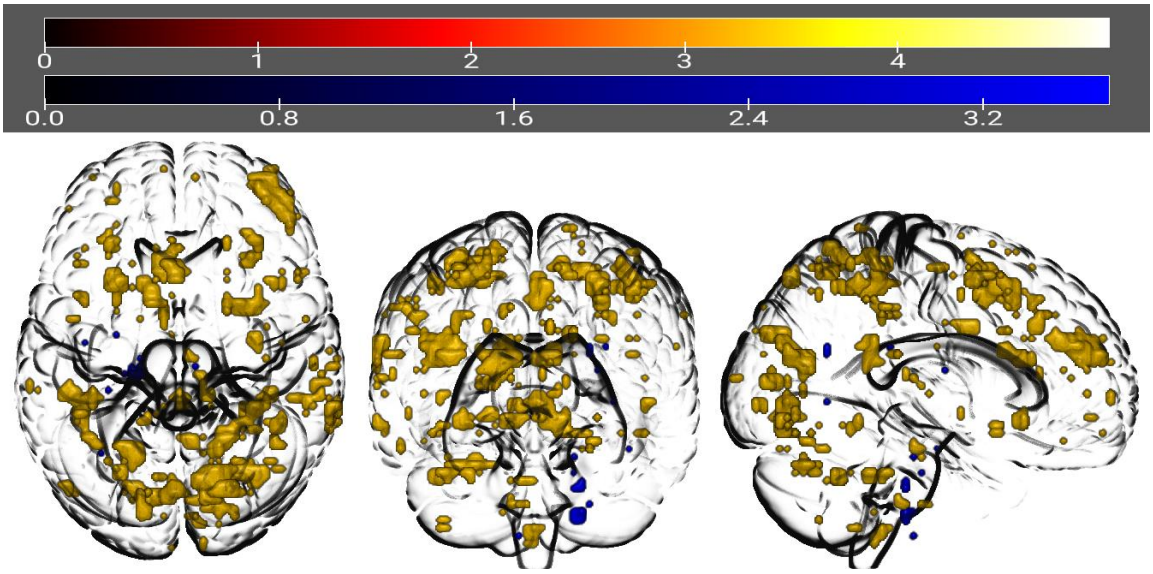
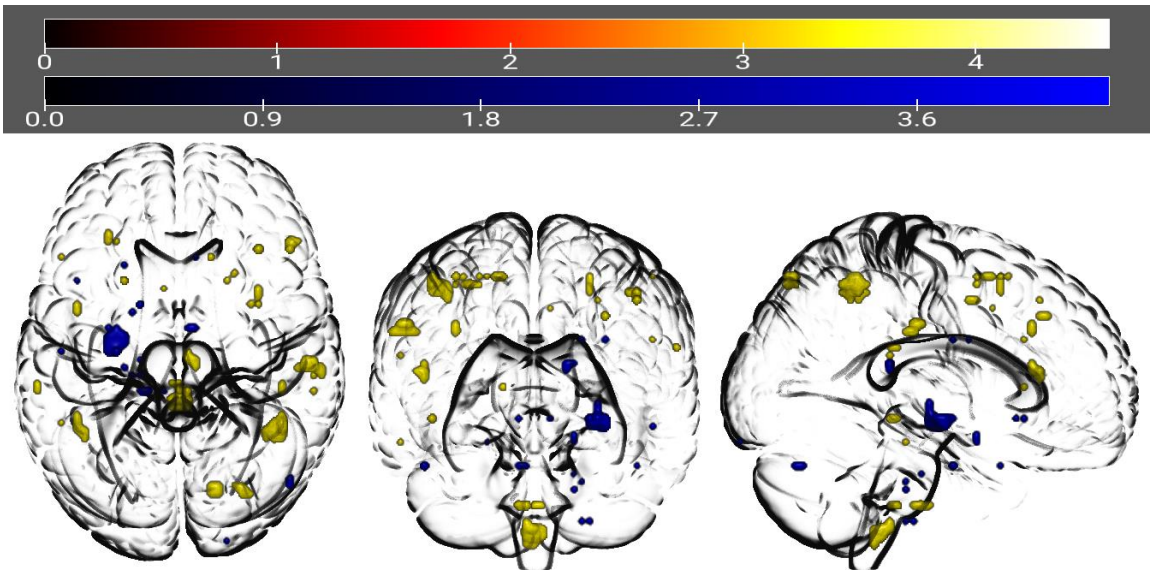
APPENDICES

APPENDIX 1: GLYMREG Study Advertisement

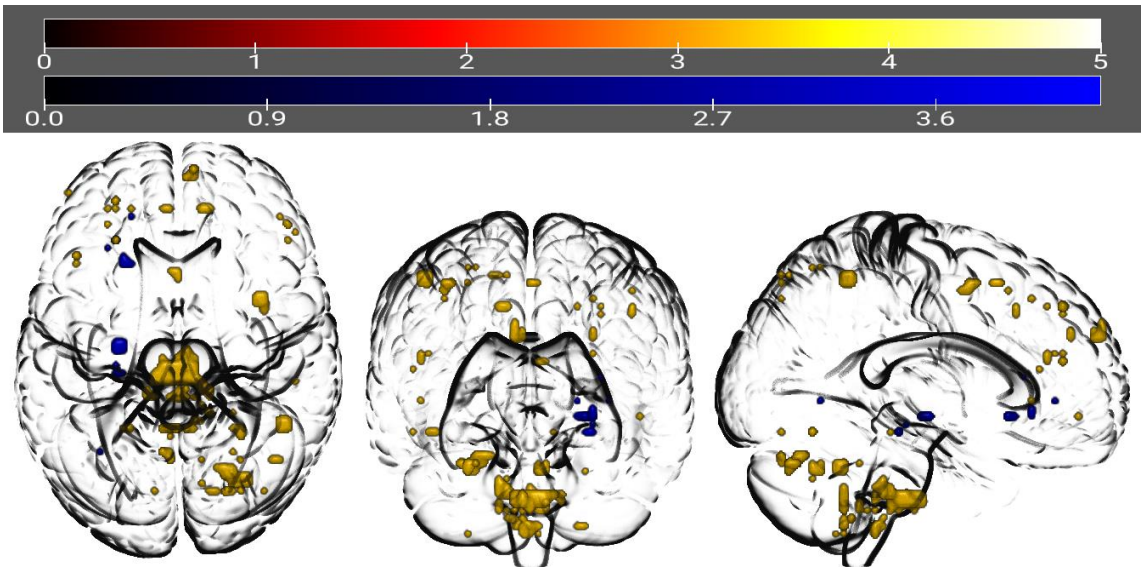
APPENDIX 2: Uncorrected ALFF Comparison



ANOVA results from ~30 minutes post-exercise SPM t-maps (POST 1). Warm color scale shows positive regions where POST 1 > PRE. Blue color scale shows the opposite. Results are uncorrected ($p < .001$, $k = 1$).



ANOVA results from ~60 minutes post-exercise SPM t-maps (POST 2). Warm color scale shows positive regions where POST 2 > PRE. Blue color scale shows the opposite. Results are uncorrected ($p < .001$, $k = 1$).



ANOVA results from ~90 minutes post-exercise SPM t-maps (POST 3). Warm color scale shows positive regions where POST 3 > PRE. Blue color scale shows the opposite. Results are uncorrected ($p < .001$, $k = 1$).