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Structural Brain Imaging of Long-Term Anabolic-Androgenic Steroid Users and Nonusing Weightlifters

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ABSTRACT

BACKGROUND: Prolonged high-dose anabolic-androgenic steroid (AAS) use has been associated with psychiatric symptoms and cognitive deficits, yet we have almost no knowledge of the long-term consequences of AAS use on the brain. The purpose of this study is to investigate the association between long-term AAS exposure and brain morphometry, including subcortical neuroanatomical volumes and regional cortical thickness.

METHODS: Male AAS users and weightlifters with no experience with AASs or any other equivalent doping substances underwent structural magnetic resonance imaging scans of the brain. The current paper is based upon high-resolution structural T1-weighted images from 82 current or past AAS users exceeding 1 year of cumulative AAS use and 68 non–AAS-using weightlifters. Images were processed with the FreeSurfer software to compare neuroanatomical volumes and cerebral cortical thickness between the groups.

RESULTS: Compared to non–AAS-using weightlifters, the AAS group had thinner cortex in widespread regions and significantly smaller neuroanatomical volumes, including total gray matter, cerebral cortex, and putamen. Both volumetric and thickness effects remained relatively stable across different AAS subsamples comprising various degrees of exposure to AASs and also when excluding participants with previous and current non-AAS drug abuse. The effects could not be explained by differences in verbal IQ, intracranial volume, anxiety/depression, or attention or behavioral problems.

CONCLUSIONS: This large-scale systematic investigation of AAS use on brain structure shows negative correlations between AAS use and brain volume and cortical thickness. Although the findings are correlational, they may serve to raise concern about the long-term consequences of AAS use on structural features of the brain.

Keywords: Anabolic-androgenic steroids, Cerebral cortex, Cortical thinning, Gray matter, Neuroimaging, Putamen

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Anabolic-androgenic steroids (AASs) comprise a large class of synthetic derivatives of the male sex hormone testosterone that are primarily used in an illicit manner for cosmetic or ergogenic purposes (1–3). Prolonged high-dose AAS use is associated with a range of adverse health consequences, including cardiovascular effects (4,5), psychiatric disorders (6–9), and cognitive deficits (10,11). Few studies have examined potential brain structural alterations (12), which is critical because AASs readily pass the blood-brain barrier and can affect the central nervous system. Testosterone's main activity in the brain occurs via binding to cytoplasmic androgen receptors (ARs) (13). ARs are widely distributed in the brain and abundantly expressed in the brain stem, hypothalamus, amygdala, hippocampus, and cerebral cortex (14–16), and these regions are implicated in a wide range of functions, including the regulation of emotion and cognition.

Supraphysiological doses of AASs may cause apoptotic effects on a variety of cell types, including neurons (17–21), may lead to impaired cognition in animal models (22,23), and are associated with lower cognitive function in humans (10). These findings, coupled with reports of AAS-induced alterations in mood and behavior (7,11), suggest that supraphysiologic AAS doses may induce neurochemical or structural alterations in the brain. This is supported by a recent neuroimaging study of 10 AAS users that suggested that chronic AAS use was associated with structural, neurochemical, and functional alterations in the brain (12). In addition, other AAS-induced medical effects may further threaten brain health. In particular, cardiovascular conditions—considered to be among the most serious risks associated with AAS use—are known to be associated with larger effects of age on brain structure (24), vascular brain disease (25), cognitive decline (26), and dementia (26,27). These cardiovascular effects associated with AAS use (5) with the potential to compromise brain and cognition include hypertension (24,28), atherosclerosis (29), and dyslipidemia (30). Therefore, many indices suggest that prolonged AAS use with supraphysiological doses may be associated with structural alterations of the brain.

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We examine the association between long-term exogenous AAS exposure and brain morphometry. Male participants engaged in heavy resistance strength training with or without experience with AASs underwent structural magnetic resonance imaging (MRI) scans of the brain. Based on the findings of neurotoxic effects of supraphysiological AAS doses, negative relationships between AAS use and regional brain volumes and cortical thickness are expected.

METHODS AND MATERIALS

Participants

The sample was drawn from the research project "Long-term androgenic anabolic steroid use on brain structure, cognitive functioning and emotional processing" coordinated from the Department of Physical Medicine and Rehabilitation, at the section of neuropsychology, Oslo University Hospital, Oslo, Norway. The participants in the study are men engaged in heavy resistance strength training belonging to one of the following groups: 1) current or previous AAS users reporting ≥1 year of cumulative AAS exposure (summarizing on-cycle periods) and 2) men who have never tried AASs or equivalent doping substances. Participants were recruited through a Facebook project page; posts on Internet forums for bodybuilding, strongman, fitness, and weightlifting; and forums (open and closed) that directly target steroid users. In addition, posters and flyers were distributed in select gyms in Oslo. All participants received an informational brochure with a complete description of the study before participation, and written informed consent was collected. The participants were compensated for their participation with 1000 Norwegian kroner (approximately $125).

