Neurovascular Response during Exercise and Mental Stress in Anabolic Steroid Users

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ABSTRACT

PORELLO, R. A., M. R. DOS SANTOS, F. R. DE SOUZA, G. W. P. DA FONSECA, A. L. C. SAYEGH, T. F. DE OLIVEIRA, C. A. AKIHO, M. YONAMINE, R. M. R. PEREIRA, C. E. NEGRÃO, and M.-J. N. N. ALVES.Neurovascular Response during Exercise and Mental Stress in Anabolic Steroid Users. *Med. Sci. Sports Exerc.*, Vol. 50, No. 3, pp. 596–602, 2018. **Purpose**: Increased resting muscle sympathetic nerve activity (MSNA) and lower forearm blood flow (FBF) were observed in young men who use anabolic androgenic steroids (AAS). However, the response of MSNA and FBF in AAS users triggered by muscle mechanoreflex and central command has never been tested. In addition, we evaluated the blood pressure (BP) and heart rate (HR) responses during these maneuvers. **Methods**: Nineteen AAS users (AASU) 31 ± 6 yr of age and 18 AAS nonusers (AASNU) 29 ± 4 yr of age were recruited. All participants were involved in strength training. AAS use was determined using a urine test (liquid chromatography with tandem mass spectrometry). MSNA was measured using the microneurography technique. FBF was measured by using venous occlusion plethysmography. BP was measured using an automatic oscillometric device. HR was recorded continuously through ECG. Isometric handgrip exercise was performed at 30% of the maximal voluntary contraction for 3 min, and mental stress was elicited by the Stroop color–word test for 4 min. **Results**: The MSNA and FBF responses during exercise were similar between AASU and AASNU, with a trend toward higher MSNA (bursts per minute; $P = 0.084$) and lower forearm vascular conductance (FVC; units; $P = 0.084$) in AASU than in AASNU. During mental stress, AASU showed a significantly higher MSNA ($P < 0.05$) and lower FBF ($P < 0.05$) compared with AASNU. During both maneuvers, HR and BP increased linearly in both groups; however, AASU showed a significantly higher HR compared with AASNU. **Conclusions**: During muscle mechanoreflex activation (isometric exercise), AASU have normal MSNA and FBF responses, whereas during central command (mental stress) stimulation, AASU have exacerbated MSNA and blunted vasodilation. Therefore, mental stress seems to exacerbate neurovascular control throughout stress reaction situations in AASU. **Key Words:** MUSCLE SYMPATHETIC NERVE ACTIVITY, FOREARM BLOOD FLOW, HANDGRIP, STROOP COLOR–WORD

Sympathetic hyperactivation is an autonomic feature in cardiovascular diseases, such as hypertension and chronic heart failure (1,2). The autonomic imbalance observed in such diseases is responsible for triggering peripheral vascular resistance and increased resting heart rate (HR) and blood pressure. Curiously, increased sympathetic activity has also been observed in healthy young men who self-administer anabolic androgenic steroids (AAS) (3).

In previous studies published by our group, we showed that the sympathetic hyperactivation in young AAS users (AASU) had a positive correlation with increased 24-h mean blood pressure (MBP) (3) and blunted HR recovery after maximal cardiopulmonary exercise testing (4). Moreover, when compared with AAS nonusers (AASNU), AASU had lower resting forearm blood flow (FBF) measured by using venous occlusion plethysmography (3). However, the measurements of neurovascular control in this study were evaluated at rest, and we do not know whether this response would persist during mental stress or isometric exercise. In animal models, AAS also impairs tonic cardiac autonomic regulation (5), which may be associated with changes in the baroreflex control of HR in male rats (6).

Isometric handgrip exercise has been used to test the mechanoreflex in humans by activating signals from muscle afferents (7). For instance, muscle mechanoreceptors and metaboreceptors reflexively control muscle sympathetic nerve activity (MSNA) by thinly myelinated group III fibers (sensitive to mechanical stimuli) and unmyelinated group IV fibers (sensitive to ischemic metabolites), respectively (8,9).

