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Exercise as a potential modulator of inflammation in patients with Alzheimer's disease measured in cerebrospinal fluid and plasma

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ABSTRACT

Background: Neuroinflammation is recognized as part of the pathological progression of Alzheimer's disease (AD), but the molecular mechanisms are still not entirely clear. Systemically, physical exercise has shown to have a positive modulating effect on markers of inflammation. It is not known if this general effect also takes place in the central nervous system in AD. The aim of this study was to investigate the effect of 16 weeks of moderate to high-intensity physical exercise on selected biomarkers of inflammation both systemically and in the CNS, in patients with AD.

Methods: Plasma and cerebrospinal fluid (CSF) from 198 patients with Alzheimer's disease participating in the Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study were analyzed for concentrations of 8-isoprostane, soluble trigger receptor expressed on myeloid cells 2 (sTREM2), and the MSD v-plex proinflammation panel 1 human containing interferon gamma (IFNγ), Interleukin-10 (IL10), IL12p70, IL13, IL1β, IL2, IL4, IL6, IL8, and tumor necrosis factor alpha (TNFα), before and after a 16-week intervention with physical exercise, and we studied whether changes were modulated by the patients' *APOE* genotype.

Results: Most inflammatory markers remained unchanged after exercise. We found an increasing effect of 16 weeks of physical exercise on sTREM2 measured in CSF. Further, IL6 in plasma increased in the exercise group after physical exercise (mean relative change 41.03, SD 76.7), compared to controls (−0.97, SD 49.4). In a sub-analysis according to *APOE* genotype, we found that in ε4 carriers, exercise had a stabilizing effect on IFNγ concentration with a mean relative change of 7.84 (SD 42.6), as compared to controls (114.7 (SD 188.3), $p = 0.038$.

Conclusion: Our findings indicate an effect of physical exercise on markers of neuroinflammation in CSF measured by an increase in sTREM2 in patients with AD. Further, there may be a small inflammatory systemic effect related to physical exercise in patients with AD.

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1. Background

Physical activity (PA) has been shown to have a positive modulatory effect on systemic inflammation by reducing pro-inflammatory cytokines, increasing anti-inflammatory cytokines (Petersen and Pedersen, 2005; Pedersen, 2000), and modulating reactive oxygen species (ROS) both in young and older adults (Kohut et al., 2006; Woods et al., 2012; Pedersen and Bruunsgaard, 2003; Wärnberg et al., 2010; Radak et al., 2016). Inflammation is an important part of the pathophysiology of Alzheimer's disease (AD) (Cameron and Landreth, 2010; Calsolaro and Edison, 2016), however, the precise pathological mechanism behind the contribution of inflammation to the pathological progression of the disease is largely unknown. It is thought that prolonged stress activation of microglia due to amyloid β (Aβ) plaque formation, can result in the development of a harmful (inflammation-promoting) increase in cytokines, chemokines and ROS (Tozzi et al., 2018). Whereas longitudinal cohort studies show an association between higher levels of PA and a reduced risk of cognitive decline and dementia (Blondell et al., 2014), there is limited evidence for an effect of PA on AD biomarkers (Jensen et al., 2015; Steen Frederiksen et al., 2018). However, PA has been shown to slow down hippocampal atrophy in healthy elderly (Erickson et al., 2011), change specific AD biomarkers in a favorable way in healthy subjects at risk of AD (Law et al., 2018), and improve cognition in older adults with mild cognitive impairment (Baker et al., 2010). It is therefore likely that PA may slow down the clinical progression of AD by reducing harmful inflammation, promoting beneficial myokines and reducing systemic metabolic risk factors of AD, e.g., insulin resistance and hypercholesterolemia (Chakrabarti et al., 2015)

In this study, we wished to analyze the effect of 4 months of moderate to high-intensity physical exercise in patients with AD, on markers of different inflammatory processes in cerebrospinal fluid (CSF) and plasma. We employed a general pro-inflammatory cytokine panel, which also includes anti-inflammatory cytokines and growth factors. Furthermore, we chose a marker of microglia activation due to cellular stress, which has been linked to AD risk, soluble trigger receptor expressed on myeloid cells 2 (sTREM2) (Suárez-Calvet et al., 2016; Colonna and Wang, 2016; Cantoni et al., 2015) and 8-isoprostane, a marker of oxidative stress and lipid peroxidation (Praticò, 2010; Praticó et al., 2000). In addition, we determined the single nucleotide polymorphism (SNP) rs75932628-T, which has been linked to genetic higher levels of sTREM2 in CSF and higher risk of developing AD (Ruiz et al., 2014; Piccio et al., 2016).

