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RESEARCH ARTICLE

Long-term anabolic steroids in male bodybuilders induce cardiovascular structural and autonomic abnormalities

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Abstract
Objective The aims of this study were to examine the hypothesis that users of anabolic androgenic steroids (AAS) would have cardiac autonomic disorders and that there is a correlation between sympathetic modulation, high blood pressure (BP) and alterations to cardiac dimensions.
Methods Forty-five male subjects were enrolled in the study. They were categorized into three groups comprising bodybuilders actively using AAS (AAS users; $n = 15$), bodybuilders who had never used AAS (nonusers; $n = 15$) and age-paired healthy sedentary controls ($n = 15$). Hemodynamic parameters, linear and nonlinear analyses of heart rate variability and electrocardiography and echocardiography analyses were performed at rest.
Results Bodybuilders in the AAS group had a higher mean BP than those in the ASS nonuser group ($p < 0.05$) and the sedentary controls ($p < 0.001$). Cardiac sympathetic modulation was higher in AAS users than in AAS nonusers ($p < 0.05$) and the sedentary controls ($p < 0.001$), and parasympathetic modulation was lower in AAS users than in nonusers and the sedentary controls ($p < 0.05$). Shannon entropy was lower in AAS users than in the sedentary ($p < 0.05$) controls, and the corrected QT interval and QT dispersion were higher in AAS users than in the sedentary controls ($p < 0.05$). The interventricular septal thickness, left ventricle posterior wall thickness and relative diastolic wall thickness were higher in AAS users than in AAS nonusers and the sedentary controls ($p < 0.001$). AAS users showed a positive correlation between increased sympathetic modulation and high BP ($r = 0.48$, $p < 0.005$), as well as sympathetic modulation and cardiac hypertrophy ($r = 0.66$, $p < 0.001$).
Conclusion There was a marked cardiac autonomic alteration in AAS users, with a shift toward sympathetic modulation predominance and vagal attenuation. The high BP observed in our group of bodybuilders using AAS was associated with increased sympathetic modulation, and this increased sympathetic modulation was associated with structural alterations in the heart. This association may constitute an important mechanism linking AAS abuse to increased cardiovascular risk.

Keywords Anabolic androgenic steroids · Autonomic nervous system · Heart rate variability · Nonlinear symbolic dynamics

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Introduction

Anabolic androgenic steroids (AAS) include both natural compounds and synthetic analogs of testosterone developed to maximize anabolic action and minimize androgenic action. AAS supplementation is a widespread strategy

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among young athletes and non-athletes alike who want to increase strength and muscle mass gain [1]. Long-term illicit AAS abuse is a public health issue, and in recent years special attention has been directed toward its role in the development of cardiovascular abnormalities, although these remain poorly understood. Results from cross-sectional studies associate AAS with hypertension [2] and suggest that AAS bind to androgen receptors to cause cardiac hypertrophy [3]. Echocardiographic studies show that supraphysiological doses of AAS lead to an increased ventricular mass index and increased thickness of the intraventricular septum in steroid-using bodybuilders in comparison to non-users and that they also cause harm to the diastolic function in the former, associated with a reduction in the velocity peak during the initial phase of diastolic filling [4, 5]. Former AAS users continue to show a concentric left ventricular (LV) hypertrophy or high blood pressure (BP), both of which seem to be related to the doses and the type of AAS used [6].

High doses of AAS increase sympathetic nervous activity, which seems to have hemodynamic implications [5]. BP levels are higher in AAS users than in AAS nonusers [7]. The effects of AAS are even more worrying due to the higher mortality rate among AAS compared to nonusers [8]. Although the cause of death in the former is unclear, postmortem investigations suggest cardiac causes in many cases [9]. Some studies have linked ventricular arrhythmias, myocardium infarction and sudden death to abusive AAS use in bodybuilders [10] which are consistent with cardiac anatomical and autonomic alterations. The resting heart rate (HR) and sympathetic nervous activity in AAS users has been found to be higher than that in nonusers, which favors the notion of a cardiac autonomic imbalance in individuals self-administering AAS [7].

The efficacy of HR autonomic control is commonly evaluated using the HR variability (HRV) method, which provides an indirect assessment of cardiac autonomic balance [11]. This approach is extensively used in cardiology to stratify the cardiovascular risk of life-threatening cardiac arrhythmias and sudden death [12, 13], due to its noninvasiveness, ease of application and high reproducibility of the results [14]. It is well documented that autonomic HR regulation is correlated with the integrity of complex interactions between electrophysiological and hemodynamic variables [14]. On the other hand, the HR is considered to present nonlinear behavior due to the complex interaction between the central nervous system, reflex mechanisms and neurohumoral factors [15–17].

Studies have demonstrated that linear analyses are appropriate for the study of HRV [18, 19]. Nevertheless, interest in nonlinear methods has grown in the last years [15–17] due to observations that HR fluctuation is subordinated to autonomic nervous system control of cardiac activity and vascular dynamics, which suggests that the mechanisms involved in cardiovascular regulation have repercussions on other organs [20].

Despite the adverse effects of high doses of AAS on the cardiovascular system, few studies have used nonlinear analysis of HRV to assess the potential association between AAS abuse and the risk of alterations to cardiac autonomic function. To our knowledge, our study is the first to evaluate cardiac autonomic control in AAS-using athletes using two different methods based exclusively on the HRV analysis and to associate these methods with BP levels and cardiac remodeling. Consequently, we examined the effects of AAS abuse on cardiac autonomic control by linear and, in particular, nonlinear methods of HRV in AAS users in comparison with drug-free bodybuilders (nonusers) and healthy sedentary controls, with special attention on the association between sympathetic modulation (evaluated by nonlinear dynamic analysis) and cardiac morphofunctional alterations in these individuals.

We further hypothesized that AAS use may lead to changes in cardiac autonomic control, in particular to an increase in sympathetic modulation and vagal reduction, and that these alterations are detectable using a nonlinear method such as symbolic dynamic and linear methods in the time and frequency domains [20]. A second hypothesis was that a higher sympathetic modulation caused by long-term use of high doses of steroids could be associated with hypertension and cardiac morphological changes in bodybuilding athletes.

Methods

Study population and AAS abuse

We recruited a male population of 30 competitive bodybuilders, among whom 15 were actively taking AAS for more than 2 years (AAS user group) and 15 had never used AAS (AAS nonuser group), by advertising in gyms, and also recruited 15 age-paired healthy sedentary controls by advertising in a university center. Those interested in participating in the study contacted us and were invited to visit our research laboratory. Using this approach, we sought to maximize the representativeness of the sample and minimize selection bias [21]. Exclusion criteria included coronary artery disease, valvular and congenital heart disease, congestive heart failure, diabetes mellitus, sinus tachycardia, smoking habit and psychiatric, respiratory or metabolic disorders. The study was approved by the appropriate institutional ethic committee, and all participants provided written informed consent before being allowed into the study.