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Likewise, mental stress, such as the Stroop color–word test, has been used to stimulate the sympathetic-adreno-medullary system by increasing plasma and urinary adrenaline concentrations (10). This response can stimulate psychological, physiological, and biochemical parameters (10). However, the response of sympathetic activity in young AASU using isometric exercise and mental stress, to the best of our knowledge, has never been tested.

The aim of this study was to evaluate the acute response of MSNA, FBF, blood pressure, and HR in AASU compared with AASNU during isometric handgrip exercise and mental stress.

METHODS

Study population. Between April 2015 and April 2017, we enrolled 57 male participants. Of those, 17 were excluded because they did not meet the inclusion criteria (see inclusion criteria hereinafter). Forty participants were evaluated; three of them did not complete all experiments and so were excluded. The final sample was 37 participants (19 AASU and 18 AASNU). All participants (AASU and AASNU) were recreational weightlifters or amateur bodybuilding athletes who were recruited from gymnasiums.

Men between 18 and 40 yr of age were included. All participants had been involved in strength training for at least 2 yr. The AASU had been self-administering AAS for at least 4 wk before the study. In addition, they had been using AAS for at least 2 yr, two to four cycles per year (Table 1). The AASU were not taking doping substances other than AAS.

The doping urine test was performed by ultraperformance liquid chromatography with tandem mass spectrometry detection in both groups to prove the presence of AAS. In addition, the testosterone/epitestosterone (T/E) concentration ratio in the urine was determined as previously reported (11). A T/E ratio greater than 6 indicates exogenous testosterone use (11).

The study protocol was approved by the Human Subject Protection Committee of the Heart Institute (InCor) and Clinical Hospital, University of São Paulo Medical School, and each participant provided written informed consent. The study was performed in accordance with the principles of the Declaration of Helsinki.

TABLE 1. Demographic characteristics in AAS users and nonusers.

Variables	AASU	AASNU	*P*
N	19	18	
Age, yr	31 ± 6	29 ± 4	0.44
Weight, kg	90.7 ± 12.0	81.0 ± 12.5	0.02
Height, m	1.76 ± 0.07	1.79 ± 0.09	0.40
BMI, kg·m^{-2}	29.1 ± 2.8	25.3 ± 2.2	<0.01
Lean mass, kg	78.1 ± 7.6	63.0 ± 7.3	<0.01
Fat, %	14.0 ± 7.7	18.9 ± 5.0	0.11
Fat mass, kg	14.0 ± 10.4	15.9 ± 6.0	0.62
Time of resistance training, yr	12 ± 5	10 ± 5	0.27
Time of AAS use, yr	8 ± 5	—	—
Type of AAS, *n*% (weekly amount)			
Nandrolone decanoate	25% (650 ± 150 mg)	—	—
Boldenone undecylenate	87% (400 ± 0 mg)	—	—
Testosterone[a]	87% (700 ± 303 mg)	—	—
Stanozolol	75% (150 ± 71 mg)	—	—
Methenolone enanthate	12% (225 ± 75 mg)	—	—

Values are means ± SD.
[a]Represents all testosterone derivatives: propionate, isocaproate, and decanoate.

Body composition. To exclude possible bias against body mass index (BMI) between AASU and AASNU, we measured body composition in 19 participants (8 AASU and 11 AASNU). Body composition measurements were performed using dual-energy x-ray absorptiometry (DXA; Discovery DXA system, Hologic Inc). DXA scan measured total lean mass and fat mass.

MSNA. MSNA was recorded directly from the peroneal nerve using the microneurography technique (12). Multiunit postganglionic muscle sympathetic nerve recordings were made using a tungsten microelectrode placed in the peroneal nerve. 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 s) to obtain a mean voltage display of sympathetic nerve activity. MSNA was expressed as burst frequency (bursts per minute) and in bursts per 100 heartbeats (HB).