In total, 159 men participated in the study, divided into 89 current or past AAS users and 70 nonusing controls. All participants in the control group underwent MRI scanning, but two participants were later excluded—one based upon the radiological evaluation, and one because he did not match the AAS group on strength and training regimens (with reported maximum bench presses, squats, and deadlifts that were 2.6, 2.8, and 3.3 SDs below the sample mean, respectively). In the AAS group, seven participants were excluded for various reasons: three did not fulfill the criteria of having ≥1 year of cumulative AAS exposure, one did not show up for the MRI session, and another did not show up for the neuropsychological evaluation. In addition, MRI scan results could not be obtained from two users, one because of a pacemaker implant and one that experienced panic when approaching the scanner (participant exclusion details can be found in Figure 1). Our final sample was 150 participants, with 82 current or previous AAS users and 68 nonusing controls.

Information about the material used and the findings in relation to mapping the characteristics of AAS use, medical history, and use of traditional use of drugs of abuse are presented in the Supplement.

Doping Analysis

Urine samples were collected during the neuropsychological evaluation and analyzed for AASs and narcotics using gas chromatography and mass spectrometry at the World Anti-Doping Agency–accredited Norwegian Doping Laboratory at the Oslo University Hospital, as described elsewhere (31). Stimulants were analyzed with liquid chromatography and mass spectrometry.

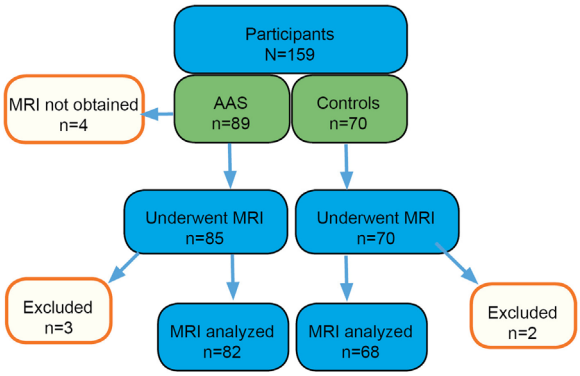

Figure 1. Flow chart. AAS, anabolic-androgenic steroid users; MRI, magnetic resonance imaging.

Briefly, the criteria used to determine the use of AASs or testosterone were as follows: 1) urine samples positive for AAS compounds and 2) a testosterone to epitestosterone (T/E) ratio > 15. A T/E ratio > 4 has been commonly applied by the World Anti-Doping Agency as a population-based criteria for samples requiring additional analysis with isotope ratio mass spectrometry or follow-up to indicate testosterone abuse (32). However, when applying this criterion in research and routine analyses, cases of naturally occurring T/E ratios > 4 do appear (33). Isotope ratio mass spectrometry analyses were not performed in this study, and the stricter T/E ratio > 15 was applied, which is equivalent according to Hullstein *et al.* (31).

Image Acquisition

MRI data were collected using a 3.0T Siemens Skyra scanner (MAGNETOM Skyra; Siemens AG, Erlangen, Germany) equipped with a 24-channel Siemens head coil. Anatomical 3-dimensional T1-weighted magnetization-prepared rapid acquisition gradient-echo sequences were used for volumetry and cortical surface analyses with the following parameters: repetition time = 2300 ms; echo time = 2.98 ms; inversion time = 850 ms; flip angle = 8°; bandwidth = 240 Hz/pixel; field of view = 256 mm; voxel size = 1.0 × 1.0 × 1.0 mm; 176 slices sagittally oriented; acquisition time = 9:50.

The magnetization-prepared rapid acquisition gradient-echo sequences were our first-priority sequence. The qualities of these scans were immediately inspected at the scanning session and rerun in case of movement in order to ensure that the scans were of good quality. For one participant who was anxious during the scanning, we could not run the sequence again. However, this participant did not show up for the neuropsychological evaluation, so he was omitted from the dataset for another reason.

