Exploring Inflammatory Predictors of Depressive Responses to Exercise

By

Benjamin Morris

Department of Psychology and Neuroscience

University of Colorado Boulder

Boulder, Colorado 80309

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Thesis Advisor: Dr. Angela Bryan, Department of Psychology and Neuroscience

Defense Committee:

Dr. Angela Bryan, Department of Psychology and Neuroscience, Thesis Advisor

Dr. Heidi Day, Department of Psychology and Neuroscience, Honors Council Representative

Dr. Monika Fleshner, Department of Integrative Physiology

Abstract

Despite its prevalence and social, economic, and emotional costs, no clear causes of depression have been identified. Two factors, pro-inflammatory cytokines and hippocampal neurogenesis, have been bidirectionally linked to depressive symptoms and interact significantly with each other. As exercise influences each of these three variables, sedentary older adults were recruited to participate in a 16-week exercise intervention in either a low intensity continuous training (LICT) or moderate intensity continuous training with interval training (MICT+IT) program to better understand how these variables covary and in particular to examine the hypothesis that cytokines effect depressive symptoms through regulatory influence on hippocampal neurogenesis. Depressive symptoms as assessed by the Beck Depression Inventory (BDI), peripheral plasma concentrations of the cytokines TNFα, IL-1β, and IL-6, and hippocampal volume from a structural MRI scan were measured pre- and post-intervention. No evidence that changes in the cytokines of interest correlate with changes in hippocampal volume or changes in BDI or that changes in hippocampal volume are correlated with changes in BDI was found. As an exploratory analysis, participants' genotype for rs16944, rs1800629, and rs1800797, three single nucleotide polymorphisms (SNPs) in the promoter regions for the IL-1β, TNFα, and IL-6 genes respectively, were assayed to investigate individual variability in depressive responses. Although no main effects of SNP genotype, exercise condition, nor genotype x exercise condition interactions were found on changes in BDI, a significant main effect of exercise condition on hippocampal volume for two of the three SNPs and a rs1800629 A-allele carrier main effect and rs1800629 A-carrier by exercise condition interaction was found. These results suggest that MICT+IT exercise may have a protective effect against hippocampal volume decrease and that A-allele carrier status with LICT exercise and Non-A-allele carrier status with MICT+IT exercise may have similar protective effects against hippocampal atrophy in older adults. More sensitive measurements of centrally-acting cytokines and development of an *in vivo* measure of neurogenesis could help further explore the cytokine-hippocampal neurogenesis-depression pathway.

Exploring Inflammatory Predictors of Depressive Responses to Exercise

Today, depression accounts for 11% of all years lived with disability globally (*Mental Health Action Plan 2013-2020*, 2013), and 17 million adults in the United States alone have experienced one or more depressive episodes in the past year ("NIMH » Major Depression," n.d.). Depression is also significantly associated with many serious chronic physical disorders such as cancer, cardiovascular disease, and hypertension (Kessler, 2012) and has a significant economic impact; from 2005 to 2010, the annual cost of major depressive disorder (MDD) in the United States rose 21.5% to $210 billion, and the individual cost of treatment provides a significant burden on low-income families (Greenberg, Fournier, Sisitsky, Pike, & Kessler, 2015). The current costs of depression pose a substantial emotional, social, and economic burden globally, and the future costs of these diseases are expected to rise within the next several decades. Despite its detrimental and wide-reaching impact, depression's etiology is poorly understood. Better understanding of factors that affect depression can lead to better and more personalized treatments while reducing this disease's impact.

Several factors have been suggested as biomarkers for depression. First, cytokines, a class of intracellular signaling proteins prominently involved in regulating the body's innate inflammatory response to infection, have been implicated in a bidirectional relationship with depressive symptoms. Depressive symptoms have been observed in patients undergoing interferon therapies (Cotler et al., 2000; Kraus, Schäfer, Csef, & Scheurlen, 2005) while cerebrospinal fluid (CSF) and plasma concentrations of Tumor Necrosis Factor-alpha (TNFα) and Interleukin (IL) 6 have been found to be elevated in depressed patients (Dowlati et al., 2010; Raison, Capuron, & Miller, 2006). Additionally, a meta-analysis of cytokine responses to a variety of pharmacological antidepressant treatments showed strong reductions in the pro-inflammatory cytokines IL-1beta (IL-1β) and IL-6. Further, treatment with a selective serotonin reuptake inhibitor significantly reduced TNFα levels (Hannestad, DellaGioia, & Bloch, 2011), while anti-TNFα drugs like celecoxib reduce depressive symptoms in combination with the antidepressant

Reboxetine (Müller et al., 2006), and the anti-TNFα drug Etanercept reduces depressive symptoms in patients with psoriasis (Tyring et al., 2006). These results highlight a strong, two-way relationship between unchecked inflammatory cytokines and depressive symptoms.

Second, decreased volume of the hippocampus, a bilateral limbic structure involved in learning and memory processes and emotion, has become a solid pathophysiological correlate of depression (Sheline, Wang, Gado, Csernansky, & Vannier, 1996; Stockmeier et al., 2004). However, no differences in neuronal atrophy rates in the hippocampus has yet been documented between depressed and non-depressed individuals (Cotter, Mackay, Landau, Kerwin, & Everall, n.d.). This suggests that differences in hippocampal volumes stem from altered growth rates (i.e. hippocampal neurogenesis), rather than different atrophy rates. Further, treatment with monoamine oxidase inhibitor-, selective serotonin reuptake inhibitor-, or selective norepinephrine reuptake inhibitor-type antidepressants increased neurogenesis in the subgranular zone (SGZ) of the adult rat hippocampus when applied chronically (2-4 weeks) but not acutely (1-5 days) (Malberg, Eisch, Nestler, & Duman, 2000). The authors note that this time course is consistent with the latency of antidepressant effects of pharmacological treatment in humans and suggest that it is the neurogenesis-promoting effects of these drugs that yield antidepressant effects. Further, Hill, Sahay, and Hen found that increased neurogenesis protects adult mice against the anxiogenic and depressive effects of chronic stress (Hill, Sahay, & Hen, 2015). Finally, Santarelli et al. demonstrated that a variety of antidepressant drugs require increases in SGZ neurogenesis for their antidepressant effects in an adult mouse model of depression (Santarelli et al., 2003). These results strongly implicate hippocampal neurogenesis and the cytokines TNFα, IL-1β, and IL-6 in depression.

Additional research indicates a link between cytokine dysregulation and neurogenesis, even suggesting a potential causative role of pro-inflammatory cytokines in hippocampal atrophy. Dysregulated TNFα (J. W. Koo, Russo, Ferguson, Nestler, & Duman, 2010), IL-6 (Monje, Toda, & Palmer, 2003) and IL-1β (Mouihate, 2014) have been implicated in decreasing proliferation, differentiation, and migration of neuronal precursor cells from the SGZ to the granule layers – essentially

inhibiting hippocampal neurogenesis. For example, IL-1β application significantly reduces Bromodeoxyuridine (BrdU)-positive neurons in the rat dentate gyrus, indicating reduced neurogenesis, but this effect is eliminated by pre-application of an IL-1β receptor antagonist (Ja Wook Koo & Duman, 2008). Further, IL-1β blockade abrogated the anhedonic and anti-neurogenic effects of repeated stress (Ja Wook Koo & Duman, 2008), while late-phase prenatal exposure to IL-6 causes neurodegeneration in the rat hippocampus (Samuelsson, Jennische, Hansson, & Holmäng, 2006). Despite established links between dysregulated cytokines like TNFα, IL-1β, and IL-6, elimination of neurogenesis in the SGZ, and depression, the direction of these links is unclear, and causality is unestablished in the direct cytokine-depression and SGZ-mediated cytokine-neurogenesis-depression pathways.