FBF. FBF was measured using venous occlusion plethysmography (13,14). The nondominant arm was elevated above the heart level to ensure adequate venous drainage. A mercury-filled silastic tube attached to a low-pressure transducer was placed around the forearm and connected to a plethysmography device (Hokanson AI6). Sphygmomanometer cuffs were placed around the wrist and upper arm. At 20-s intervals, the upper cuff was inflated above venous pressure (60 mm Hg) for 10 s followed by 10 s of release. FBF (mL·min^{-1} per 100 mL) was determined on the basis of a minimum of four separate readings, and the mean value of each minute was used. Forearm vascular conductance (FVC) was calculated as FBF/MBP × 100 and expressed in "units" (=100 mL·dL^{-1} tissue·min^{-1}·mm Hg^{-1}).

Isometric handgrip exercise. After the maximal voluntary contraction (MVC; average of three attempts), handgrip isometric exercise was performed at 30% of MVC with the dominant arm using a handgrip dynamometer for 3 min (15). The participants were instructed to breathe normally during exercise to avoid the Valsalva maneuver.

Mental stress testing. Mental stress was elicited through the Stroop color–word test administered for 4 min (10). During this test, participants were shown a series of names of colors written in different color ink from the color specified. The participants were asked to identify the color of the ink, not read the word. Each participant was asked to assess task difficulty on completion of the protocol, using a standard 5-point scale: 0, not stressful; 1, somewhat stressful; 2, stressful; 3, very stressful; and 4, very, very stressful. A single observer conducted the mental stress tests in all participants.

HR and blood pressure. HR was monitored through lead II of the ECG. At the same time, noninvasive, beat-to-beat blood pressure (systolic blood pressure (SBP), diastolic

blood pressure (DBP), and MBP) was monitored using the Finometer® (Finapres Medical Systems).

Experimental protocol. MSNA, FBF, HR, and blood pressure were evaluated simultaneously with the participant in the supine position. After the electrocardiogram was placed on the chest, the arm was positioned for venous occlusion plethysmography. On the right leg, a tungsten microelectrode was inserted into the peroneal nerve. After the participant rested quietly for 15 min, MSNA, FBF, HR, and blood pressure were recorded for 3 min of baseline in a quiet, temperature-controlled (21°C) room. After baseline measurements, the isometric handgrip exercise was immediately performed at 30% of MVC for 3 min.

After the first experiment, the participants rested again for 15 min. Then, another 3 min of baseline measurement was recorded followed by 4 min of mental stress (Stroop color–word test; Fig. 1).

Statistical analysis. The sample size calculation was based on at least 80% power to detect a mean difference in MSNA in any of the two groups using two-way ANOVA for repeated measures with a 5% significance level. We evaluated a total of 37 participants in the present study to identify a 30% difference in MSNA.

Data are presented as mean ± SD. The Kolmogorov–Smirnov test was used to evaluate the normal distribution of the variables studied. The paired Student *t*-test was used to compare parametric variables. ANOVA for repeated measures was used to compare neurovascular and hemodynamic responses. When a significant difference was found, the Bonferroni *post hoc* comparison test was used. $P < 0.05$ indicates statistical significance. The Statistical Package for the Social Sciences (SPSS) version 23 was used to perform all statistical analyses.

RESULTS

The doping control assessment was negative in the AASNU group (mean T/E <1). In contrast, the following steroids were identified in the AASU group: nandrolone decanoate, boldenone undecylenate, testosterone, stanozolol, and methenolone enanthate (Table 1). In addition, the mean of T/E concentration ratio in the urine was 42.41 ± 38.47.

As expected, AASU had higher weight and BMI compared with AASNU (both $P < 0.05$; Table 1). However, this increased body weight was due to higher fat-free mass in AASU compared with that in AASNU ($P < 0.05$; Table 1), because both groups showed similar fat mass and fat percentage (both $P > 0.05$; Table 1). Demographic characteristics and body composition are provided in Table 1.

Neurovascular responses during isometric handgrip exercise. At baseline, AASU had a significant increase in MSNA (bursts per minute and bursts per 100 HB) compared with AASNU ($P < 0.05$; Fig. 2A, B, respectively). From baseline to the peak of exercise, both groups had significant increases in MSNA (within group, $P < 0.05$), whereas we did not observe significant differences between groups (Fig. 2A, B).