We hypothesized that PA would have an upregulating effect on anti-inflammatory cytokines, and down-regulating effect on pro-inflammatory cytokines. Further, we analyzed whether PA exhibited modulating effects on markers of microglia activation and ROS, measured via sTREM2 and 8-isoprostane respectively. Finally, we analyzed whether the apolipoprotein E (*APOE*) genotype influenced the impact of PA on inflammation.

2. Material and methods

2.1. Study population

One hundred and nighty eight home-dwelling patients with clinically diagnosed mild AD according to NINCDS-ADRDA criteria (McKhann et al., 1984) and an MMSE > 19 were enrolled in the multicenter ´Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise' or in short, the ADEX study. For a detailed description of the

Table 1
Baseline characteristics of the study cohort. A) All patients. B) CSF sub-group. ¥ Given as mean ± (standard deviation), #independent *t*-test, ¤ Chi-squared test, € controls versus exercise, $ controls versus high exercise. From the exercise group, participants who participated > 80% of the session and had a mean intensity of 70% or high, was further analyzed as a '*high exercise group*'.

A) Whole group	Controls (n = 92)	Exercise (n = 106)	p-Value€	High exercise sub-group (n = 66)	p-Value$
Age, years¥	71.4 (7.3)	69.9 (7.5)	0.14#	70.2 (7.5)	0.31#
Gender, n			0.13¤		0.41¤
Males	57	56		34	
Females	35	50		32	
Characteristics					
Disease duration, years from diagnosis¥	1.3 (1.0)	1.0 (1.0)	0.1#	0.94 (0.9)	0.05#
MMSE¥	24.2 (3.9)	23.8 (3.4)	0.54#	24.1 (3.3)	0.96#
Education, years¥	11.8 (2.8)	11.9 (2.7)	0.84#	12.2 (2.7)	0.42#
Weight, kg¥	71.4 (12.1)	73.0 (13.5)	0.36#	72.5 (13.8)	0.18#
Height, cm¥	172.0 (8.9)	170.2 (9.1)	0.15#	170.1 (9.2)	0.18#
BMI¥	24.1 (3.6)	25.2 (4.0)	0.04#	23.0 (4.0)	0.12#
APOE ε4 alleles, n			0.35¤		0.22¤
0	21	34		19	
1	45	45		26	
2	26	27		21	

B) CSF subgroup	(n = 29)	(n = 29)		(n = 19)	
Age, years¥	68.8 (8.0)	68.83 (6.7)	0.83#	68.6 (7.0)	0.91#
Gender, n			0.17¤		0.36¤
Males	21	16		11	
Females	8	13		8	
Characteristics					
Disease duration, years from diagnosis¥	1.5 (1.1)	1.0 (0.9)	0.07#	1.2 (1.0)	0.25#
MMSE¥	25.1 (3.9)	25.4 (2.8)	0.70#	25.1 (2.6)	0.99#
Education, years¥	13.0 (2.9)	12.7 (2.8)	0.62#	12.5 (2.5)	0.54#
Weight, kg¥	73.3 (2.4)	73.3 (13.0)	0.51#	75.4 (16.5)	0.63#
Height, cm¥	174.8 (8.8)	172.5 (9.0)	0.33#	172.8 (9.3)	0.45#
BMI¥	23.9 (3.5)	25.4 (4.2)	0.15#	25.1 (4.1)	0.28#
APOE ε4 alleles, n			0.25¤		0.21¤
0	13	7		3	
1	10	14		9	
2	6	8		7	

study, please see (Hoffmann et al., 2013). In short, patients with mild to moderate AD were randomized to either a control group with treatment as usual or a 16-week 60 min three times per week moderate-to-high intensity aerobic physical exercise (treadmill, stationary bike, cross trainer) group. Subjects were tested at baseline and at 16-week follow-up with a comprehensive battery of tests of cognitive function, Activities of Daily Function, Quality of Life, physical activity and neuropsychiatric symptoms.

Primary and secondary clinical outcomes have previously been published showing an effect of physical exercise on neuropsychiatric symptoms as well as on cognitive and physical function (Hoffmann et al., 2015; Sobol et al., 2016). Before and after the intervention, blood samples were collected from all subjects, and CSF was collected from a subgroup of patients. The baseline characteristics of the whole study population and the CSF subgroup are shown in Table 1.

