Cerebrospinal Fluid Amyloid Beta and Tau Concentrations Are Not Modulated by 16 Weeks of Moderate- to High-Intensity Physical Exercise in Patients with Alzheimer Disease

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Abstract

Introduction

Dementia is a growing epidemic affecting more than 45 million people worldwide, where Alzheimer's disease (AD) accounts for approximately 60% of all cases of diagnosed dementia [1]. AD is incurable and progressive and the symptoms of AD are loss of memory and other cognitive skills, as well as loss of ability to perform activities of daily living [2]. The pathological hallmarks of AD are the extracellular accumulation of the protein amyloid beta (A□) in plaques as well as the protein tau that accumulates inside the neurons as neurofibrillary tangles. Both deposits spread throughout the brain as the disease progresses [3].

The effect of pharmacological treatments has so far been limited, with no new drugs approved in the last decade [4]. Therefore there is a crucial need to investigate non-pharmacological treatment opportunities.

From animal studies we know that physical activity can improve cognitive performance [5] and even reduce the deposition of A□ in the brain [6–8], and previous human longitudinal cohort studies have established a connection between physical exercise and reduced risk of AD [9,10]. One non-pharmacological approach to reduce A□ in the human brain could therefore be to increase physical activity.

The 42 amino acid-long aggregation-prone A□ protein (Aβ42) is derived from amyloid precursor protein (APP), which is a type I transmembrane protein expressed in the cell membrane of neurons and several other cell types, by proteolytic processing involving β- and γ-secretase. This processing pathway is sometimes referred to as "the amyloidogenic pathway", which may be regarded a misnomer since it also gives rise to soluble 17-40 amino acid long Aβ forms, along with β-cleaved soluble APP (sAPPβ), all of which are secreted from the cell. APP can also undergo non-amyloidogenic processing by α-secretase, which results in the release of sAPPα, and by β- and α-secretase, which results in the release of 13-16 amino acid-long, soluble Aβ species that do not form plaques. (ref: Portelius E, Gustavsson MK, Zetterberg H, Andreasson U, Blennow K. Neurodegener Dis. 2012;10(1-4):138-40)

The stoichiometry of sAPP □ and □ have not been established, however it is speculated that it is generated in as 1:1 manner [14].

A potential mechanism for the treatment of AD could be a reduction in A□ plaque formation by modulating the APP processing by □□, □□ and □-secretases. We speculated that physical exercise could be such a modulator. In a multicentre randomized controlled trial, the Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study, we have recently established that physical exercise can alter the cognitive function and reduce neuropsychiatric symptoms in patient with mild AD [9, 10] [15]. However, the underlying molecular mechanisms are not known [16]. Our hypothesis was that moderate-to-high intensity physical exercise would increase sAPPα and soluble Aβ species and reduce CSF markers of neurodegeneration (T-tau and P-tau) in patients with clinically diagnosed mild AD.

Materials and methods

Participating subjects

Two-hundred community-dwelling patients with clinical diagnosed mild AD were included and randomized to either a control group with treatment as usual or a 16 week 60 min 3 times per week moderate-to-high intensity physical exercise group. All subjects donated a blood sample before and after intervention, and 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. For details of inclusion and exclusion criteria and methods, please see [9]. A subgroup of 44 subjects recruited from 3 of the 8 centers had a lumbar puncture performed to collect CSF samples before and after intervention. For this sub-study, only subjects with biomarker-positive AD were included (A□42<550 pg/ml, positive PiB-PET amyloid binding) (n=?).

Intervention

The physical exercise intervention used in this study was a aerobic exercise consisting of three weekly sessions of 1hour duration in groups of 2-5 participants, supervised by an experienced physiotherapist. The first four weeks of exercise consisted of an adaption period, and the subsequently 12 weeks participants performed aerobic exercise of moderate-to-high intensity (in each session a total 3*10 minutes on an ergometer bicycle, cross trainer and treadmill with 2-5 min rest in between). Heart Rate (HR) was monitored during aerobic exercise, including the rest intervals. Target intensity was 70-80% of maximal HR (220-age).

The participants assigned to the control group received treatment as usual.

