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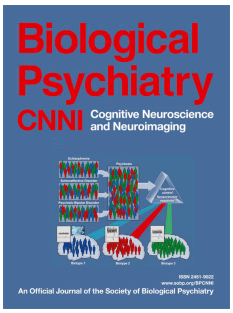

Long-term anabolic androgenic steroid use is associated with deviant brain aging

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Title: *Long-term anabolic androgenic steroid use is associated with deviant brain aging*

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Abstract

Background High-dose long-term use of anabolic-androgenic steroids (AAS) may cause a range of adverse effects, including brain and cognitive abnormalities. We performed age prediction based on brain scans to test whether prolonged AAS use is associated with accentuated brain aging.

Methods T1-weighted MRI (3D MPRAGE) scans were obtained from male weightlifters with a history of prolonged (n=130) or no (n=99) AAS use. We trained machine learning models on combinations of regional brain volumes, cortical thickness and surface area in an independent training set of 1838 healthy males (18-92 years) and predicted brain age for each participant in our study. Including cross-sectional and longitudinal (mean interval 3.5 years, n=76) MRI data, we used linear mixed effects (LME) models to compare the gap between chronological age and predicted brain age (the brain age gap, BAG) between the two groups, and tested for group differences in the rate of change in BAG. We tested for associations between apparent brain aging and AAS use duration, pattern of administration and dependence.

Results AAS users had higher BAG compared to weightlifting controls, which was associated with dependency and longer history of use. Group differences in BAG could not be explained by other substance use, general cognitive abilities or depression. While longitudinal analysis revealed no evidence of increased brain aging in the overall AAS group, accelerated brain aging was seen with longer AAS exposure.

Conclusions The findings suggest that long-term high dose AAS use may have adverse effects on brain aging, potentially linked to dependency and exaggerated use of AAS.

Introduction

Anabolic-androgenic steroids (AAS) are a family of hormones that comprise testosterone, and hundreds of synthetic derivatives of testosterone (1). Administration of supraphysiological doses of AAS in combination with strength training increases lean muscle mass and strength (2). These are desired effects for athletes and bodybuilders where widespread use was seen from the 1950s, before it spread to the general population around the 1980s. AAS use has a range of adverse health and social consequences (3, 4). Yet, the long-term effects on brain health and cognition are understudied, which is paradoxical since sex steroids readily pass the blood-brain barrier and affect the central nervous system (CNS).

The biological action of AAS and their metabolites are primarily mediated via the androgen receptors (AR), however many will also exert physiological effects via estrogen receptor pathways, upon aromatization (5, 6). Sex steroid receptors are widely expressed in the brain, and abundantly in regions such as the brainstem, hypothalamus, amygdala, striatum, hippocampus and the cerebral cortex (7-9). High-dose AAS administration typically involves a complex pattern where testosterone compounds and other AAS are co-administered with doses equivalent to 250-5000 mg/week, which is 5-100 times greater than the natural male production (10). Administration of supraphysiological doses of AAS suppresses the hypothalamic-pituitary gonadal axis and reduces the endogenous production of testosterone, luteinizing and follicle-stimulating hormones. The administration typically continues for several weeks or months, separated by drug-free intervals with the intention to allow the hormonal system to recuperate (11). However, it seems that continuous use persisting for years have become more common (12-16), likely to avoid abstinence symptoms that often occur upon cessation (17, 18).

While neuroprotective effects of physiological doses of testosterone have been observed (19, 20), growing evidence suggests that high-dose long-term AAS use harms the brain. Neurotoxic effects of various sorts of AAS in response to high dosages such as those administered by bodybuilders and recreational athletes have been shown (21-26). Moreover, AAS use frequently causes cardiomyopathy

(27, 28), atherosclerotic disease (27), prolonged hypogonadism (upon withdrawal) (29, 30), lower LDL cholesterol level (31), impaired insulin sensitivity (32), and occasionally toxicity to liver and kidney (33), with potential implications for brain health (34, 35).

Emerging evidence from field studies suggests that prolonged high-dose AAS use is associated with aberrant brain aging. For instance brain imaging has revealed that long-term AAS-use is associated with structural, neurochemical (36), and functional brain differences (36-38), including smaller gray matter, cortical and putamen volume, and thinner cerebral cortex (37). Also, compared to non-using weightlifters, AAS-exposed weightlifters performed poorer on tests assessing working memory (12, 39, 40), executive functions (12, 40, 41), learning and memory (12, 39, 41), processing speed and problem solving (12, 40). Although correlational, such findings have led to the hypothesis that high-dose AAS users are at risk for accelerated brain aging (42, 43).