Imaging Analysis

All datasets were automatically processed and analyzed using FreeSurfer software (version 5.3; http://surfer.nmr.mgh.harvard.edu), which is described in detail elsewhere (34–39) (see

Supplemental Methods for details). The cortical surface was reconstructed for each subject to measure both surface area and thickness at each surface location or vertex. The individual thickness maps were smoothed using a Gaussian kernel of 15 mm. Subcortical volumes were obtained from the automatic volume segmentation procedure (40,41), and we selected a limited number of regions to limit the number of comparisons that we needed to control for. The selection was done before the statistical tests. The most commonly applied subcortical regions that made sense theoretically were selected, including the amygdala, accumbens area, thalamus, caudate, putamen, pallidum, and hippocampus, and more global measures, such as cortical volume, the lateral ventricle, cerebellum cortex, total gray matter, and corpus callosum volume. The volumes from both hemispheres of these structures were combined to generate a bilateral volume value. In addition, estimated intracranial volume (ICV) (42) was computed and included in the analyses. All reconstructed datasets were visually inspected and inaccuracies corrected when required.

Statistical Analyses

Group differences in demographic and neuropsychological data were evaluated with two-tailed independent sample t tests and χ^2 tests for categorical data. Group differences in neuroanatomical volumes were tested using general linear models (GLMs) with regional volume as the dependent variable, group as the fixed factor, and age and ICV as covariates. Bonferroni-adjusted α-level for correlated measures of 0.012 per test was applied (.05/13, similar to a Pearson's $r = .43$). For cortical thickness, we fitted a GLM at each vertex using thickness as the dependent variable and age as the covariate. To reduce the possibility of type I errors, a clusterwise correction using Z Monte Carlo simulations with 10,000 iterations was performed. A cluster-forming threshold of $p < .05$ (two-sided) was applied because we expected anatomically broad and less spatially specific effects. The stability of the main findings was also tested using a cluster-forming threshold of $p < .01$ (two-sided) and by a false discovery rate (FDR) of 5%. Similar exploratory analyses were conducted for different subgroups of the sample (i.e., for participants with prolonged AAS exposure, fulfilling the criteria of AAS dependence, and for those with no additional non-AAS drug abuse). To control for the possible influence of general cognitive functions, aspects of mental health, and alcohol or drug use on brain variables, we conducted analyses where the Wechsler Adult Intelligence Scale vocabulary performance, weekly reported alcohol consumption, Achenbach System of Empirically Based Assessment Adult Self-Report T-scores for anxious/depressed syndrome, drug use, attention problems, and total problems were included as additional covariates (one at a time). Moreover, within the AAS group, analyses were performed to test for possible effects of degree of AAS exposure, use of other drugs, or the status of use on brain measures.

RESULTS

Demographics and User Characteristics

Demographic data can be found in Table 1, and characteristics of AAS usage are shown in Supplemental Table S1. The groups did not differ in age, but both IQ and years of education were lower in the AAS group. In the AAS group, 59 were current and 23 were previous users of AASs. A large proportion of the AAS users and controls did not engage in organized sports and could therefore be classified as recreational athletes. For the other study participants, weight training was associated with participation in organized sports, with bodybuilding, powerlifting, and combat sports being the most popular categories. There were more bodybuilders in the AAS group and powerlifters in the control group. Almost a quarter (23.2%) of the AAS group and 35.3% of the control group included participants who had placed in the top five competitors in either national or international competitions. The AAS group had a more frequent use of prescribed psychotropic medications than the control group, with antidepressants and anxiolytics being the most frequently prescribed. Of note, the majority of AAS users and nonusers had never used prescribed psychotropic medications of any kind (Table 1). The groups also differed with regard to the presence of previous or present comorbid substance abuse problems, and these were more frequently observed in the AAS group. Information about the use of illicit drugs is shown in Supplemental Table S3. In general, the most frequently used substances were cocaine, psychostimulants, and marijuana, whereas opiates were uncommon, as confirmed by the drug analyses (described in the Supplement). Comparing the groups and including subcategories with current or previous non-AAS substance abuse problems is suggestive of different drug habits in the different groups. Several of the drugs, including tranquilizers, gamma-hydroxybutyric acid, and hallucinogens, such as ecstasy and lysergic acid diethylamide, were exclusively being used by the AAS drug subgroup.

On average, AASs had been used for 9.1 years, and AASs were commonly initiated during the participants' early 20s (mean age ± SD, 21.2 ± 6.2 years). The reported weekly AAS doses ranged from 125 to 7000 mg/week, with an average of 1278 mg/week (Supplemental Table S1).

Of the current AAS users ($n = 59$), including those having used AASs within the past 12 months, 80.7% had urine samples that were positive for steroids (the T/E ratio was not taken into account in this measure) (Table 2). Of the 11 current users with urine samples that were negative for AASs, three had T/E ratios of 6.3 (this test was also positive for antiestrogens), 9, and 47, respectively, that were consistent with their reports of exogenous testosterone administration. For seven participants, a few months had passed since their last AAS cycle, thus compatible with the test observations. One participant reported using low doses of trenbolone that could not be documented and might reflect the use of counterfeit steroids at the time of testing. This test was positive for antiestrogens and there was no reason to doubt his reports of use.