All participants were asked to complete self-administered questionnaires aimed at eliciting a self-reported history of AAS use regarding specific drug(s), dosages and duration. All AAS users had been self-administering AAS

for at least 4 weeks before the study. Bodybuilders in both groups were not taking other performance-enhancing drugs (e.g. human growth hormone, etc.). Additionally, those in the AAS user group reported not using any form of aromatase inhibitor, selective estrogen receptor modulator or human chorionic gonadotropin. Venous blood samples were drawn from each subject, always in the morning between 1000 and 1200 hours, to evaluate serum hormone levels of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol hormone (E_2) and dehidroepiandrosterona sulfate (DHEA-S) to confirm the consumption of anabolic steroids. Each AAS user admitted to currently self-administering multiple AAS by intramuscular injection and/or oral intake.

Experimental design

The study used a randomized cross-sectional experimental design with two experimental visits. The experimental visits were completed within 4 weeks and separated by at least 2 days. In the week that the data collection protocols were performed, both groups of bodybuilders were instructed to reduce the training load by 50% in order to minimize the effects of resistance training on HRV. During the subjects' first visit to our laboratory we obtained blood samples, data on anthropometrical and body composition, information on lifestyle habits and a detailed history of classical illicit substance use. During the second visit, we evaluated basic physiological data, HRV analyses and electrocardiogram (ECG) and echocardiogram data. For these analyses, all subjects were instructed to abstain for at least 24 h prior to the visit from the use of stimulants (e.g. drugs, caffeine, tobacco, alcoholic beverages, high-fatty food) and medicines, as well as physical activity. The experimental sessions were performed in baseline condition (supine position) in a quiet clinical laboratory room at ambient temperature (21–25 °C) in the morning period, 2 h after the first regular breakfast, at approximately the same time of day. As the first step, the hemodynamic parameters of BP and HR were obtained after the subject had rested for 10 min in a quiet temperature-regulated room. The autonomic tests were then performed, and the ECG signal was recorded for 5 min. Subjects were not allowed to sleep. The ECG signal was continuously amplified by an ECG recorder (model ECG-5; EcafixFunbec, São Paulo, Brazil), collected by analog to a digital converter board (DI-194 Starter kit; Dataq Instruments, Akron, OH) at a sampling rate of 240 Hz and stored on a personal computer for posterior offline analysis. In the acute autonomic recording sessions, the individuals breathed spontaneously and regularly and had their respiratory rate counted; only those with more than 9 rpm were included in the analysis to avoid a modulatory impact of respiratory variables on HRV power spectra. The final procedure consisted of conducting resting ECG and echocardiographic recordings.

Body composition, BP and HR measures

Height, weight and body mass index (BMI) were measured and body surface estimated from a nomogram. The percentage of body fat and the resulting fat-free body mass were estimated using the three-skinfold method with calipers. The BP was monitored noninvasively with an automatic and oscillometric cuff (M3 Intellisense HEM-7051-E; Omron Healthcare, Kyoto, Japan). The HR was monitored through lead II of an electrocardiogram (ECG-5; Ecafix Funbec, São Paulo, Brazil) beat by beat.

Time and frequency domains analysis of HRV

The overall variability of RR intervals was assessed in the time domain by means of the time-series variance. Subsequently, frequency domain analysis of HRV (spectral analysis) was performed with an autoregressive algorithm [12] on the same sequences used for symbolic dynamics of HRV. In brief, a modeling of the time-series RR of intervals was calculated based on Levinson–Durbin recursion, with the order of the model chosen according to Akaike's criterion. The temporal indices analyzed were: RR intervals (in milliseconds) and variance. The variance was estimated as a marker of total variability in the time domain. The power spectral density was calculated for each RR series. Three spectral components were considered: very low frequency, ranging from 0 to 0.03 Hz; low frequency (LF), ranging from 0.03 to 0.15 Hz; high frequency (HF), ranging from 0.15 to 0.40 Hz. The spectral components were expressed in absolute (ms^2) and normalized units (nu). The LF/HF ratio was also calculated to reflect a shift to sympathetic or parasympathetic predominance. Normalization consisted of dividing the power of a given spectral component by the total power minus the power below 0.03 Hz and multiplying the product by 100 [12]. All temporal and spectral parameters were quantified in the baseline condition.

Symbolic dynamics analysis of HRV

Symbolic dynamics is based on a coarse-graining of the measurements, that is, the data are transformed into a new time series with only a few elements (letters from an alphabet). The method has been fully described and validated [16]. Briefly, RR interval sequences of length $n = 300$ were selected. The length (L) was kept fixed in all analyses. The full range of the sequences was uniformly spread on six levels (from 0 to 5), and patterns of length $L = 3$ were constructed. Therefore, each subject and each experimental condition had its own range of RR intervals. The Shannon

entropy [15] of the distribution of the patterns was calculated to provide a quantification of the complexity of the pattern distribution. All possible patterns (i.e. 216) were grouped without any loss into three families referred to as: patterns without variation (0 V; i.e. all 3 symbols were equal); patterns with one variation (1 V; i.e. 2 consequent symbols were equal and the remaining symbol was different); patterns with two variations (2 V; i.e. all symbols were different from the previous one). The percentage of patterns 0 and 2 V is reported in the Results section. To test the presence of deterministic structures in the series, we carried out a surrogate data analysis [20]. Fifteen surrogate series for each original series were created by shuffling the temporal order of the samples. The percentages of 0 and 2 V patterns derived from the original series were compared with those derived the surrogate series.

ECG and echocardiography measurements

A standard 12-lead ECG was recorded with the subject in the supine position during quiet respiration using an Ergo98 electrocardiograph (Heart Ware; Belo Horizonte, Brazil) at a paper speed of 25 mm/s. P wave and QRS complex duration, PR interval and ST segment parameters were measured manually in each of the 12 leads using the average of three consecutive cardiac cycles. The QT interval (QTi) was determined from the beginning of the Q wave to the end of the T wave. QT dispersion (QTd) was calculated as the difference between the longest and the shortest QTi. Both QTi and QTd were corrected for HR using Bazett's formula (cQT and cQTd = QTi and QTd divided by the square root of the preceding RR interval). The mean of cardiac electrical axis was obtained to determine the vector in a horizontal plane. The clinical usefulness of the Sokolow–Lyon voltage criteria in the assessment of ECG LV hypertrophy is addressed.

The echocardiographic recordings and measurements were obtained using a Philips model IE33 echocardiograph (Philips Medical Systems, Andover, MA) equipped with a 2–5 MHz transducer, according to the guidelines of the American Society of Echocardiography, with the subject in the supine left lateral position. All recordings and measurements were made by the same experienced investigator who was blinded to AAS use and to the results of the physical examination. The parameters measured were LV end-diastolic and end-systolic diameters, interventricular septal thickness, LV posterior wall thickness, relative diastolic wall thickness, LV muscle mass (calculated according to American Society of Echocardiography criteria) and normalized for body surface area (BSA), LV end-diastolic and end-systolic volume and LV ejection fraction, calculated from the LV volumes measured using Simpson's biplane method. Cardiac output was also measured.