2.2. Samples

A total of 198 patients participated in the study, all gave blood samples, and 58 patients donated CSF samples. Due to technical reasons, not all analytes were measured in all the patients. CSF sTREM2 was measured in 55 samples (27 controls and 28 exercises). Paired plasma and CSF cytokine profile was measured in 51 samples (27 controls and 24 exercises) with the MSD v-plex proinflammation panel 1 human. CSF 8-isoprostane was measured in 55 samples (28 controls and 27 exercises) and in 187 plasma samples (87 controls and 100 exercises). All samples were collected and processed according to international guidelines (Teunissen et al., 2014).

2.3. Assays

Commercially available kits were used for measuring the concentrations of plasma and CSF cytokines containing interferon gamma (IFNγ), Interleukin-10 (IL10), IL12p70, IL13, IL1β, IL-2, IL4, IL6, IL8, tumor necrosis factor alpha (TNFα) (MSD v-plex proinflammation panel 1 human, Meso Scale Discovery, MA, USA), and plasma and CSF 8-isoprostane (8-isoprostane ELISA kit, Cayman Chemicals, MI, USA) by following the manufacturers' enclosed procedure, in technical duplicates.

CSF sTREM2 was measured with an in-house MSD assay. In short, to streptavidin-coated 96-well plates (Meso-Scale discovery (MSD) a biotinylated polyclonal goat anti-human TREM2 capture antibody (0.25 μg/ml R&D Systems BAF1828) was bound, and subsequently incubated with the CSF or a standard protein solution constructed from recombinant human TREM2 protein (4000–62.5 pg/ml Sino Biological Inc. 11084-H08H). Further, a detector antibody, monoclonal mouse anti-human TREM2 antibody (1 μg/ml Santa Cruz Biotechnology; B-3, sc373828), was added, and thereafter incubated with a secondary antibody (SULFO-TAG–labeled anti-mouse secondary antibody, MSD). Lastly, the plates were developed by adding MSD Read buffer and the light emission measured using the MSD SECTOR Imager 6000. The concentration of sTREM2 was calculated using a five-parameter logistic curve fitting method with the MSD Workbench software package. Intra-assay coefficients of variation (CVs) were < 10%, and all samples were measured on the same day using the same reagents. *TREM2* genotype for SNP rs75932628-T was analyzed using TaqMan assay (TaqMan SNP assay MTO human SMC_100657057_10, Life Technologies, CA, USA).

2.4. Concentration change calculations

The changes in concentration of the biomarkers measures, were presented as relative change from baseline and calculated as

$$\frac{[Follow\ up] - [Baseline]}{[Baseline]} * 100 = relative\ change\ from\ baseline$$

This calculation was used to take into account marginal levels at baseline (very low or very high levels). Further, it gives more emphasis on small changes in individuals with smaller baseline levels, than small changes in individuals with higher baseline levels. As a result, relative change from baseline < 0, equals lower concentration at follow up. Relative change from baseline = 0, equals no difference concentration at follow up compared to baseline. Relative change from baseline > 0, equals higher concentration at follow up.

Table 2
Changes in plasma and CSF biomarkers after 16 weeks of intervention. Baseline and follow-up values of sTREM2, INFγ, IL10, IL6, IL8, IL13, IL2, TNFα, and 8-isoprostane. In addition, analysis of between group difference in mean change from baseline exercise versus controls and per-protocol high-exercise versus controls. [¥] Given as mean ± (standard deviation). [#] A positive value means greater positive mean change from baseline in the exercise group.

