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## **A pilot study to assess the effect of acute exercise on brain glutathione**

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## **A pilot study to assess the effect of acute exercise on brain glutathione**

The brain is highly susceptible to oxidative stress due to its high metabolic demand. Increased oxidative stress and depletion of glutathione (GSH) are observed with aging and many neurological diseases. Exercise training has the potential to reduce oxidative stress in the brain. In this study, nine healthy sedentary males (aged  $25 \pm 4$  years) undertook a bout of continuous moderate intensity exercise and a high intensity interval (HII) exercise bout on separate days. GSH concentration in the anterior cingulate was assessed by magnetic resonance spectroscopy (MRS) in four participants, before and after exercise. This was a pilot study to evaluate the ability of the MRS method to detect exercise-induced changes in brain GSH in humans for the first time. MRS is a non-invasive method based on nuclear magnetic resonance, which enables the quantification of metabolites, such as GSH, in the human brain *in vivo*. To add context to brain GSH data, other markers of oxidative stress were also assessed in the periphery (in blood) at three time points [pre-, immediately post-, and post (~1 hour)- exercise]. Moderate exercise caused a significant decrease in brain GSH from  $2.12 \pm 0.64$  mM/kg to  $1.26 \pm 0.36$  mM/kg ( $p = 0.04$ ). Blood GSH levels increased immediately post-HII exercise,  $580 \pm 101$   $\mu$ M to  $692 \pm 102$   $\mu$ M ( $n=9$ ,  $p = 0.006$ ). The findings from this study show that brain GSH is altered in response to acute moderate exercise, suggesting that exercise may stimulate an adaptive response in the brain. Due to the challenges in MRS methodology, this pilot study should be followed up with a larger exercise intervention trial.

Keywords: exercise; glutathione; brain; oxidative stress; magnetic resonance spectroscopy

Word count: 7,857

## **Introduction**

The human brain consumes approximately 20% of the oxygen utilized by the body even though it only constitutes approximately 2% of total body weight [1]. This makes it highly susceptible to oxidative stress, a state in which an imbalance exists between antioxidants and oxidants. Increased oxidative stress has been reported in normal aging [2], in chronic diseases such as cancer [3], and neurodegenerative diseases such as Alzheimer's disease [4,5], and may arise due to increased reactive oxygen or nitrogen species (RONS), decreased antioxidants, or both. Glutathione (GSH) is the most abundant endogenous antioxidant in the body and its depletion can significantly contribute to oxidative stress; indeed, decreased brain GSH has been reported in aging and in diseases such as Alzheimer's Disease, epilepsy, Parkinson's disease and schizophrenia [6–10]. Interventions that facilitate GSH synthesis in the brain would therefore be a promising therapeutic strategy to treat and potentially prevent neurodegenerative disease.

There is strong evidence linking regular physical activity to higher cognitive function [11,12], decreased cognitive decline [13], and reduced risk of numerous diseases such as dementia and cancer [14–16], however, the mechanism by which exercise has these effects is still uncertain. A single bout of exercise, dependent on factors including exercise intensity and duration, can induce a transient oxidative stress via increased RONS production. An increase in RONS, caused by exercise, can stimulate adaptive change [17,18], if exercise is undertaken repeatedly, as RONS act as critical signalling molecules. Therefore, taking part in regular bouts of exercise can therefore provoke an adaptive response of increased antioxidant capacity, including increased GSH, both at baseline and in response to exercise, following exercise training [17,19–22]. As the whole glutathione redox cycle is responsive to exercise, the increase in GSH following exercise training may be attributed to increased synthesis of

endogenous GSH [20,23] and increased expression of key antioxidant enzymes involved in the glutathione redox cycle, such as glutathione peroxidase and glutathione reductase [24–26]. Indeed, there are numerous studies in humans that have shown that acute exercise can alter redox status in the peripheral circulation and in muscle [27], and one could extrapolate this to suggest that exercise can help maintain redox balance in the brain. However, to date, few studies have assessed the direct effect of exercise on brain redox status, in part due to the difficulty in acquiring samples from the brain.

Magnetic resonance spectroscopy (MRS), is a technique which enables the non-invasive quantification of metabolites and is the only method available to measure GSH in the human brain *in vivo* [9]. Trabesinger et al. [28] were the first to detect GSH in the human brain by MRS. MRS is based on nuclear magnetic resonance, a phenomenon that occurs when an atomic nucleus with a magnetic moment, or spin, exhibits resonance behaviour when placed in a magnetic field [29]. In the MRS signal obtained, the molecular structure of a particular metabolite is reflected by a typical peak pattern, and the area of a particular peak is directly proportional to the concentration of the metabolite. MRS becomes particularly useful when the region of tissue being investigated is difficult to access, such as is the case in the brain, however, there are limitations to the method.

The popular hormesis theory, relating to a dose-response relationship for many different stimuli, has been extended to include RONS and the process of adaptation to exercise. The theory suggests that a low-dose stimulation will lead to beneficial effect, whereas a high-dose stimulation may cause a harmful or toxic effect [18]. Exercise intensity is one of the determinants of the magnitude of RONS release and thus the magnitude of oxidative stress in response to a single bout of exercise [30–32]. Assessing the effect of exercise intensity on redox balance may be a crucial step in determining the ‘best’ exercise

strategy for optimising brain health [33]. High intensity interval (HII) exercise is a form of exercise that comprises short bursts of high intensity exercise interspersed with recovery periods of rest or low intensity exercise. Although HII involves substantially lower time commitment compared to traditional form of continuous moderate exercise, HII has been shown to provide equivalent or superior benefits for metabolic, cardiac, vascular and oxidative stress adaptations [19,31,33,34].

Increases in exercise intensity, up to around 60% of maximal oxygen uptake, produce elevations in cerebral blood flow, after which blood flow decreases toward baseline due to hyperventilation induced cerebral vasoconstriction, despite the cerebral metabolic demand [35]. Periods of intense exercise can cause temporary ischemia and hypoxia in certain regions of the body, and during reperfusion, the reintroduction of oxygen can result in burst of RONS, such as superoxide and hydrogen peroxide ( $H_2O_2$ ), which could be expected to induce oxidative stress [36]. As ischemia-reperfusion can induce RONS production, a decrease in antioxidant capacity is likely. Indeed, brain tissue GSH has been shown to decrease as a result of brain ischemia in rodents [37,38].

The aim of this pilot study was to assess MRS as a non-invasive method to detect potential exercise-induced changes in brain GSH. We hypothesized that HII would provoke a different response in GSH when compared to moderate intensity exercise. Cerebral blood flow was assessed during the intense intervals of HII to enable further understanding of any exercise induced changes in brain GSH in relation to brain blood flow.