Through manipulation of an exogenous variable, we aimed to simultaneously alter cytokine regulation, hippocampal neurogenesis, and depressive symptoms in order to better understand their interactions. Exercise was identified as an ideal exogenous variable, as it has been demonstrated to normalize cytokine regulation, increase hippocampal neurogenesis, and alleviate depressive symptoms. While exercise has an acute inflammatory effect (Castell et al., 1996), cross-sectional studies have revealed an association between inactivity and elevated inflammation in older adults (Bruunsgaard et al., 2003). Additionally, in TNF-receptor knockout mice, exercise regulated over-expression of TNFα (Keller, Keller, Giralt, Hidalgo, & Pedersen, 2004) and in human subjects dosed with an *E. coli* endotoxin to mimic chronic, low-grade inflammatory stimuli, exercise eliminated the elevated TNFα response (Starkie, Ostrowski, Jauffred, Febbraio, & Pedersen, 2003). A variety of rodent studies have also demonstrated the stimulatory effects of exercise on neurogenesis. For example, exercise has been found to increase neurogenesis in rats (van Praag, Kempermann, & Gage, 1999), with high-intensity exercise reducing anti-neurogenic factors (E, Burns, & Swerdlow, 2014) and low-intensity exercise promoting neurogenesis and new neuron survival (Inoue et al., 2015). Finally, numerous meta-analyses of randomized controlled studies have consistently found a moderate effect of exercise, especially aerobic exercise, on amelioration of depressive symptoms (Kvam, Kleppe, Nordhus, & Hovland, 2016; Silveira et

al., 2013). Thus, exercise was selected to simultaneously manipulate the cytokines TNFα, IL-1β, and IL-6, hippocampal neurogenesis, and depressive symptoms in order to better understand the relationships between these variables.

Questions about the role of cytokines in the hippocampal neurogenesis – depression pathway seem particularly important for older adults. First, depression remains a common disorder in older adults, with estimates around 4% prevalence across various populations (Byers, Yaffe, Covinsky, Friedman, & Bruce, 2010; Ritchie et al., 2004). Further, the phenomenon of "inflammaging" has recently been recognized, with chronic, low-grade inflammation a near-ubiquitous feature of aging (Franceschi & Campisi, 2014). TNFα and IL-6 show marked increases during aging (Bruunsgaard, Skinhøj, Pedersen, Schroll, & Pedersen, 2000), while IL-1β, TNFα, and IL-6 production from peripheral blood mononuclear cells in healthy older adults is increased (Fagiolo et al., 1993). Evidence has also been found of decreased neurogenesis in older adults (Lucassen, Stumpel, Wang, & Aronica, 2010). Older adults are also the most sedentary group in the United States, making exercise a particularly relevant manipulation (Matthews et al., 2008). These factors make older adults an ideal population to study the connections between cytokine expression, hippocampal neurogenesis, and depression via manipulation of exercise.

Several hypotheses were developed in order to better understand these connections in the context of an exercise intervention consisting of random assignment to a moderate intensity or low intensity exercise condition in sedentary older adults. First, exercise was predicted to decrease peripheral plasma concentrations of the cytokines IL-1β, TNFα, and IL-6, with moderate-intensity exercise coupled with interval training inducing a larger decrease than low-intensity exercise. Changes in hippocampal volume and BDI were also predicted to be exercise-condition dependent. Second, pre-intervention levels of IL-1β, TNFα, and IL-6 were expected to be negatively correlated with pre-intervention hippocampal volume and pre-intervention depressive symptoms. Third, pre-intervention hippocampal volume was predicted to be negatively associated with pre-intervention depressive symptoms. Fourth, changes in levels of IL-1β,

TNFα, and IL-6 from pre- to post-intervention were expected to be associated with changes in hippocampal volume and depressive symptoms and changes in hippocampal volume to be associated with changes in depressive symptoms. Finally, exercise condition and changes in cytokine levels were expected to predict changes in hippocampal volume, while exercise condition, changes in cytokines, and changes in hippocampal volume will predict depressive symptoms.

Prompted by evidence that the heritability of depression is between 32% and 41% and individuals vary in their depressive responses, several exploratory genetic hypotheses regarding single nucleotide polymorphisms (SNPs) related to cytokine regulation and depression were proposed (summarized in Figure 1). Of particular interest are rs1800629, rs1800797, and rs16944, three promoter SNPs for the genes encoding TNFα, IL-6, and IL-1β respectively. rs1800629 G/G homozygotes, who exhibit decreased TNFα expression, were more likely to be depressed than their G/A and A/A counterparts in a small (n=50) study of older adults (Cerri et al., 2009). Additionally, the A allele of rs1800797 results in elevated IL-6 expression and has been identified as a risk factor for depression (Zhang, Wu, Zhao, Wang, & Fang, 2016). Finally, the G allele of the rs16944 SNP in the IL1-1β gene is associated with reduced amygdala and anterior cingulate cortex activity in response to emotionally arousing stimuli and increased remission of depression (Baune et al., 2010). Given the relationships between exercise, cytokines, and depression, it seems likely that individual differences in these SNP alleles could moderate the influence of exercise on depressive symptoms through effects on cytokines and thus hippocampal neurogenesis.

Specifically, the following hypotheses were proposed. It was hypothesized that rs1800629 and rs1800797 A-carrying individuals would have larger changes in plasma concentrations of TNFα and IL-6 in response to exercise than G/G homozygotes of these SNPs due to elevated TNFα and IL-6 expression. Further, rs1800629 and rs1800797 A-carrying individuals were expected to exhibit larger improvements in BDI and increases in hippocampal volume as a result of the intervention due to larger changes in cytokine concentrations. Finally, G/G homozygotes were expected to have lower BDI scores and larger hippocampi than their A-carrying counterparts due to lower cytokine levels. Each of these changes was

expected to vary in response to a SNP by exercise condition interaction. rs16944 G-carrying individuals were also expected to have altered IL-1β levels and depression symptoms at baseline, and exhibit altered BDI and hippocampal responses to exercise in an exercise condition-dependent manner.

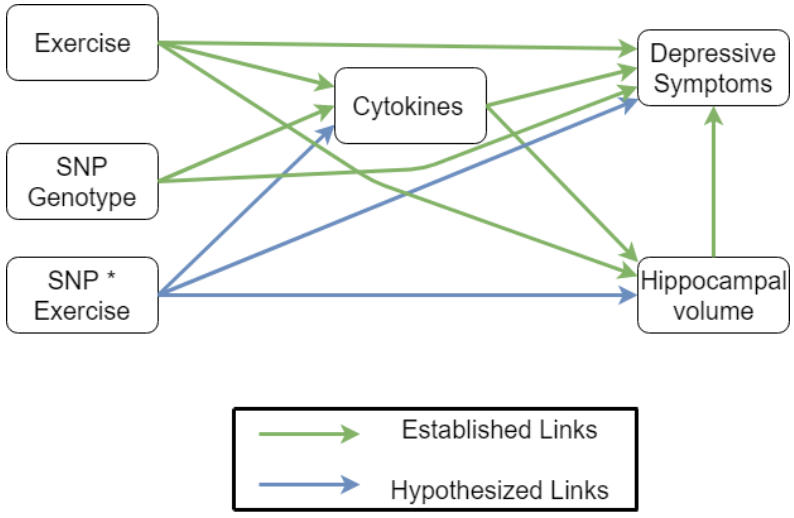


Figure 1 Summary of previously established and proposed relationships mediating the neuroinflammatory hypothesis.

Methods

Subjects

Adults (n= 115) aged 60 and over who consistently engaged in fewer than 60 minutes of aerobic exercise in the 6 months prior to study enrollment were recruited to participate in a 16-week NIH/NIA funded randomized clinical trial testing two different exercise interventions. Heavy smokers (> 20 pack-years), individuals with bipolar disorder, schizophrenia, Alzheimer's Disease, dementia, hypertension (systolic BP > 160 mmHg, diastolic BP >100 mmHg), or uncontrolled diabetes (hemoglobin AIC > 7%) or individuals who were ineligible for an MRI scan were excluded from the study. Additionally, individuals deemed unable to safely exercise by a physician or with abnormal heart activity during a Graded Exercise Test were excluded unless cleared by a physician. The study sample consisted of primarily females (n=68) and primarily individuals identifying as white (n= 107) (Table 1).