None of the control participants tested positive for steroids, whereas two positive urine tests were found in the previous user group. The positive tests could be related to the long detection times of the compounds used. Still, to minimize potential false reporting, replication analyses were carried out, excluding one control subject with a suspiciously high T/E ratio, current users with negative tests ($n = 9$), and previous users with positive tests ($n = 2$). Excluding these only marginally influenced the findings (Supplemental Figure S1).

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

	AAS Group (n = 82)		Control Group (n = 68)			
Attribute	Mean	SD	Mean	SD	t	p Value
Age (Years)	33.0	8.2	31.4	9.1	–1.15	.252
Education (Years)	14.1	2.5	15.9	2.7	4.10	.000
IQ	104.9	12.0	112.9	9.4	4.53	.000
Cigarettes per Day	1.7	4.3	0.3	2.4	–2.42	.016
Alcohol Units per Week	1.7	3.2	3.3	4.8	2.38	.017
Height (cm)	180.7	6.9	180.9	6.7	0.23	.817
Weight (kg)	96.7	13.7	90.4	14.0	–2.87	.005
Body Mass Index (kg/m^2)	29.6	4.1	27.6	4.0	–3.01	.003
Strength Training per Week (min)	351.5	206.7	467.0	241.9	3.13	.002
Endurance Training per Week (min)	124.7	194.1	92.0	112.8	–1.79	.204
Squats Max	217.2	57.4	172.3	41.5	–4.99	.000
Bench Max	168.6	30.8	135.6	32.0	–6.21	.000
Deadlift Max	231.8	49.1	198.8	45.1	–3.81	.000
Training Reason, n (%)					χ^2	
Bodybuilding	20 (24.4)		4 (6.0)		9.26	.002
Fitness	1 (1.2)		2 (2.9)		0.21	.885
Weightlifting	0 (0)		3 (4.5)		3.75	.053
Powerlifting	1 (1.2)		15 (22.4)		17.24	.000
Combat sports	11 (13.4)		4 (6.0)		2.26	.133
Athletics	1 (1.2)		0 (0.0)		0.82	.364
Strongman	5 (6.1)		1 (1.5)		2.02	.155
Recreational	37 (45.1)		31 (46.3)		0.02	.889
Other (e.g., ball sports)	6 (7.3)		8 (11.9)		0.93	.336
Top 5 Achievement (Sport/Bodybuilding), n (%)	19 (23.2)		24 (35.3)		2.67	.102
Non-AAS Substance Abuse (Previous or Current), n (%)	33 (40.2)		3 (4.5)		25.74	.000
Psychopharmaca (Previous or Current Use), n (%)						
Antidepressants	16 (20)		2 (3.0)		9.82	.002
Anxiolytics	14 (17.3)		0 (0.0)		12.79	.000
Opioids	4 (4.9)		0 (0.0)		3.41	.065
>1 type	6 (7.5)		0 (0.0)		5.16	.023
None reported	58 (71.6)		65 (97.0)		16.58	.000

AAS, anabolic-androgenic steroid.

The frequencies of various steroids found in the urine sample are shown in Supplemental Figure S2.

Associations Between AAS Use and Regional Brain Volumetry

Neuroanatomical volumes in each group (whole AAS sample) are presented in Table 3. Furthermore, neuroanatomical volumes with effects surviving Bonferroni correction and results when covarying for potential confounders and for different subsamples are presented in Table 4. There were no significant differences in ICV between the groups, but ICV was still regressed out from all group comparisons to ensure that this did not influence the results. The AAS group had significantly smaller volumes on measures of total gray matter volume, cortical volume, putamen volume ($p \leq .012$), and corpus callosum ($p = .013$), although the latter did not reach the Bonferroni-adjusted α-level. No group differences were found for hippocampal or amygdala volume (Table 3).

The findings of smaller total gray matter and cortical volume in the AAS group were marginally influenced by controlling for potential confounders. In addition, the effect sizes remained relatively stable across different AAS subsamples comprising various degrees of exposure to AASs and other drugs of abuse. Importantly, the differences were still significant in subsamples excluding participants with concurrent substance abuse, but the effect sizes were somewhat reduced (Supplemental Figure S3). The finding of smaller putamen varied more across different sample refinements and was not

Table 2. Percentage of Urine Samples Positive for AASs and Mean T/E Ratio in Controls, Current, and Previous AAS Users

Group	Analyzed, n (Missing)	AAS-Positive, n (%)	T/E Ratio	Range
Control	66 (2)	0	1.5 (1.5)	0.10–8.5
AAS Current	57 (2)	46 (80.7)	32.9 (42.9)	0.10–225.9
AAS Previous	22 (1)	2 (9.1)	2.5 (3.2)	0.6–14.7

AAS, anabolic-androgenic steroid; T/E, testosterone/epitestosterone.