The effects of AAS use show substantial inter-individual heterogeneity. Some users exhibit little or no symptoms, while others demonstrate multiple psychological and medical consequences following long-term use (11, 44). The range and severity of adverse effects may increase with the burden of use (19), and are particularly pronounced in users fulfilling the criteria for AAS dependence (1, 15, 45). This includes seemingly more pronounced effects on MRI-based measures of cerebral cortical structure (37, 45), self-reported memory problems (12, 41), and impaired executive (40) and memory functions (12, 39) in dependent users. However, group-level differences may disguise substantial individual differences.

Machine learning offers individual predictions based on neuroimaging data (46). For example, training a model to find relationships between brain scans and chronological age allows you to predict the age from unseen brain images with high accuracy (47, 48). The difference between the predicted and chronological age, termed the *brain age gap* (BAG), serves as a surrogate marker of brain health and individual differences in brain maturation and aging (49, 50). In adults, an older brain age compared to chronological age has been linked with cognitive impairment (51), cardiovascular risk factors (34),

mortality (52), dementia (53), and several other common brain disorders, with regionally differing patterns (54). Conversely, a healthy lifestyle has been associated with a younger looking brain, with correlations between BAG and level of education and physical activity, as indicated by the daily number of flights of stairs climbed (55). Contrary, drug abuse and addiction has been associated with premature brain aging (56-58) and early onset of age-related disease (59). While recent studies have documented associations between cumulative exposure to sex hormones and brain age in middle-aged and elderly women (60), the effects of long-term exposure of supraphysiological doses of testosterone and AAS on brain aging have not been studied.

In a sample of 130 AAS users and 99 weightlifting controls (WLC), we used cross-sectional (n=229) and longitudinal (n=76) data to test the hypothesis of higher relative brain age and higher rates of brain aging in AAS users compared to WLC. We also tested for associations between brain age and AAS use severity, duration, administration (cycling versus continuous use) and dependence.

METHODS AND MATERIALS

Participants

Demographics and clinical characteristics of the sample are summarized in table 1. The sample is part of a longitudinal study investigating effects of long-term AAS use on brain morphology, cognitive functioning, and emotional processing. Data collection was performed during 2013-2015 and 2017-2019. We recruited males engaged in heavy resistance strength training who were either current or previous AAS users reporting at least one year of cumulative AAS exposure (summarizing on-cycle periods) or who had never tried AAS or equivalent doping substances. Participants were recruited through webpages and forums targeting people partaking in heavy weight training, bodybuilding, and online forums (open and closed) directly addressing AAS use. In addition, posters and flyers were distributed at select gyms in Oslo. Prior to enrollment all participants received an information brochure with a complete description of the study. The study was approved by the Regional

Committees for Medical and Health Research Ethics South East Norway (REC) (2013/601), all research was carried out in accordance with the Declaration of Helsinki, and written informed consent was collected from all subjects. The participants were compensated with 1000 NOK at time point 1 (TP1) and 500 NOK at time point 2 (TP2).

In total 139 AAS users and 109 WLC underwent brain MRI. 19 participants were excluded. Among AAS users, two participants did not fulfill the inclusion criteria of at least 1 year cumulative exposure, one was excluded due to a previous head injury that had caused coma, one due to poor scan quality, two due to IQ <80, and two due to missing background information. Among WLC one was excluded due to epilepsy, two did not match the AAS group on strength training regimens, and three were excluded due to missing background information. Furthermore, three WLC were excluded due to clinical significant abnormalities based on a neuroradiological examination. In addition one 73 year old AAS user and a 75 year old WLC were excluded due to their substantially higher age than the rest of the sample, which may influence the brain age models and the findings. Therefore, our final sample comprised 130 AAS users and 99 WLC. Among those, 36 AAS users and 40 WLC were scanned at TP2, on average 3.5 years after TP1.

Image Acquisition

MRI was performed using a 3.0T Siemens Skyra scanner (MAGNETOM Skyra; Siemens AG, Erlangen, Germany) equipped with a 20-channel head coil. Anatomical 3D T1-weighted magnetization-prepared rapid acquisition gradient-echo (MPRAGE) sequences with the following parameters were used for volumetry and cortical surface analyses: repetition time 2300 ms; echo time 2.98 ms; inversion time 850 ms; flip angle 81°; bandwidth 240 Hz/pixel; field of view 256 mm; voxel size 1.0 * 1.0 * 1.0 mm; 176 sagittal slices; acquisition time 9:50 min. Scan quality was inspected at the scan session and rerun in case of movement.

MRI processing and brain age estimation

All datasets were processed using Freesurfer v.5.3 (https://surfer.nmr.mgh.harvard.edu/; (61), and segmentations and reconstructions were visually inspected and edited if needed.

