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Retrograde and oscillatory shear rate in young anabolic androgenic steroid users

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[Correction added on 7 December 2018, after first online publication: The panel A in figure 1 has been updated.]

Disturbed shear rate (SR), characterized by increased retrograde and oscillatory SR in the brachial artery, is associated with inflammation, atherosclerosis, endothelial dysfunction, and sympathetic hyperactivity. Young subjects do not have disturbed SR; however, elderly subjects do, which seems to be associated with sympathetic hyperactivity. Anabolic androgenic steroids (AAS) abuse in young is associated with increased muscle sympathetic nerve activity (MSNA). We hypothesized that AAS users might have disturbed SR. We tested the association between retrograde and oscillatory SR with MSNA. In addition, we measured the high-sensitivity C-reactive protein (hs-CRP). We evaluated 10 male AAS users, age 27 ± 4 years, and 10 age-matched AAS nonusers, age 29 ± 5 years. At rest, retrograde and oscillatory SR were evaluated by Doppler ultrasound, MSNA was measured with microneurography, and hs-CRP was measured in blood sample. Flow-mediated dilation (FMD) was also assessed. AAS users had higher retrograde SR (24.42 ± 17.25 vs 9.15 ± 6.62 s^{-1}, $P = 0.01$), oscillatory SR (0.22 ± 0.13 vs 0.09 ± 0.07 au $P = 0.01$), and MSNA (42 ± 9 vs 32 ± 4 bursts/100 heart beats, $P = 0.018$) than nonusers. MSNA (bursts/100 heart beats) was correlated with retrograde SR ($r = 0.50$, $P = 0.050$) and oscillatory SR ($r = 0.51$, $P = 0.042$). AAS users had higher hs-CRP [1.17 (0.44-3.63) vs 0.29 (0.17-0.70) mg/L, $P = 0.015$] and decreased FMD (6.42 ± 2.07 vs $8.28\% \pm 1.53\%$, $P = 0.035$) than nonusers. In conclusion, AAS abuse is associated with retrograde and oscillatory SR which were associated with augmented sympathetic outflow. In addition, AAS seems to lead to inflammation characterized by increased hs-CRP. These alterations may have the potential of increasing the early risk of atherosclerotic disease in young AAS users.

KEYWORDS
anabolic steroid, muscle sympathetic nervous activity, retrograde shear rate

1 | INTRODUCTION

Shear rate (SR) is an important mechanical factor that contributes to the control of blood flow and endothelial function.[1] However, disturbed SR, characterized by increased retrograde and oscillatory SR, favors a pro-atherogenic endothelial cell phenotype associated with endothelial dysfunction.[1,2] A study of elderly individuals provides evidence of increased retrograde and oscillatory SR, which seems to be associated with increased muscle sympathetic nerve activity (MSNA) and reduced nitric oxide bioavailability.[4]

Anabolic androgenic steroids (AAS) have been used as an illicit source linked to skeletal muscle hypertrophy and performance.[5] However, AAS abuse is associated with autonomic dysfunction. Previously, it was demonstrated that AAS users have increased MSNA and lower forearm blood flow measured by venous occlusion plethysmography.[6] Moreover, endothelial dysfunction seems to be present in AAS users, because they have blunted flow-mediated dilation (FMD).[7]

Increased retrograde SR promotes secretion of factors that contribute to inflammation processes leading to the development of atherosclerotic disease.[1] Curiously, high-sensitivity C-reactive protein (hs-CRP), which is associated with a higher risk of future cardiovascular events in the general population,[8] is significantly increased in AAS users.[7] Thus, it is possible to speculate that the disturbed SR could lead to vascular inflammation in young AAS users; the increased sympathetic outflow might be one of the mechanisms involved in this vascular disturbance. However, the association between disturbed SR, MSNA, and inflammation in AAS users is unknown.

Based on these assumptions, we hypothesized that young AAS users might have increased retrograde and oscillatory SR. Furthermore, these alterations could be associated with MSNA and hs-CPR.