At baseline, both groups had similar FBF and FVC (Fig. 2C, D). Although FBF increased significantly from baseline to the peak of exercise in both groups (within group, $P < 0.05$), this response was not sustained for FVC (Fig. 2C, D).

At baseline, HR was increased in AASU compared with AASNU ($P < 0.05$; Table 2), but blood pressure (SBP, DBP, and MBP) was similar between groups (Table 2). Both groups had a significant linear increase in HR, SBP, DBP, and MBP (within group, $P < 0.05$; Table 2). However, AASU had an exacerbated chronotropic response compared with AASNU

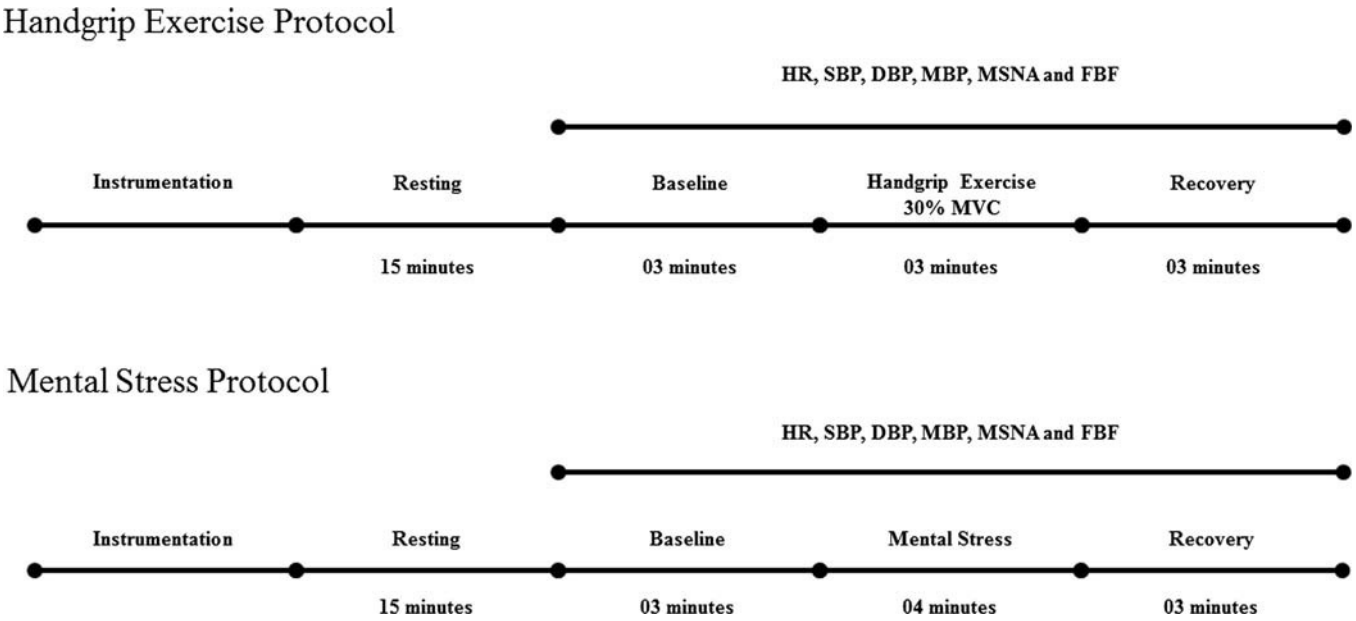

FIGURE 1—Timeline of the experimental protocols.