Table 1. Baseline demographics, exercise habits, BDI, hippocampal volume, and SNP allele frequencies by exercise group.

	LICT	MICT+IT
N	55	60
Age (M±St. Dev.)	68.89 ± 1.61	68.28 ± 1.39
%Female	56.36	61.67
%White	92.7	93.3
BDI (M±St. Dev.)	8.61 ± 5.96	7.58 ± 6.92
Hippocampal Volume / ICV	0.0049 ± 0.00074	0.0051 ± 0.00092
	rs16944	
% A/A	5.5	16.7
%A/G	36.36	38.33
%G/G	41.82	35
%Undetermined	16.36	15
	rs1800629	
% A/A	3.6	1.67
%A/G	20	23.33
%G/G	67.27	70
%Undetermined	9	5
	rs1800797	
% A/A	18.18	20.5
%A/G	38.18	23.08
%G/G	24.54	43.57
%Undetermined	9.1	12.8

Experimental Design

Eligible participants provided informed consent and were given opportunities to ask questions about the study. Participants wishing to continue the study were then examined by a physician to ensure they could safely exercise. Participants gave a four-hour fasted blood sample, completed a variety of self-report measures, including the Beck Depression Inventory-II (BDI) (Beck, Steer, Ball, & Ranieri, 1996) and demographic questions, and completed a treadmill administered VO_2 max test to establish aerobic fitness and estimate the participant's maximum heart rate. At a subsequent appointment, a structural MRI was taken at the University of Colorado's Intermountain Neuroimaging Consortium using a Siemens 3T MRI scanner (Seimens, Munich, Germany). Subjects were then randomly assigned to either the moderate-

intensity continuous training plus interval training (MICT+IT) exercise condition or the low-intensity continuous training (LICT) exercise condition. Subjects were instructed to conduct research assistant-supervised treadmill exercise three times per week for 16 weeks at the University of Colorado's Center for Innovation and Creativity. Over 16 weeks, the MICT+IT group participated in increasing levels of interval training, comprising of a two-minute warm-up followed by 15 minutes of 75-80% HR_{max} exercise followed by three cycles of 3 minutes of 85-95% HR_{max} exercise followed by two minutes of active recovery. In weeks 1-3, individuals assigned to the MICT+IT group exercised at 60% of their HR_{max} for 30 minutes. In weeks 4-6, MICT+IT participants had 1 interval session per week with the other two non-interval sessions consisting of continuous exercise at 75-80% HR_{max}. Weeks 7-9 had two interval sessions per week and one non-interval session. Weeks 10-16 consisted of three interval training sessions per week. LICT subjects exercised at 50% HR_{max} for 30 minutes for 16 weeks. Heart rate was assessed using Polar H10 heart rate monitors (Polar, Kempele, Finland) and monitored throughout exercise by research assistants. Following 16 weeks of exercise, subjects again completed the BDI, a VO_2 max test, structural MRI, and blood draw.

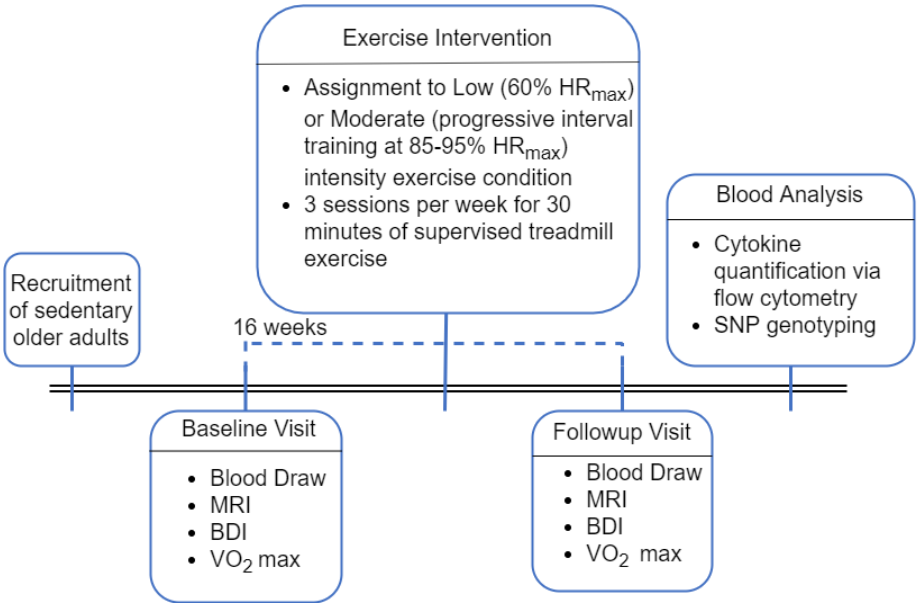


Figure 2 Summary of experimental design

Cytokine Quantification

Plasma was obtained through centrifugation of 10mL of whole blood stored in EDTA tubes (Beckton, Dickinson and Company, Franklin Lakes, New Jersey) at 1000xg for 10 minutes, aliquoted into 0.5mL microcentrifuge tubes, and stored at -80^{o} C until analysis. 96-well LegendPlex Human Inflammation Panels (Biolegend, San Diego, California) were stored at +4^{o}C prior to use and prepared using thawed plasma samples in duplicate according to manufacturer instructions. Plates were then covered in tin foil and transported to the Propel Labs Yeti flow cytometer (Propel Labs, Fort Collins, Colorado) made available through the University of Colorado Cancer Center's shared resources program. Data exports from the flow cytometer were analyzed using the LEGENDPlex (v8.0) application for Windows. All software defaults were used for curve fitting. During gating, Bead Size X was changed to FS00-A, Bead Size Y was changed to FS00-H, Bead Classification was set to FL29-A, and Report Signal was set to FL11-A. A versus B beads were identified by hand using the highest bead density regions in the plot generated by the above settings. Following gating and export, cytokine quantifications below the Lower Limit of Quantification (LLOQ) were replaced with (LLOQ/2) following (Beal, 2001) and duplicates were averaged. Coefficients of Variance (CV) were calculated using:

$$CV = \frac{\sigma}{(s_1 + s_2)/2}$$

High CVs (>20%) were noted, but not excluded. 14.6% of IL-1β samples, 12.2% of TNFα, and 16.2% of IL-6 samples were below the LLOQ and were imputed using LLOQ/2. No intra-plate standards were used to examine quality between plates.

DNA extraction

Blood samples collected in EDTA tubes were stored at 4^{o}C until extraction. The Gentra Puregene kit (QIAGEN, Hilden, Germany) for whole blood was used following the manufacturer's instructions optimized for 10mL of blood with several exceptions: the RNase A optional step was not taken, DNA

was vortexed in DNA hydration solution until no longer visible, and DNA was immediately stored at +4°C for at least 24 hours prior to quantification, rather than the 65°C and room temperature incubation steps. DNA concentrations were assessed using the Quant-iT Qubit 1.0 fluorometer (ThermoFisher Scientific, Waltham, Massachusetts) and protocol following all manufacturer instructions. Samples were then stored at -80°C until analysis.

SNP analysis

From the whole blood DNA extracted, SNP genotyping analyses were conducted on rs1800797, rs1800629, and rs16944. TaqMan SNP Genotyping Assays and TaqMan Genotyping Master Mix (ThermoFisher Scientific, Waltham, Massachusetts) were used to prepare 384-well plates with samples in duplicate. The genotyping PCR reaction was conducted on the QuantStudio 6 Flex Real-Time PCR system (ThermoFisher Scientific, Waltham, Massachusetts) on VIC/FAM genotyping settings for a 5ul reaction and 42 cycles. Data were analyzed using QuantStudio Real-Time PCR Software (ThermoFisher Scientific, Waltham, Massachusetts). Samples with at least one duplicate called as undetermined were re-run. All examined SNPs were in Hardy-Weinberg equilibrium.