2 | MATERIAL AND METHODS

2.1 | Subjects

The local Human Subject Protection Committee approved this cross-sectional study under the number 3945/13/070. Ten male AAS users (AASU) and 10 male AAS nonusers (AASNU) 18-40 years of age were invited to participate. All participants (AASU and AASNU) were recreational weightlifters or amateur bodybuilding athletes who were recruited from gymnasiums. Anamnesis was performed in all participants to obtain information about their physical activity status, AAS administration, medications, and sports supplements. All individuals had been involved in strength training for at least 2 years. AASUs should be self-administering AAS in periodic cycles lasting from 8 to 12 weeks for at least

2 years with 2-4 cycles per year. All AASUs were on a cycle over the course of the study. Exclusion criteria for all participants were smoking, alcohol consumption, use of diuretic and/or antihypertensive medications, and liver and kidney disease.

2.2 | Measures and procedures

All participants signed a written informed consent. The evaluations were performed on two consecutive days. On the first day, a blood sample was drawn and body composition was measured. On the next day, blood pressure, heart rate, MSNA, and vascular measurements were assessed. On both days, all participants abstained from sports supplements, caffeine-containing products, and exercise training for 48 hours.

2.3 | Blood measures

Blood sample was collected in the morning (between 8:00 and 10:00 AM) after 12 hours of fasting and after 30 minutes of resting for lipid assessment (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides), glucose, and hs-CRP.

2.4 | Body composition

Body composition was assessed by dual-energy X-ray absorptiometry (DXA), (Discovery DXA system, Hologic Inc) to measure total fat-free mass, fat mass, and fat percentage in all participants. DXA was used to exclude possible bias of body mass index (BMI) between AASU and AASNU.

2.5 | Blood pressure and heart rate

Blood pressure and heart rate were recorded after 10 minutes of rest in a quiet, temperature-controlled (21°C) room in the morning at approximately the same time each day. Arterial blood pressure was measured using the auscultatory method with the participant in a seated position. Heart rate was measured through 12 derivative electrocardiograms with the participant in the supine position.

2.6 | Muscle sympathetic nerve activity

After blood pressure and heart rate measurements, the MSNA was recorded directly from the fibular nerve using the microneurography technique. A tungsten microelectrode was placed in the peroneal nerve, and a sympathetic neurogram was recorded. Nerve signals were amplified by a factor of 50 000 to 100 000 and band-pass filtered (700-2000 Hz). For recording and analysis, nerve activity was rectified and integrated (time constant, 0.1 second) to obtain a mean voltage display. MSNA was expressed as burst frequency (in bursts

per minute) and burst incidence (in bursts per 100 heart beats). All MSNA recordings met previously established and described criteria.[6]

2.7 | Brachial artery imaging

After the MSNA technique, the participants rested for 15 minutes. Under supine resting conditions, the brachial artery images (2-12 cm above the antecubital fossa) were obtained through duplex mode, and ultrasound equipment (Vivid E9, General Electric, Horten, Norway) equipped with a 13 MHz linear probe. The left arm was positioned with the shoulder abducted at approximately 80° and the forearm supinated. The contrast resolution, depth, and gain were adjusted to optimize the longitudinal images of the lumen/arterial wall interface. Brachial artery diameter and insonation angle-corrected (≤60°) blood velocity spectra were simultaneously recorded via the pulsed-wave mode at linear frequencies of 13 and 6.0 MHz, respectively. The probe location was marked on the skin and did not change position throughout the study. The sample volume was located at the center of the brachial artery and then adjusted to cover the vessel width. Diameter and blood velocity waveforms were continuously recorded for a 3-minute period.

2.8 | Flow-mediated dilation (FMD)

Before starting the reactive hyperemia, the participants rested for 10 minutes in a supine position. Brachial artery FMD was then evaluated in the left arm, with the shoulder abducted at approximately 80° and the forearm supinated. An appropriate-sized rapid inflation/deflation pneumatic cuff (E-20 Rapid Cuff Inflator, DE Hokanson) was placed around the left forearm, immediately distal to the olecranon process. For the evaluation of FMD, the same ultrasound equipment of SR patterns was used (Vivid E9, General Electric, Horten, Norway). Baseline diameter and blood velocity waveforms were continuously recorded over 60 seconds. Reactive hyperemia was assessed immediately after relief of 5 minutes of total ischemia, which was induced by external compression of the arm using an inflatable cuff at 250 mm Hg. After this period, the cuff was rapidly deflated, and the changes in artery diameters from baseline were expressed as percentages.