## **Methods**

### ***Participants***

Nine untrained males (aged  $25 \pm 4$  years,  $\text{VO}_2$  max  $40 \pm 8$  ml.kg.min<sup>-1</sup>) who undertook <2 hours of moderate exercise per week were recruited to this study. Four participants were assessed by MRS for brain GSH following the exercise bouts. Due to MRI-scanning contraindications, five participants were unable to be scanned for brain GSH. Participants were non-smokers, had not taken vitamin supplements for at least six weeks prior to recruitment and were healthy as assessed by a general health questionnaire. All participants gave their informed written consent and the study was approved by the Science, Technology and Mathematics ethics review committee at the University of Birmingham.

### ***Experimental design***

This study comprised 3 experimental visits, which were each separated by at least 3 days. Participants were asked to refrain from exercising and drinking alcohol for 24 hours prior to each visit and be fasted at least 4 hours prior to each visit. For the first visit, participants were accompanied to the Birmingham University Imaging centre (BUIC), where they undertook an MRS scan (baseline scan) to assess brain GSH. After the scan, the participants returned to the exercise laboratory to carry out an incremental exercise test to exhaustion (detailed below).

During the second and the third visit, the participants took part in either HII exercise or moderate exercise. All participants undertook both forms of exercise, the order of which was counterbalanced. An indwelling catheter (that was kept patent by flushing regularly with saline) was placed into an antecubital vein in the forearm and a pre-exercise blood sample was taken. At the end of the exercise bout a blood sample (immediately post-exercise) was taken via the catheter. A post-exercise MRS scan was then undertaken to assess brain GSH. This

scan was performed within 15 minutes after the end of exercise. A final blood sample (post-exercise) was taken by venepuncture approximately 1 hour after the end of exercise.

In order to measure blood flow velocity in the middle cerebral artery (MCAv) during high intensity phase of the HII bout, four participants were fitted with a transcranial Doppler (TCD) system (Doppler BoxX, DWL, Sipplingen, Germany) before starting HII exercise trial. Bilateral MCAv was obtained via two probes placed over the temporal window and held in place with an adjustable head piece. Standard procedures to locate and confirm the MCAv were followed, as described by [39].

### ***Exercise bouts***

All exercise tests were performed on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Netherlands). Maximal oxygen consumption ( $\text{VO}_2 \text{ max}$ ) of participants was assessed by an incremental exercise test to exhaustion, which consisted of a 3-minute warm up at 60 watts after which workload increased by 35 watts every 3 minutes until volitional exhaustion. A breath-by-breath system (Oxycon Pro, Jaegar, Germany) was used for continuous measurement of oxygen uptake and the heart rate was monitored using a heart rate monitor (Polar Vantage, Finland). Participants were asked to maintain a constant pedal rate and encouragement was given by an experimenter. A respiratory exchange ratio (carbon dioxide consumption/oxygen consumption)  $> 1.10 - 1.15$ , plateau in participant oxygen consumption or a maximal heart rate  $> 220 \text{ beats min}^{-1} - \text{age}$  were all factors used to indicate  $\text{VO}_2 \text{ max}$  and thus the termination of the test [40]. Maximal workload was calculated as

$$\text{Workload max} = \text{last completed work load} + (\text{time spent in the final non-completed work rate} \\ \times \text{work rate increment}) \quad (1)$$



A schematic representation of the HII and the continuous moderate exercise trials are shown in Figure 1. The HII exercise trial consisted of 4 x 30-seconds high intensity sprints at 200% of workload max, separated by 4 minutes of active recovery during which participants cycled at 40% of workload max. The continuous moderate exercise trial consisted of cycling at 65% workload max for 20 minutes and then at 55% workload max for the last 20 minutes (the work load was decreased in the last 20 minutes to match the oxygen consumption in the first 20 minutes [41]). Both exercise bouts were preceded with a 3-minute warm up and followed by a 2-minute cool down of cycling at 40% workload max.

### ***Magnetic resonance spectroscopy***

All MRS scans were undertaken using  $^1\text{H}$ -MRS with a Philips 3.0 T Achieva scanner using MEGA-PRESS adapted for detection of the cysteinyl resonance of GSH (Mescher et al., 1998). An editing pulse which edited the cysteinyl  $\beta\text{-CH}_2$  protons of GSH was applied in alternative fashion, thus two alternate datasets that differed in the treatment of the GSH spin systems were collected. The experimental parameters were TR = 1800 ms, TE = 130 ms, dynamics = 128, NSA = 8, excitation frequency = 4.56 ppm, volume of interest (VOI) =  $30 \times 30 \times 20$  mm.

In order to optimise the MEGA-PRESS MRS method, *in vitro* scans were carried out first using GSH ‘phantoms’, or standard solutions in 250 ml round bottom flasks. These GSH phantoms were made with various concentrations of GSH (14 mM, 20 mM, 30 mM and 60 mM, Sigma-Aldrich, G4251) together with other common brain metabolites brain (20 mM creatine, 6 mM choline, 25 mM glutamate, 12mM glutamine, 10 mM lactate, 3 mM  $\gamma$ -aminobutyric acid and 25 mM N-acetyl-L-aspartic acid) at a physiologically representative pH of 7.2. Additionally, in order to assess GSSG signal and any potential overlap with the

GSH signal in the MRS spectrum, a GSH phantom was oxidised by the addition of 30 mM  $\text{H}_2\text{O}_2$  (stored at 4 °C). Scans were acquired before and 2 days after addition of  $\text{H}_2\text{O}_2$ .

Post-exercise *in vivo* MRS scans were acquired with the participant in the supine position with the region of interest set in the anterior cingulate cortex as shown in Figure 3A. The anterior cingulate cortex was chosen since the activation of this region has been linked to the physical fitness of individuals [42], as well as shown to be detrimentally impacted by ageing and neurodegenerative conditions (e.g., dementia [43]). Optimisation of the method was undertaken in one young healthy male participant, in which three different excitation frequency positions of the selective 180° pulse for cysteine residue were assessed. The reproducibility of the method to detect brain GSH was assessed by repeating a scan on a different day. All conditions concerning, physical activity, and diet of participant were kept the same between days.

#### *Quantification of GSH*

All of the MRS data were analysed using the freely available software jMRUI. GSH was quantified after subtraction of the two alternate datasets that differed in the treatment of the GSH (edit-on and edit-off spectra). GSH concentrations in phantoms were estimated using water as internal standard. Brain GSH concentrations were estimated using tissue water as concentration standard (while taking into account the combination of the grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) water fractions) as described by Gasparovic et al. [44]. The  $T_1$ -weighted images were segmented using SPM8 (Wellcome Trust Centre for Neuroimaging, University College London, UK) to determine the percentage of GM, WM and CSF in each VOI.