A machine learning model was trained to predict brain age based on volume, area and thickness data following a recent implementation (54). The training set for brain age estimation included MRI scans from 1838 healthy males from different cohorts (mean age 46 years, (±) sd 20 years, age range 18-92 years) obtained from several publicly available datasets and processed in the same pipeline. The age distributions for the training set and our cohort is shown in Figure 1a, and information about included datasets are shown in Supplementary table 1. The MRI features were derived from the Human Connectome Project cortical parcellation (62), comprising 180 regions of interest per hemisphere for thickness, area, and volume. In addition, we used subcortical and cerebellar volumes from Freesurfer. The full set comprised 1,118 features in total. We used the extreme gradient boosting package xgboost in R to train machine learning models for brain age estimation. In line with recent work, the learning rate was pre-set to eta=0.01 and the optimal number of rounds (nrounds) were determined in a nested cross-validation loop (54).

For all participants, brain age and BAG were estimated using either features from the whole brain or subregions (54, 63), including occipital, frontal, temporal, parietal, cingulate, insula, or cerebellar/subcortical features, based on the lobe parcellation labels from Freesurfer (61). We corrected for a well-known bias in age prediction (64) using a procedure described in (65). Briefly, the association between BAG and age was estimated using linear models including relevant covariates, and the resulting parameter estimate reflecting the linear association between BAG and chronological age was used to adjust the individual brain age estimates prior to recalculation of BAG.

Interviews and screening instruments

Demographics and clinical data were assessed using self-report questionnaire and a semi-structured interview. Current and previous non-AAS substance use were assessed with Alcohol Use Disorders

Identification Test (AUDIT) (66), the Drug Use Disorders Identification Test (DUDIT) (67), and the drug and alcohol dependence scales from the Millon Clinical Multiaxial Inventory-III (MCMI-III), where a composite scores of substance use were computed from the mean score of these z-transformed subtests. The depression scale from the MCMI-III was used to covary for depressive symptoms. "Total lifetime AAS dose" ingested was calculated as the life-time average weekly dose reported and life-time weeks of AAS exposure, in line with previous studies (1, 68, 69). Intelligence Quotient (IQ) was assessed using the Wechsler Abbreviated Scale of Intelligence (WASI) (70).

Doping analysis

Urine samples were collected and analyzed for AAS and some antiestrogens using gas and liquid chromatography coupled to mass spectrometry at the WADA accredited Norwegian Doping Laboratory at Oslo University Hospital (71). The criteria used to determine AAS use were: 1) urine samples positive for AAS compounds 2) a T/E ratio > 15 equivalent to previous work (37, 71). Other compounds, including stimulants and remaining antiestrogens were analyzed with liquid chromatography and mass spectrometry.

Statistical Analysis

Group differences in demographic data were evaluated with two-tailed independent sample t-tests and Chi-square and Fisher's exact tests for categorical data. To assess group differences in global and regional BAG linear mixed effects (LME) models were tested using the *lme* function in the R (72) package lme4 (73). In fitting the model, we entered time point (TP) and age as fixed effects. Participant ID was entered as a random effect (intercept). Visual inspection of residual plots did not reveal obvious deviations from homoscedasticity or normality. The significance threshold was set at $p < 0.05$, corrected for multiple comparisons using false discovery rate (FDR) adjustment (74).

Sensitivity analyses

Similar LME models including a group by time interaction was run to test for differences in the rate of change between AAS users and WLC. In addition, to test for confounding effects of cognitive ability, depression and non-AAS substance use, the main analyses were rerun including IQ, depressive scores and a composite score of non-AAS substance use as additional covariates. As we were primarily interested in long-term exposure and since stricter inclusion criteria have previously been applied (29, 32), we rerun the main analyses after including only AAS users with more than two years of AAS use.

Next, similar LME analyses were conducted to test for differences between subgroups of AAS users. 1) Use category: WLC, AAS users fulfilling the criteria for AAS-dependence and non-dependent users, 2) Use pattern: WLC, AAS users practicing a continuous way of administrating AAS versus users administrating AAS in cycles, and 3) Use state: WLC, current and previous AAS users. 4) Use length: AAS users with < 10 years of exposure and users with ≥10 years history of AAS exposure.

Lastly, as only ~50% of the sample took part at TPII, we conducted linear models controlling for age to examine if BAG at baseline was associated with study dropout.

Results

Demographics and user characteristics

Table 1 summarizes key clinical and demographic characteristics. Years of education and IQ were higher among WLC, and AAS users were heavier and stronger than WLC. The use of prescribed psychotropic medication was significantly higher among AAS users, where antidepressants and anxiolytics were the typical preparations prescribed (not shown). The majority of users (65%) and non-users (93%) reported no previous or current use of prescribed psychotropic medication.