2.9 | FMD and shear rate analysis

The Doppler ultrasound video signal was real-time encoded and captured at a frequency of 30 Hz. The video files were compatible with commercial automated edge-detection and wall-tracking software (Cardiovascular Suite, FMD Studio, Quiposrl), which was used for offline analysis. The initial phase of the software analysis consisted of identifying regions of interest on the optimal portion of the brachial artery image and its blood velocity spectra. R-wave gaiting function was applied to continuously assess brachial artery diameter or blood velocity. Brachial artery diameter was calculated by an average of 3 seconds. FMD was calculated as the percentage rise of this peak diameter from the preceding baseline diameter. We also performed the allometric modeling solution proposed by Atkinson et al.[9] SR was calculated as four times the ratio between mean blood velocity (V_{mean}; in cm/s) and artery diameter (in cm) [ie, $SR = 4 \times (V_{mean}/diameter)$]. For calculations of antegrade and retrograde SR, antegrade and retrograde mean blood velocities were used, respectively. In addition, oscillatory SR index, a variable that estimates the magnitude of oscillation in the vascular bed was calculated as (IRetrograde shearI/(IAntegrade shearI + IRetrograde shearI)). Oscillatory SR index values range from 0 to 0.5, with 0 corresponding to unidirectional shear throughout the cardiac cycle, and 0.5 representing pure oscillation with time-average shear equal to 0. Oscillatory SR is expressed in arbitrary units (au). After deflation of the cuff, the cumulative SR was determined by a 3-minute period through the area under the curve (AUC; s^{-1}·). An additional experiment on FMD reliability revealed high levels of intersession reproducibility (n = 10, FMD intraclass correlation coefficient 0.92 [95%CI 0.83-0.979]).

2.10 | Doping urine test

Doping urine test was performed with ultra-performance liquid chromatography with tandem mass spectrometry detection (UPLC-MS/MS) in both groups to document the presence of AAS. In addition, the testosterone/epitestosterone (T/E) concentration ratio in the urine was obtained as previously reported.[10] A T/E ratio greater than 6 indicates testosterone abuse.[11]

In addition, cocaine, tetrahydrocannabinol (THC), amphetamine/methamphetamine, methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyamphetamine (MDA) were tested in the urine by gas chromatography-mass spectrometry (GC-MS) analysis as previously described.[12]

2.11 | Statistical analysis

Data are presented as the mean ± SD or median (interquartile range [IQR] 25%-75%). Normality (Shapiro-Wilk) test was conducted to determine the distribution of the data. Possible differences between groups were analyzed using an unpaired Student t test and Mann-Whitney-Wilcoxon test for parametric or nonparametric data. Spearman's correlation coefficient was used to test the correlation between MSNA and retrograde SR, MSNA and oscillatory SR, and hs-CRP levels with retrograde SR $P < 0.05$ were considered statistically significant.

TABLE 1 Physical, hemodynamic, lipids profile, biomarkers, and autonomic characteristics in anabolic androgenic steroids users (AASU) and anabolic androgenic steroids nonusers (AASNU)