## ***Laboratory assays***

### *Glutathione assay*

GSH in whole blood was measured using a commercially available kit (GSH-Glo Glutathione Assay V6911, Promega, USA) according to the manufacturer's instructions. The assay quantifies GSH using bioluminescence, based on the conversion of luciferin derivative into luciferin in the presence of GSH, catalysed by glutathione S-transferase.

### *Total antioxidant capacity*

Total antioxidant capacity in plasma was assessed using the ferric reducing ability of plasma (FRAP) assay [45]. Samples and standards (ascorbic acid) (10 µl) were added to the respective wells of a multiwell plate. This was followed by addition of 300 µl FRAP reagent (300 mM sodium acetate, 10 mM 2,4,6-tripyridyl-s-triazine and 20 mM ferric chloride). After incubating for 8 minutes, the absorbance values were measured at 650 nm.

### *Lipid peroxidation*

Lipid peroxidation in plasma samples was assessed using the malondialdehyde (MDA) assay and 8-isoprostane assay as described below.

#### **MDA assay**

Plasma samples and standards (1,1,3,3-tetramethoxypropane) (100 µl) were mixed with 100 µl trichloroacetic acid (410 mM) and 800 µl colour reagent (4.6 mM thiobarbituric acid, 1.74 M glacial acetic acid and 0.67 µM butylated hydroxytoluene) in Eppendorf tubes. After boiling vigorously in water (100 °C) for one hour, the reaction was stopped by placing the tubes in an ice bath for 10 minutes. Supernatant were transferred to a multiwell plate and the

absorbance was measured at 540 nm.

### 8-Isoprostane

The concentration of 8-isoprostane in plasma samples was determined by a competitive enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (8-Isoprostane EIA kit 516351, Cayman Chemical, USA) according to the manufacturer's instructions.

### *Protein carbonylation*

The protein carbonyl concentration was measured in plasma samples using the ELISA method [46]. Briefly, the protein concentration of the plasma samples was determined using the bicinchoninic acid assay and protein carbonyls were expressed per mg of protein [47]. Samples and standards (50 µl) were allowed to bind to the respective wells of multi-sorb plates (Nunc, Fisher Thermo Scientific) for 1 hour. Bound protein was incubated with 1 mM 2,4-dinitrophenylhydrazine for 1 hour. Next, the wells were blocked with 200 µl tris-buffered saline (TBS) with 0.1 % Tween-20 overnight at 4 °C. Wells were first incubated with mouse IgE anti-dinitrophenyl antibody (50 µl, 1:1000 dilution) for 2 hours at 37 °C and then with anti-mouse IgE horse radish peroxidase conjugate antibody (50 µl, 1:5000 dilution) for 1 hour. All steps were followed by three washes with TBS with 0.05 % Tween. Then, 50 µl substrate (0.5 M citrate phosphate buffer, hydrogen peroxide and 2 mg o-phenyldiamine) was added and the reaction was stopped with 50 µl sulphuric acid (2 M) after 1 hour incubation in the dark. Absorbance values were measured at 490 nm.

### *Brain derived neurotropic factor*

Brain derived neurotropic factor (BDNF) in plasma was measured using a sandwich ELISA method with a commercially available kit (BDNF Quantikine ELISA kit, R&D systems, Minneapolis, USA) according to the manufacturer's instructions.

### *Statistical analysis*

Data were analysed using SPSS 22.0 statistical package for Windows (SPSS Inc, USA). The Shapiro-Wilk test was used to check for normality of distribution of data. Parameters which were not normally distributed were transformed for normal distribution prior to statistical analyses. Analysis on the effect of exercise bouts on brain GSH were assessed using one-way repeated measure analyses of variance (ANOVA). Analyses on the effect of two exercise trials (HII and moderate exercise trial) at each of the three time points (pre-, immediately post- and post (~1hour)- exercise) on markers of oxidative stress and BDNF levels in the blood/plasma were performed using a two-way repeated measure ANOVA. Where appropriate, post hoc analyses were conducted to explore the main effects in more detail. The effect of HII exercise on MCAv during the high intensity phase was assessed using one-way repeated measure ANOVA. Statistical significance was accepted at the  $p < 0.05$  level and results are presented as means  $\pm$  standard deviation.

## **Results**

### *Optimisation of MRS method*

One of the most significant challenges of *in vivo* MRS is insufficient signal-to-noise ratio (SNR), and one way to improve the SNR is to increase the acquisition time. This allows a sufficient number of repeat signals to be recorded and averaged in order to obtain a spectrum

of sufficient quality. However, this process significantly increases scan time, which has its own practical limitations [48]. As a consequence of these limitations, MRS is restricted in its ability to measure transient changes of the metabolite of interest. In addition, it is challenging to measure brain GSH in particular, because of the spectral overlap seen around the GSH peak, and the comparatively small concentration of GSH compared to other metabolites in particular the creatine peak (examples of these considerations are shown in Figure 2 and Figure 3). MEGA- PRESS (MEshcher-Garwood Point RESolved Spectroscopy) [49,50] is becoming the standard technique used in MRS measurement of GSH. This spectral editing technique allows the discrimination of GSH signals from stronger overlying signals of other metabolites, in particular the creatine peak, by utilising known couplings within the GSH molecule. This technique involves collection of two interleaved datasets that differ in their treatment of the GSH spin system.

Figure 2 and Figure 3 show measurement of GSH using the MRS method *in vitro* (phantoms) and *in vivo* (brain) respectively. GSH signal measured by the MRS method was proportional to the concentration of GSH in the phantoms (Figure 2B). Oxidation of GSH phantom by addition of H<sub>2</sub>O<sub>2</sub> resulted in decreased GSH signal at 2.95 ppm, and generation of a new peak at 3.24 ppm, proposed as GSSG (Figure 2C). *In vivo*, the highest GSH signal was obtained with excitation frequency position of the selective 180° pulse for cysteine residue of GSH at 4.56 ppm (Figure 3C). Repeating the scan at 4.56 ppm excitation on a different day gave a coefficient of variation (CV) of 17%.

### ***Brain GSH***

Figure 4 shows brain GSH post-exercise (HII and moderate bout) compared to control GSH concentration. One-way repeated measure ANOVA showed that the concentration of brain

GSH detected by MRS was altered following exercise  $F(2, 6) = 9.16, p = 0.01$ . Bonferroni post-hoc analysis showed a significant decrease in GSH level post-moderate exercise compared to baseline,  $p = 0.04$ .