Insert table 1

Characteristics of AAS use

The average duration of AAS use at baseline was 10.6 years (SD=7.7), and mean age of onset was 22 years (SD=6.6, range 12-52). Mean weekly AAS dosage was 1023 mg (SD=656, range 100-3750), and mean calculated lifetime dose was 444 g (SD=452 g, range 20-2016 g). Continuous AAS administration was reported by 43 (33.1 %) users, and 78 (60.0%) reported a cycling pattern, rotating between periods "on" and "off" AAS. The remaining 9 (6.9%) were either on testosterone replacement therapy, had missing details regarding administration pattern or was difficult to classify. 77 (59.2%) AAS users fulfilled the criteria for AAS-dependence and 87 (67%) had used AAS within the past 6 months and defined as current users. Current users had longer history of AAS and higher age of onset compared to past AAS users. No differences in extent of use measures were seen between cyclical versus continuous users, whereas dependent users had used longer, debuted earlier and used higher AAS doses compared to non-dependent users (Table S2).

None of the 99 WLC tested positive for AAS or had T/E ratio above threshold, whereas tests indicative of AAS use were seen in 78.2% (n=68) of current users, and in 7.5 % (n=3) of previous users. The positive tests among previous users could be compatible with previous use, stated by the participants, and one test with elevated T/E ratio was consistent with reported medical use of TRT. The mean T/E ratio for the groups were 1.4 (SD=1.6, range 0.1 – 10.0) for WLC (n=99), 44.8 (SD=50.6, range 0.1 – 226.0) for current users (n=82), and 2.8 (SD=7.9, range 0.0 – 50.4) for previous users, where previous users and WLC were significantly different from current users (df=220, t=-7.2, p<.001). The frequency of the specific anabolic-androgenic steroids found in the urine sample are summarized in Figure S1 along with a summary of the most popular compounds based on self-reports.

Brain age prediction

A 10-fold cross validation on age prediction in the training set confirmed high accuracy of the model, with correlations between chronological age and predicted age ranging from *r*=.93 (MAE=5.76,

RMSE=7.57,) for the global model to *r*=.76 (MAE=10.05, RMSE=12.94) for the model based on occipital features (Table. S3). Figure 1 shows predicted age plotted as a function of chronological age for the test set of AAS users and WLCs, and Table S3 summarizes the prediction accuracies.

Insert Figure 1

Associations between AAS use and BAG

Table 2 summarizes the results from the LME models. Significant main effects for group were found for the global BAG (β) (305) = 3.29, t=3.58, p_{FDR}<.001), and for the frontal, temporal, insula, cingulate and occipital BAGs. An examination of the fixed effects estimates showed higher BAG in AAS users compared to WLC in all regions. There were no significant main effects of time or age.

When including an interaction term between group (and subgroups of AAS) and time, few significant main effects were found (table S4-S8). One global BAG model survived FDR correction and revealed significant group by time interaction, indicating accelerated aging in users with ≥10 years of use compared to WLC (β (305) = 3.68, t=3.06, p_{FDR}=.024, not displayed in Table S8). The longitudinal findings are depicted in Figure 2 for global brain age gap.

Insert table 2

Sensitivity analyses

Sensitivity analyses revealed that the main effect of group remained significant for the global BAG when IQ, non-AAS substance use and depression were included as fixed effects in the model (Table 3). Frontal and subcortical BAG differences were found at an FDR corrected threshold of p<.05, whereas group differences for the temporal, insula, cingulate and occipital model were no longer significant when adding covariates. Further, the sensitivity analyzes omitting AAS users with <2 years

of AAS-use revealed significant main effects of group, with higher BAG for AAS users in all but the subcortical models (Table S9).

Sensitivity analyses with WLC and subgroups of AAS users revealed significant main effects of use category with higher BAG in dependent compared to WLC for all regions, whereas non-dependent AAS users showed no significant differences from WLC (Table S10). Significant main effects were also found for use pattern with higher BAG in fullbrain and some regional models for cyclic and continuous AAS administration compared to WLC (Table S11). Also, significant main effects of use state were seen, where current AAS users had significantly higher BAG in most regions compared to WLC (Table S12). Previous users (>6 months since last use) were not significantly different from WLC, although differences were seen at an uncorrected significance level for some models including the fullbrain measure ((302) = 2.55, t=2.24, p=.03). Lastly, splitting the groups into shorter (<10 years) versus longer (≥10 years) history of AAS use revealed significant main effects of use length and higher BAG compared to WLC for the fullbrain model and some subregions, with most pronounced differences seen with longer exposure (Table S13).

Insert figure 2

BAG associated with dropout

56.7% of the WLC and 46.3% of the AAS users from TPI participated at TPII. Frontal and cingulate BAG at baseline was significantly higher in participants who dropped out of the study compared to those with complete longitudinal data, whereas no significant differences were seen for other regions and the global BAG (Table 4).