MRI Analysis

Structural MRIs were taken at the University of Colorado's Intermountain Neuroimaging Consortium using a Siemens 3T MRI scanner. Acquisition parameters were: TR/TE/TI=2530/1.64/1200 ms, flip angle=7°, FOV=256x256 mm, Slab thickness=192 mm, matrix size=256x256, 3D voxel resolution=1×1×1 mm, Pixel bandwidth=651 Hz, Total scan time=6 min 3s. To estimate and reduce radio frequency (RF) inhomogeneities and spatial distortion, a fieldmap was also acquired: TR/TE=400ms/4.92ms, field of view=238×238 mm, matrix size=68×68, in-plane voxel resolution =3.5×3.5×3.55 mm, 48 slices, total scan time=54s.

The Freesurfer Software Suite was used to analyze MRI data. Analyses included motion correction, removal of non-brain tissue, automated Talairach transformation, subcortical white matter and

deep gray matter volumetric structure segmentation, intensity normalization, gray matter/white matter boundary tessellation, automated topology correction, and surface deformation to optimize tissue class separation. Further analyses of the cortical models include surface inflation, registration to a spherical atlas, cerebral cortex parcellation into gyral/sulcal structure-dependent units, and creation of curvature and sulcal depth maps.

Results

Baseline correlations

None of the baseline measures of IL-6 (r_p = -0.03, p = 0.79), IL-1β (r_p = -0.11, p = 0.26), or TNFα (r_p = -0.02, p = 0.81) were significantly correlated with pre-intervention hippocampal volume (Fig. 6). IL-6 (r_p = 0.21, p <0.05) was significantly correlated with pre-intervention BDI in the predicted direction, such that higher levels of IL6 were associated with greater depressive symptoms. Neither IL-1β (r_p = -0.04, p = 0.68), nor TNFα (r_p = 0.06, p = 0.55) was significantly correlated with baseline BDI. Baseline hippocampal volume was not correlated with baseline BDI (r_p = -0.01, p = 0.94) (Table 2).

Table 2. Pearson's correlation matrix between cytokines, exercise, BDI, and hippocampal volume at the baseline visit. * indicates p <0.05, *** indicates p <0.001.

	IL-1β	IL-6	TNFα	Hippocampal Volume
IL-6	0.53***			
TNFα	0.72***	0.75***		
Hippocampal Volume	-0.11	-0.03	-0.02	
BDI	-0.04	0.21*	0.06	-0.01

Note. * indicates p < 0.05, *** indicates p <0.001

Exercise regulation of cytokines

Peripheral plasma concentrations of the cytokines TNFα, IL-6, and IL-1β were assessed pre- and post-intervention. In a paired t-test the intervention did not significantly affect plasma cytokine concentrations ($p > 0.05$). No statistically significant differences were found between LICT and MICT+IT exercise groups for changes in IL-6 (t = 0.66, p =0.5) or TNFα (t = 0.47, p = 0.64) in paired t-tests. However, the effect for IL-1β was marginal, IL-1β (t = 1.84, p = 0.068), such that this cytokine increased in the LICT group and decreased slightly in the MICT+IT group (see Figure 3).

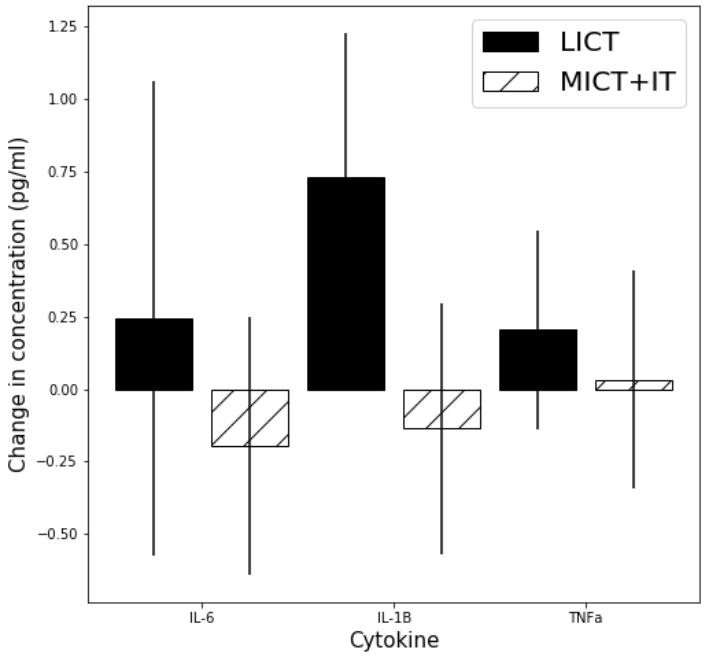


Figure 3 Impact of exercise condition on changes in peripheral plasma concentrations of the cytokines IL-6, IL-1β, or TNFα pre- and post-intervention. Data are presented as mean +/- SEM. LICT: low intensity continuous training group (n=55). MICT+IT: moderate intensity continuous training plus interval training group (n = 60).

Exercise regulation of hippocampal volume

Left and right hippocampal volumes from the structural MRI were summed and divided by intracranial volume (ICV). In a paired t-test comparing pre- and post-intervention hippocampal volume, hippocampal volume significantly decreased following the exercise intervention (t(108) = 2.6387,

$p < 0.01$) (Figure 4A). In an independent t-test, no statistically significant differences were found in change in hippocampal volume between exercise conditions (t = 1.05, $p = 0.30$) (Figure 4B).

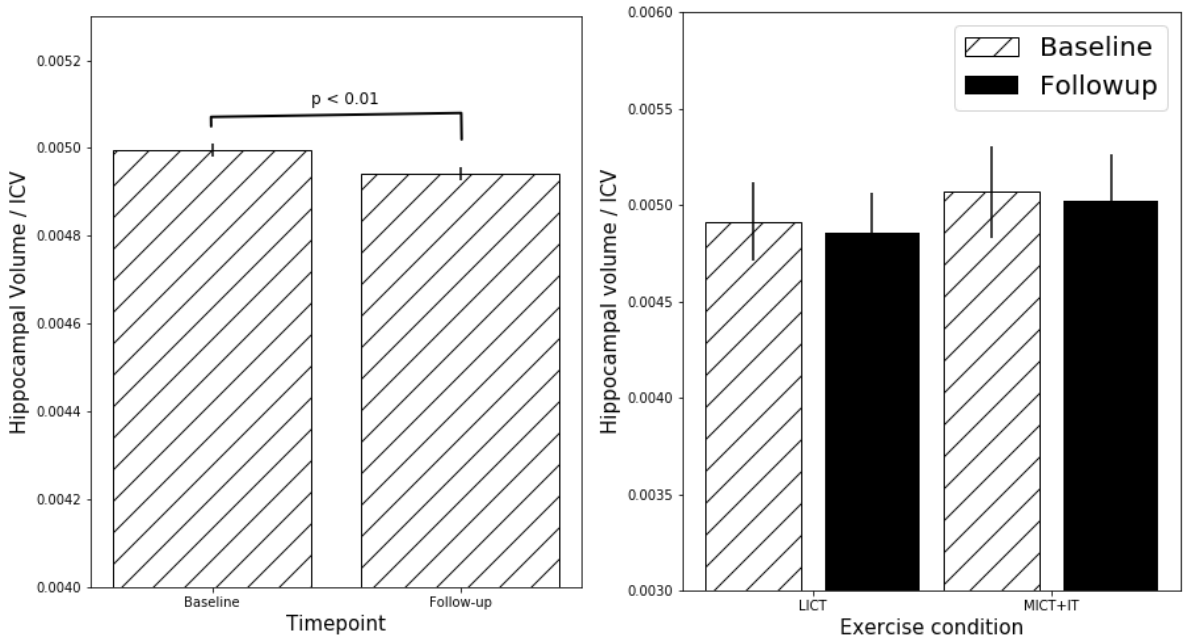


Figure 4 A. Decrease in hippocampal volume from pre- to post- intervention (t(108) =2.6387, p <0.01). **B.** No difference in hippocampal volume pre- and post-intervention by exercise condition (p >0.05). Data are presented as mean +/- SEM. LICT: low intensity continuous training group (n=55). MICT+IT: moderate intensity continuous training plus interval training group (n = 60). ICV: Intracranial volume.