Table 3. Group Differences in Brain Volumes Between AAS Users and Controls

	Controls (n = 68)		All AAS users (n = 82)				
	Mean	SD	Mean	SD	F	p Value	Partial η^2
Cerebral Cortex	530,158	47,327	506,914	46,871	11.06	.001	0.07
Total Gray Matter	705,416	58,536	678,682	56,569	10.36	.002	0.07
Intracranial Volume	1,670,649	130,821	1,655,202	109,347	0.32	.575	0.00
Lateral Ventricles	17,100	8437	18,881	10,699	1.55	.215	0.01
Thalamus	15,874	1462	15,511	1347	1.14	.288	0.01
Caudate	8358	1093	7997	975	3.11	.080	0.02
Putamen	13,529	1681	12,801	1477	6.55	.012	0.04
Pallidum	3268	439	3236	453	0.09	.768	0.00
Hippocampus	9351	846	9280	895	0.00	.960	0.00
Amygdala	4088	468	4014	496	0.29	.588	0.00
Accumbens	1627	239	1541	237	3.07	.082	0.02
Corpus Callosum	3456	460	3274	409	6.27	.013	0.04
Cerebellar Cortex	110,392	10,421	108,528	11,441	0.16	.69	0.00

Values are mm^3.

General linear models were performed with neuroanatomical volume as the dependent variable, group as the fixed factor, and age and intracranial volume as continuous covariates. All effects were in the form of smaller volumes in the anabolic-androgenic steroid (AAS) group.

significant when controlling for recent use of illegal drugs, symptoms of anxiety/depression, and a summarized measure of behavioral problems. It was also not significant when restricting the analyses to previous AAS users or AAS users with ≥10 years of AAS exposure (Table 4).

Associations Between AAS Use and Cortical Thickness

Figure 2 and Table 5 show the results from the corrected GLM analyses comparing differences in cortical thickness between the AAS group and control subjects. In the main analysis (upper panel), which included all participants, AAS users had significantly thinner cortex bilaterally. Five clusters were found in the left hemisphere and three clusters in the right hemisphere. The largest cluster in the left hemisphere covered part of the inferior and superior parietal lobe as well as lateral and medial occipital regions, and the cuneus. Other clusters with thinner cortex in the AAS users comprised (ranked from largest to smallest) superior temporal areas, the posterior cingulate, precentral and medial frontal gyrus, and finally a cluster covering a smaller part of the postcentral gyrus corresponding to Brodmann area 43.

The significant clusters in the right hemisphere corresponded to some degree to the clusters of the left

Table 4. Brain Volumes and Group Differences Shown for Controls and Various Anabolic-Androgenic Steroid User Subsamples

	Cerebral Cortex			Total Gray Matter			Putamen		
	mm^3 (SD)	F	p Value	mm^3 (SD)	F	p Value	mm^3 (SD)	F	p Value
All VIQ Regressed out (n = 82)	506,914 (46,871)	8.25	.005	678,682 (56,569)	8.092	.005	12,801 (1477)	6.655	.011
All Weekly Alcohol Regressed Out (n = 80)	506,421 (47,297)	12.69	.001	678,324 (57,218)	11.38	.001	12,779 (1476)	7.31	.008
All ASR Drugs Regressed Out (n = 67)	508,167 (46,068)	7.71	.006	680,380 (54,152)	6.92	.010	12,953 (1402)	.94	.334
All ASR Anxious/Depressed Regressed Out (n = 69)	507,716 (46,384)	5.22	.024	679,948 (55,509)	4.55	.035	12,991 (1404)	1.40	.239
All ASR Attention Problems Regressed Out (n = 69)	507,444 (45,398)	8.75	.004	679,703 (53,327)	7.34	.008	13,020 (1402)	3.25	.074
All ASR Total Problems Regressed Out (n = 65)	505,126 (45,168)	5.17	.025	676,808 (52,914)	4.74	.031	12,904 (1353)	1.98	.160
Current Users (n =59)	501,319 (48,625)	11.85	.001	671,367 (58,016)	12.01	.001	12,679 (1463)	6.51	.012
Previous Users (n = 23)	521,267 (39,445)	2.53	.116	697,445 (48,958)	1.63	.206	13,114 (1499)	1.98	.163
≥5 Years (n = 65)	503,060 (44,278)	13.80	.000	674,876 (53,936)	11.75	.001	12,758 (1512)	5.04	.027
≥5 Years With No Additional Drug Addiction[a] (n = 38)	506,042 (43,627)	4.25	.042	676,133 (53,074)	4.25	.042	12,504 (1448)	7.03	.009
≥10 Years (n = 32)	499,203 (45,041)	7.13	.009	668,009 (54,761)	7.40	.008	12,824 (1575)	.21	.650
≥10 Years With No Additional Drug Addiction[a] (n =18)	492,786 (42,037)	1.52	.221	655,133 (47,815)	3.20	.078	12,369 (1277)	.48	.491
AAS Dependence (n =44)	502,838 (46,158)	13.90	.000	676,604 (54,303)	10.94	.001	12,749 (1457)	6.49	.012
AAS Dependence With No Additional Drug Addiction[a] (n = 22)	503,386 (47,455)	4.97	.028	675,333 (55,018)	4.18	.044	12,468 (1428)	6.60	.012