Insert table 4

Discussion

Accumulating evidence suggests that prolonged AAS use harms the brain (12, 36, 37, 39, 42, 43). Using brain scans and brain age prediction based on an independent training set we found evidence of

higher relative global, frontal, temporal, occipital and insular brain age in 130 male AAS users compared to 99 male WLC. Further, among AAS users we found that long-term use and dependence were associated with higher relative brain age. Longitudinal analysis revealed no evidence of accelerated BAG over time in the overall AAS group, however AAS users with more than 10 years of AAS exposure showed accelerated aging compared to WLC, with a significant increase in BAG between the time points in this subgroup. These findings suggest that long-term high dose AAS use may have adverse effect on brain aging, potentially linked to dependency and exaggerated use of AAS.

AAS use associated with apparent brain aging

More evident brain aging in long term AAS users is consistent with *in vitro* studies suggesting that various sorts of AAS might have neurotoxic effects (19-24), and recent findings of impaired cognitive performance (12, 39, 40), smaller brain volumes (37), and metabolites abnormalities (36) in long-term AAS users. Older appearing brains in AAS-dependent compared to non-dependent users is consistent with a mega-analyses pooling data from 23 cohorts, suggesting that dependence shares a common neural substrate across a range of substances, indicating smaller brain volumes and thinner cortex in dependent relative to non-dependent individuals (75). The group difference in global BAG suggests widespread effects, although regional models revealed significant differences in several regions, most pronounced frontally. Interestingly, the insula and part of the frontal cortex have been implicated in substance dependence (76-79), and our findings align with structural MRI studies showing reduced insula and frontal gray matter volume in drug users (75, 80).

AAS dependence, current use and longer history of AAS of use were associated with higher BAG. The apparent difference in BAG between past and current AAS users should be regarded with caution, and could be confounded by the considerable shorter duration of use among the past users. The links between AAS use and brain aging are likely complex and reflecting individual vulnerability, properties with the compounds being administered and potential links to medically induced side-effects. In line with this, users with ≥10 years of AAS exposure or AAS dependence, which is

characterized by more exaggerated use, the presence of psychological and/or medical side-effects, and continued use despite negative impact on life (1, 15, 40), showed the most prominent accelerated aging over time compared to WLC.

Moreover, we found that AAS users who had dropped out of the study after TPI had older appearing brains in frontal and cingulate regions, compared to those who completed. Hence with a dropout rate of 49% in the total sample and 54% in the AAS user group, it is likely that our longitudinal findings are biased.

Some limitations should be noted. Whereas we included both cross-sectional and longitudinal data, the high drop-out rate and non-random attrition might have limited the generalizability of the longitudinal models. This is in line with previous longitudinal studies of brain aging and dementia, showing that study drop-out is associated with past worse executive and memory functioning (81) and MRI findings suggestive of higher future dementia risk (82). Furthermore, due to the age distribution of the sample the generalizability to the older AAS population is unclear. Moreover, while the total sample size is relatively large considering barriers of recruiting participants when studying clandestine and illegal behaviors, our sensitivity analyses resulted in small subsamples and estimates with high uncertainty. For instance, while previous users did not differ from WLC, which could suggest part or full recovery after ceasing AAS use, larger follow-up studies of past users covering a wide age-range are warranted to make plausible conclusions about recovery. It will also be important to study a potential link between long-term AAS use on white matter measures, e.g. measured using diffusion MRI, and, given the strong vascular effects of AAS (27, 83, 84), with measures of cerebral blood flow and slowly progressive vascular pathology such as small vessel disease.

The group differences could not be explained by general cognitive abilities, depression or non-AAS substance use. Still, AAS use is commonly combined with a variety of drugs, such as aromatase inhibitors, human chorionic gonadotropin (hCG), tamoxifen, 5-α-reductase inhibitors, growth hormone (GH), insulin-like growth factor (IGF-1), dietary supplements, as well as narcotics and stimulants (85). In addition, the intricate administration pattern of AAS typically includes different doses and stacking of multiple classes of AAS with different molecular and cellular effects (86). Such complexity makes it extremely difficult to distinguish the unique contributions of single factors on measures of brain health and behavior. Moreover, a range of psychological and medical effects linked to AAS use might influence brain health (15). Hence, future interdisciplinary studies are needed in order to better understand mechanisms linking AAS use and brain aging.

Conclusively, in line with mounting evidence of adverse health effects of AAS use, using brain age prediction we found evidence of increased apparent brain aging in long-term high-dose AAS users, seemingly linked to dependency and exaggerated use of AAS.

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Disclosures

The authors report no biomedical financial interests or potential conflicts of interest.