Statistical analysis

Data are presented as the mean ± standard error of the mean. The unpaired Student's *t* test was used to analyze the differences in training time in years and weekly training volume between both groups of bodybuilders. One-way analysis of variance followed by Tukey's adjusted post hoc test was used to correct for multiple comparisons when the analyses revealed significant differences in age, anthropometric profile, physiological hemodynamic parameters, HRV and electrocardiographic and echocardiograph data between the three groups. Pearson correlation coefficients were used to assess the association of sympathetic modulation with mean BP and echocardiography parameters. Probability values of < 0.05 were considered to be statistically significant. All data were entered and analyzed using the SigmaStat program (Jandel Scientific Software, SPSS, Chicago, IL).

Results

Clinical characteristics of the study population

The mean duration of AAS use was 3.8 ± 0.3 years, as determined by the drug combination method over two to four cycles per year. The mean weekly dosage of AAS was 646.6 ± 34.2 mg. The most highly used anabolic steroids were nandrolone decanoate (80%), testosterone propionate (73%), stanozolol (57%) and testosterone cypionate (31%). Individuals in both bodybuilding groups had similar weight-lifting practice times and number of hours per week currently spent on weight training.

Anthropometric characteristics, information on exercise training profiles and endocrinological measurements are shown in Table 1. No age and height differences between groups emerged. The weight and BMI were higher in AAS users (99.1 ± 2.1 kg and 30.8 ± 04 kg/m^2) than in nonusers [85.6 ± 3.1 kg ($p < 0.05$) and 27.7 ± 0.8 kg/m^2 ($p < 0.05$)] and the sedentary controls [81.6 ± 2.9 kg ($p < 0.001$) and 27.0 ± 0.8 kg/m^2 ($p < 0.05$)]. Total fat content was lower in the AAS user (12.4 ± 1.1 kg) and nonuser (12.4 ± 1.2 kg) bodybuilding groups than in the sedentary controls (17.5 ± 1.1 kg; $p < 0.05$). As expected, total muscle mass was higher in AAS users (63.0 ± 1.8 kg) and nonusers (52.9 ± 1.5 kg) than in the sedentary controls (44.8 ± 1.5 kg; $p < 0.001$) and significantly lower in AAS nonusers than in AAS users ($p < 0.05$).

Analysis of the collected data indicated that serum testosterone and E_2 levels were higher in the AAS users (99.8 ± 7.7 pg/ml) than in the nonuser (40.2 ± 2.1 pg/ml; $p < 0.001$) and sedentary controls (39.3 ± 3.0 pg/ml; $p < 0.001$). In addition, AAS users were found to have drastically reduced levels of LH (0.4 ± 0.1 mUI/L), FSH

Table 1 Anthropometric characteristics, training profile and endocrine profile

Anthropometric characteristics	Study group		
	Sedentary controls ($n = 15$)	AAS nonusers ($n = 15$)	AAS users ($n = 15$)
Age (years)	30.2 ± 0.8	30.0 ± 1.0	29.2 ± 1.1
Years of regular weightlifting (years)	–	6.3 ± 0.5	7.0 ± 0.2
Hours of exercise per week (h)	–	6.6 ± 0.2	7.4 ± 0.3
Weight (kg)	81.6 ± 2.9	85.6 ± 3.1	99.1 ± 2.1*§
Height (cm)	1.73 ± 0.0	1.75 ± 0.0	1.79 ± 0.0
BMI (kg/m^2)	27.0 ± 0.8	27.7 ± 0.8	30.8 ± 0.4‡§
Total fat (kg)	17.5 ± 1.1	12.4 ± 1.2‡	12.4 ± 1.1‡
Total MM (kg)	44.8 ± 1.5	52.9 ± 1.5‡	63.0 ± 1.8*†
Testosterone (ng/dL)	406.2 ± 34.4	417.0 ± 22.4	665.8 ± 87.6‡§
LH (mUI/L)	3.5 ± 0.2	2.9 ± 0.2	0.4 ± 0.1*†
FSH (mUI/ml)	3.4 ± 0.3	2.9 ± 0.2	0.8 ± 0.2*†
E_2 (pg/ml)	39.3 ± 3.0	40.2 ± 2.1	99.8 ± 7.7*†
DHEA-S (mcg/dL)	232.8 ± 18.7	238.8 ± 25.5	98.8 ± 5.5*†

Values in table are presented as the mean ± standard error of the mean (SEM)

AAS Anabolic androgenic steroids, *BMI* body mass index, *MM* muscle mass, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, E_2 estradiol hormone, *DHEA-S* dehidroepiandrosterona sulfate

*$p < 0.001$ vs. sedentary men; †$p < 0.001$ vs. AAS nonusers; ‡$p < 0.05$ vs. sedentary controls; §$p < 0.05$ vs. AAS nonusers

(0.8 ± 0.2 mUI/L) and DHEA-S (98.8 ± 5.5 mcg/dL) in comparison to the nonusers (2.9 ± 0.2 mUI/L, 2.9 ± 0.2 mUI/L, 238.8 ± 25.5 mcg/dL; $p < 0.001$) and sedentary controls (3.5 ± 0.2 mUI/L, 3.4 ± 0.3 mUI/L, 232.8 ± 18.7 mcg/dL; $p < 0.001$) (Table 1). Analysis of the baseline characteristics of all subjects, such as family history of atherosclerotic disease, eating habits and work activities, revealed no significant differences between the three groups of subjects.

Hemodynamic parameters

Resting HR and BP are shown in Fig. 1. There were no significant between-group differences in baseline HR (sedentary