### ***Oxidative stress markers in the blood***

Figure 5 A-E shows the response of the different peripheral markers of oxidative stress to exercise. GSH was assessed in whole blood and protein carbonyls, antioxidant potential, MDA and 8-isoprostanes were assessed in plasma at the three time points (pre-, immediately post- and post (~1 hour)-exercise) following the two different exercise bouts. For blood GSH, a two-way repeated measure ANOVA a significant interaction effect between the type of exercise and the time points on GSH level  $F(2, 14) = 9.22, p = 0.003$  (significant difference between pre-exercise and immediately post-exercise comparing the two exercise trials,  $F(1, 7) = 31.4, p = 0.001$ ). Further analysis of each exercise bout revealed that in response to HII exercise there was a significant increase in blood GSH immediately post-exercise compared to pre-exercise level,  $p = 0.001$ .

Two-way ANOVA showed a significant interaction effect between the type of exercise (HII and moderate exercise) and time points (pre-, immediately post- and post (~1 hour)-exercise) for antioxidant capacity  $F(1.1, 8.08) = 6.3, p = 0.03$  and for MDA levels,  $F(2, 12) = 4.72, p = 0.03$ . Contrasts revealed that for antioxidant capacity the difference was between pre- and post-exercise levels,  $F(1, 7) = 9.18, p = 0.02$  and for MDA levels the difference was between immediately post- and post- exercise level,  $F(1, 6) = 6.23, p = 0.047$ . Two-way ANOVA showed no significant interaction effect between the type of exercise bout and the time points for isoprostane levels,  $F(1.04, 6.26) = 2.64, p = 0.15$ , and for protein carbonyl,  $F(2, 14) = 0.62, p = 0.55$ .

Further analysis of the individual exercise bouts showed that for the HII bout there was an increase in antioxidant capacity post-exercise relative to pre-exercise ( $p = 0.003$ ), and a decrease in MDA levels post-exercise compared to immediately post-exercise ( $p = 0.04$ ). For moderate exercise, there was a significant decrease in protein carbonyl levels immediately post-exercise from pre-exercise level ( $p = 0.04$ ).

### ***BDNF***

Two-way repeated measure ANOVA showed that there was no significant interaction in BDNF level between time and type of exercise,  $F(2, 16) = 1.77$ ,  $p = 0.20$  (data shown in Figure 6). Further analysis of the individual exercise bouts revealed that for the HII bout, there was a significant increase in BDNF at immediately post-exercise and post (~1 hour)-exercise compared to pre-exercise,  $p = 0.02$  and  $p = 0.03$  respectively.

### ***Cerebral blood flow***

Figure 7 shows MCAv changes during the high intensity phases of the HII bout (10 s increments) along with warm up/ recovery before the high intensity phase (1 minute average) and after the high intensity phase (10 s increments for 60 s after the high intensity phase). One-way repeated measure ANOVA showed a significant effect of HII exercise on MCAv  $F(9, 27) = 8.90$ ,  $p < 0.001$ . Post-hoc tests revealed that compared to the first 10s of the high intensity phase, there was a significant decrease in MCAv during the second 10s ( $p = 0.003$ ) and the third 10s ( $p = 0.006$ ) of the high intensity phase.

### **Discussion**

In this small cohort study, the MRS method described detected a difference in brain GSH in response to a moderate bout of steady state exercise. Altered brain GSH was not observed in



response to HII. In the periphery, the blood GSH response to HII was significantly different to the blood GSH response to moderate exercise. In the blood, GSH showed greatest response to the HII exercise, whereas in the brain GSH showed greatest response to the moderate exercise. It is accepted that the contribution from the vasculature to the GSH levels measured in the brain using the MRS method is negligible [7,51] as GSH does not directly reach the brain due to blood-brain barrier (BBB) [52]. The transient increase seen in blood GSH after an acute bout of intense exercise has been reported by other studies [53–56]. This transient increase seen in blood is probably due to hepatic efflux of GSH [57,58] in order to compensate for the ROS production during exercise. However, not all studies report increased GSH following exercise. Fisher-Wellman and Bloomer [27] reviewed several studies that either report a decrease or no change in blood/plasma GSH levels during, or at the end of exercise. These differences across studies could well be explained by training status of the participants, and duration, mode and intensity of exercise.

A review by Camiletti-Moiron et al. [59] found that moderate aerobic exercise training in rodents promoted the antioxidant capacity of brain, while high-intensity or aerobically-exhaustive exercise training could deteriorate the antioxidant response. The authors of the review did not find any relevant studies in humans but they hypothesised that aerobic moderate training is the most appropriate exercise to positively enhance the brain antioxidant response. The results presented herein are in agreement with this hypothesis, as moderate exercise, rather than HII exercise, caused a greater decrease in brain GSH in all of the participants tested. A single bout of exercise can induce a transient oxidative stress via increased RONS production. This is sometimes associated with a decrease in antioxidant, as the antioxidant is utilised in quenching RONS. This process can stimulate adaptive change [17,18], especially if it is repeated, as RONS act as critical signalling molecules. It is likely

therefore that the decrease in brain GSH reported herein, in response to this acute bout, is part of the accepted process of adaptation to exercise. Taking part in regular bouts of exercise is known to provoke an adaptive response of increased antioxidant capacity, including increased GSH, by first stimulating a transient oxidative stress [17, 19-23].

In this study a MEGA-PRESS MRS method was optimised for the detection of brain GSH. *In vitro* experiments showed that the measured GSH signal was proportional to the GSH concentrations in the phantoms, which confirmed that the observed signal was GSH and that GSH could be successfully quantified using this technique. Oxidation of the GSH phantom with H<sub>2</sub>O<sub>2</sub> resulted in decreased GSH signal at 2.95 ppm and the appearance of a new peak at 3.24 ppm in the spectrum, which suggested that GSH had been oxidised to GSSG and that GSSG signal did not contribute to the detected GSH signal, in agreement with previous findings [62,63]. Repeated measurement of brain GSH between days in one male participant gave a CV of 17 %, which suggests that brain GSH measured by MRS is reproducible using this method.

As presented in the introduction, the MRS method is limited due to its sensitivity. However, although it is limited in its ability to measure transient changes in brain metabolites (due to poor signal to noise ratio), a small proof-of-concept pharmacodynamics study did report measurement of increased brain GSH using an MRS method in humans [51]. A study involving larger numbers of individuals is needed in order to confirm the potential exercise-induced changes in brain GSH observed in the current study. A power analysis (G\*power, University of Dusseldorf, Germany) indicated that 46 participants would be required to observe a 0.80 power (i.e.  $1 - \beta = 0.8$ ) with  $\alpha = 0.05$  and partial  $\eta^2 = 0.1$ . A further limitation of the study is that brain GSH was measured post-exercise only once, within the hour. Therefore, any changes in brain GSH in response to exercise at a later time was not assessed in this

study. Slivka and Cohen [37] observed that GSH decreased in rodent brain in response to brain ischemia only after 4 hours post-ischemia, which suggest that brain GSH may have been altered at a later time in response to HII exercise.