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Tables and legends

Table 1. Demographics, Sports Information, Substance Abuse, and Use of Psychopharmaca

Sample characteristics	WLC n=99		AAS users n=130		t	95% CI		p
	Mean	SD	Mean	SD		*LL*	*UL*	
Age	35.0	8.8	36.2	9.4	-0.97	-3.6	1.2	.332
Edu[a]	16.3	2.5	14.4	2.7	5.51	1.3	2.7	<.001
IQ[b]	115.4	9.2	106.0	10.9	7.06	6.9	12.3	<.001
Alcohol (units/week)[c]	3.5	4.7	2.6	3.3	1.45	-0.3	2.1	.148
Height	180.3	12.3	181.4	6.8	-0.88	-3.7	1.4	.378
Weight	90.6	11.7	97.7	15.2	-3.85	-10.7	-3.5	<.001
BMI	28.7	11.9	29.7	4.2	-0.88	-3.2	1.2	.379
Strength training (min/week)[d]	399.0	223.2	360.9	226.7	1.22	-23.4	99.6	.223
Endurance training (min/week)[d]	98.0	129.2	122.9	183.3	-1.15	-67.4	17.6	.25
Bench Max[e]	138.3	30.7	171.9	33.6	-7.70	-42.3	-25.0	<.001
Training Classification[f]	%		%		X2		p	
Bodybuilding /fitness	18.9		44.8		15.8		<.001	
Weightlifting	26.3		19.8		1.3		.363	
Combat Sports	20.2		23.1		0.7		.414	
Recreational exercise	30.5		32.8		0.1		.729	
Psychopharmaca[g]	7.2		35.0		23.6		<.001	
Smoker[g]	1.0		13.6		11.3		.001	

Of note, data availability for the different measures varies to some degree. Mean values are based on n of non-exposed/AAS-exposed participants a 98/124, b 99/128, c89/91, d >95/114, e96/119, f 98/118. G Fisher's exact test was applied due to few responses for a category.

Table 2: Main model

Linear mixed effect model results for estimates of brain age gaps (BAGs) for fullbrain and subregions, where variables are displayed with corresponding fixed effect estimates (β), (standard error), t-statistic, and FDR corrected *P* value. "Group" levels= WLC (reference, N=139) and AAS (N=166).* p-value (uncorrected) <.05, ** p-value (uncorrected) <.01, *** p-value (uncorrected) <.001.

	Fullbrain	**Frontal**	**Temporal**	**Insula**	**Cingulate**	**Parietal**	**Occipital**	**Subcortical**
Group AAS	3.288***	3.743**	2.573*	2.483*	2.613*	2.033*	2.885*	1.906
	(0.918)	(1.177)	(1.046)	(0.979)	(1.185)	(1.025)	(1.154)	(1.064)
	3.580	3.180	2.460	2.535	2.205	1.984	2.499	1.790
	<.001	0.008	0.024	0.024	0.037	0.055	0.024	0.075
Time	0.260	0.221	-0.846	-0.879	-0.436	0.008	0.874	0.796
	(0.513)	(0.720)	(0.563)	(0.552)	(0.685)	(0.554)	(0.701)	(0.522)
	0.507	0.307	-1.504	-1.592	-0.636	0.015	1.246	1.527
	0.817	0.867	0.36	0.36	0.817	0.988	0.432	0.36
Age	-0.021	-0.007	-0.033	-0.020	0.007	-0.015	-0.025	-0.056
	(0.050)	(0.064)	(0.056)	(0.053)	(0.064)	(0.055)	(0.062)	(0.057)
	-0.415	-0.105	-0.590	-0.385	0.113	-0.280	-0.394	-0.975
	0.916	0.916	0.916	0.916	0.916	0.916	0.916	0.916
Observations	305	305	305	305	305	305	305	305
Log Likelihood	-986.417	-1,070.714	-1,022.649	-1,007.022	-1,067.389	-1,016.988	-1,064.043	-1,019.256
Akaike Inf. Crit.	1,984.834	2,153.427	2,057.298	2,026.044	2,146.777	2,045.976	2,140.085	2,050.512
Bayesian Inf. Crit.	2,007.155	2,175.749	2,079.620	2,048.366	2,169.099	2,068.298	2,162.407	2,072.834

Table 3: Main model with covariates

Linear mixed effect model results for estimates of brain age gaps (BAGs) for fullbrain and subregions, where variables are displayed with corresponding fixed effect estimates (β), (standard error), t-statistic, and FDR corrected *P* value. "Group" levels= WLC (reference) and AAS.* p-value (uncorrected) <.05, ** p-value (uncorrected) <.01, *** p-value (uncorrected) <.001.