Variables	AASU (n = 10)	AASNU (n = 10)	P
Age (years)	27 ± 4	29 ± 5	0.295
Weight (kg)	100.6 ± 12.2	79.9 ± 10.1	<0.001
Height (m)	1.77 ± 0.04	1.78 ± 0.08	0.891
Body mass index (kg.m^{-2})	32.07 ± 3.47	25.34 ± 2.25	<0.001
Fat percentage (%)	14.2 ± 6.3	18.8 ± 4.7	0.111
Lean Mass (kg)	82.3 ± 8.0	61.4 ± 7.9	<0.001
HR (beats/min)	77 ± 11	57 ± 8	0.002
SBP (mm Hg)	130 (130-140)	110 (100-120)	0.001
DBP (mm Hg)	80 (80-90)	70 (68-80)	0.009
MBP (mm Hg)	100 (97-103)	84 (78-93)	0.001
Total cholesterol (mg/dL^{-1})	194 ± 54	159 ± 26	0.079
LDL cholesterol (mg/dL^{-1})	166 ± 52	96 ± 17	<0.001
HDL cholesterol (mg/dL^{-1})	14 (12-18)	44 (41-60)	<0.001
Triglycerides (mg/dL^{-1})	61 (55-103)	71 (52-78)	0.966
hs-PCR (mg/L)	1.17(0.44-3.63)	0.29 (0.17-0.70)	0.015
MSNA (bursts/min)	32 ± 3	19 ± 1	<0.001
MSNA (bursts/100 heartbeats)	42 ± 9	32 ± 4	0.018

Data are presented as mean ± SD or median ± IQR (25%-75%; IQR = interquartile range).
DBP, diastolic blood pressure; HDL, high density lipoprotein; HR, heart rate; hs-PCR, high-sensitivity C-reactive protein; LDL, low density lipoprotein; MBP, mean blood pressure; MSNA, muscle sympathetic nerve activity; SBP, systolic blood pressure.

3 | RESULTS

The doping control assessment was negative in the AASNU group (mean T/E lower than 1). In contrast, nandrolone decanoate, boldenone undecylenate, testosterone, and stanozolol were found in the AASU group. Furthermore, the mean T/E concentration ratio in the urine was 50.75 ± 24.46. The lifetime years of AAS use was 5 ± 3 years. The drug test assessment was positive to MDMA and amphetamine for one participant in the AASU group, and positive to cocaine for one participant in the AASNU group. In addition, both groups had similar lifetime years of resistance training (10 ± 4 vs 10 ± 4 years, $P = 0.90$).

The physical characteristics, hemodynamics, lipid profiles, biomarkers, and autonomic characteristics are shown in Table 1. No significant differences were found between AASU and AASNU in age, height, and fat percentage ($P > 0.05$). However, AASU had greater weight, body mass index, and lean mass compared with AASNU ($P < 0.05$). AASU had higher heart rate, systolic blood pressure, diastolic blood pressure, mean blood pressure, LDL cholesterol, hs-PCR, MSNA (bursts/min), and MSNA (bursts/100 heart beats) compared with AASNU

($P < 0.05$). However, no significant differences existed between AASU and AASNU for total cholesterol and triglycerides ($P > 0.05$).

Brachial artery characteristics at rest are shown in Table 2. AASU had higher resting diameter, peak diameter retrograde velocity compared with AASNU ($P < 0.05$). In contrast, FMD and allometrically scaled FMD were reduced in AASU compared with AASNU ($P < 0.05$). No significant differences were found between AASU and AASNU for time to peak, SR_{AUC}, anterograde velocity, anterograde SR, and mean SR ($P > 0.05$).

As illustrated in Figure 1, AASU had higher retrograde SR (24.42 ± 17.25 vs 9.15 ± 6.62 s^{-1}, $P = 0.01$; Panel A) and oscillatory SR (0.22 ± 0.13 vs 0.09 ± 0.07 au; $P = 0.01$; Panel B) than AASNU. An example of SR in the brachial artery in one AASU and one AASNU is shown in Figure 1C and D, respectively.

Further analysis showed a significant correlation between retrograde SR and MSNA (bursts/100 heart beats) (Figure 2A); oscillatory SR and MSNA (bursts/100 heart beats) (Figure 2B). However, no significant correlation between retrograde and oscillatory SR with FMD was found (Table 3; $P > 0.05$).