n Denotes the number of participants in the AAS subgroup included in each subanalysis. Values are mm^3 (SD).

ASR, Adult Self-Report; VIQ, verbal IQ.

[a]Analyses where anabolic-androgenic steroid (AAS) users and controls with concurrent substance abuse are omitted. For the control group, n = 68 aside from the no additional drug addiction conditions (n = 65).

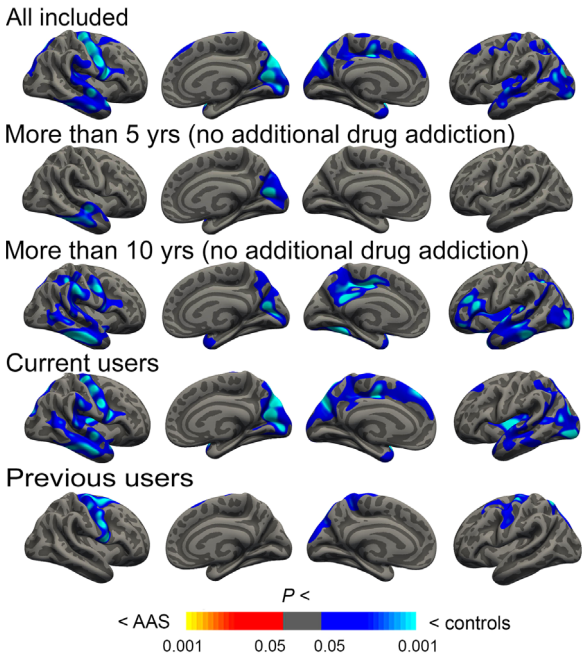

Figure 2. Vertexwise comparisons of cortical thickness between the control group and various anabolic-androgenic steroid (AAS) subsamples. The results show comparisons between the following groups: AAS users (n = 82) and controls (n = 68) (row 1); controls (n = 64) and AAS users exceeding 5 (n = 38) (row 2) or 10 years (n = 18) (row 3) of AAS use without concurrent non-AAS substance abuse; and controls (n = 68) with current (n = 59) (row 4) and previous (n = 23) (row 5) AAS users. Shades of blue indicate clusters with thinner cortices in the AAS group. No effects were seen in the opposite direction (i.e., thicker cortices).

hemisphere, but with some exceptions. The three clusters were of similar size and were located in the cuneus, the precentral gyrus, and some minor parts of the inferior frontal and superior frontal gyrus, and in temporal areas, including the temporal pole and the inferior, medial, and superior temporal gyrus. Regressing out verbal IQ, drug use, weekly alcohol consumption, symptoms of anxiety and depression, attention problems, and total problem scores had a marginal influence of the differences in thickness between users and nonusers (Supplemental Figure S4).

Exploratory analyses based on refinements of the AAS sample—done in order to focus on those with a longer history of AAS use—were suggestive of more widespread effects. This was particularly apparent in users with ≥10 years of AAS exposure and for those fulfilling the criteria for AAS dependence (Supplemental Figure S5 and Table S4). Characteristic findings after longer and more severe exposure were larger clusters in occipital, temporal, parietal, and frontal areas, which suggest that group differences after protracted AAS exposure are global. Group differences were now also seen in large frontal cortical regions and in the left cingulate. Similar findings were seen when excluding participants with concurrent substance abuse, particularly for those with ≥10 years of AAS exposure, although some clusters were smaller or did not survive corrections. The main findings employing a cluster-forming threshold of $p < .01$ and FDR correction are shown in Supplemental Figures S6 and S7 and Supplemental Table S5.