	Baseline[¥]		16 week follow up[¥]		Mean relative change from baseline, ((16 week follow up – baseline) / baseline) * 100, [¥,#]				
pg/mL	Controls	Exercise	Controls	Exercise	Controls	Exercise	*p*-Value	High exercise	*p*-Value
	(*n* = 27)	(*n* = 28)	(*n* = 24)	(*n* = 23)	(*n* = 22)	(*n* = 22)		(*n* = 16)	
sTREM2, CSF	8172.51 (2656.2)	8193.32 (2992.5)	7717.16 (2488.7)	8596.5 (3267.5)	−2.4 (13.8)	5.0 (12.9)	0.075	6.9 (14.0)	0.05
	(*n* = 27)	(*n* = 24)	(*n* = 27)	(*n* = 19)	(*n* = 26)	(*n* = 19)		*(n = 14)*	
IFNγ, CSF	0.6 (0.2)	0.5 (0.2)	0.6 (0.1)	0.5 (0.2)	9.0 (34.0)	1.6 (52.8)	0.57	−4.1 (51.9)	0.34
IL10, CSF	0.1 (0.0)	0.1 (0.1)	0.1 (0.1)	0.1 (0.6)	13.5 (52.4)	18.0 (67.5)	0.80	15.2 (70.8)	0.93
Il13, CSF	0.7 (0.5)	0.7 (0.3)	0.7 (0.3)	0.8 (0.5)	7.9 (68.7)	55.8 (115.4)	0.09	48.1 (107.2)	0.16
IL2, CSF	0.2 (0.2)	0.2 (0.12)	0.1 (0.1)	0.1 (0.1)	11.7 (96.1)	4.6 (69.3)	0.79	−0.1 (78.4)	0.70
IL6, CSF	1.2 (0.4)	1.1 (0.3)	1.3 (0.6)	1.1 (0.3)	21.7 (50.1)	1.9 (28.2)	0.13	3.5 (29.5)	0.22
IL8, CSF	44.8 (13.0)	42.4 (9.2)	45.1 (15.4)	40.7 (10.5)	2.7 (24.7)	−5.0 (16.6)	0.25	−2.5 (15.9)	0.48
TNFα, CSF	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	4.5 (31.9)	−4.7 (38.8)	0.39	−6.8 (41.5)	0.34
	(*n* = 23)	(*n* = 24)	(*n* = 23)	(*n* = 21)	(*n* = 19)	(*n* = 21)		*(n = 14)*	
IFNγ, plasma	5.1 (3.4)	4.9 (2.2)	5.0 (3.2)	5.2 (2.2)	61.4 (144.6)	22.1 (63.7)	0.27	11.6 (44.2)	0.22
IL10, plasma	0.3 (0.2)	0.3 (0.2)	0.5 (0.8)	0.3 (0.2)	6.2 (58.6)	31.2 (95.6)	0.33	35.2 (109.8)	0.33
IL6, plasma	2.9 (7.4)	0.7 (0.3)	3.3 (8.6)	0.8 (0.3)	−1.0 (49.4)	41.0 (76.7)	0.049	48.0 (85.8)	0.047
IL8, plasma	4.8 (1.6)	4.8 (1.4)	5.6 (2.3)	5.4 (1.5)	27.9 (65.5)	20.7 (38.9)	0.67	25.4 (41.7)	0.90
TNFα, plasma	1.6 (0.6)	1.7 (0.4)	1.8 (0.6)	1.7 (0.4)	33.2 (83.1)	1.2 (39.6)	0.12	−3.7 (41.9)	0.14
	(*n* = 28)	(*n* = 27)	(*n* = 23)	(*n* = 22)	(*n* = 20)	(*n* = 22)		*(n = 15)*	
8-Isoprostane, CSF	8.7 (4.0)	9.93 (5.4)	8.97 (3.6)	16.28 (4.1)	33.2 (99.8)	80.1 (192.4)	0.33	95.4 (225.9)	0.28
	(*n* = 87)	(*n* = 100)	(*n* = 80)	(*n* = 90)	(*n* = 75)	(*n* = 86)		*(n = 55)*	
8-Isoprostane, plasma	70.2 (94.0)	68.4 (86.8)	70.8 (88.9)	60.3 (75.8)	213.9 (582.7)	109.9 (354.2)	0.17	98.8 (296.7)	0.18