Predicting changes in hippocampal volume

Three linear regressions were conducted to examine the effects of changes in IL-1β (F(2, 112) = 0.79, $p = 0.46$, R^2adj = -0.004), IL-6 (F(2, 106) = 0.78, $p = 0.46$, R^2adj = -0.004), and TNFα (F(2, 106) = 0.73, $p = 0.48$, R^2adj = -0.005) and exercise condition on changes in adjusted hippocampal volume. None of the predictors were statistically significant (p >0.05).

Exercise regulation of BDI

Depressive symptoms as measured by the self-report BDI were assessed pre- and post-intervention. In a paired t-test comparing pre- and post-intervention BDI, depressive symptoms

significantly decreased following the exercise intervention (t(114) =3.894, p <0.001) (Figure 5A). In an independent t-test, no statistically significant differences were found in changes in BDI between exercise conditions (t = 0.69, p = 0.49) (Figure 5B).

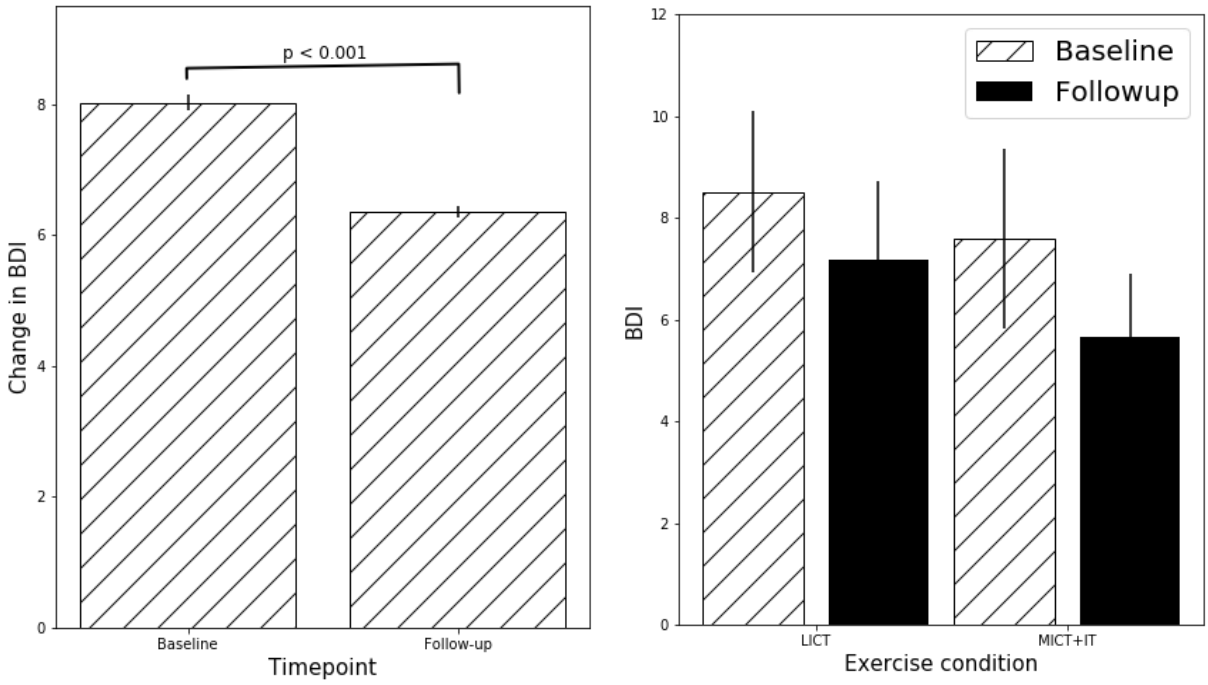


Figure 5 A. Depressive symptoms as measured by the self-report Beck Depression Inventory (BDI) decreased significantly after exercise (t(114) =3.894, p <0.001). **B.** No differences in change in BDI by exercise condition. Data are presented as mean +/- SEM. LICT: low intensity continuous training group (n=55). MICT+IT: moderate intensity continuous training plus interval training group (n = 60).

Predicting Change in BDI

Three linear regressions were conducted to examine the effects of changes in IL-1β (F(3, 105) = 1.343, p = 0.26, R^2adj = 0.009), IL-6 (F(3, 105) = 0.88, p = 0.46, R^2adj = -0.003), and TNFα (F(3, 105) = 1.5, p = 0.21, R^2adj = 0.014), changes in hippocampal volume, and exercise condition on changes in BDI. None of the predictors were statistically significant (p >0.05).

Change correlations

Similarly, changes in IL-6 (r_p = 0.06, p = 0.55), baseline IL-1β (r_p = 0.05, p = 0.61), and baseline TNFα (r_p = -0.06, p = 0.52) were not significantly correlated with changes in hippocampal volume.

Changes in IL-6 (r_p = -0.06, p = 0.42), IL-1β (r_p = 0.1, p = 0.27), and TNFα (r_p = 0.12, p = 0.19) were also not significantly correlated with changes in BD. Changes in hippocampal volume were not significantly correlated with changes in BDI (r_p = -0.13, p = 0.17) (Table 3).

Table 3 Pearson's correlations between changes in BDI, hippocampal volume, IL-1β, IL-6, and TNFα. ^ indicates p <0.1, *** indicates p <0.001.

	IL-1β	IL-6	TNFα	Hippocampal Volume
IL-6	0.17^			
TNFα	0.65***	0.34***		
Hippocampal Volume	0.05	0.06	-0.06	
BDI	0.1	-0.08	0.12	-0.13

Note. ^ indicates p < 0.1, *** indicates p <0.001

Exploratory analyses

SNP genotype and cytokine regulation

At baseline, no significant differences in peripheral plasma concentrations of IL-1β were found between G-carrying and non-G-carrying individuals of the rs16944 SNP (p>0.05). No significant differences were found in peripheral plasma concentrations of IL-6 or TNFα between A-carrying and non-A-carrying individuals of the rs1800797 or rs1800629 SNPs respectively (Table 4).

Table 4. Independent t-tests of baseline concentrations of IL-1β, IL-6, and TNFα and baseline BDI and hippocampal volume between allele carrier groups for the rs16944, rs1800797, and rs1800629 SNPs.

	rs16944		rs1800629		rs1800797	
	G-carrier	Non-G-carrier	A-carrier	Non-A-carrier	A-carrier	Non-A-carrier
IL-1β	4.02 ± 3.72	5.51 ± 3.49				
TNFα			3.66 ± 3.16	3.29 ± 2.27		
IL-6					4.1 ± 2.08	3.5 ± 2.63

BDI	8.52 ± 6.8	7.6 ± 4.4	9.93 ± 7.67^	7.41 ± 5.85^	8.57 ± 6.6	7.56 ± 6.13
Hippocampal Volume/ICV	0.0050 ± 0.00085	0.0048 ± 0.00093	0.0052 ± 0.00095	0.0049 ± 0.0078	0.0050 ± 0.00077	0.0050 ± 0.00095

Note. ^ indicates $p < 0.1$

A series of 2x2 exercise condition by allele carrier status ANOVAs for the rs16944, rs1800797, and rs1800629 SNPs were conducted with changes in IL-1β, IL-6, and TNFα as the dependent measures in three separate analyses. No significant main effects of exercise condition or allele carrier status were found, nor were any significant exercise condition x genotype interactions observed (p >0.05) (Figure 6).