	Fullbrain	**Frontal**	**Temporal**	**Insula**	**Cingulate**	**Parietal**	**Occipital**	**Subcortical**
Group AAS	3.631**	3.737*	2.458	1.997	2.568	1.836	1.571	3.516*
	(1.177)	(1.550)	(1.354)	(1.301)	(1.546)	(1.274)	(1.551)	(1.381)
	3.085	2.411	1.815	1.535	1.662	1.441	1.013	2.547
	0.016	0.045	0.142	0.169	0.157	0.173	0.312	0.045
Time	-0.194	-0.751	-0.727	-1.046	-1.291	0.510	1.302	0.536
	(0.627)	(0.916)	(0.693)	(0.672)	(0.846)	(0.698)	(0.891)	(0.673)
	-0.309	-0.820	-1.049	-1.556	-1.526	0.731	1.461	0.796
	0.758	0.534	0.534	0.392	0.392	0.534	0.392	0.534
Age	-0.011	-0.006	-0.016	0.022	0.060	-0.041	-0.012	-0.025
	(0.056)	(0.073)	(0.065)	(0.062)	(0.074)	(0.061)	(0.074)	(0.066)
	-0.198	-0.076	-0.253	0.353	0.820	-0.669	-0.168	-0.374
	0.939	0.939	0.939	0.939	0.939	0.939	0.939	0.939
IQ	0.009	-0.012	-0.074	0.015	-0.009	-0.101*	-0.152*	0.050
	(0.047)	(0.063)	(0.053)	(0.051)	(0.062)	(0.051)	(0.063)	(0.054)
	0.194	-0.187	-1.389	0.300	-0.140	-1.986	-2.434	0.935
	0.889	0.889	0.443	0.889	0.889	0.192	0.128	0.702
Substance use	-0.479	-0.277	-0.034	0.759	-1.067	-0.302	0.434	-1.476*
	(0.584)	(0.808)	(0.657)	(0.635)	(0.777)	(0.641)	(0.798)	(0.651)
	-0.821	-0.342	-0.052	1.195	-1.373	-0.472	0.544	-2.266
	0.826	0.837	0.958	0.624	0.624	0.837	0.837	0.2
Depression	-0.136	-0.096	-0.628	-0.797	-0.205	-0.260	-0.284	-0.453
	(0.460)	(0.630)	(0.520)	(0.502)	(0.611)	(0.503)	(0.624)	(0.518)
	-0.296	-0.152	-1.209	-1.589	-0.335	-0.516	-0.455	-0.874
	0.877	0.879	0.877	0.877	0.877	0.877	0.877	0.877
Observations	217	217	217	217	217	217	217	217
Log Likelihood	-692.323	-758.245	-720.425	-712.307	-753.042	-711.166	-756.652	-721.886
Akaike Inf. Crit.	1,402.645	1,534.491	1,458.850	1,442.614	1,524.085	1,440.331	1,531.303	1,461.772
Bayesian Inf. Crit.	1,433.065	1,564.910	1,489.270	1,473.033	1,554.504	1,470.751	1,561.722	1,492.191

Table 4. Baseline brain age gap for dropouts (after TP1) and completers across groups
Linear model results for brain age gap (BAGs) estimates for fullbrain and subregions, where variables are displayed with corresponding estimates (β), (standard error), and FDR corrected *P* value.
"Dropout" levels= Dropout (reference) and completer.
* p-value (uncorrected) <.05, ** p-value (uncorrected) <.01, *** p-value (uncorrected) <.001.