Variables	AASU (n = 10)	AASNU (n = 10)	P
Resting diameter (mm)	4.8 ± 0.28	3.9 ± 0.22	<0.001
Peak diameter (mm)	5.1 ± 0.28	4.3 ± 0.26	<0.001
Time to peak (s)	66.13 ± 41.07	60.83 ± 42.21	0.817
FMD (%)	6.42 ± 2.07	8.28 ± 1.53	0.035
Allometrically scaled FMD (%)	6.91 ± 2.37	9.05 ± 1.82	0.036
$SR_{AUC}(s)10^3$	35.41 ± 8.45	31.35 ± 11.20	0.721
Retrogade velocity (cm/s)	-2.94 ± 1.97	-0.89 ± 0.65	0.005
Anterograde velocity (cm/s)	10.95 ± 3.36	10.39 ± 3.58	0.722
Anterograde SR (s^{-1})	90.36 ± 28.56	106.12 ± 37.21	0.302
Mean SR (s^{-1})	65.94 ± 38.30	96.97 ± 41.18	0.098

Data are presented as mean $\pm$ SD.

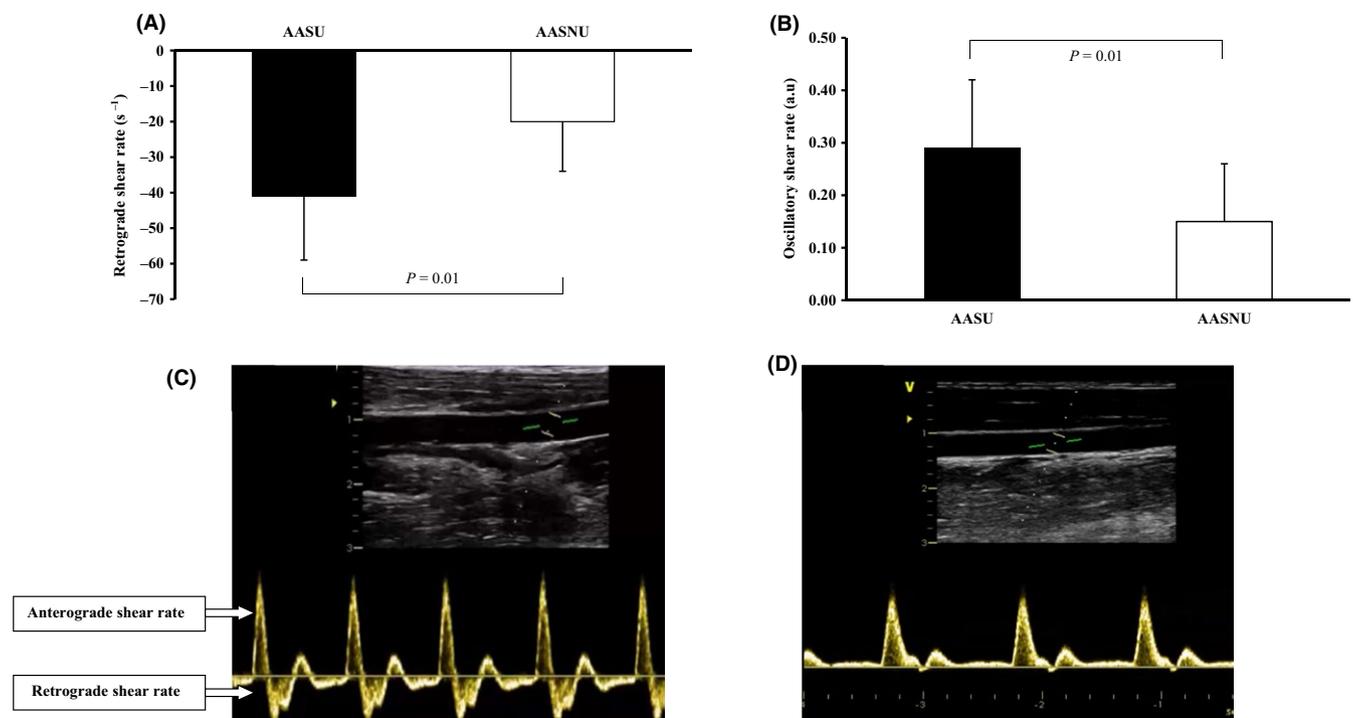FMD, flow-mediated dilatation; SR_{AUC}, shear rate area under the curve, SR, shear rate.