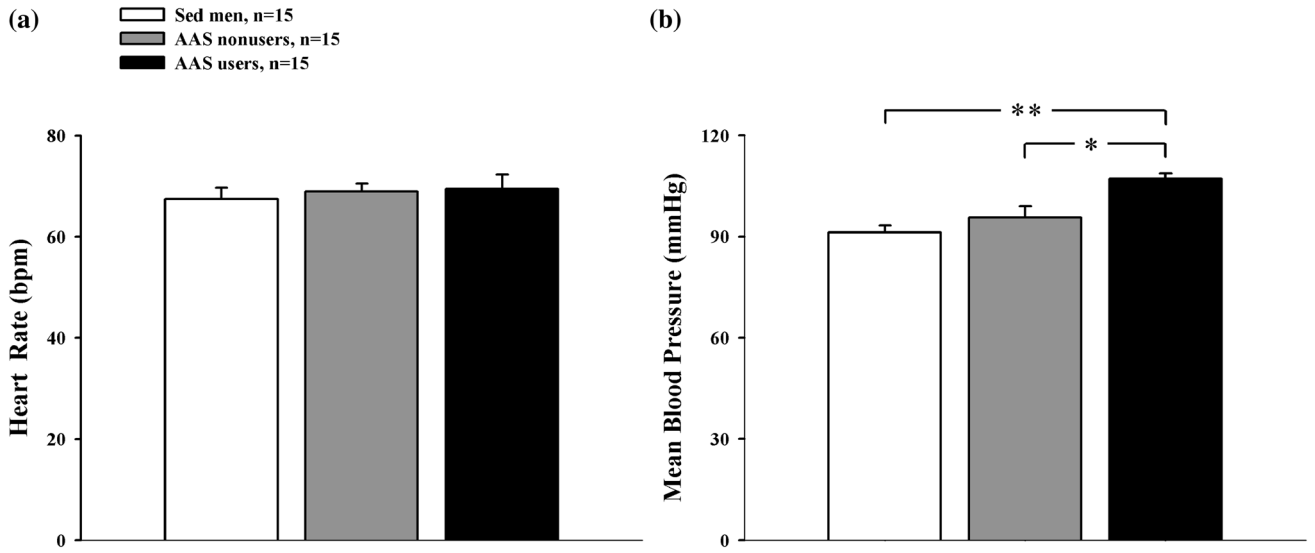

Fig. 1 Graphs showing resting heart rate (HR) (**a**) and baseline mean blood pressure (MBP) (**b**) in anabolic androgenic steroid (AAS) users ($n = 15$), AAS nonusers ($n = 15$) and sedentary controls (Sed men, $n = 15$). Data are presented as the mean ± standard error of the mean (SEM). Note that MBP at rest was significantly increased in AAS users compared with the other groups *$p < 0.05$; **$p < 0.001$

controls: 67.5 ± 2.2 bpm; nonusers: 68.9 ± 1.6 bpm; AAS users: 69.4 ± 2.9 bpm) (Fig. 1a). In contrast, the mean BP at rest was higher in the AAS user group (107.1 ± 2.8 mmHg) than in the nonuser and sedentary control groups [95.7 ± 3.3 mmHg ($p < 0.05$) and 91.2 ± 2.1 mmHg ($p < 0.001$), respectively] (Fig. 1b).

HR variability

The HRV determined by linear analysis is shown in Table 2. There was a significant increase in absolute (1711.0 ± 133.6 ms^2) and normalized (73.0 ± 3.5 nu) LF oscillations of HRV in AAS users compared to nonusers (711.7 ± 66.7 ms^2 and 48.8 ± 4.3 nu; $p < 0.001$) and the sedentary controls (802.7 ± 95.0 ms^2 and 48.9 ± 4.3 nu; $p < 0.001$). AAS users also had a significantly increased LF/HF ratio (6.5 ± 1.4) compared to nonusers (1.9 ± 0.5; $p < 0.001$) and the sedentary controls (0.8 ± 0.1; $p < 0.001$). In contrast, the HF band was lower in AAS users (647.7 ± 266.6 ms^2) than in the sedentary controls (1265.7 ± 248.8 ms^2; $p < 0.05$). The normalized values of HF were significantly lower in AAS users (26.9 ± 3.5 nu) than in nonusers (48.5 ± 5.0 nu; $p < 0.001$) and the sedentary controls (50.9 ± 4.3 nu; $p < 0.001$).

Symbolic nonlinear analysis

The percentage of 0 V dynamics was significantly higher in AAS-using bodybuilders (43.5 ± 4.3%) than in nonusers (30.8 ± 2.9%; $p < 0.05$) and the sedentary controls (21.1 ± 2.0%; $p < 0.001$) (Fig. 2a). In contrast, the percentage of 2 V dynamics was significantly lower in AAS users (10.8 ± 1.4%) than in AAS nonusers and the sedentary controls [17.1 ± 2.3% ($p < 0.05$) and 20.7 ± 1.2% ($p < 0.001$), respectively] (Fig. 2b). The Shannon entropy analysis in AAS users was lower (3.1 ± 0.1) than that in the sedentary controls (3.6 ± 0.0; $p < 0.05$) (Fig. 2c).

Electrocardiographic and echocardiographic parameters

The electrocardiographic data are shown in Table 3. The mean duration of the QT interval did not differ between groups. However, when the data were adjusted for HR, among the two groups of bodybuilders, the AAS users had the longest corrected QT interval (375.5 ± 6.7 ms) in comparison to the sedentary controls (354.9 ± 4.0 ms; $p < 0.05$). What also was shown up in the corrected QT dispersion (67.6 ± 5.2 ms in users) versus (42.9 ± 2.2 ms in sedentary; $p < 0.001$). The mean of cardiac electrical QRS axis was significantly higher in user group (17.8 ± 7.4°) when compared to nonusers (46.9 ± 5.1°; $p < 0.001$) and sedentary (54.7 ± .1°; $p < 0.001$). The Sokolow–Lyon voltage criteria in the assessment of electrocardiographic LV hypertrophy were higher in users (29.0 ± 0.4 mm) than in nonusers (21.1 ± 0.6 mm; $p < 0.001$) and sedentary men (15.6 ± 0.7 mm; $p < 0.001$).

Standard echocardiographic results are listed in Table 4. The interventricular septal thickness (12.3 ± 0.4 mm), LV posterior wall thickness (11.5 ± 0.3 mm), LV mass (243.7 ± 4.2 g), LV mass normalized to BSA (127.7 ± 3.9 g/m^2) and relative diastolic wall thickness (0.46 ± 0.0) were significantly greater in AAS users than in nonusers (8.6 ± 0.2 mm, 8.7 ± 0.2 mm, 178.9 ± 5.6 g, 89.6 ± 2.2 g/m^2 and 0.34 ± 0.0, respectively; $p < 0.001$) and the sedentary controls (8.0 ± 0.2 mm, 7.7 ± 0.2 mm, 139.3 ± 7.3 g, 71.8 ± 3.5 g/m^2 and 0.32 ± 00, respectively; $p < 0.001$). The LV end-diastolic volume was higher in AAS users (131.1 ± 3.0 mm) than in the sedentary controls (110.7 ± 6.0 mm; $p < 0.05$). The LV end-diastolic volume, LV mass and LV mass normalized to BSA were significantly higher in AAS nonusers than in the sedentary controls ($p < 0.05$).