Similar to previous studies [22,30,32], the results presented herein found that the appearance of markers of oxidative stress in the periphery, in response to exercise, is dependent on exercise intensity. Total antioxidant capacity and lipid peroxidation were significantly altered post (~1 hour)-exercise in response to HII bout, while moderate exercise elicited a decrease in protein carbonyl immediately post exercise. Plasma BDNF increased in response to HII exercise but not moderate exercise, and thus, considering the role of BDNF in neurogenesis, exercise intensity may indeed be important in brain health. BDNF is the most abundant growth factor in the brain and it is often thought to be a link between exercise and improved brain health.

The blood flow data from the high intensity phase of the HII exercise suggest that cerebral blood flow seems to be protective at high intensity exercise against hyperperfusion. The significant decrease in MCAv seen during the 2<sup>nd</sup> 10 seconds of the high intensity phase of HII, compared to the 1<sup>st</sup> 10 seconds was probably due to exercise-induced cerebral vasoconstriction due to hyperventilation hypocapnic effect. Hence this constrictive effect may serve as a neuroprotective response to prevent BBB disruption and hyperfusion injury [33]. Similarly, a recent study [64] also found a decrease in cerebral blood flow during high intensity phase of HII. These findings suggest that there might be intrinsic mechanisms which protect the brain during high intensity exercise including against hyperperfusion.

## **Conclusion**

The results presented herein suggest that MRS is capable of detecting changes in brain GSH following an acute bout of exercise, and if the mechanisms of adaptation to oxidative stress

are the same in the brain as they are in other tissues, then such response may contribute to brain health. However, due to the small sample size and the limitations of the MRS method, the magnitude of change and the relationship to exercise intensity should be evaluated with caution. A bout of HII exercise consisting of short phases of a very high intensity exercise elicited a greater oxidative stress in the periphery compared to moderate exercise. Compared to moderate exercise, HII with its reduced time and energy cost may be an attractive exercise modality for a variety of populations with specific goals such as increased aerobic capacity. The results do not suggest that HII places any higher oxidative stress on the brain than a moderate exercise bout.

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## Disclosure statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

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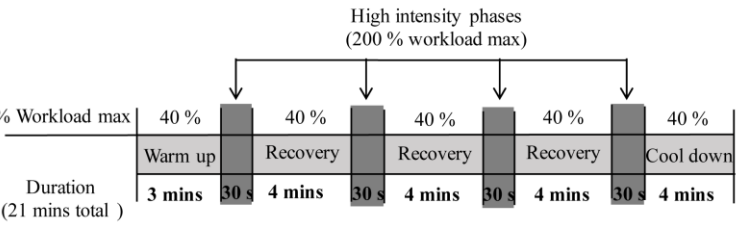
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**HIIT exercise**



**Moderate exercise**

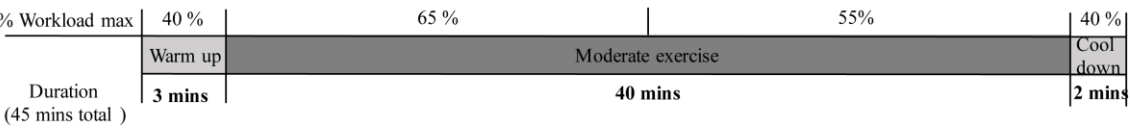


Figure 1. Schematic representation of the two exercise trials.

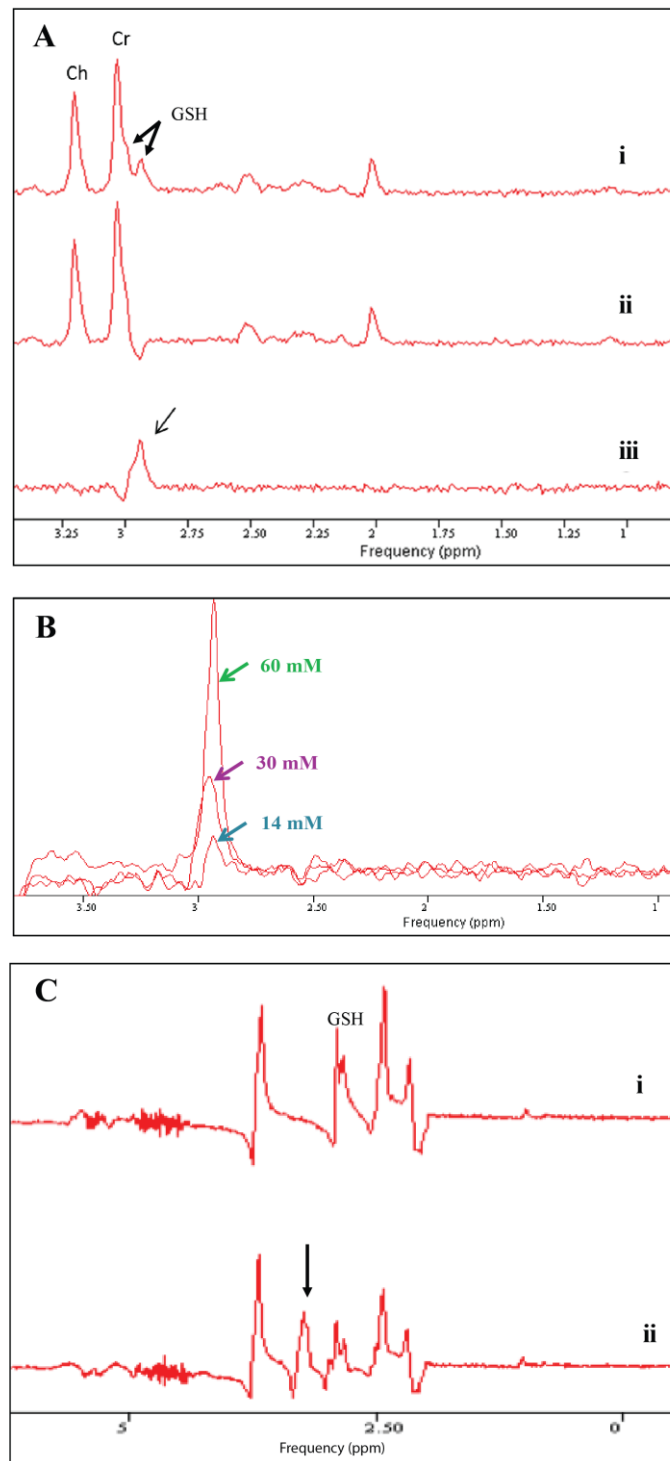


Figure 2 *In vitro* measurement of GSH in phantoms using MRS. (A) GSH editing in a 20 mM GSH phantom in spectra acquired using the optimised sequence parameters: i) spectrum acquired without editing GSH (edit-off spectrum), ii) spectrum acquired with editing of GSH with MEGA-suppression (edit-on spectrum) and iii) edited spectrum obtained on subtraction of spectrum ii from i (arrow pointing to the GSH signal)

obtained on subtraction). Ch, choline ; Cr, creatine **(B)** Superimposed spectra of edited GSH signals (edit-on and edit-off spectra subtracted) of three phantoms of varying GSH concentrations showed that GSH signal measured by MRS method was proportional to the GSH concentration in the phantoms. **(C)** A 30 mM GSH phantom before (i) and after (ii) oxidation by addition of  $\text{H}_2\text{O}_2$ . On oxidation, the GSH peak was decreased and a new peak was observed at 3.24 ppm (shown by arrow).