FIGURE 1 Retrograde SR (A) and Oscillatory SR (B) in AAS users and AAS nonusers. Example of SR in the brachial artery in the AASU group (C) and in the AASNU group (D)

Similarly, no significant correlation between retrograde and oscillatory SR with hs-CRP was found (Table 3; $P > 0.05$).

4 | DISCUSSION

The main and new findings of the present study are as follows: (a) AAS users have increased retrograde and oscillatory SR in the brachial artery compared with AASNU; and (b) both retrograde and oscillatory SR are associated with increased MSNA.

The clinical impact of these results is noteworthy. Disturbed SR has been documented in elderly populations, in whom vascular dysfunction is expected, but not in a young population.[13] Interestingly, our study highlights early vascular alterations in young individuals who abuse AAS. However, the underlying mechanisms that may explain this alteration are still unclear. For instance, several studies suggest that an increase in MSNA and/or a decrease in bioavailability of nitric oxide may justify this pathophysiological phenomenon in the aging process.[14,15] In fact, a previous study showed that MSNA was increased in AAS users.[6] This finding is also
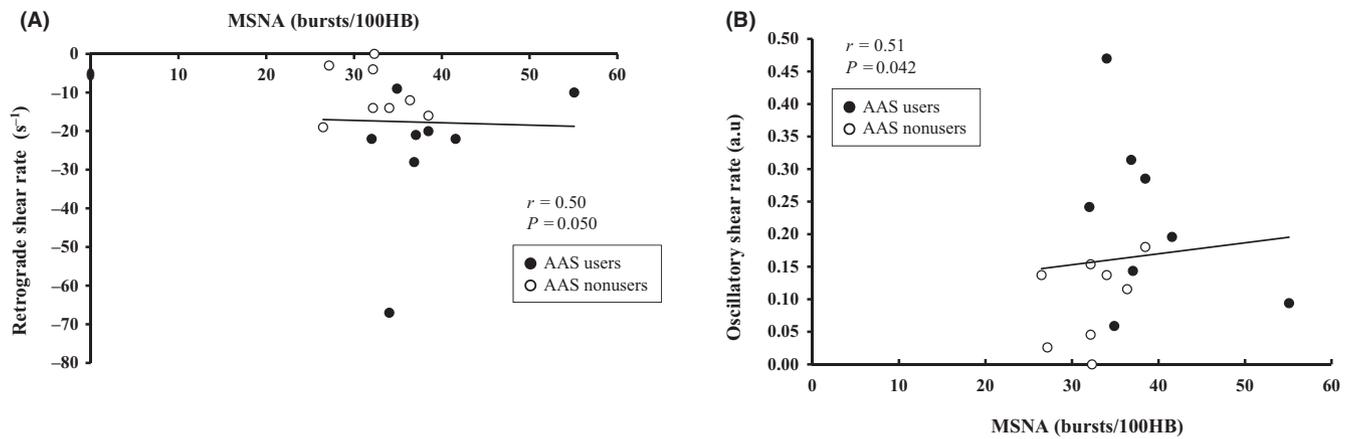
FIGURE 2 Positive linear correlation between retrograde SR and MSNA (A) (MSNA, AAS users n = 8 and AAS nonusers n = 8), Oscillatory SR and MSNA (B) in AAS users and AAS nonusers

TABLE 3 Correlation between retrograde and oscillatory SR, FMD, allometrically scaled FMD and hs-CRP

Variables	r	P
Retrograde SR and FMD	0.173	0.466
Retrograde SR and allometrically scaled FMD	0.173	0.466
Oscillatory SR and FMD	−0.244	0.300
Oscillatory SR and allometrically scaled FMD	−0.244	0.300
Retrograde SR and hs-CRP	−0.188	0.427
Oscillatory SR and hs-CRP	0.217	0.357

FMD, flow-mediated dilatation; hs-CRP, high-sensitivity C-reactive protein; SR, shear rate.

confirmed in the present study, in a subgroup of participants. More interestingly, a positive correlation between retrograde SR and MSNA in bursts/100 heart beats ($r = 0.50$, $P = 0.050$; Figure 2A) and oscillatory SR and MSNA in bursts/100 heart beats was found ($r = 0.51$, $P = 0.042$; Figure 2B).