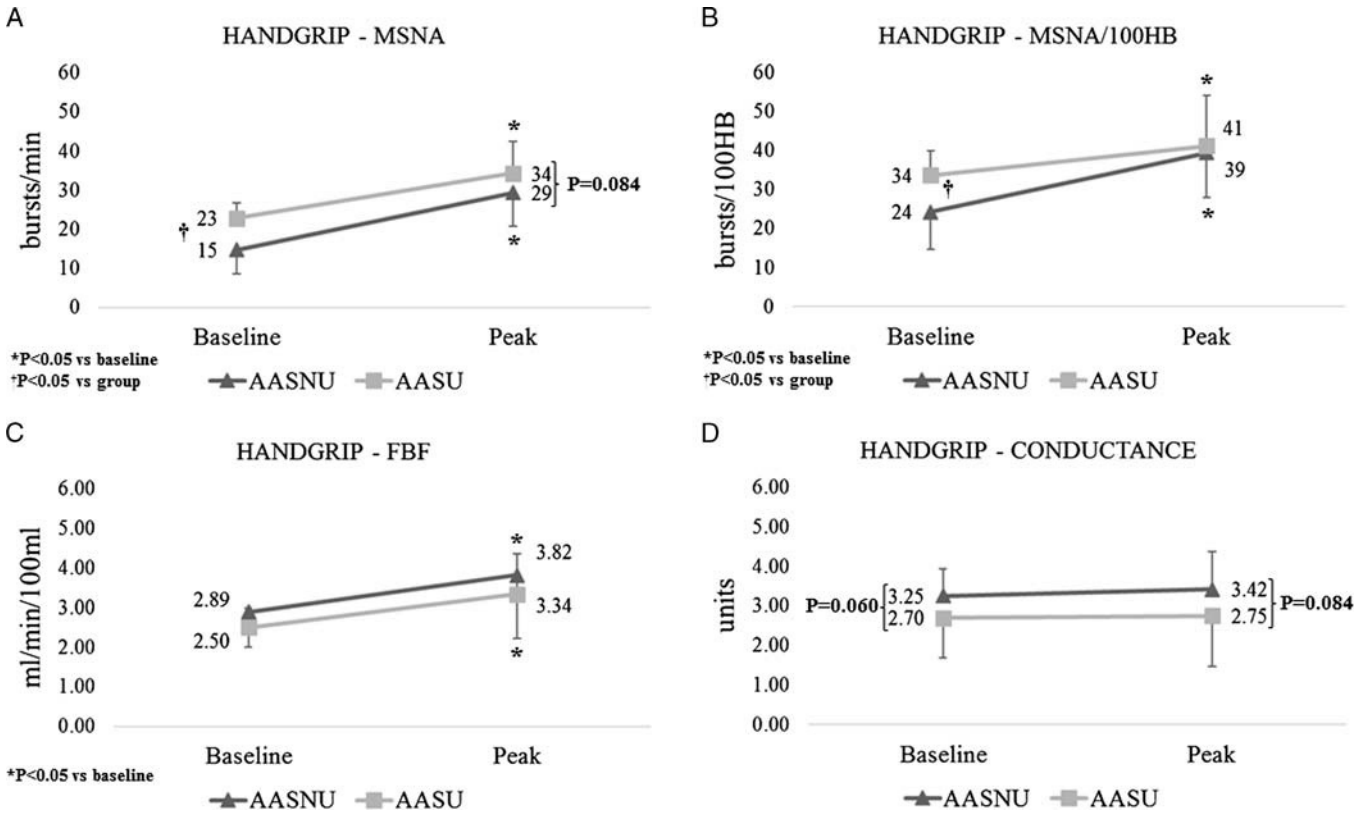

FIGURE 2—MSNA and FBF responses during handgrip exercise. A, MSNA in bursts per minute. B, MSNA in bursts per 100 HB. C, FBF in mL·min^{-1} per 100 mL. D, FVC in units.

(between groups, $P < 0.05$; Table 2). In addition, AASU had higher MPB at the peak of the exercise compared with AASNU (between groups, $P < 0.05$; Table 2).