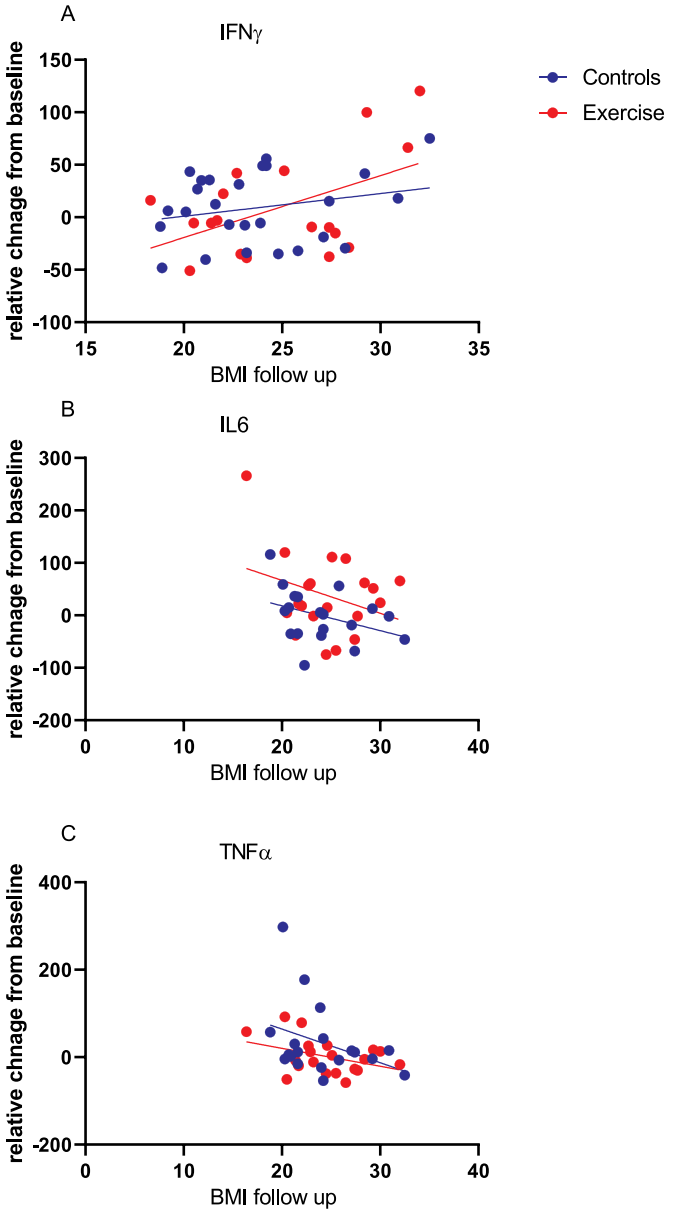

Fig. 1. correlation between BMI at follow up and relative change in biomarkers, with randomization groups as a covariate A) relative change in CSF IFNγ, B) relative change in plasma IL6, and C) relative change in plasma TNFα.

2.5. Statistics

The baseline characteristics were compared between the two groups using *t*-tests. The baseline and follow up concentrations were analyzed using paired *t*-test within each group respectively, and the mean relative change from baseline in biomarker concentrations was analyzed by Student's *t*-test. These analyses were done both as an intention to treat (ITT) analysis where all participants were analyzed, and as a per-protocol analysis where only the subjects that exercised with a mean intensity of > 70% of maximal heart rate (HR) and attended > 80% of the sessions, were included, this sub-group is called high exercise group, and consists of 66 subjects from the exercise group (Hoffmann et al., 2013; Hoffmann et al., 2015). Additionally, linear regression between BMI at follow up and change from baseline was performed for the cytokine panel. Furthermore, the *APOE* genotype contribution to the effect on the biomarkers was analyzed in non-carries versus carriers of the AD risk relevant ε4 allele. The significance level was set to 0.05 and outlier analysis was performed.

3. Results

Baseline characteristics in the control group, exercise group, and high exercise sub-group were comparable, as shown in Table 1. Body Mass Index (BMI) was slightly, but significantly higher in controls versus exercise group, and disease duration in the high exercise sub-group was slightly shorter as compared to the control group. Table 1B displays the baseline characteristics of the groups and high exercise sub-group with CSF samples and in these sub-groups, no differences were found between controls and exercise subjects. Further, there was no difference in baseline levels of any of the analytes when comparing controls to exercise, and controls to the high exercise group (data not shown).

Over all, most of the analyzed biomarkers were not affected by PA, and remained stable over the intervention period, see Table 2. However, when analyzing relative change from baseline, the IL6 plasma concentration increased significantly in the exercise group compared to the control group with a mean change from baseline at 41.03 (SD 76.7) compared to −0.97 (SD 49.4) in the control group ($p = 0.049$). When analyzing the high exercise group vs controls, the same outcome was found with a mean change of 48.01 (SD 85.8) and –0.97 (SD 49.4), respectively ($p = 0.047$). Similar results with borderline significance were found for sTREM2 in CSF. Here, sTREM2 mean relative change from baseline was 6.9 (SD 14.0) in the high exercise group compared to −2.4 (SD 13.8) in controls, ($p = 0.05$). *TREM2* SNP assay showed no genotype that corresponds with genetically altered sTREM2 levels.

Furthermore, analysis whether BMI at follow up and relative change from baseline were correlated showed significant correlations between BMI and relative change in IFNγ in CSF and in TNFα in plasma. Further analysis with exercise as a groups variable showed that in CSF IFNγ the correlation was due to BMI and independent of randomization group ($p = 0.017$). The same result was in plasma TNFα ($p = 0.03$). In plasma IL6 there was not an overall correlation between BMI and relative change in IL6 however, analysis with groups variable showed a significant effect of exercise and not BMI ($p = 0.04$) on the relative change from baseline, see Fig. 1.

In addition, when stratifying for *APOE* genotype in carriers and non-carriers of the ε4 allele, we found that plasma IFNγ concentration increased more in controls who were ε4 carriers (114.7 [SD 188.3] compared to 7.8 (SD 42.8) in the ε4-positive exercise group, $p = 0.038$). The opposite was observed when analyzing sTREM2 based on *APOE* genotype. Here, there was an increase in exercising *APOE* ε4 carriers, 5.7 (14.4), compared to a decrease in the controls −6.4 (15.5), $p = 0.037$, see Table 3.