Cell culture and isolated perfused artery studies have demonstrated that unidirectional SR stimulates anti-atherogenic gene expression and inhibits atherogenic gene expression.[16,17] In contrast, exposure of endothelial cells to increased oscillatory SR promotes a pro-atherogenic phenotype.[18] In our study, compared with AAS nonusers, AAS users had a twofold increase in oscillatory SR We recognize that the mechanisms of this alteration and their relationship with atherosclerosis need further investigation. However, this alteration seems to be in line with post-mortem studies, which suggest that sudden cardiac death in athletes who abuse AAS is due to coronary artery disease.[19] In fact, in a recent study of men between 34 and 54 years of age, long-term AAS accelerated coronary atherosclerosis, and the lifetime AAS dose was strongly associated with coronary atherosclerotic burden.[20]

Interestingly, as well as oscillatory SR, retrograde SR is also associated with endothelial dysfunction.[21] Thijssen and collaborators showed blunted FMD after 30 minutes of dose-dependent increase in retrograde SR by using cuff inflation in

healthy men.[21] We observed decreased FMD and allometrically scaled FMD in the brachial artery at rest in AAS users. However, no correlation between retrograde SR and FMD and retrograde SR and allometrically scaled FMD was found (Table 3).

Grace and Davies in a short report showed higher CRP levels in AAS users.[22] Similarly, Severo and colleagues also showed that AAS users have elevated levels of hs-CRP.[7] CRP is an inflammatory marker and a strong predictor of cardiovascular events in the general population.[23] In addition, hs-CRP is a marker of vascular wall inflammation with a high sensitivity for predicting coronary events.[24] In the present study, we also observed increased hs-CRP levels in AAS users, which ranked them at moderate cardiovascular risk.[25] However, no correlation between retrograde and oscillatory SR and hs-CRP was found. Higher hs-CRP is observed in obese when compared with lean subjects.[26] In the present study, although AASU present higher body weight, which could be associated with increased hs-CRP, we found no correlation between hs-CRP and fat mass ($r = -0.061$, $P = 0.798$), hs-CRP and lean mass ($r = 0.345$, $P = 0.150$), and hs-CRP and weight ($r = 0.345$, $P = 0.136$).

In conclusion, AAS abuse may increase retrograde and oscillatory SR which were associated with increased MSNA.

In addition, AAS users have higher inflammatory profile characterized by increased hs-CRP. All these alterations may expose young AAS users to the early risk of atherosclerotic disease.

4.1 | Limitations

We recognize potential limitations of our study. Polypharmacy among AASU is often associated with AAS use.[27] Therefore, we assessed several drugs as described previously; however, we did not evaluate others non-AAS substances such as heroin, alcohol, and clenbuterol, which could also lead to adverse effect. We studied a small sample size for generalizable inferences of the harms of AAS use. Our study is descriptive and correlational, and therefore it does not allow us to be conclusive regarding the mechanisms proposed. We measured the SR pattern in the brachial artery, but we do not know its influence in the femoral artery. Although the brachial artery is a resistant vascular bed to the development of atherosclerosis, a previous study showed that both brachial and femoral arteries demonstrated a similar relationship between changes in retrograde SR.[3] MSNA was measured only in a subgroup of participants, and the mechanism involved between sympathetic outflow and vascular shear rate deserves more study.

4.2 | Perspective

Our study indicates that young individuals under AAS abuse have impaired brachial artery retrograde and oscillatory SR, which are associated with increased MSNA. Therefore, these findings are clinically relevant because it suggests that young AAS users may be at risk of early development of atherosclerotic cardiovascular disease. Future studies using intra-arterial drugs infusions are needed to elucidate the role of sympathetic activity and inflammation on endothelial function in AAS users.

CONFLICT OF INTEREST

None to declare.

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