Statistics

The baseline characteristics age, MMSE, weight, height and BMI, and the outcome measures at baseline, were compared between the two groups using independent t-tests. The difference between changes in outcomes from baseline to follow-up after 16 weeks of intervention between the groups, were analyzed in two independents analyses, an intention to treat (ITT) analysis where all participant were analyzed, and in a per protocol analysis (*high exercise subjects*), where only the subjects that exercised with a mean intensity of > 70% of maximal HR and attended >80% of the sessions were included. The significance level was set to 0.05.

Outcomes

The primary outcome measures were group differences in changes from baseline in the CSF concentration of A isoforms, tau, phosphorylated tau, and the two proteolytic isoforms of the A precursor protein APP and . Furthermore ApoE genotype for polymorphism 334 and 472 was included in analysis.

Sample material

CSF was collected from a total of 44subjects, before and after intervention. CSF was obtained by lumbar puncture in polypropylene tubes. After collection the CSF samples were centrifuged at 2000 g at 4C and aliquoted in to 250ul aliquots in cryotube, within 2 hours of collection, and frozen at -80C for later use as recommended [17].

Aβ Triplex

CSF concentrations of Aβ38, Aβ40 and Aβ42 were measured using V-plex Peptide Panel 1 Kits A38, A40, A42 (Meso Scale Discovery system, Rockville, MD, USA) according to the manufacturer's protocol. In brief, 60 ul of CSF was diluted 2-fold in diluent provided with the kit. Calibrators and controls were prepared according to protocol. On a capture antibody pre-coated 96 well MSD plate, 25 ul of sample, calibrator or controls were added, 25 ul of detection antibody, and incubated at room temperature for 2 hours. The plate was then washed in washing buffer provided with the kit, and read in a MSD imager at appropriate wavelength.

Total-tau

CSF T-tau was measured by the hTAU Ag ELISA assay (INNOTEST, Fujirebio, Japan) according to the manufacturer's protocol. On an anti-human tau antibody pre-coated 96 well microtiter plate, 25 ul samples, controls and standards ranging from 50-2500 pg/mL were added to the microtiter plate and incubated in each well with a biotinylated detection antibody to tau, followed by addition of peroxidase-conjugated streptavidin. The reaction was developed with tertamethyl benzidine (TMB) chromogen solution, and subsequently stopped with 0.9M sulfuric acid, and quantified at 450 nm in a microplate reader.

Phosphorylated-tau

CSF tau phosphorylated at amino acid 181 (p-tau) was measured by the PHOSPHO-TAU (181p) ELISA assay (INNOTEST, Fujirebio, Japan) according to manufacturer's protocol. On an anti-human p- tau antibody pre-coated 96 wells microtiter plate, 75 ul samples, controls and standards ranging from 15.6-1000 pg/mL were added to the microtiter plate and incubated in each well with a biotinylated detection antibody to p-tau, followed by addition of peroxidase-conjugated streptavidin. The reaction was developed with TMB chromogen solution, and subsequently stopped with 0.9M sulfuric acid, and quantified at 450 nm in a microplate reader.

Immunoprecipitation mass spectrometry of Aβ

Immunoprecipitation mass spectrometry (IP-MS) of the full spectrum of Aβ forms present in CSF was performed as previously described [18]. In brief, … Erik.

Aβ1-13/14/15/16 represent the non-amyloidogenic β-/α-processing pathway, whereas longer fragments represent β- and γ-dependent pathways. Aβ fragments ending at amino acid 42 are the only markers of plaque pathology, whereas all other Aβ forms are soluble species.

Soluble amyloid precursor protein and

sAPPα and sAPPβ were measured using Human sAPP and Human sAPP kits, respectively (IBL, Japan) according to the manufacturer's protocol. In brief, 100 ul of 1:40 diluted CSF, controls and standards (ranging from 0.78 to 50 ng/mL) were added to wells of anti-human sAPP or sAPP antibody pre-coated microtiter plates and incubated over night at 4°C. Following incubation, 100 uL HRP-conjugated detection antibody was added to the wells. The reaction was developed with TMB chromogen solution, and subsequently stopped with 1M sulfuric acid, and quantified at 450 nm in a microplate reader.