Table 2 Spectral parameters of heart rate calculated from time series using autoregressive power spectral analysis at rest

Spectral parameters	Study group		
	Sedentary controls ($n = 15$)	AAS nonusers ($n = 15$)	AAS users ($n = 15$)
Variance (ms^2)	4293.8 ± 814.4	3199.2 ± 535.4	2301.0 ± 386.3
LF (ms^2)	802.7 ± 95.0	711.7 ± 66.7	1711.0 ± 133.6*†
LF (nu)	48.9 ± 4.3	48.8 ± 4.3	73.0 ± 3.5*†
HF (ms^2)	1265.7 ± 248.8	742.3 ± 156.8	647.7 ± 266.6‡
HF (nu)	50.9 ± 4.3	48.5 ± 5.0	26.9 ± 3.5*†
LF/HF ratio	0.8 ± 0.1	1.9 ± 0.5	6.5 ± 1.4*†

Values in table are presented as the mean ± SEM

LF Low-frequency spectral component of heart rate variability (HRV), *HF* high-frequency spectral component of HRV, *nu* normalized units

*$p < 0.001$ vs. sedentary men; †$p < 0.001$ vs. AAS nonusers; ‡$p < 0.05$ vs. sedentary men; §$p < 0.05$ vs. AAS nonusers

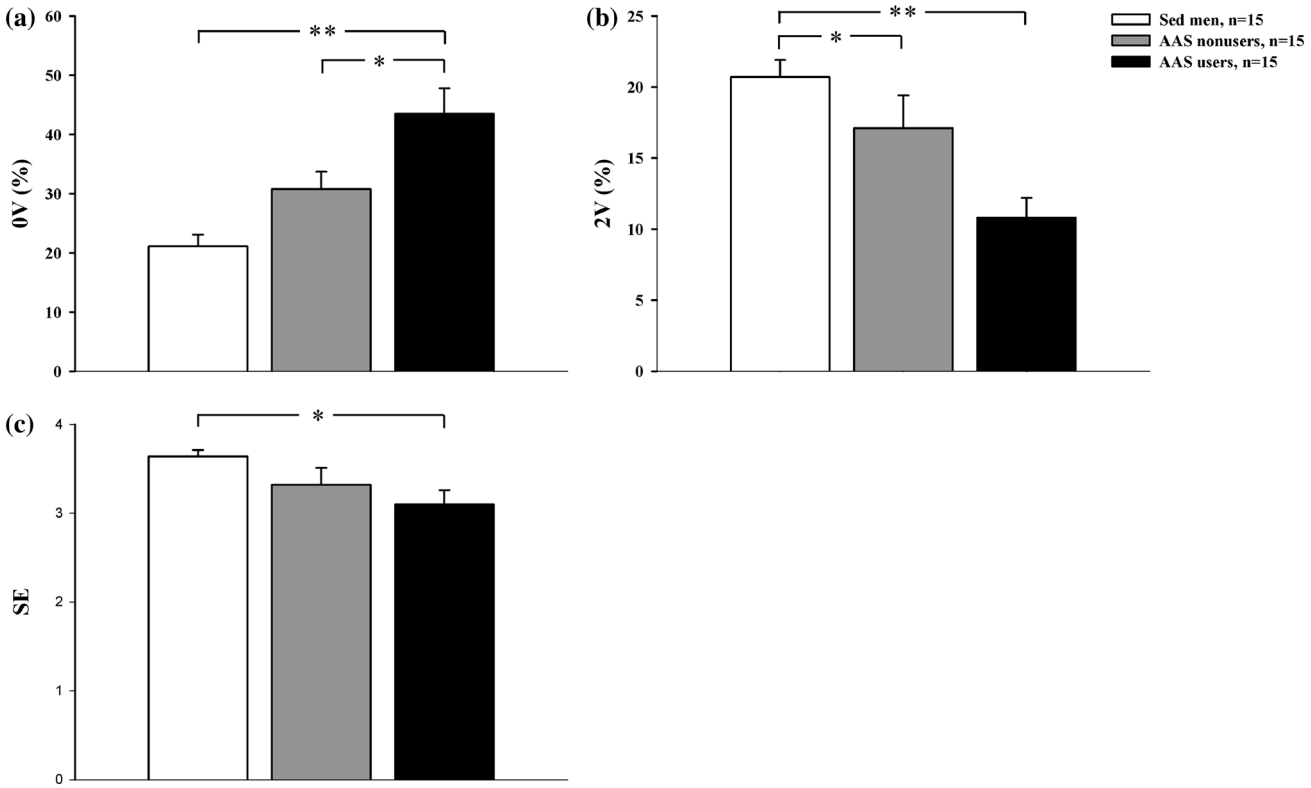

Fig. 2 Bar graphs (mean ± SEM) showing the percentage of patterns without variation (0 V; flat) (**a**) and of patterns with two variations (2 V) (**b**) in subjects at rest, and the results of the Shannon entropy (SE) analysis in subjects at rest (**c**). *$p < 0.05$; **$p < 0.001$

Table 3 Electrocardiographic data

Electrocardiographic parameters	Study group		
	Sedentary ($n = 15$)	AAS nonusers ($n = 15$)	AAS users ($n = 15$)
P wave duration (ms)	82.3 ± 2.2	84.7 ± 3.0	87.4 ± 1.8
PR interval (ms)	136.1 ± 5.7	140.6 ± 6.5	153.4 ± 8.3
QRS duration (ms)	58.6 ± 4.0	62.8 ± 4.2	63.7 ± 4.3
ST segment (ms)	116.0 ± 6.6	117.6 ± 6.2	118.3 ± 6.6
QTi (ms)	343.7 ± 3.3	342.8 ± 6.0	358.8 ± 8.8
Corrected QTi (ms)	354.9 ± 4.0	360.6 ± 5.0	375.5 ± 6.7‡
QTd (ms)	36.4 ± 2.7	41.7 ± 5.1	48.8 ± 2.8
Corrected QTd (ms)	42.9 ± 2.2	54.4 ± 3.9	67.6 ± 5.2*
P axis (°)	51.5 ± 5.1	43.2 ± 4.7	44.0 ± 5.0
QRS axis (°)	54.7 ± 7.1	46.9 ± 5.1	17.8 ± 7.4*†
T axis (°)	35.4 ± 5.3	30.8 ± 5.7	24.9 ± 6.4
Sokolow–Lyon index (mm)	15.6 ± 0.7	21.1 ± 0.6*	29.0 ± 0.4*†

Values in table are presented as the mean ± SEM

QTi QT interval, *Qtd* QT dispersion

* $p < 0.001$ vs. sedentary men; $^{\dagger}p < 0.001$ vs. AAS nonusers; $^{\ddagger}p < 0.05$ vs. sedentary men; $^{\S}p < 0.05$ vs. AAS nonusers

Association between sympathetic modulation and cardiovascular alterations

Further analysis showed a significant correlation between normalized LF oscillations of HRV and mean BP levels ($r = 0.48$, $p < 0.005$) (Fig. 3a) and percentage of 0 V dynamics and mean BP ($r = 0.31$, $p < 0.05$) (Fig. 3b). In addition, a significant correlation occurred between the