Difference in patient numbers in the reported results, and in follow up versus mean relative change from baseline, is due to samples falling below limit of detection in either baseline or follow up measurements, or due to technical reasons e.g. lysis of the sample.

4. Discussion

In this explorative sub-study, we investigated the effect of an intervention of 16 weeks of moderate-to-high intensity exercise in patients with AD on selected biomarkers of inflammation measured in plasma and CSF, to attempt to elucidate the biochemical effect of PA, as several large population-based studies have shown that greater physical exercise is associated with lower levels of inflammation (Beavers et al., 2010). We have previously published the effect on cognition (Hoffmann et al., 2015) and physical measures (Sobol et al., 2016). Here, we have measured a variety of known biomarkers of inflammation to investigate molecular underpinnings of the previously reported beneficial effect on cognition and physical parameters in this population. The approach resulted in many negative results, but we did find some moderate modulating effects of our intervention on a number of inflammatory proteins. Besides this study we have published our results on cognition, neuropsychiatric (Hoffmann et al., 2015), physical parameters (Sobol

Table 3
The effect of exercise on biomarker concentrations, stratified according to ApoE genotype. Group differences in mean change from baseline high exercise versus control depending of *ApoE* ε4 carrier status (0 alleles versus 1 or 2 alleles). [¥] Given as mean ± (standard deviation). [#] A positive value means greater positive mean change from baseline in the exercise group.

	APOE e4 non-carriers			APOE e4 carriers		
	Mean relative change from baseline, ((16 week follow up – baseline) / baseline) * 100[¥,#]					
pg/mL	Controls	Exercise	*p*-Value	Controls	Exercise	*p*-Value
	(*n* = 9)	(*n* = 5)		(*n* = 13)	(*n* = 17)	
sTREM2, CSF	3.5 (8.1)	2.5 (3.89)	0.81	−6.4 (15.5)	5.7 (14.4)	0.037
	(*n* = 12)	(*n* = 4)		(*n* = 14)	(*n* = 15)	
IFNγ, CSF	12.6 (38.1)	−2.4 (42.2)	0.52	5.8 (31.1)	9.4 (51.1)	0.82
IL10, CSF	25.4 (66.5)	23.6 (74.6)	0.96	3.3 (36.3)	16.5 (68.2)	0.53
Il13, CSF	29.1 (73.8)	62.3 (117)	0.31	−10.3 (61.0)	46.3 (103.4)	0.08
IL2, CSF	45.2 (129.9)	18.1 (40.4)	0.69	−17.0 (40.0)	1.0 (75.9)	0.44
IL6, CSF	39.3 (59.2)	−14.7 (26.8)	0.11	5.6 (36.3)	6.3 (27.9)	0.98
IL8, CSF	1.9 (21.8)	−11.6 (21.4)	0.30	3.4 (27.7)	−3.3 (15.5)	0.43
TNFα, CSF	16.2 (38.8)	−9.1 (33)	0.26	−5.6 (21.0)	2.9 (33.3)	0.42
	(*n* = 10)	(*n* = 5)		(*n* = 9)	(*n* = 16)	
IFNγ, plasma	13.5 (72.1)	67.9 (100.2)	0.24	114.7 (188.4)	7.8 (42.8)	0.038
IL10, plasma	−3.6 (45.5)	35.46 (68.4)	0.21	17.1 (71.7)	29.8 (104.4)	0.75
IL6, plasma	−17.15 (47.4)	1.4 (71.3)	0.56	17.0 (47.7)	53.4 (76.4)	0.21
IL8, plasma	31.2 (80.6)	3.0 (23.3)	0.46	24.2 (48)	26.2 (41.6)	0.91
TNFα, plasma	26.6 (68.3)	1.3 (46.3)	0.47	40.5 (101.1)	1.1 (39.2	0.17
	(*n* = 10)	(*n* = 5)		(*n* = 10)	(*n* = 17)	
8-Isoprostane, CSF	9.7 (94.9)	139.0 (143.8)	0.058	56.5 (101.5)	62.8 (204.9)	0.93
	(*n* = 19)	(*n* = 31)		(*n* = 56)	(*n* = 55)	
8-Isoprostane, plasma	279.0 (492.1)	179.9 (479.4)	0.48	191.8 (612.9)	71.0 (255.9)	0.18

et al., 2016; Sobol et al., 2015), and other relevant biomarkers (Jensen et al., 2017; Steen Jensen et al., 2016)