Apolipoprotein E genotyping

APOE genotyping for the 2, ε3,and ε4 alleles was performed with a TaqMan qPCR assay as described by Koch et al., 2002 [19]. DNA was isolated with Promega Maxwell DNA purification kits (Promega, WI, USA), according to the manufacturer's protocol from 250 uL of buffy coat from 6 mL EDTA vials.

Results

Baseline participating subjects

From the 200 randomized subjects of the intervention study, CSF samples were obtained from 56 subjects by lumbar puncture in addition to blood samples from all participants. Of the 44 subjects with a CSF sample available 53 met criteria regarding A42 below 550 pg/mL for those 3 with levels >450 pg/mL, a PiB-PET scan was analyzed. In all three cases, the PiB-PET scan was negative, and the patients were excluded. There were no significant difference in baseline characteristics between the intervention group and control group in any of the parameters (table 1). Of the 53 subjects, 18 were female (34.1 %), the mean age was 68.7 years and the mean MMSE score was 25.3. In the intervention group, 18 subjects (69.2%) exercised with an intensity of more than 70% of maximal HR during the sessions (*high exercise subjects*). There was no significant difference in the three groups at baseline (supplementary table 1).

The baseline characteristics of the subjects with CSF samples are shown in table 1.

	Controls (n=27)	**Intervention (n=26)**	
	Mean (SD)	**Mean (SD)**	**p-value**
Age, yeans	69.2 (3.89)	68.12 (6.76)	0.59
Gender			0.06 (chi^2)
Male, n (%)	21 (39.6)	14 (26.4)	
Female, n (%)	6 (11.3)	12 (22.6)	
Disease duration, years	1.5 (1.0)	1.08 (1.0)	0.13
MMSE	25.1 (3.9)	25.5 (2.28)	0.62
Characteristics			
Weight, kg	72.7 (13.7)	76.2 (16.1)	0.40
Height, cm	175.7 (8.3)	173.4 (9.0)	0.33
BMI	23.5 (3.7)	25.3 (4.4)	0.11
ApoE e4			0.22
0, n (%)	11 (20.8)	5 (9.4)	

1, n (%)	9 (17.0)	13 (24.5)	
2, n (%)	7 (13.2)	8 (15.1)	

Table 1: *Subjects baseline characteristics.*

Baseline concentrations of AD biomarkers

The concentration of A□□□□□□□□□□, t-tau, p-tau and sAPP □ and □, are shown in table 2. There was no statistical significant difference in baseline mean concentration of these markers when comparing controls and intervention groups, see table 2a and b. Furthermore, there was no difference in baseline ratios (Aβ42/Aβ40 or sAPPβ/sAPPα) when comparing controls and intervention, see table 2a and b. Analysis of the relative expression of A□ isoforms long/short ratio analyzed by IP-MS revealed no statistical significant difference at baseline (see table 2b).

Intention to treat analysis

Mean between-group difference in outcomes after 16 weeks of intervention are shown in table 2. There was no significant difference in changes from baseline to follow-up between the control and intervention groups in any of the outcome measures. Furthermore, changes in the Ab42/40 ratio were similar in controls and intervention group with a mean difference of 0.003 (95% CI: -0.0009;0.006). Similarly, no group difference was found for the sAPP□/sAPP□ ratio (mean difference of change 0.21 (-0.46;0.89). Analysis of the changes in relative expression of A□ isoforms long/short ratio from baseline to follow-up between the groups was 0.35 (-0.88;1.59) and was not statistically significant. Figure 1 displays the difference in outcome measure form baseline to follow-up.

Per protocol analysis

Dividing the intervention group into low and high exercise subjects did not alter the results. At follow-up, the *high exercise subjects* (n=18) did not differ from the control group in any of the outcome measures (table 2 A and B).

Discussion

The current study investigated the effects of physical exercise on A□ isoforms, cleaved APP subtypes, and the neuronal injury markers t-tau and p-tau. We found that there was no significant effect of physical exercise on any of the most important CSF AD biomarkers, either in a IIT analysis of all subjects, or in a per protocol analysis, where only subjects that adhered to the exercise program were included.