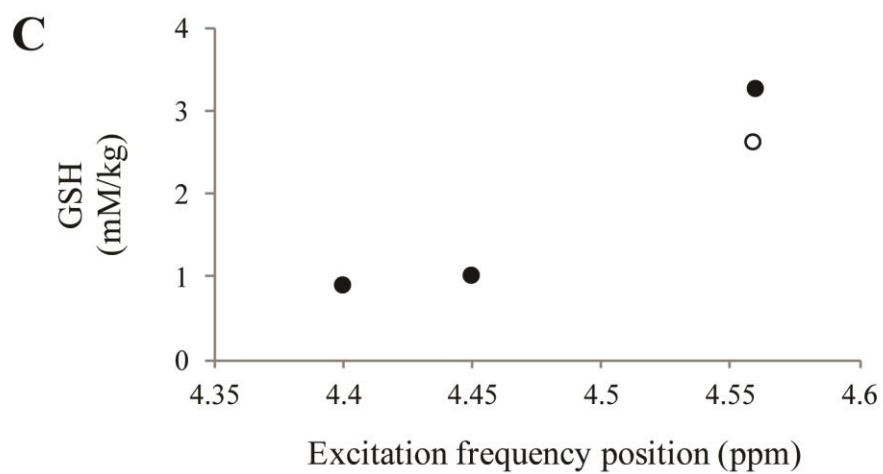
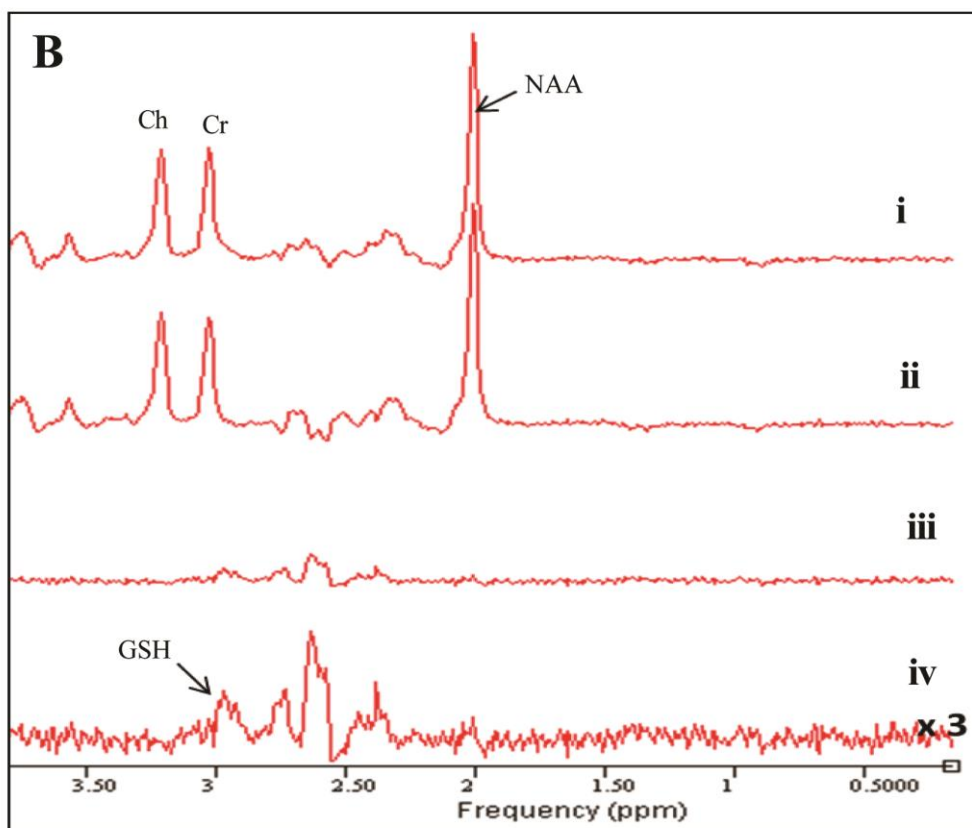
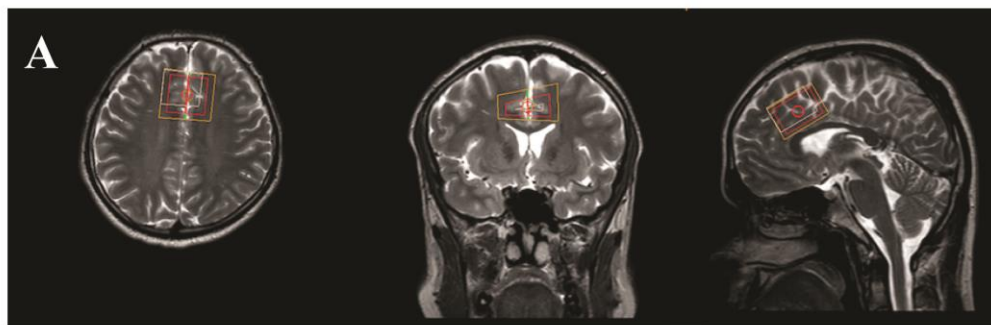


Figure 3. *In vivo* measurement of GSH in the human brain using MRS. **(A)** Placement of voxel at region of interest in the brain. Representative T1 weighed MRI images of a participant brain illustrating the voxel placement at the region of interest in the anterior cingulate cortex with volume of interest (VOI)  $30 \times 30 \times 20$  mm. Boxes representing the VOI at the axial, frontal and sagittal view from left to right. **(B)** Editing of brain GSH signal in spectra acquired using the optimised sequence parameters: i) spectrum acquired without editing GSH (edit-off spectrum), ii) spectrum acquired with editing of GSH with MEGA-suppression (edit-on spectrum), iii) edited spectrum obtained on subtraction of spectrum ii from i, and iv) spectrum iii multiplied by a factor of three. Ch, choline; Cr, creatine; and NAA, N-aceylaspartate **(C)** Optimisation of method *in vivo*. Graph showing the detection of GSH at various excitation frequency position of the selective  $180^\circ$  pulse for cysteine residue while keeping other experimental parameters the same. Closed circles data were collected on the same day, while open circle was collected on a different day