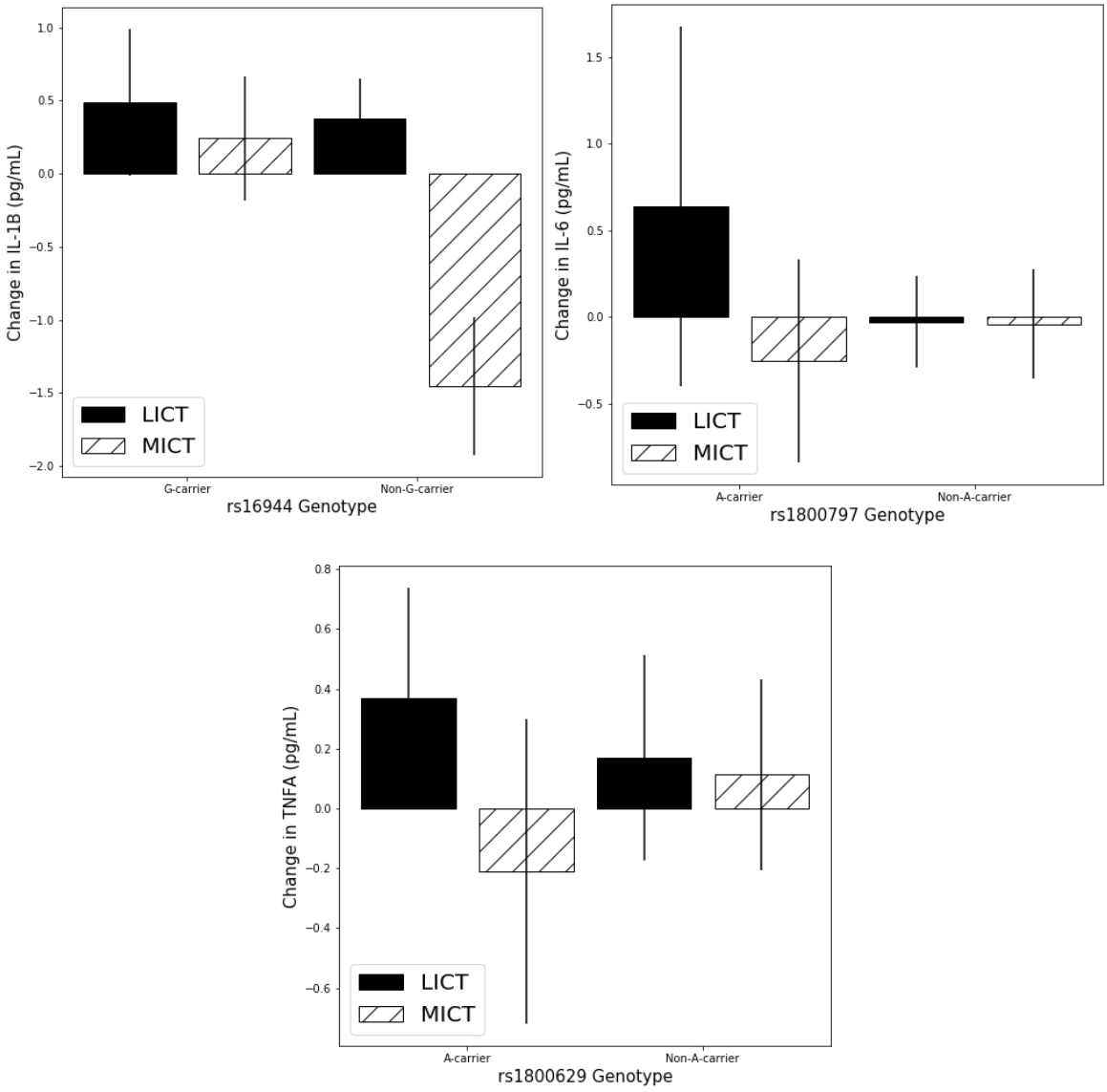


Figure 6 2x2 Exercise condition by SNP allele ANOVAs on mean change in cytokines. Data are presented as mean +/- SEM **A.** No significant main effects of rs16944 G carrier status or exercise, and no significant interaction on changes in peripheral concentrations of IL-1β. **B.** No significant main effects of rs1800797 A carrier status or exercise, and no significant interaction on changes in peripheral concentrations of IL-6. **C**. No significant main effects of rs1800629 A carrier status or exercise, and no significant interaction on changes in peripheral concentrations of TNFα. LICT: low intensity continuous training group (n=55). MICT+IT: moderate intensity continuous training plus interval training group (n = 60).

SNP genotype and hippocampal volume

At baseline, no differences in hippocampal volume were found between A-carriers and non-A carriers of the rs1800629 and rs1800797 SNPs or between G-carriers and non-G-carriers of the rs16944 SNP (Table 4).

rs16944

In a 2x2 rs16944 G-allele carrier status by exercise condition ANOVA, a significant main effect of exercise condition on change in hippocampal volume ($F(3, 89) = 6.72$, $p<0.05$) was found, indicating that MICT+IT exercisers ($M = -2.69x10^{-5}$, $SD = 0.00016$) had a lesser decrease in hippocampal volume than did LICT exercisers ($M = -6.13x\ 10^{-5}$, $SD = 0.00018$) (Figure 7A).

A linear regression was calculated to examine the effects of changes in IL-1β, rs16944 G-allele carrier status, exercise condition, and exercise by rs16944 interaction, on changes in hippocampal volume. Exercise condition was a significant predictor of change in hippocampal volume ($p <0.05$) and the predictors accounted for 3.6% of the variance in hippocampal volume change.

rs1800797

In a 2x2 rs1800797 A-allele carrier status by exercise condition ANOVA, no significant main effects or interactions where found on change in hippocampal volume. The main effect of exercise condition approached significance ($F(3, 93) = 3.90$, $p =0.051$) such that MICT+IT exercisers had marginally lesser decreases in hippocampal volume than did LICT exercisers (Figure 7B).

A linear regression was calculated to examine the effects of changes in IL-6, rs1800797 A-allele carrier status, exercise condition, and exercise by rs1800797 interaction on change in hippocampal volume. Exercise condition was again a significant predictor of change in hippocampal volume (p <0.05) and the predictors accounted for 2.2% of the variance in hippocampal volume change.

rs1800629

In a 2x2 rs1800629 A-allele carrier status by exercise condition ANOVA on change in hippocampal volume, a significant main effect of exercise was found ($F(3,99) = 4.65$, p <0.05). MICT+IT exercisers ($M = -2.69x10^{-5}$, SD = 0.00016) had a lesser decrease in hippocampal volume than did LICT exercisers ($M = -6.13x\ 10^{-5}$, SD = 0.00018). A significant interaction was found between rs1800629 A-carrier status and exercise condition on changes in hippocampal volume ($F(3, 99) = 11.09$, p <0.01). LICT non-A-carriers had significantly larger decreases in hippocampal volume ($M = -1.1x10^{-4}$, SD = 0.00019) than did LICT A-carriers ($M = 3.42x10^{-5}$, $SD = 9.06x10^{-5}$) (p <0.05, Tukey HSD). MICT+IT non-A-carriers ($M = 1.31x10^{-5}$, $SD = 1.25x10^{-4}$) (p <0.05, Tukey HSD) had significantly smaller decreases in hippocampal volume than did LICT non-A-carriers ($M = -1.09x10^{-4}$, $SD = 1.86x10^{-4}$) (Figure 7C).

A linear regression was calculated to examine the effects of changes in TNFα, rs1800629 A-allele carrier status, exercise condition, and exercise by rs1800629 interaction on change in hippocampal volume. The interaction between SNP genotype and exercise condition (p <0.01) and the main effect of rs1800629 A-carrier status (p <0.01) significantly predicted changes in hippocampal volume. These predictors accounted for 10.9% of the variance in hippocampal volume change.