Neurovascular responses during mental stress. At baseline, AASU had a significant increase in MSNA (bursts per minute) compared with AASNU ($P < 0.05$; Fig. 3A). However, we did not observe a similar result at baseline MSNA in bursts per 100 HB (Fig. 3B). From baseline to the peak of mental stress, both groups had a significant increase in MSNA (within group, $P < 0.05$), and AASU compared with AASNU exhibited a higher MSNA (in bursts per minute; between groups, $P < 0.05$; Fig. 3A). On the other hand, MSNA in bursts per 100 HB tended to be higher in AASU than in AASNU (between groups, $P = 0.076$; Fig. 3B).

At baseline, both groups had similar FBF and FVC (Fig. 3C, D). From baseline to the peak of mental stress, both groups experienced a significant increase in FBF (within group, $P < 0.05$); however, AASU had lower FBF compared with AASNU (between groups, $P < 0.05$; Fig. 3C). Interestingly, FVC increased significantly only in AASNU, whereas AASU experienced no change in FVC (Fig. 3D).

At baseline, HR was increased in AASU compared with that in AASNU ($P < 0.05$; Table 3), but blood pressure (SBP, DBP, and MBP) was similar between groups (Table 3). Both groups had a significant linear increase in HR, SBP, DBP, and MBP (within group, $P < 0.05$; Table 3). However, AASU had an exacerbated chronotropic response compared with AASNU (between groups, $P < 0.05$; Table 3), particularly at the third and fourth minutes of mental stress.

DISCUSSION

To the best of our knowledge, this is the first study to assess the acute response of neurovascular control by means of afferent muscle mechanoreflex (isometric exercise) and efferent central command (mental stress) in young men self-administering AAS. The main findings of our study are that, compared with AASNU, AASU had normal MSNA and FBF responses during muscle mechanoreflex activation, whereas

TABLE 2. Handgrip exercise and hemodynamic responses in AAS users and nonusers.

	Handgrip Exercise at 30% MVC			
	Baseline	1 min	2 min	3 min
HR, bpm				
AASU	69 ± 6*	78 ± 9*,**	79 ± 7*,**	84 ± 8*,**
AASNU	61 ± 6	68 ± 9*	71 ± 8**	76 ± 11**
SBP, mm Hg				
AASU	137 ± 13	147 ± 12**	155 ± 15**	170 ± 18**
AASNU	131 ± 10	142 ± 9**	157 ± 12**	164 ± 13**
DBP, mm Hg				
AASU	72 ± 8	79 ± 9**	86 ± 11**	96 ± 14**
AASNU	71 ± 11	77 ± 10**	85 ± 12**	89 ± 12**
MBP, mm Hg				
AASU	94 ± 15	101 ± 9**	109 ± 11**	122 ± 14*,**
AASNU	90 ± 11	97 ± 8**	107 ± 12**	113 ± 11**

Values are means ± SD. AASU, $n = 19$; AASNU, $n = 18$.
* $P < 0.05$ versus AASNU.
** $P < 0.05$ versus baseline.