Table 4 Standard echocardiographic parameters

Echographic parameters	Study groups		
	Sedentary ($n = 15$)	AAS nonusers ($n = 15$)	AAS users ($n = 15$)
LV end-diastolic diameter (mm)	48.4 ± 1.1	50.4 ± 1.3	51.8 ± 1.0
LV end-systolic diameter (mm)	29.4 ± 1.0	31.9 ± 0.9	32.3 ± 1.5
LV end-diastolic volume (mm)	110.7 ± 6.0	130.5 ± 4.8‡	131.1 ± 3.0‡
LV end-systolic volume (mm)	27.5 ± 2.1	35.4 ± 2.3	35.3 ± 3.8
Interventricular septal thickness (mm)	8.0 ± 0.2	8.6 ± 0.2	12.3 ± 0.4*†
LV posterior wall thickness (mm)	7.7 ± 0.2	8.7 ± 0.2	11.5 ± 0.3*†
LV mass (g)	139.3 ± 7.3	178.9 ± 5.6*	243.7 ± 4.2*†
LV mass/BSA (g/m^2)	71.8 ± 3.5	89.6 ± 2.2*	127.7 ± 3.9*†
Relative diastolic wall thickness	0.32 ± 0.0	0.34 ± 0.0	0.46 ± 0.0*†
Cardiac output (ml/min)	5.5 ± 0.2	6.4 ± 0.2	6.5 ± 0.4
Ejection fraction (%)	75.5 ± 1.3	73.0 ± 1.3	73.2 ± 1.6

Values in table are presented as the mean ± SEM

LV Left ventricular, *BSA* body surface area

* $p < 0.001$ vs. sedentary men; † $p < 0.001$ vs. AAS nonusers; ‡ $p < 0.05$ vs. sedentary men; § $p < 0.05$ vs. AAS nonusers

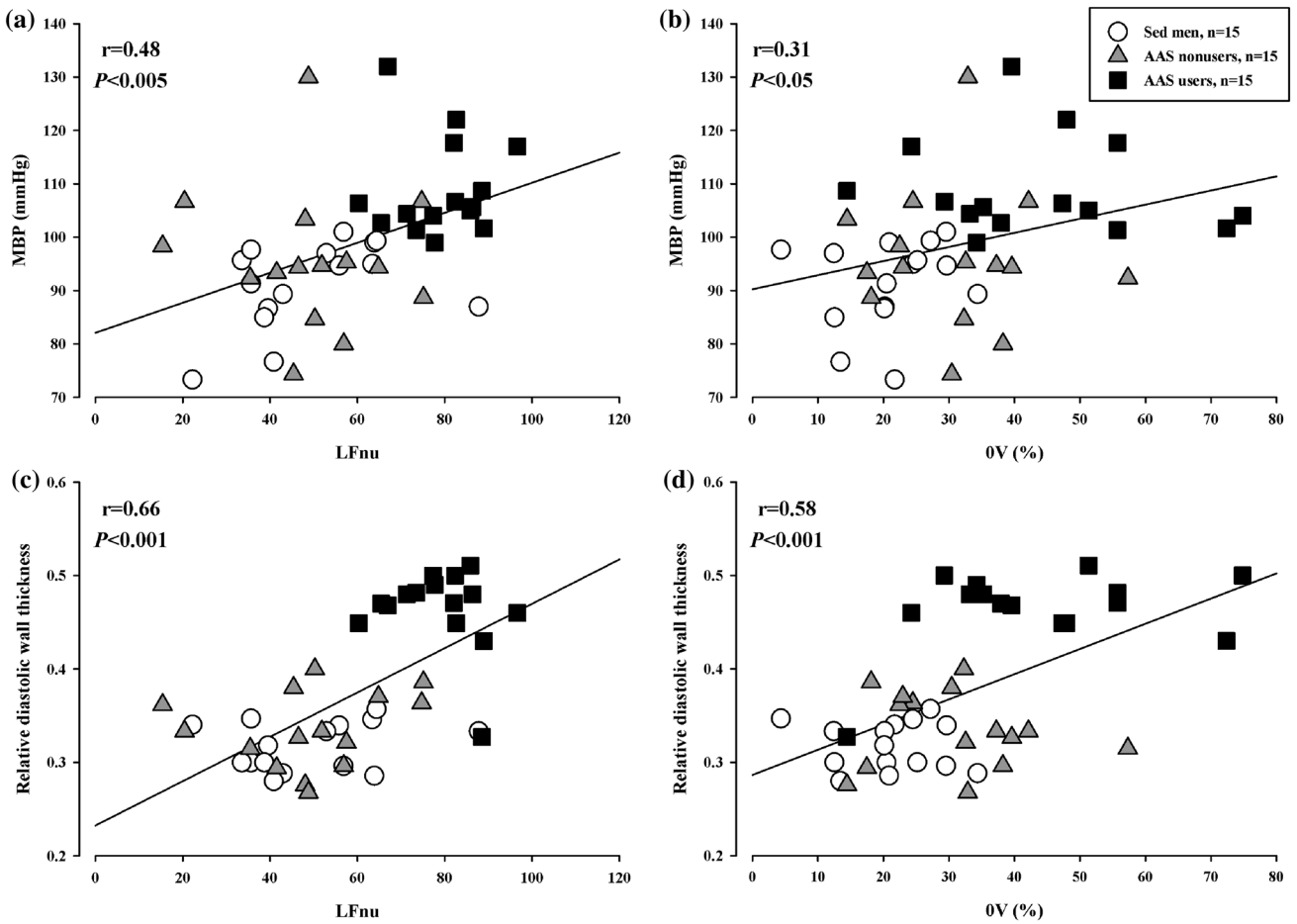

Fig. 3 Correlation coefficient between the normalized low-frequency spectral component of HRV (LFnu) and mean blood pressure (MBP; **a**), percentage of 0 V patterns and MBP (**b**), normalized low-frequency spectral component of HRV (LFnu) and relative diastolic wall thickness (**c**) and percentage of 0 V patterns and relative diastolic wall thickness (**d**) in AAS users ($n = 15$), AAS nonusers ($n = 15$) and sedentary controls (Sed men; $n = 15$)

normalized LF component of HRV and relative diastolic wall thickness ($r = 0.66$, $p < 0.001$) (Fig. 3c) and between the percentage of 0 V dynamics and relative diastolic wall thickness ($r = 0.58$, $p < 0.0001$) (Fig. 3d).

Discussion

The results of our study show for the first time that in athletes on an active bodybuilding training program who chronically self-administer AAS LV hypertrophy is significantly associated with a higher sympathetic modulation and elevated BP in comparison to age-matched athletes on an active bodybuilding training program who do not self-administer AAS. These findings indicate that the increase in the LF normalized component of HRV and the higher percentage of 0 V% symbolic dynamics (both indicators of elevated sympathetic modulation) found in AAS users may be a key determinant of human LV myocardium growth, thereby providing evidence for the malefic impact of the association between AAS and the cardiovascular system. Our findings relate to the influence of supraphysiological levels of AAS on sympathetic modulation and parasympathetic decreasing oscillations in humans in causing a substantial amount of human subject variability in all the measures of linear and nonlinear dynamics of HRV that were estimated in our study. These results are supported by those from the study of Parssinen and Seppalaother [7] who showed an increased muscle sympathetic nerve activity in bodybuilders using AAS.