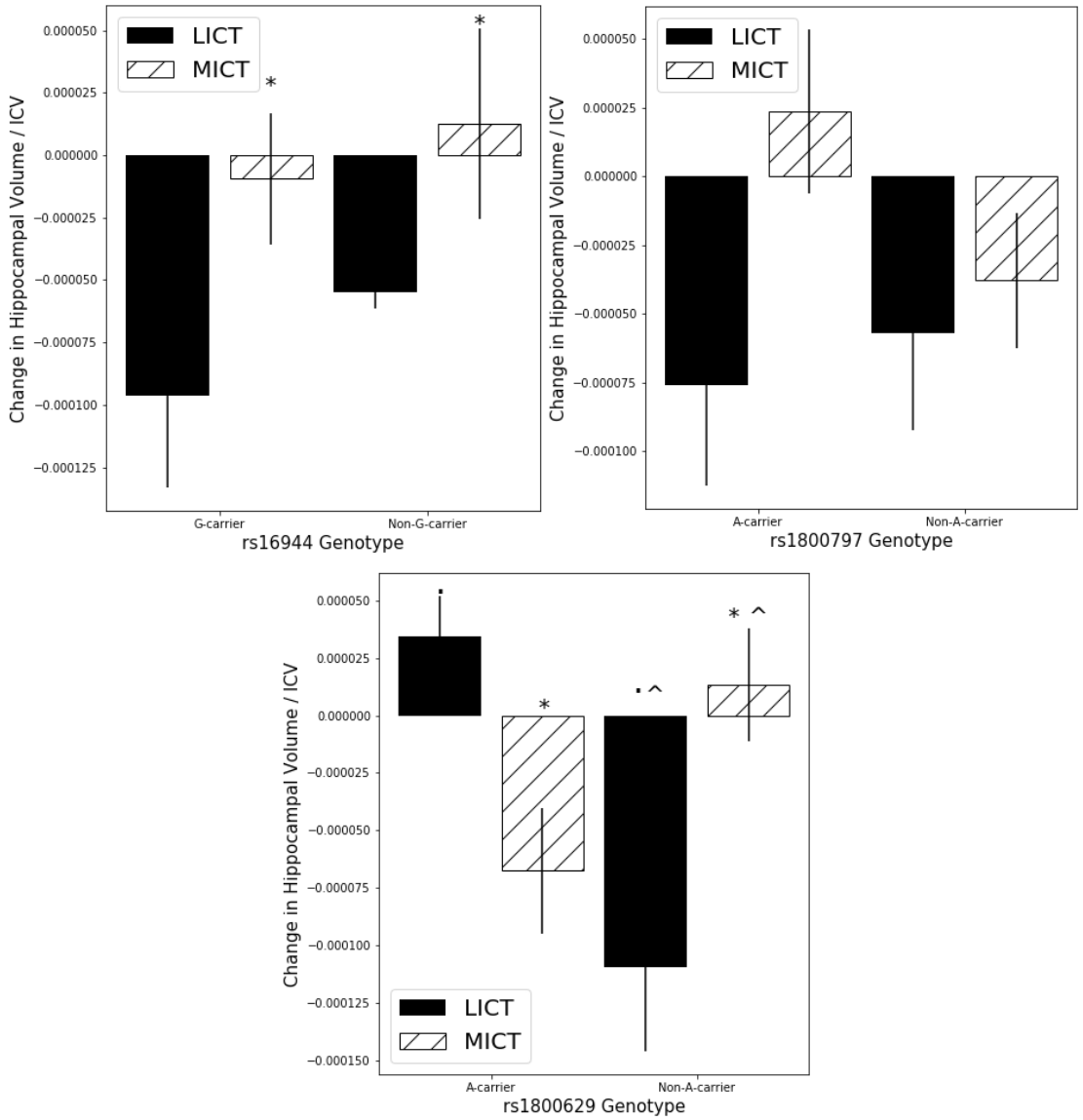


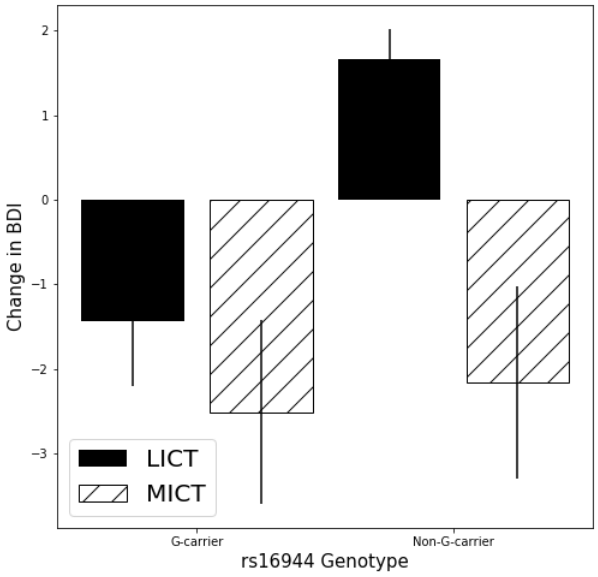
Figure 7 2x2 Exercise condition by SNP allele ANOVAs on mean change in hippocampal volume/ICV. Data are presented as mean +/- SEM **A.** Change in hippocampal volume by exercise condition and rs16944 G allele carrier status * indicates difference between MICT+IT and LICT exercise groups *p<0.05* ***B.*** Change in hippocampal volume by exercise condition and rs1800797 A allele carrier status **C**. Change in hippocampal volume by exercise condition and rs1800629 A allele carrier status. * indicates LICT vs. MICT+IT differences (*p<0.05),* . indicates LICT genotype differences (*p* <0.01), and ^ indicates significant exercise condition by genotype differences (*p* <0.01). LICT: low intensity continuous training group (n=55). MICT+IT: moderate intensity continuous training plus interval training group (n = 60).

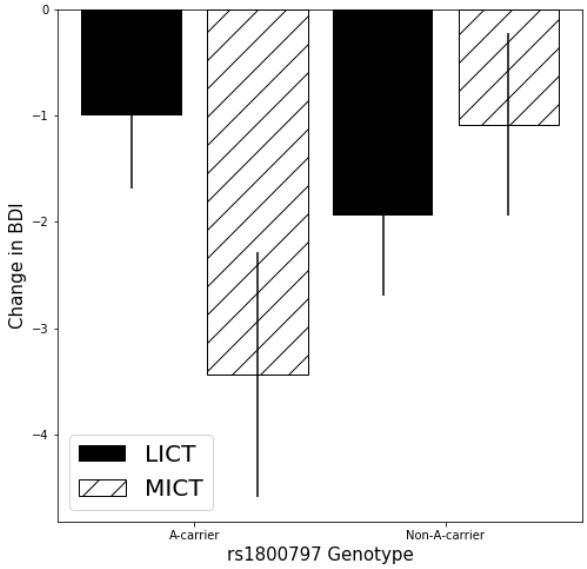
SNP genotype and depression

At baseline, no differences in BDI scores were found between A-carriers and non-A carriers of the rs1800629 or rs1800797 SNPs or between G-carriers and non-G-carriers of the rs16944 SNP (Table

4). rs1800629 A-carriers had marginally significantly higher BDI scores than rs1800629 non-A-carriers (p = 0.078), contrary to Cerri et al.

A series of 2x2 exercise condition by allele carrier status ANOVAs for the rs16944, rs1800797, and rs1800629 SNPs were conducted on BDI change. No significant main effects of exercise condition or allele carrier status were found, nor were any significant interactions between exercise and allele observed (p >0.05) (Figure 7). The interaction between rs1800797 A allele carrier status and exercise condition approached significance (F(3, 93) = 3.25, p = 0.075) (Figure 8).