We found PA to increase the levels of sTREM2 in CSF and IL6 in plasma. In a subgroup analysis of *APOE* ε4 carriers, plasma IFNγ increased in the control group whereas it remained unchanged in the exercise group. Additionally, in *APOE* ε4 carriers, sTREM2 increased in the exercise group, while decreased in the control group. Furthermore, analysis of BMI correlated with relative change from baseline with randomization groups as a covariate showed, that some of the relative change from baseline detected was due to increased BMI. Both negatively and positively. However only a very small percentage (ranging from 13 to 18%) of the relative change from baseline in the biomarkers could be explained by BMI.

Inflammation has been established as an important contributor to AD pathology (Ransohoff, 2016). It is however not clear if inflammation is the cause or the outcome of pathological Aβ plaque accumulation, in other words, whether Aβ plaques cause inflammation or whether neuroinflammation increases Aβ buildup (Bagyinszky et al., 2017). Nonetheless, markers of inflammation that can be measured in CSF and even plasma have the potential to aid in diagnosis, monitor disease progression and help to understand the beneficial effects of treatment strategies (Galasko and Montine, 2010; Thambisetty and Lovestone, 2010).

Many previous studies have found altered cytokine levels in patients with AD. IL1 has been investigated in brains from AD patients and in blood, where increased levels in brain and blood have been linked to Aβ formation (Griffin et al., 1989; Licastro et al., 2000). Furthermore, increased levels of other members of the IL1 family, IL18, IL33, and IL12 have been linked to increased AD risk (Su et al., 2016). In addition to findings of increased pro-inflammatory cytokines, treatments with the anti-inflammatory cytokine IL4 have shown reduced Aβ accumulation in AD mice models (Kawahara et al., 2012).

The cytokine IL6 has historically been viewed as a pro-inflammatory molecule, released in the acute phase of an infection. However, IL6 is increasingly being recognized to exhibit both pro-inflammatory as well as anti-inflammatory abilities, supported by the lack of induction of among others nitric oxide with IL6 release, as seen with the release of the pro-inflammatory cytokines IL1 and TNFα (Pedersen, 2000; Scheller et al., 2011). AD research has focused on IL6 both systemically and in the CNS. Blood levels of IL6 have been found to be increased in AD as compared to cognitively healthy controls, and brain tissues from AD patients stained for IL6 showed increased levels of IL6 adhesive to senile plaque inclusion (Licastro et al., 2000; Eriksson et al., 2011; Strauss et al., 1992). In relation to exercise and physical activity, studies have shown that exercise induces an acute phase response, similar to sepsis and trauma (Pedersen, 2000). Especially, IL6 is produced in high amounts in response to exercise in healthy subjects, with a rapid decline after end of the exercise (Petersen and Pedersen, 2005; Pedersen, 2000). In addition, IL6 is also produced locally in the muscles exhibiting growth factor properties (Pedersen, 2000). These findings are in line with the present results where we also see an increase in plasma IL6 in the exercise group, compared to controls, suggesting that exercise have similar effects on IL6 in AD as in healthy subjects.

IFNγ is a key player in the adaptive immune system, in promoting T1-helper cells and clear infections (Zhu and Paul, 2010). IFNγ has been shown to increase Aβ and tau pathology in a mouse model for AD (Janelsins et al., 2008), but on the other hand to improve cognition in AD patients (Tobinick et al., 2006). We found a significantly higher concentration at follow up in IFNγ among the *APOE* ε4 carriers in the control group. However, this result should be interpreted with great caution, due to the very small sample number, as it could be a false positive result. We would assume that if IFNγ is suppressed by PA in AD, we would see a change in both *APOE* ε4 carriers as well as non-carriers. However, studies in apoE$^{-/-}$ mice models, have shown that these mice have an increased immune response including increased IFNγ production (Getz and Reardon, 2009). Human *APOE* ε4 carriers have low plasma apoE protein levels (Rasmussen et al., 2015), similar to the apoE$^{-/-}$ mice, and studies have shown apoE to be tightly related to the immune system response (Zhang et al., 2010), suggesting that apoE protein helps suppress the activity of natural killer t-cells (NKT) cells, and the IFNγ release (Zhang et al., 2010). In other words, lower levels of apoE protein could result in elevated levels of IFNγ. This, it seems, is inhibited by exercise, displaying an anti-inflammatory effect of PA in *APOE* ε4 carriers, but not in non-carriers.