A substantial limitation of the current study is the small sample size. Only 53 subjects were analyzed for levels of outcome markers in CSF. This low samples size might not generate enough power to statistically calculate a significant difference in mean levels. Another question to discuss is the duration of the exercise intervention. In a review from 2015 by our group, multiple exercise interventions in dementia or MCI patients where reviewed. The intervention ranged from a single burst of exercise to duration of 26 weeks, with various effects on biomarkers. In our study the intervention was of 16 weeks, which might not be long enough to see a significant alteration in A□ and tau. Furthermore, there has been for logistic reasons a time lag from the last exercise session to the date of lumbar puncture. The effect of physical exercise could be transient or only be present when the individuals exercise regularly, the effect therefore might not been measurable in our samples.

One of the strengths in our study is the good adherence to the study in general (96%) and a good per protocol adherence (69.2%) to the targeted exercise of over 70% of maximal HR. All exercise sessions have been supervised by professional and experienced trainers and the levels of activity have additionally been monitored by pulse watches. Furthermore, we have biological samples before and after the intervention.

Our findings are in disagreement to previous studies in animal models for AD. Both A□ and p-tau have been shown to be reduced in the brain as a response to moderate-intensity exercise in transgenic mice models for A□ and tau respectively [6,20]. Our study is the first to investigate the effect of physical exercise on biomarkers of AD in a controlled setting. Therefore, evidence for an effect on AD pathology in humans is sparse.

The amyloid mouse models that are used in Um *et al.,* 2008[8] and Adlard *et al.,* 2005[6] are transgenic models (Tg) overexpressing APP [21,22]. The overexpression of proteins in models raises some question concerning protein integrity, such as that over expression of proteins in itself could be toxic for the cells, and cell toxicity therefore is not protein specific. In addition, over expression generates vast amounts of protein that could enable a not representative large detectable reduction after intervention studies. Furthermore, the Tg NSE/APPsw mouse model used in Um et al., 2008 does not develop plaques, as seen in human patients with AD [23]. The above mentioned could indicate that the pathology of A□ in the mouse brain differs in such a great way from the human, that direct comparison of the effect of an exercise intervention can be problematic and could account for the disagreement between our findings and previous findings.

There could be other explanations for the lack of significant changes in CSF measures of AD pathology. We assumed that decreased amyloid accumulation in the brain would be reflected in an increased clearance of A□ from the brain to CSF. However the physical exercise may not lead to an increased clearance via the CSF, but to an increase in on-site clearance by proteases. Previous studies have found the enzymes Insulin degrading enzyme (IDE) and neprilysin might contribute to the clearance of A□ [24,25]. A recent study found that exercise training reduced extracellular soluble A□ in the brains of Tg2576 mice in a dose-dependent manner through an up-regulation of A□ clearance caused by an induction of IDE and neprilysin [26]. However, such a clearance should be reflected in the CSF by increased levels of Aβ1-X (Erik, which Aβ forms would you regard as the most established IDE and NEP products?).

We analyzed whether physical exercise had the potential to shift towards the non amyloidogenic pathway of APP processing by analyzing the sAPP □ and □ forms and the ratio between sAPP□/□. We did not find a significant difference in the sAPP□/□□ratio in our study□ sAPP□ and □ give rise to isoforms of A□ shorter than 16 amino acids (aa) and longer than 16 aa, respectively [27,28]. We analyzed whether the ratio between long and short isoforms of A□ was affected by physical exercise, by analyzing the relative expression of the A□□isoforms that represent amyloiogenic ad non-amyloidogenic APP-processing, respectively. We found no significant difference in the mean ratios indicating that physical exercise does not seem to influence the cleavage of APP towards the non- amyloidogenic pathways. Further, there was no relative increase in C-terminally truncated Aβ isoforms, *e.g.*, Aβ1-37 and Aβ1-38, that are thought to be markers of beneficial γ-secretase modulation.

Drug trials have also addressed A□ as a target for AD pathology. Lately there have been trials with antibody treatment and immunizations against A□ to prevent plaque formation. However, without overwhelmingly positive results [29]. A new target in preventing A□ plaque formation is the prevention of production of the isoforms of A□ that are prone to aggregate

Another matter to consider is that the subjects in this study and in previous studies are already diagnosed with AD. An intervention to reduce amyloid plaque formation might be more effective several years before the fulfillment of criteria for dementia, since A□ plaque formation precedes these criteria. In a study by Baker *et al.,* 2010 [30], there was a trend that physical exercise could alter the levels of A□ in plasma in mild cognitive impaired (MCI) subjects, although this finding was not significant.