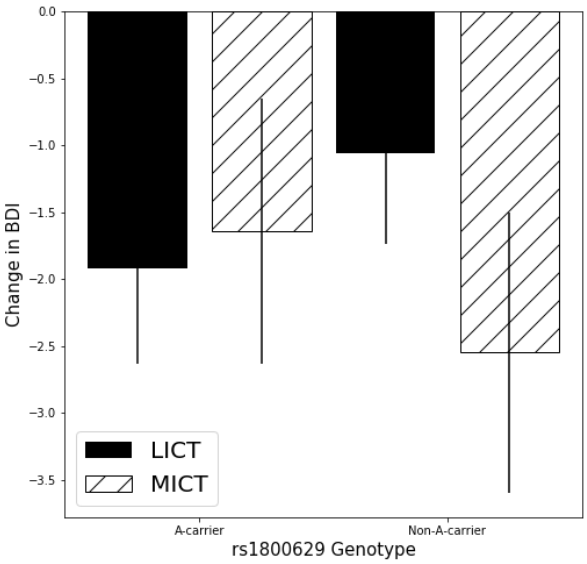


Figure 8 2x2 Exercise condition by SNP allele ANOVA on mean change in BDI. Data are presented as mean +/- SEM **A.** No significant main effects of rs16944 G carrier status or exercise or interactions on change in BDI. **B.** No significant main effects of rs1800797 A carrier status or exercise or interactions on change in BDI. **C**. No significant main effects of rs1800629 A carrier status or exercise or interactions on change in BDI. LICT: low intensity continuous training group (n=55). MICT+IT: moderate intensity continuous training plus interval training group (n = 60).

For each of the three cytokines of interest (IL-1β, IL-6, and TNFα), a regression was calculated to predict changes in depressive symptoms from SNP genotype for the cytokine, change in hippocampal volume, change in the cytokine of interest, exercise condition, and exercise condition by SNP genotype interaction. While changes in IL-1β ($p = 0.077$), exercise condition ($p = 0.059$), and the rs1800797 by exercise condition interaction ($p = 0.082$) approached significance, main effects of SNP genotype, exercise condition, the SNP by exercise interaction, change in hippocampal volume, or change in cytokine concentration were not significant predictors in any model.

Discussion

This study aimed to better understand the relationships between regulation of the pro-inflammatory cytokines IL-1β, IL-6, and TNFα, hippocampal neurogenesis, and depressive symptoms using a 16-week exercise intervention to simultaneously manipulate each of these variables. Two exercise groups engaging in either a low intensity continuous training (LICT) or moderate intensity continuous training with interval training (MICT+IT) program were studied in order to examine any differences between moderate and low intensity aerobic exercise.

Contrary to prior research showing that chronic exercise has a net anti-inflammatory effect, the exercise intervention did not significantly alter peripheral plasma concentrations of any of the cytokines of interest, nor were any differences observed in changes in cytokine concentrations between the LICT and MICT+IT exercise groups. Previous research has indicated that aerobic exercise promotes neurogenesis in rodents and increases hippocampal volume in humans. In contrast to this literature, we found a decrease in hippocampal volume adjusted for ICV from pre- to post-intervention, with no differences in changes in hippocampal volume between MICT+IT and LICT conditions. As there were no

age-matched controls that did not change their exercise habits over 16 weeks, it is unclear whether this decrease in hippocampal volume is an artifact of the normal aging processes or exercise dependent. In line with previous work, a significant decrease in depressive symptoms was noted over the course of the intervention, but no difference in changes in depression was observed between exercise conditions. These results provide an inconsistent picture considering previous research documenting the inflammatory, neuroanatomical, and psychological benefits of exercise.

Associations between cytokines, hippocampal volume, and BDI were expected pre-intervention in light of previous evidence suggesting links between them. Aligned with previous research suggesting that elevated IL-6 could serve as a biomarker for depression, IL-6 was significantly positively correlated with depressive symptoms at baseline. However, neither TNFα nor IL-1B were correlated with depressive symptoms, and none of these inflammatory markers were significantly correlated with hippocampal volume as would be expected if these cytokines work to impede hippocampal neurogenesis. It was further expected that changes in depressive symptoms and hippocampal volume would vary over time in association with changes in cytokines, but no evidence of such a relationship was found. Finally, it was hypothesized that changes in hippocampal volume would vary with changes in depressive symptoms, but no evidence was found of this relationship either. It is important to note that this was a non-clinical sample, and endorsement of depressive symptoms was therefore very low. Thus, restriction of range in the measure of depression may have impacted these findings.

As a holistic examination of how exercise modulates the tripartite system of interest, it was expected that changes in cytokines and exercise condition would predict changes in hippocampal volume and that changes in cytokines and hippocampal volume and exercise condition would predict changes in depressive symptoms, however no evidence of these connections was found. These results fail to support the idea that cytokines impact depressive symptoms through mediation of hippocampal neurogenesis.

Several exploratory analyses were conducted to better understand individual variation in depressive outcomes. Despite evidence that several of the SNPs assayed influence cytokine regulation and

susceptibility to depression, no differences were found in depressive symptoms or cytokine concentrations by genotype, nor was any main effect of exercise condition or SNP x exercise condition interaction found. Although no prior study has indicated differences in hippocampal volume between carriers of different alleles for the three SNPs, it was expected that the SNPs' effects on cytokine regulation would translate to altered rates of neurogenesis and thus differences in hippocampal volume. No such differences in hippocampal volume by SNP genotype were found. However, main effects of exercise condition were found in two of the three SNPs such that MICT+IT exercisers had smaller decreases in hippocampal volume than did LICT exercises. Further, there was a significant rs1800629 by exercise interaction such that LICT A-carriers and MICT+IT non-A-carriers had lesser decreases in hippocampal volume than did LICT non-A-carriers and MICT+IT A-carriers. This result suggests that rs1800629 A-carrier status could be protective against hippocampal atrophy in combination with low intensity aerobic exercise, while non-A-carrier status could offer similar protection in combination with moderate intensity aerobic exercise coupled with interval training. The mechanism of this effect, however, remains unclear as neither exercise condition nor rs1800629 genotype had a significant effect on TNFα expression in plasma and TNFα appears the most likely mediator of hippocampal volume in the context of rs1800629.

Jointly, these results offer little support for the theory that cytokine regulation is an important mediator of depression through effects on hippocampal neurogenesis. However, this study had several limitations that should be noted. Chiefly, current MRI techniques are not yet able to determine whether changes in hippocampal volume are due to production and integration of new neurons. A better proxy for measuring neurogenesis could be MRI measurement of cerebral blood volume (CBV) in the SGZ, which Periera et al. have identified as a viable *in vivo* correlate for neurogenesis due to its relationship with angiogenesis (Pereira et al., 2007). Additionally, there was no age-matched control group that remained sedentary for 16 weeks. Both LICT and MICT+IT groups had decreases in hippocampal volume relative to ICV, begging the comparison to non-exercising individuals to assess the rate of normal, aging-related

decreases in hippocampal volume. Further, despite evidence of cytokine transport across the BBB and vagal and endothelial induction of central cytokine production, measurement of peripheral plasma cytokines is an inherently low-sensitivity measure when the true markers of interest are centrally-produced and centrally-acting cytokines. Measurement of cytokines in the cerebrospinal fluid, while a far more invasive procedure than a peripheral blood draw, could better explain the rs1800629 by exercise condition interaction on changes in hippocampal volume in the absence of rs1800629 by exercise condition regulation of TNFα in peripheral plasma. Combined with a larger sample size, more sensitive central cytokine measurements could further interrogate the significance of several of the trending effects noted in this study. The actions of cytokines are also currently considered more pleiotropic than strictly pro- or anti-inflammatory, and they often work synergistically in a tissue-dependent manner to produce their effects. No attempts to examine the interactions between TNFα, IL-1β, and IL-6 were made. Finally, several factors suggest unreliability of the cytokine dataset. These limitations suggest clear routes to test cytokine mediation of neurogenesis and depression with more sensitivity in future studies.

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