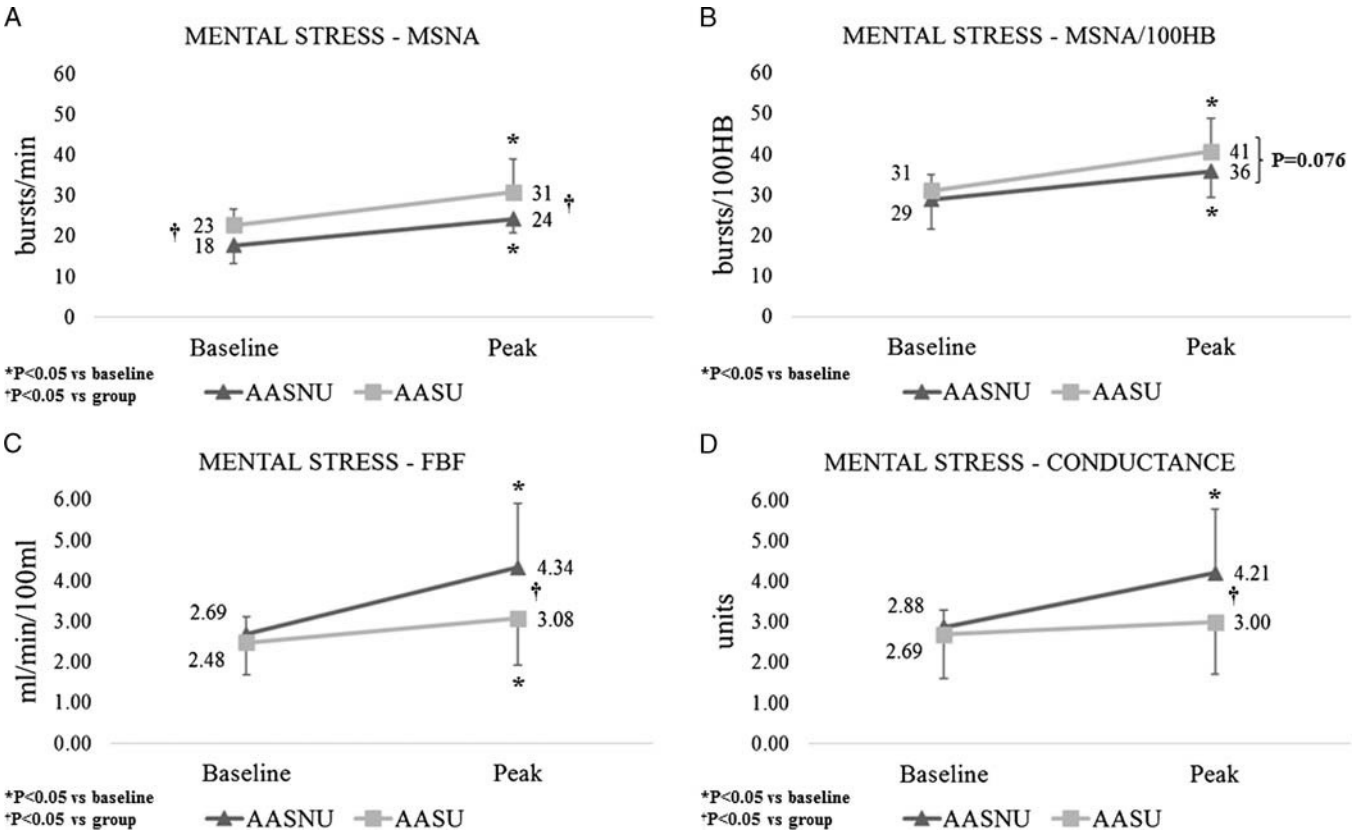

FIGURE 3—MSNA and FBF responses during mental stress. A, MSNA in bursts per minute. B, MSNA in bursts per 100 HB. C, FBF in mL·min^{-1} per 100 mL. D, FVC in units.

during central command stimulation, AASU had exacerbated MSNA and blunted vasodilation.

AAS has been used to increase skeletal muscle hypertrophy and strength in athletes and recreational practitioners of resistance training. In fact, AAS combined with an appropriate exercise-training regime has great potential for improving physical performance in humans (16). In this context, many athletes worldwide refer to this illicit resource; however, several physiological alterations have been postulated in this population (16). For instance, AAS is linked to cardiovascular impairment, with increased blood pressure, endothelial dysfunction (3,17), cardiac hypertrophy (18), and sudden cardiac arrest (19). Moreover, a supraphysiological dose of AAS has a free pathway through the blood–brain barrier, where it binds to specific regions such as γ-aminobutyric acid type A (GABAA) receptors (20). AAS changes GABAergic transmission in neural circuits that play a crucial role in the expression of aggression (21).

Previously, we demonstrated that AASU have increased resting MSNA and lower muscle vasodilation (3). However, we did not know whether this neurovascular alteration would perpetuate during some types of stress, such as exercise or mental challenge. Herein, we observed that during both experiments, AASU exhibited exacerbated MSNA in bursts per minute, with a significant trend toward MSNA corrected by 100 bpm. Interestingly, the absence of vasodilation in the AASU group was more pronounced during mental stress than during isometric exercise. Some potential hypotheses could explain this result. Probably, AASU have both mechanoreceptors and metaboreceptors preserved during isometric exercise, which stimulate group III and IV fiber afferents. This preserved response may be associated with the specific exercise training performed by the participants (resistance exercise training).