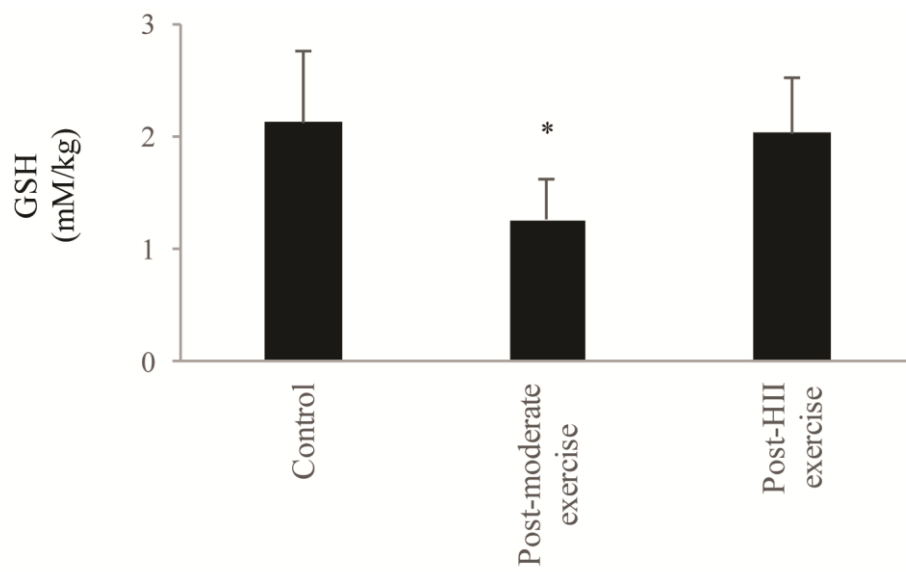


Figure 4. Brain GSH response to the two exercise bouts (n=4 participants). Pairwise comparisons showed GSH level post-moderate exercise was lower to control level ( $p = 0.04$ ).

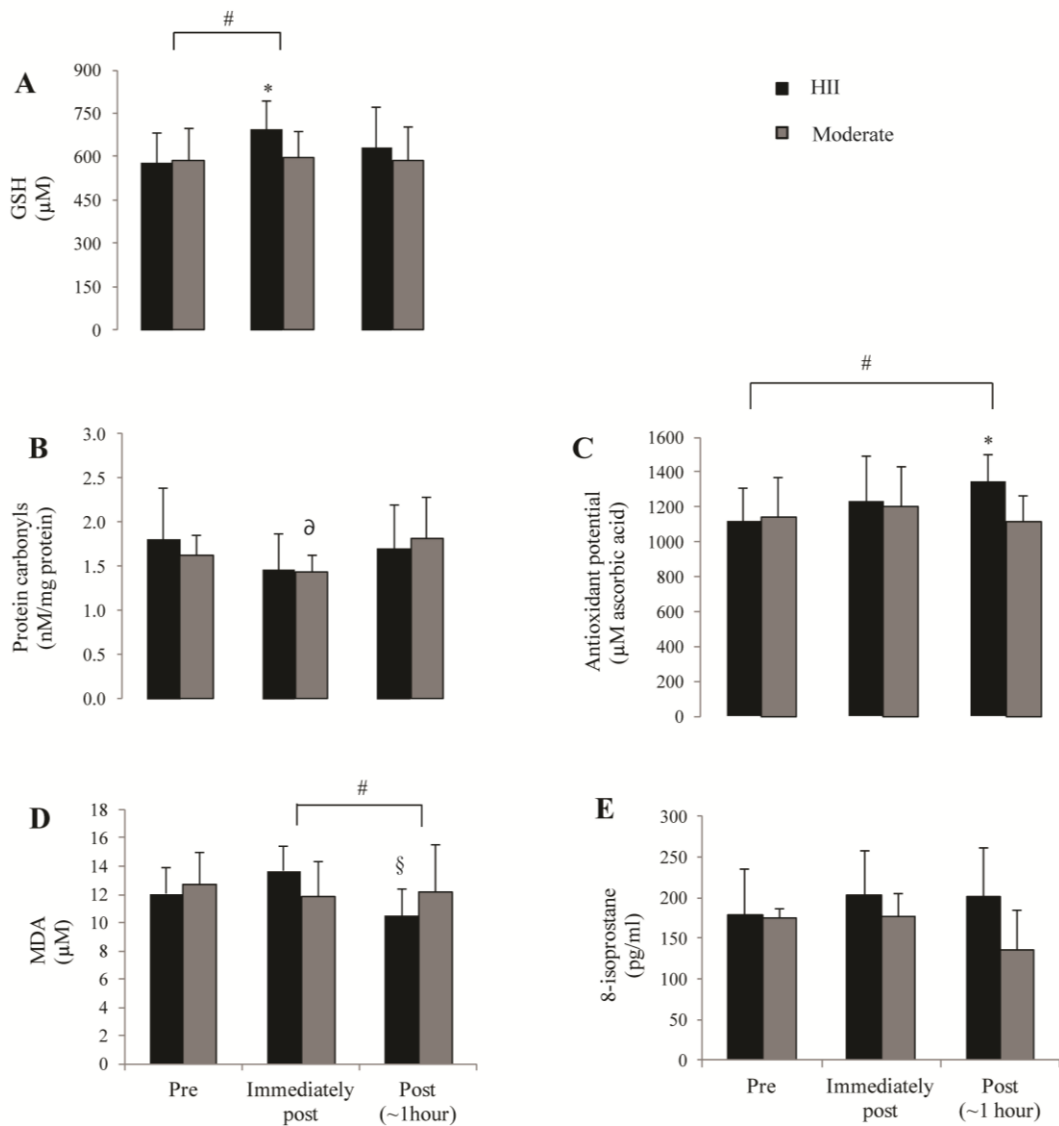


Figure 5. Acute oxidative stress responses to the two exercise bouts (HII and moderate exercise). Bars represent mean values of GSH (**A**), protein carbonyl (**B**), antioxidant potential (**C**), MDA (**D**) and 8-isoprostane (**E**) in response to the two exercise bouts at the 3 different time points (pre-, immediately post- and post (~1 hour)- exercise). Error bars are standard deviation. Two-way ANOVA: # indicates significant interaction effect between the type of exercise and the time. Further analysis of individual exercise trial- for HII exercise, \* indicates significant difference to pre-exercise level and § to immediately post-exercise level; and for moderate exercise, ∂ indicates significant difference to pre-exercise level.