Evidence of increased sympathetic activity after the administration of AAS in human and animal models has recently been reported. Alves et al. showed that high BP levels in young strength-trained men using AAS are associated with increased sympathetic nerve activity [7]. Pereira-Junior et al. found that chronic treatment with supraphysiological doses of nandrolone decanoate provoked cardiac sympathetic hyperactivity in rats [22]. High doses of AAS can lead to cardiovascular abnormalities, thus contributing to the development of ventricular arrhythmias and sudden death [23–25]. Chronic AAS abuse has been shown to induce a sympathovagal imbalance in the cardiac autonomic regulation with sympathetic dominance, which may be proarrhythmic in the early period [26].

The increased sympathetic modulation associated with chronic AAS use may be explained, in part, by a puzzling physiological alteration. Previous studies suggest that the chronic administration of AAS may induce a profile of increased responsiveness to catecholamines [27]. However, the activation of the adrenergic system by AAS cannot be attributed only to the increased release of catecholamines as anabolic steroids cross the blood–brain barrier and act on specific androgen receptors in the central cardiovascular regulatory regions [28]. In addition, there is evidence that supraphysiological steroid doses can enhances β-adrenoceptor expression, selectively inhibit extraneuronal uptake of neuroamines and increase the vascular response to catecholamines [29].

Sympathetic hyperactivity plays an important role in the progression of hypertension and damage to target organs. In our study, concentric LV hypertrophy was evidenced in AAS users by the increase in interventricular septal thickness, in LV posterior wall thickness and in relative diastolic wall thickness. The increased Sokolow–Lyon index and QRS axis toward the left side in the frontal plane of the heart also coincides with concentric LV hypertrophic diagnostics. Similar findings were obtained in humans by Hartgens et al. [30], who observed that high doses of AAS induced cardiac hypertrophy and attenuated myocardial contractility. De Piccoli et al. showed that AAS abuse is associated with pathological LV hypertrophy and altered diastolic filling [29]. The discrepancies between the results obtained in other studies conducted in humans [31] could be attributed to the patterns of AAS abuse and to the difficulty in finding athletes who will admit to having taken AAS and who are concomitantly willing to participate in scientific investigations [32].

Cardiac hypertrophy is often associated to sudden death and arrhythmias in endurance athletes and AAS users [33, 34] and, in addition, a number of autonomic dysfunctions may be generated by cardiac remodeling [35]. LV hypertrophy can lead to diastolic dysfunction, and if high BP is not adequately controlled diastolic heart failure can develop.

AAS on their own have been found to directly induce myocardial lesion, with the main pathological finding in autopsied hearts of AAS users being LV hypertrophy in frequent association with myocyte hypertrophy, augmented matrix collagen deposition, increased cardiac angiotensin-converting enzyme activity and myocardial fibrosis [36]. Previous studies have reported the presence of endogenous pathways of androgen actions in the development of cardiac hypertrophy and a higher expression of androgen receptors in cardiac cells of hypertrophied hearts in humans and rats [2, 37, 38]. AAS appear to act directly on the heart through the action of nuclear receptors, increasing the expression of mRNA and stimulating cardiac protein synthesis, resulting in myocardial hypertrophy [5].

The etiology of LVH is multifactorial. The main mechanisms are pressure overload and neurohumoral factors, both of which play a role in hypertension. Mediators such as angiotensin II, aldosterone and norepinephrine accelerate cardiac hypertrophy and fibrosis. Clinical studies have shown significant correlations between circulating norepinephrine levels and LV mass [38]. In our study, LV hypertrophy was linked to greater normalized LF band of HRV in the AAS user group.

The increase in BP in humans with aging has been attributed to the effects of androgens [39]. Therefore, it

can be expected that the administration of supraphysiological doses of AAS would result in increased BP in humans. This hemodynamic alteration was noted in our study, with the AAS users having higher BP levels than AAS nonusers and the sedentary controls. An elevated resting BP in AAS users has been previously reported by other investigators. In a cross-sectional study, Urhausen et al. [3] found that systolic BP levels at rest was higher in current AAS users than in non-AAS users. Palatini et al. reported that AAS users had a smaller reduction in BP during the sleep period compared with AAS nonusers [40].

In humans, increased BP or even hypertension has been linked to high levels of circulating androgens, induced either by various endocrine diseases [41] or by AAS abuse in athletes or amateur bodybuilders [42]. However, the controversy regarding the effects of AAS abuse in the sporting environment remains ongoing [43, 44], with some earlier observational studies indicating either an elevation of BP [3, 44] or no significant BP changes in AAS users [43]. This variety of outcomes may result from the lack of homogeneity in type of AAS, dosage and timing of AAS administration and the exercise regimen and/or to the difficulty in controlling drug abuse in the AAS group [8].

Increased BP levels have been reported in AAS users. While we acknowledge that the mechanism by which AAS increases BP is not fully understood, there is published evidence of the impact of AAS on the testosterone aromatization process that can result in high BP levels. Testosterone esters undergo aromatization in the body, and the rate at which testosterone is aromatized into estrogen is directly related to the AAS dose used. In users of higher AAS doses, testosterone undergoes a higher rate of aromatization. Thus, bodybuilding doses can and do elicit moderate aromatase activity. In this specific case, the aromatization of testosterone into estradiol in peripheral fat can cause elevations in BP due to water retention. In addition, changes in the lipid profile may attenuate nitric oxide bioavailability and impair endothelial function. A number of published studies have clearly demonstrated that lipid disorders provoke endothelial dysfunction in humans [45–47].

On the other hand, these alterations in BP in AAS users are also due to changes in sympathetic control. One study has shown an association between augmented BP levels and sympathetic nerve activity [7]. A link between BP levels and muscular sympathetic nervous activity has also been observed in hypertensive patients [48]. We also observed an association between BP levels and sympathetic modulation of HRV by linear and nonlinear methods. The elevated LF component of sympathetic nerve activity in AAS users is consistent with disequilibrium in the vasoconstrictor forces. The early observation that testosterone increases the vascular response to norepinephrine [49, 50] strengthens the notion that sympathetic exacerbation increases the BP in AAS users.

In our study, we found that AAS users presented increased sympathetic modulation and increased BP levels compared to AAS nonusers. Intriguingly, AAS use solely did not promote any changes in the resting HR, probably due to modulation of the autonomic nervous system on the cardiac chronotropism during the resting state. Under physiological conditions, β1-adrenergic receptors are considered to be the main mediators of cardiac chronotropism, with β2-adrenergic receptors also playing a key role in this process. However, under certain conditions, changes in the homeostasis of β-adrenergic receptors may occur in the right atrium, with an elevated participation of β2-adrenergic receptors on the control of cardiac chronotropism [51]. In association with pathological cardiac hypertrophy, these alterations may also increase the cardiac afterload and change BP control [52].

Neves et al. demonstrated that Wistar rats treated with nandrolone presented higher levels of β1- and β2-adrenergic receptors without changes in the resting HR [51]. These increased levels of β-adrenergic receptors suggest that the hearts of nandrolone-treated rats develop compensatory mechanisms in response to the functional damage caused by the pathological cardiac hypertrophy. However, the influences of AAS on cardiac electrophysiology as well as on the cardiac chronotropism remain poorly understood.