On the other hand, during mental stress, AASU may have greater stimulation of the sympathetic-adreno-medullary system, which in turn increases catecholamine spillover. Indeed, AASU had more intensified MSNA than AASNU had, showing the exacerbated effect of mental stress on the

TABLE 3. Mental stress and hemodynamic responses in AAS users and nonusers.

	Mental Stress				
	Baseline	**1 min**	**2 min**	**3 min**	**4 min**
HR, bpm					
AASU	74 ± 10*	80 ± 9**	76 ± 7	76 ± 7*	76 ± 7*
AASNU	62 ± 6	75 ± 14**	71 ± 11**	69 ± 10**	69 ± 10**
SBP, mm Hg					
AASU	138 ± 17	145 ± 22**	150 ± 23**	150 ± 24**	151 ± 22**
AASNU	131 ± 11	133 ± 14	142 ± 17**	142 ± 17**	142 ± 18**
DBP, mm Hg					
AASU	76 ± 13	81 ± 14**	83 ± 15**	83 ± 15**	84 ± 14**
AASNU	75 ± 10	78 ± 13**	82 ± 14**	82 ± 14**	83 ± 16**
MBP, mm Hg					
AASU	94 ± 14	101 ± 16**	104 ± 16**	103 ± 17**	104 ± 15**
AASNU	94 ± 9	96 ± 12	102 ± 14**	103 ± 14**	103 ± 16**

Values are means ± SD. AASU, n = 19; AASNU, n = 18.
* $P < 0.05$ versus AASNU.
** $P < 0.05$ versus baseline.

sympathetic-adreno-medullary system in this group. Moreover, stress reactions can also stimulate psychological parameters, such as the state of anxiety and aggression (10). An experimental study showed that Sprague–Dawley rats treated with nandrolone (20 $mg \cdot wk^{-1}$ for 4 wk) had increased levels of aggression compared with controls (22). In addition, anxious behavior is also associated with AAS use in experimental studies via activation of the central androgenic receptors and mediated by the GABAA receptor (21). All these psychological and physiological alterations may have a role in increasing sympathetic activity in AASU. Interestingly, in the present study, the progressive increase of sympathetic activity during mental stress was accompanied by blunted vasodilation in those participants who used AAS.

The cardiac and hemodynamic response was also evaluated in the present study; it was observed that, compared with AASNU, the chronotropic cardiac response during isometric exercise was increased in AASU over the course of 3 min, whereas during mental stress, HR response was significantly different between groups at rest and at the third and fourth minutes of mental challenge. The exacerbated chronotropic cardiac response can be explained by the autonomic imbalance observed in AASU, as evidenced by the increased sympathetic nerve activity (3) and the blunted vagal reactivation as demonstrated previously (4). Unlike cardiac response, blood pressure (SBP, DBP, and mean) was similar between groups during both experiments. This was an unexpected result, because we thought that blood pressure would also be exacerbated in AASU during the maneuvers. In fact, only during mental stress did the AASU show a tendency toward increased SBP. The mechanisms that may be involved in this response are out of the scope of the present study, and this topic needs to be addressed in the future. Furthermore, long-term studies are needed to evaluate the clinical impact and burden of neurovascular alterations in AASU.

In conclusion, during muscle mechanoreflex activation (isometric exercise), AASU have normal MSNA and FBF responses, whereas during central command (mental stress) stimulation, AASU have exacerbated MSNA and blunted vasodilation. Therefore, mental stress seems to exacerbate neurovascular control throughout the stress reaction situations in AASU.

LIMITATIONS

Our study has limitations. We studied only young men; therefore, the results should be interpreted with caution in older subjects and in women. We tested isometric exercise and mental stress, and we do not know the autonomic and hemodynamic response during other experiments like the cold stress test.

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The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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