In conclusion, the molecular mechanism behind the cognitive effect of a 16-week exercise intervention could not be explained by modulation of A□ or Tau levels in the CSF of patients with AD. More studies investigating other likely pathways are to be performed.

Table 2

A

	Baseline			16 week follow-up		Between group difference in mean change from baseline			
	Control (n=25)	Intervention (n=26)		Control (n=20)	Intervention (n=22)	Intervention versus control		High exercise (n=16) versus control	
	Mean (SD)	Mean (SD)	p-value	Mean (SD)	Mean (SD)	□ (95%CI)	p-value	□ (95%CI)	p-value
□-*amyloid 38*	1754 (616)	1947 (727)	0.27	2034 (991)	1934 (627)	44.4 (-157.7;246.5)	0.69	114.5 (-111.2;340.2)	0.31
□-*amyloid 40*	3720 (1221)	4148 (1319)	0.24	4549 (1802)	4187 (1158)	336.3 (-135.1;807.8)	0.16	410.5 (-119.8;940.7)	0.12
□-*amyloid 42*	144 (68)	175 (107)	0.23	195 (80)	200 (121)	19.8 (-12.79;52.4)	0.23	27.1 (-7.69;61.94)	0.12
□-*amyloid 42/40 ratio*	0.038 (0.012)	0.041 (0.021)	0.52	0.044 (0.013)	0.046 (0.022)	0.003 (-0.0009;0.006)	0.14	0.003 (-0.0008;0.007)	0.11
Total-tau	692 (373)	721 (385)	0.79	639 (136)	664 (141)	-53.1 (-291.3;185:0)	0.65	-87.4 (-369.7;194.8)	0.52
Phosphorylated-tau	72 (30)	82 (31)	0.23	32 (6.9)	35 (7.3)	5.6 (-3.011;14.30)	0.19	4.8 (-3.62;13.22)	0.25

B

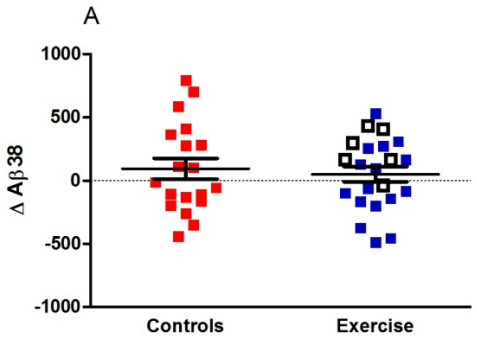
	Baseline			16 week follow-up		Between group difference in mean change from baseline			
	Control (n=22)	Intervention (n=23)		Control (n=19)	Intervention (n=20)	Intervention versus control		High exercise (n=14) versus control	
	Mean (SD)	Mean (SD)	p-value	Mean (SD)	Mean (SD)	□ (95%CI)	p-value	□ (95%CI)	p-value
sAPP□□	362 (239)	412 (198)	0.45	289 (66)	151 (33)	55.5 (-71.23;182.2)	0.38	66.6 (-78.01;211.3)	0.35
sAPP □	859 (726)	942 (444)	0.64	409 (94)	422 (94)	69.1 (-141.7;279.9)	0.51	27.6 (-196.9;252.1)	0.80
sAPP □/□ ration	2.14 (0.41)	2.38 (0.71)	0.52	2.29 (0.92)	2.26 (0.53)	0.21 (-0.46;0.89)	0.52	0.03 (-0.65;0.33)	0.92

Table 2. ***Changes in CSF AD biomarkers after 16 weeks of intervention***. A: Values for □-amyloid, tau and phosphorylated -tau. B: Measures of amyloid precursor protein isoforms □ and □, and the ration between the isoforms.