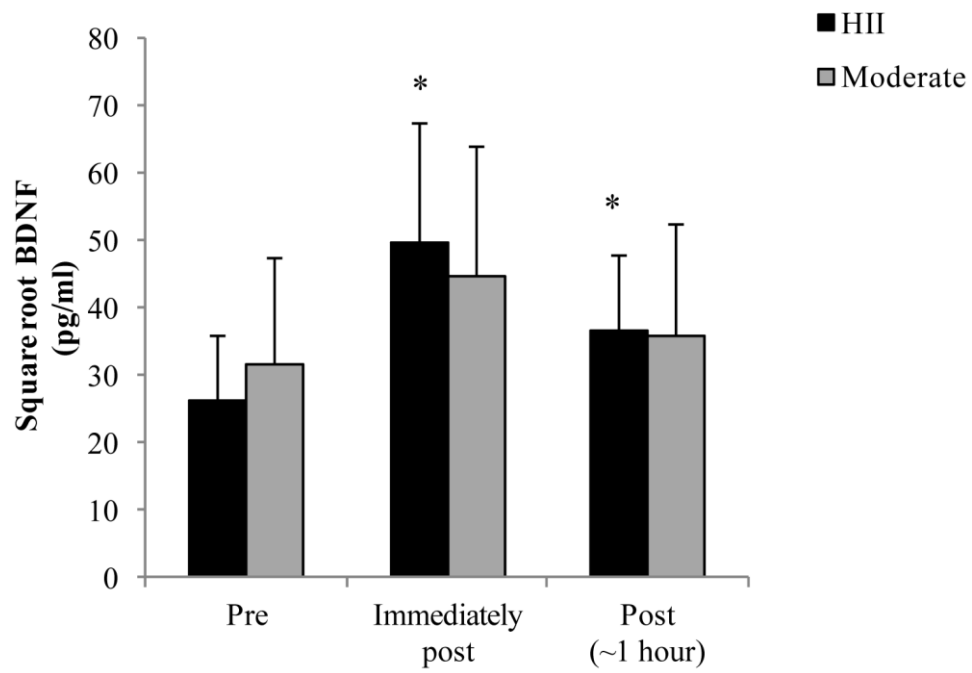


Figure 6. Mean BDNF levels in responses to the two exercise bouts, with standard deviation as error bars. Pairwise comparisons: \* indicates significant difference compared to pre-exercise levels in response to the HII trial.



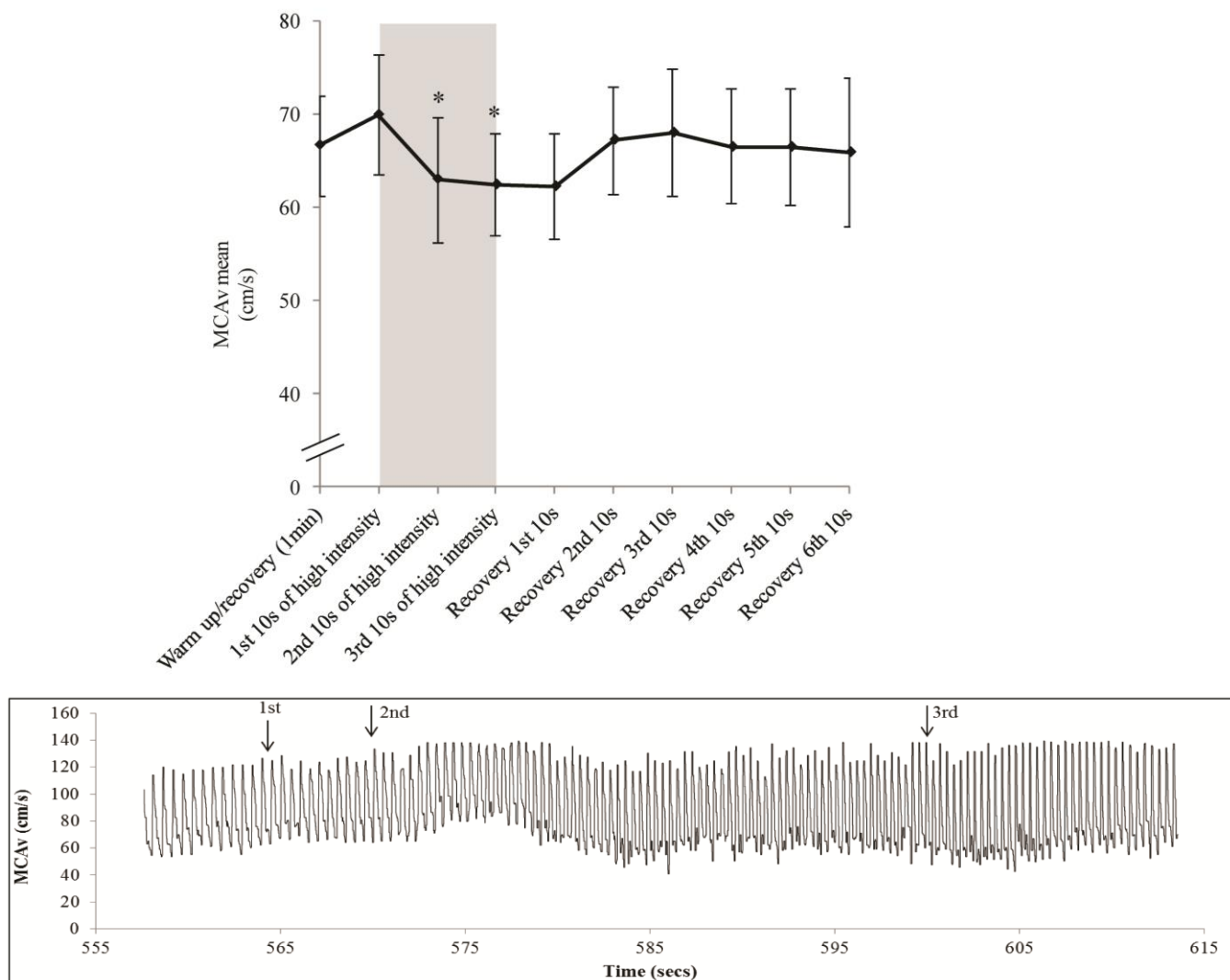


Figure 7. **Graph.** Mean MCAv changes during the high intensity phase of the HII bout (n=4 participants). MCAv changes during high intensity phases (grey box) (10s increments) were compared with warm up/ recovery before the high intensity phases (1 minute average) and after the high intensity phases (10 s increments for 1 minute after the high intensity phase). Pairwise comparisons: \* indicates significant difference from 1<sup>st</sup> 10 s of high intensity phase (p<0.01). **Box.** Raw time series of MCAv is shown during one of the high intensity phases in one of the participants. The first, second and third arrows indicate pre 5 seconds before the high intensity phase, start of the high intensity phase and end of the high intensity phase respectively.

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