Moreover, as the choice of smoothing kernel might influence the findings, the main findings using the higher smoothing level of 30 mm (both uncorrected and FDR-corrected) are shown in Supplemental Figure S8. Note that more effects survive FDR correction by applying this higher smoothing level.

Similar clusters were also found when restricting the analysis to current AAS users. For previous users, thinner cortex was seen bilaterally in the precentral gyrus and in the left superior parietal cortex (Figure 2 and Table 5). The effects were more widespread in current users, but statistical power was higher for this group. Analyses comparing previous (n = 23) and current (n = 59) users showed only minor differences, with thinner cortex in the current users in parts of the parahippocampal and lateral occipital cortex in the left hemisphere (Supplemental Figure S9 and Supplemental Table S6).

Analyses comparing AAS users with long-term AAS exposure (≥10 years; n = 32) to those with shorter exposure (≤5 years; n = 23) showed that longer exposure was associated with thinner cortex in widespread regions, including the isthmus and posterior cingulate, middle and inferior temporal regions, and a frontal cluster, including rostral middle frontal regions, pars opercularis, and the medial orbitofrontal cortex. Finally, the combination of drug abuse with AAS use was associated with more widespread thinning, particularly in the cuneus, superior frontal, and orbitofrontal regions of the left hemisphere, and one cluster in the supramarginal gyrus of the right hemisphere (Supplemental Figure S9).

DISCUSSION

In the first large systematic neuroimaging investigation of AAS users, we found smaller overall gray matter, cortical and putamen volume, and thinner cortex in widespread regions in AAS users compared to nonusing weightlifters. Generally, stronger effects were seen with an increasing burden of AAS exposure and in users without any other substance abuse problems. Possible implications of the results are discussed below.

Thinner and Smaller Cortex Associated With AAS Use

AAS users had smaller overall cortical volume and thinner cortex in widespread regions, and this was relatively stable across different subsamples. The effects were more widespread after longer use. The effects on cortical thickness and volume could not be explained by concurrent substance abuse, although drug abuse in combination with AAS use was associated with even larger cortical effects than AAS use alone.

One previous study reviewed associations between long-term AAS use and brain morphometry, and suggested that chronic AAS use could be associated with enlargement of the right amygdala (12), consistent with the primary action of steroids acting on androgen receptors, which are highly expressed in the amygdala (15). In the present study, we could not replicate the findings regarding the amygdala. Instead, smaller volumes and thinner cortex throughout were seen in the AAS group. For brain volumes, the strongest group effects

Table 5. Differences in Cortical Thickness Between Various AAS User Subsamples and the Control Group

Cortex Area	Cluster Size (mm^2)	Talairach Coordinates			CWP
		x	y	z	
All Included					
Left inferior parietal	11,253.03	−36.5	−84.4	21.7	0.00010
Left posterior cingulate	2332.27	−11.1	−11.6	40.0	0.00170
Left superior temporal	4176.67	−44.0	6.8	−24.6	0.00010
Left superior frontal	1540.41	−7.0	38.9	48.4	0.02770
Left precentral	2059.62	−22.4	−23.2	63.7	0.00500
Right cuneus	6796.20	9.1	−66.0	13.6	0.00010
Right precentral	5358.84	53.9	−2.7	28.7	0.00010
Right superior temporal	5884.78	57.9	−10.8	−5.1	0.00010
≥5 Years With No Drugs					
Right cuneus	3437.14	4.1	−69.0	15.6	0.00010
Right superior temporal	2405.88	53.6	−13.9	−6.9	0.00110
≥10 Years With No Drugs					
Left posterior cingulate	4254.27	−12.7	−15.7	38.5	0.00010
Left fusiform	19,776.99	−27.9	−55.8	−16.1	0.00010
Right middle temporal	9072.23	54.1	−30.8	−17.2	0.00010
Right cuneus	3591.47	12.1	−67.6	16.2	0.00010
Right precentral	2371.46	55.8	−2.4	36.0	0.00140
Right middle temporal	1607.63	51.8	−57.6	1.2	0.02350
Current Users					
Left inferior parietal	13,995.15	−35.7	−82.7	24.4	0.00010
Left superior temporal	5169.37	−46.0	3.9	−21.6	0.00010
Left fusiform	1702.65	−27.3	−50.1	−17.0	0.01510
Left superior frontal	1405.15	−6.2	42.0	44.9	0.04500
Right cuneus	7477.24	10.1	−65.9	13.1	0.00010
Right precentral	3426.04	54.1	−2.8	29.3	0.00010
Right insula	8132.27	36.0	−17.5	12.1	0.00010
Previous Users					
Left precentral	4061.93	−32.1	−16.2	67.0	0.00010
Left superior parietal	2375.72	−22.0	−61.7	60.9	0.00010
Right precentral	4611.91	57.1	5.3	13.2	0.00010