Table 3

	Baseline			16 week follow-up		Between group difference in mean change from baseline			
	Control (n=24)	Intervention (n=26)		Control (n=21)	Intervention (n=23)	Intervention versus control		High exercise (n=13) versus control	
	Mean (SD)	Mean (SD)	p-value	Mean (SD)	Mean (SD)	□ (95%CI)	p-value	□ (95%CI)	p-value
□-*amyloid long/short*	6.57 (1.99)	6.65 (2.26)	0.90	8.60 (7.97)	6.84 (2.69)	0.35 (-0.88;1.59)	0.57	0.28 (-1.02;1.58)	0.66

Table 3. ***Ration between relative expressions of long versus short forms of □-amyloid.*** Long isoforms of A□ >17 amino acids, short isoforms of A□<16 amino acids


A
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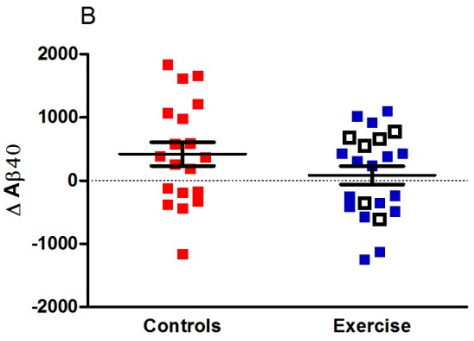
0
-500
-1000
Δ Aβ38
Controls
Exercise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0
-1000
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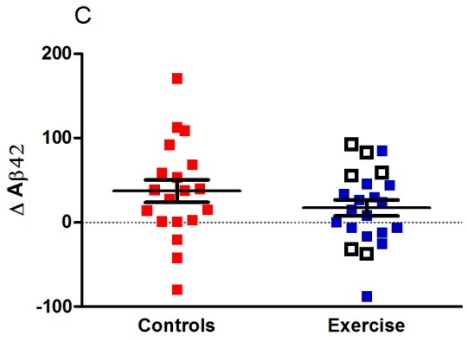
Δ Aβ40
Controls
Exercise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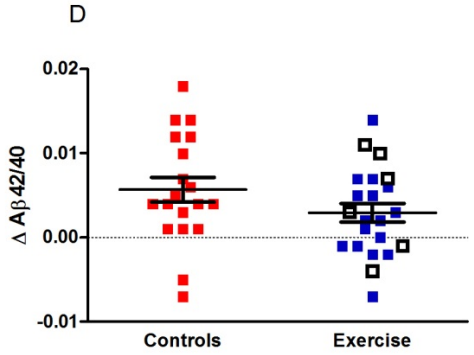
Δ Aβ42
Controls
Exercise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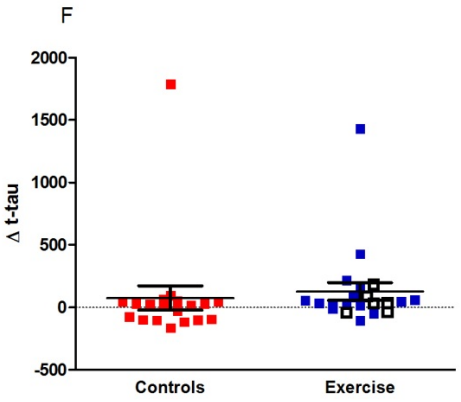
0.01
0.00
-0.01
Δ Aβ42/40
Controls
Exercise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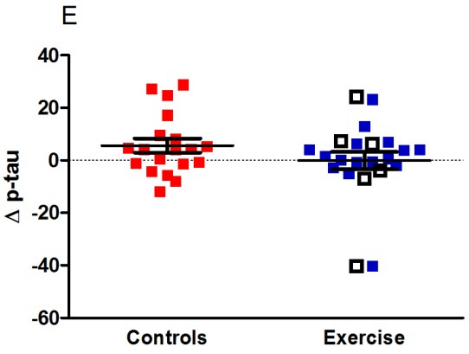
1000
500
0
-500
Δ t-tau
Controls
Exercise