In the gene encoding TREM2, SNPs have been linked to AD risk, and studies have found conflicting results in the analysis of sTREM2 in CSF, some with higher amount of sTREM2 in CSF in patients with AD, and some with lower levels, compared to healthy controls (Suárez-Calvet et al., 2016; Heslegrave et al., 2016; Kleinberger et al., 2014; Finelli et al., 2015). We found that exercise increased levels of sTREM2, and that non-exercise controls had a decrease in sTREM2 levels over the 16 weeks of intervention. This difference was driven by the *APOE* ε4 genotype, as seen in our sub-analysis according to *APOE* genotype. As with the results found with IFNγ, the stratification in APOE genotype generates very small sample sizes, and the results should be interpreted with caution. Considering the conflicting findings of the previous studies, it is hard to conclude if the increase seen in sTREM2 in the *APOE* ε4 exercise group, is beneficial or harmful.

TREM2 expressed on the cell surface acts as an immune receptor, a soluble form of the protein sTREM2 can be cleaved from the cell surface under conditions of microglial activation and stress (Wunderlich et al., 2013). It has been found in mice studies that sTREM2 acts as a microglia activator through the Akt-GSK3β-β-cetenin pathway (Zhong et al., 2017). The elevated levels seen in AD, may be due to the need for an acute survival response for the microglia. However, later in the process of AD sTREM2 might be more harmful than useful. In addition to its proinflammatory function, sTREM2 has been shown to induce IL6 transcription in a mouse model (Zhong et al., 2017). One study even suggests that modulating sTREM2 levels could be an efficacious therapeutic approach to treat AD (Zhong et al., 2017). This is further underlined with the finding that loss of function mutation in the gene for TREM2, leads to the early onset dementia disease Nasu-Hakola disease (Piccio et al., 2016). In that case we could postulate that exercise may in fact be such a therapeutic approach, since the exercise group displays increased sTREM2 in CSF after 16 weeks of physical exercise.

A general limitation of this study is the size of the CSF group, and subgrouping these patients according to *APOE* ε4 genotype generated even smaller groups. The very small group sizes increase chances of a statistical type II error (Banerjee et al., 2009). This is only solved by having big sample sizes or very small variations in data (Biau et al., 2008). However, variations in biomarkers were high, due to biological or analytical variations. In addition, the disease stage may have impacted our results. Certain biomarker profiles may be abnormal in MCI stages and may change towards normal values as the disease progress into dementia stages (Duits et al., 2018), more over the level of inflammation may vary throughout the disease. This could indicate that physical exercise intervention studies should have the MCI stage as the target, as pathophysiological events may be more amenable to change at this stage. This is further emphasized by the findings from studies of PA, where effects on cognition generally have been larger in MCI subjects than in AD subjects (Öhman et al., 2014).

5. Conclusion

In conclusion, IL6 and sTREM2 were modulated in patients with AD by 16 weeks of PA. In addition, IFNγ was increased in *APOE* ε4 carriers who exercised. However, most other inflammatory markers in plasma and CSF remained unchanged following PA. The use of PA as a therapeutic approach for AD and other dementias is an attractive add-on to conventional medical treatment (Groot et al., 2016), but the underlying biochemical effects remain largely unknown (Jensen et al., 2015). Our study addresses part of this and reports promising pilot data on a number of inflammatory/microglia-related biomarkers from a hypothesis-generating explorative analysis, not corrected for multiple comparisons. More studies, preferably also examining effects of type and length of the physical exercise, are needed to replicate these findings and elucidate more of the molecular effects of PA in AD. In larger patient cohorts, it would be relevant to stratify according to disease severity and analyze the impact of PA on inflammation in subgroups with subjective cognitive impairment MCI, mild, moderate and severe AD.

List of abbreviations

AD	Alzheimer's disease
ADEX	Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's diseaseThe Effect of Physical Exercise
APOE	apolipoprotein E
CNS	central nervous system
CSF	cerebrospinal fluid
TREM2	trigger receptor expressed on myeloid cells 2
sTREM2	soluble trigger receptor expressed on myeloid cells 2
IFNγ	interferon gamma
IL	interleukin
TNFα	tumor necrosis factor alpha
APOE	apolipoprotein E
PA	physical activity
ROS	reactive oxygen species
Aβ	amyloid beta
SNP	single nucleotide polymorphism
NINCDS-ADRDA	National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association
MMSE	mini mental status examination
MSD	Meso Scale Discovery
ITT	intention to treat
HR	heart rate
BMI	body max index
SD	standard deviation
NKT	natural killer t cells
MCI	mild cognitive impaired
CV	coefficient of variation

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The authors have nothing to declare.

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