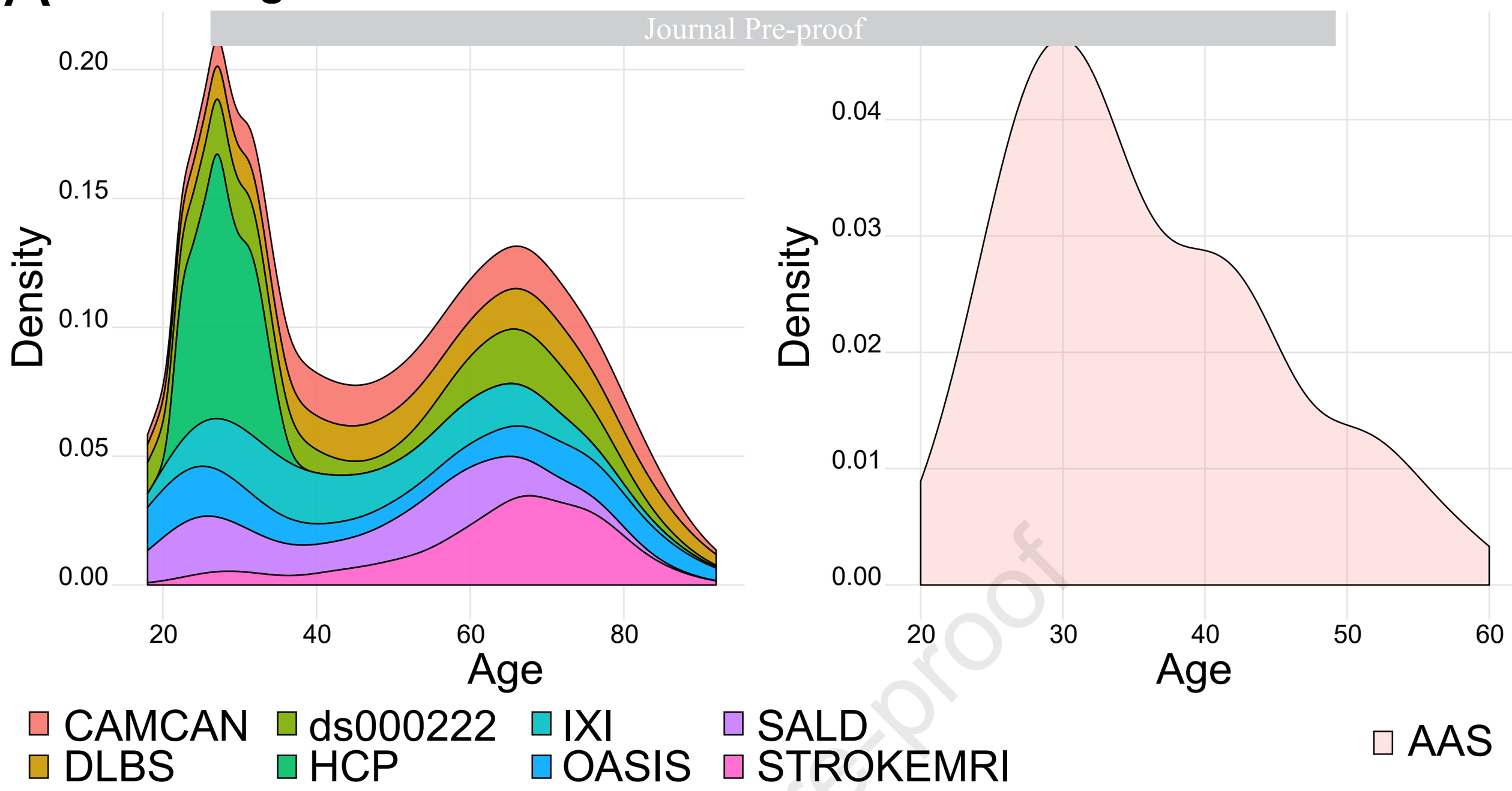
	Fullbrain	**Frontal**	**Temporal**	**Insula**	**Cingulate**	**Parietal**	**Occipital**	**Subcortical**
Dropouts	-2.145	-4.321**	1.040	0.238	-3.799**	-2.015	-0.851	0.792
Completer	(1.165)	(1.538)	(1.332)	(1.265)	(1.431)	(1.297)	(1.413)	(1.360)
	0.181	0.036	0.642	0.852	0.036	0.246	0.642	0.642
Age	0.024	0.043	0.052	0.047	0.171	0.017	-0.037	0.032
	(0.073)	(0.097)	(0.084)	(0.080)	(0.090)	(0.082)	(0.089)	(0.086)
	0.839	0.839	0.839	0.839	0.839	0.839	0.839	0.839
Observations	139	139	139	139	139	139	139	139
R^2	0.025	0.056	0.007	0.003	0.073	0.018	0.004	0.004
Adjusted R^2	0.011	0.042	-0.007	-0.012	0.059	0.003	-0.011	-0.011
Residual Std. Error (df = 136)	6.867	9.065	7.852	7.457	8.431	7.642	8.327	8.015
F Statistic (df = 2; 136)	1.749	4.046*	0.493	0.194	5.336**	1.229	0.268	0.241

Figure legends

Figure 1. Age distribution and predicted brain age as a function of age A) The age distributions for the training set and our cohort. B) Predicted global brain age corrected for age, as a function of chronological age. The fit lines represent the best linear fit within each group, and the points connected by lines represent individual change in BAG between the two MRI scans for each individual.

Figure 2. Brain age gap in subgroups. Panel A-E shows group*time (x-axis) interaction for corrected brain age gap (BAG) (y-axis) of subgroups of participants with two scans approximately 3.5 years apart. Fitted lines made with lme-derived predicted values. Shaded gray areas represent CI of 95%. Panel F shows box-plot of corrected BAG at baseline for participants who completed or dropped out of the study. Horizontal lines represents median of sample. Abbreviations: BAG; brain age gap, WLC; weightlifting controls, AAS; anabolic androgenic steroids, Non-dep; non-dependent, Cont; continuous use.

A



B