The cortical area, the size of the significant cluster, Talairach coordinates corresponding to the most significant vertex within each cluster, and clusterwise *p* values (CWPs) are shown. All findings are in the direction of thinner cortices in the anabolic-androgenic steroid (AAS) group.

applied to the global measures of cerebral cortex and total gray matter volume. Group differences were also found for putamen, a large structure of the basal ganglia. These effects could reflect an association between long-term AAS exposure and less brain tissue in general rather than with more region-specific effects. In general, stronger effects were observed after prolonged exposure, and this was particularly evident in those who met the requirements for AAS dependence. Within-group analyses also confirmed that a longer history of AAS exposure was associated with thinner cortex in the frontal, temporal, parietal, and occipital regions compared to subjects with shorter exposures. Our findings might indicate that use of AAS is associated with a risk of progressive deterioration of cerebral tissue, and that this is particularly evident after prolonged heavy use. It has been shown that supraphysiological doses of testosterone and commonly abused AASs can induce apoptosis on a variety of mammalian cell types, including neurons (17,18,20,43). The mechanisms behind possible AAS-induced neurotoxicity are unclear, but amyloid-beta aggregation and increased susceptibility to oxidative stress have been suggested (17–20,44–46). Cardiovascular effects are among the most serious adverse effects associated with AAS use (24,28–30) that again have been linked with accelerated brain aging (24,47,48), vascular brain disease (25), cognitive decline (26), and dementia (26,27). Another potential mechanism behind our findings could be related to AAS-induced cardiovascular effects and associated risks to compromise brain health.

Prolonged AAS use has been associated with a range of psychiatric symptoms and disorders (6–9) and recently also with cognitive deficits (10), and our findings could potentially constitute brain correlates of such deviations. However, the nature of such relations are complex, not least of all because it is difficult to distinguish what is caused by premorbid psychological characteristics and what is a direct cause of AAS use. Our knowledge of adverse health consequences of AASs primarily comes from field studies (49). In addition, for a

proportion of users, AASs are used in conjunction with other drugs of abuse (50–52), and studies usually do not separate users with more clean AAS use from those with combined AAS and non-AAS substance abuse. This is of importance not only in order to understand the consequences of AAS use per se, but also to grasp the potential health consequences of a lifestyle consisting of hard weight training in combination with hormone and polydrug abuse.

Our findings could not be explained by differences in verbal cognitive function (i.e., verbal intelligence) or ICV. Verbal intelligence can be argued, at least to some degree, to reflect premorbid cognitive functions. ICV reflects premorbid brain volume, and the lack of ICV differences between the groups indicates that the volumetric effects seen emerged after the volume of the brain was fully developed. The fact that the cortical group effects survived controlling for verbal intelligence and ICV may indicate that the AAS exposure itself could be causing the effects on the cerebral cortex. In addition, there were also group differences when users with additional non-AAS substance abuse were omitted, and after controlling for other potential confounders, such as weekly alcohol consumption, recent use of illegal drugs, anxiety/depression, attention problems, or global indices of behavioral problems. In addition, the within-group findings of thinner cortex after longer history of AAS use raise the ominous possibility that the observed group differences are the result of AAS-induced cerebral atrophy. The underlying mechanisms are not known but could involve direct AAS-induced neurotoxicity or more indirect mechanisms through AAS side effects on the cardiovascular system. However, these are speculations, and the present design does not allow drawing definite conclusions regarding causality beyond the correlational results described.

Limitations

Important limitations of the study include the use of a cross-sectional, retrospective design, which means that we do not know whether differences in brain morphometry also existed before AAS initiation. We also cannot rule out the influence of genetic effects or the possibility that AAS use is associated with other lifestyle risk factors that might influence brain volume.

Conclusions

Although correlational, our findings of thinner and smaller cortices associated with AAS exposure raise concerns about possible deleterious effects of long-term AAS use on brain health. The cortical effects seemed to persist after stopping AAS use. Understanding the impact of AAS use on brain and its cognitive and psychiatric correlates is important in order to safeguard the needs of the growing numbers of long-term AAS users now entering middle age. Large-scale longitudinal—and ideally prospective—studies are warranted to address the possible implication of accelerated cerebral atrophy caused by long-term AAS exposure.

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