Another important result of our investigation is the observed attenuation of cardiac vagal modulation in AAS users. The AAS group presented a marked impairment of parasympathetic cardiac modulation compared to the other two groups. In the AAS group, the 2 V parameter of symbolic dynamics was also reduced, which corroborates the parasympathetic cardiac dysfunction shown by the spectral analysis methodology. Cardiac vagal impairment, detected by reduced parasympathetic modulation, is a marker of cardiac electrical instability and has been shown to constitute an independent prognostic factor of ventricular arrhythmia and sudden cardiac death in cardiac patients [53].

In the clinical setting, sudden death resulting from cardiac arrhythmias is an important cause of mortality [43]. The onset of major arrhythmias is generally considered to be an unpredictable phenomenon. Cardiovascular risk factors can help to identify subjects at high risk of arrhythmias [54]. The standard linear HRV methods of analysis do not seem to be adequate for the study of any short-term instability that may precede major arrhythmias, possibly due to the presence of only brief and transient instabilities in the RR interval dynamics, thus leading to controversial results [55].

The nonlinear method of HRV analysis (symbolic dynamics) to quantify the prevalence of sympathetic or parasympathetic cardiac modulation under conditions in which the linear HRV approach can be used [12] is limited or even

disputable. Our results show that the percentage of symbolic patterns in bodybuilding AAS users changed in distinct directions, consistent with expected changes in sympathetic or vagal cardiac modulation at rest. Specifically, an increase in sympathetic modulation and vagal withdrawal elicited both an increase in the 0V pattern and a decrease in the 2 V pattern. This method of analysis was able to take into account short instabilities in the HRV series that may provide insights into autonomic cardiac regulation by taking into consideration the different latencies and time courses of the fast parasympathetic and slow sympathetic modulations [56].

The symbolic dynamics of the RR series has been found to forecast life-threatening arrhythmias [57]. Shusterman et al. recognized disturbances in "core" patterns, indicating progressive destabilization of cardiac rhythm, which would predict the onset of spontaneous sustained ventricular tachyarrhythmias [58]. The possible concomitance of both vagal and sympathetic actions, most likely on a reflex basis, could facilitate arrhythmias by a complex interplay [59]. A symbolic analysis seems to be able to detect the coexistent excitation of the two systems, weakening the concept that vagal and sympathetic outflows under pathophysiological conditions work exclusively in a sort of reciprocal arrangement.

Other nonlinear approaches, such as Shannon entropy, are clearly decreased in AAS users at rest, corresponding to a reduced number of different patterns (i.e. less complexity). Finally, the symbolic dynamics method was able to identify experimental changes in cardiac autonomic modulation. Porta et al. demonstrated that six levels for a sequence of three beats is the most appropriate compromise to best detect the relative changes induced by the autonomic modulation on HRV [12].

In our study, the strength-building athletes who used AAS had the greatest amount of both corrected QT interval and QT dispersion. This same result has observed been observed in other studies in humans [59, 60] and experimental animals [61]. The QTc interval has been associated with an increased risk of all-cause mortality and sudden cardiac death. The authors of several studies have suggested that increased QTd is associated with increased mortality in hypertensive patients [62]. The LV hypertrophy induced by anabolic substances reflects an increase in the QTd that is similar to the one found in hypertensive patients with LV hypertrophy [62]. This observation possibly indicates an increase of arrhythmic risk in AAS users [63]. In our study we observed an association between increased sympathetic modulation of HR by linear and nonlinear methods and LV cardiac hypertrophy.

Long-term power training is a potent cardiac hypertrophic stimulus. In previous reports, top-level strength-trained athletes, mainly involved in static isometric anaerobic exercise, showed increased LV wall thickness and relative wall thickness, with a pattern of LV concentric geometry caused by pressure overload typical of this kind of effort. In addition, power athletes are often exposed to high doses of AAS, which is known to cause early impairment of the cardiac function [64].

Study limitations

For ethical reasons, we were unable to conduct a controlled randomized study. However, the endocrine blood test confirmed that our subjects were in fact self-administrating AAS. Our study is limited by a small sample size and a study group comprised of only males, so further research should be extended to a larger AAS user population and to both genders. The history of AAS use, information on the use of other performance-enhancing drugs and the use of any forms of inhibition of aromatization were obtained by requesting the participants in the study to complete self-administered questionnaires. We believe that the information provided by the participants of this investigation is correct. The abusive doses of AAS were studied during the cycle of self-administration but not after discontinuation. Thus, our cross-sectional study cannot assess longitudinal changes in cardiac parameters among AAS users. Earlier studies have reported persisting effects of AAS even after discontinuation of AAS use [3, 5]. In our study, we did not conduct tests to detect AAS use by any means other than asking the participants if they had used AAS. This approach reflects typical daily clinical practice. Whereas it is hard to imagine non-users admitting AAS use, it is possible that AAS users may not wish to admit to using AAS and were erroneously placed in the nonuser group. However, this would not duly detract from our findings as it would lead to an underestimation of the observed differences. Another limitation of our study is that the HRV protocol used does not directly assess autonomic dysfunction or sympathetic overactivity. The linear analysis performed in our study showed that AAS users at rest had higher values for normalized LF. The interpretation of these results is not easy because it is known that the LF component is not predominantly sympathetic, a phenomenon which has been thoroughly investigated in several studies [21, 64–66]. Other methods and analyses should be studied regarding the linear analysis of the HRV to arrive at a clearer interpretation of the results. Linear methods allow an interpretation of the system as a whole (reductionist). However, most natural systems display nonlinear behavior, and the cardiac system is not any different. From these nonlinearities arise chaotic and complex behaviors with fractal characteristics which enable the human body to adapt to different environments [67]. The nonlinear analysis can detect nonreciprocal autonomic change [68–70], and we have performed the first nonlinear analysis of HRV in AAS users. The nonlinear analysis using symbolic analysis indicated

higher sympathetic modulation in AAS users. The AAS users had high 0 V% values, which is associated with sympathetic modulation [15, 69]. The analysis of complexity was also a differential in our investigation. The Shannon entropy of the HRV data of the AAS users was lower than that for nonusers and the controls. The Shannon entropy is a measure of the complexity of the pattern distribution. Additionally, the complexity of heart period dynamics depends on the autonomic nervous system, i.e., it decreases in the presence of increased sympathetic modulation [71].

Conclusions

Athletes on a bodybuilding training program who use supraphysiological doses of AAS showed marked cardiovascular autonomic abnormalities shifted toward increased sympathetic modulation and vagal modulation attenuation; these were detected by both linear and nonlinear HRV methods. In addition, the AAS users presented an association between higher sympathetic modulation with increased BP levels as well as an association between this autonomic modulation and concentric cardiac hypertrophy. These findings may help raise awareness of the consequences of AAS use.

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Compliance with ethical standards

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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