E
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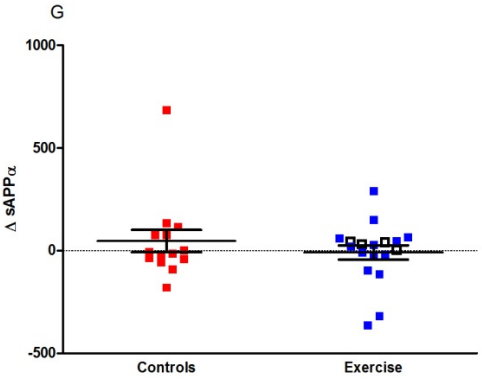
20
0
-20
-40
-60
Δ p-tau
Controls
Exercise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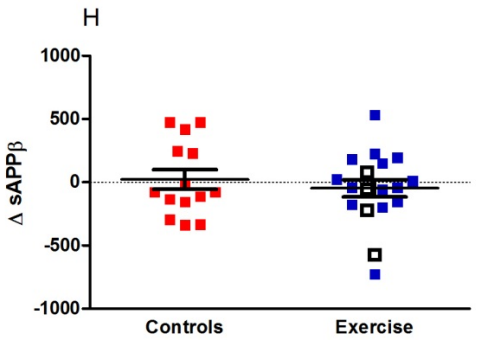
Δ sAPPα
Controls
Exercise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Δ sAPPβ
Controls
Exercise

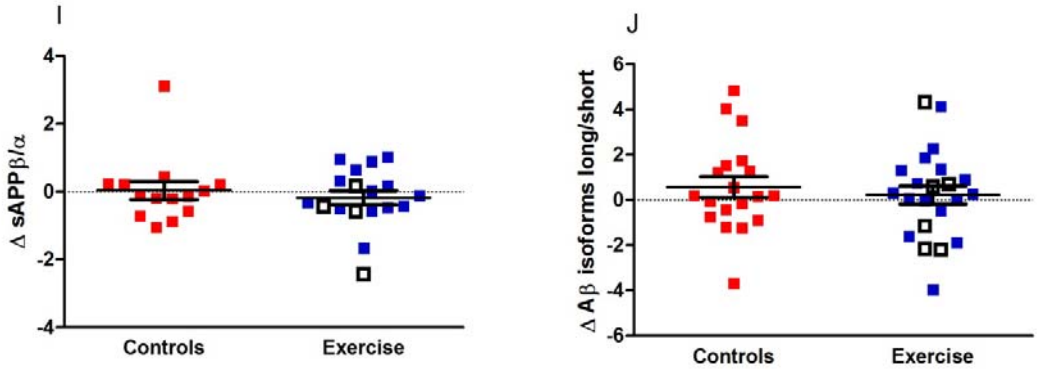

Figure 1. ***Scatterplot of changed from baseline to 16 weeks follow-up in outcome measure***. Controls are in red, the exercise group in blue. The open dots in the exercise group belong to the low exercise group (HR<70% of maximal HR, see supplementary table 1). A) □-amyloid 1-38, B) □-amyloid 1-40, C) □-amyloid 1-42, D) □-amyloid 42/40 ration, E) total- tau, F) phosphorylated-tau, G) sAPP □, H) sAPP □, I) sAPP□/□ and J) □-amyloid long/short isoforms.

Supplementary

Supplementary table 1

	Controls (n=27)	**Low exercise (n=8)**	**High exercise (n=18)**	
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**p-value**
Age, yeans	69.2 (3.9)	66.8 (4.3)	68.72 (7.6)	
Gender				0.18
Male, n (%)	21 (39.6)	4 (7.5)	10 (18.9)	
Female, n (%)	6 (11.3)	4 (7.5)	8 (18.9)	
Disease duration, years	1.5 (1.0)	0.88 (0.99)	1.17 (0.99)	0.25
MMSE	25.1 (3.9)	26.8 (2.8)	25.0 (2.6)	0.42
Characteristics				
Weight, kg	72.7 (13.7)	77.8 (14.9)	75.6 (17.0)	0.66
Height, cm	175.7 (8.3)	173.6 (9.1)	173.3 (8.6)	0.63
BMI	23.5 (3.7)	25.9 (5.0)	25.0 (4.2)	0.25
ApoE e4				0.23
0, n (%)	11 (20.8)	3 (5.7)	2 (3.8)	
1, n (%)	9 (17.0)	4 (7.5)	9 (17.0)	
2, n (%)	7 (13.2)	1 (1.9)	7 (13.2)	

Supplementary table 1: *Subjects baseline characteristics controls, low exercise and high exercise.*

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