

## ORIGINAL ARTICLE

# Heart Rate Reactivity to Acute Mental Stress is Associated With Parasympathetic Withdrawal and Adiposity in Firefighters

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## Abstract

**Background:** Firefighters are regularly exposed to stress and have a high incidence of cardiovascular events. Investigating cardiovascular and autonomic reactivity to acute mental stress (AMS) and its association with adiposity may contribute to explaining the increased cardiovascular risk in these professionals.

**Objectives:** To evaluate cardiovascular and autonomic reactivity to AMS in firefighters while considering adiposity parameters.

**Methods:** This study recorded the blood pressure and heart rate (HR) of twenty-five firefighters (38±8 years) at rest, while performing the Stroop color-word test to induce AMS, and recovery. Cardiac autonomic modulation (HR variability), baroreflex sensitivity (BRS – sequential method), and adiposity (electrical bioimpedance) were assessed. One-way or two-way analysis of variance followed by Tukey's post hoc test and multiple linear regression were performed. The significance level was  $P<0.05$ .

**Results:** The AMS increased mean arterial pressure (MAP –  $\Delta 16\pm 13$  mmHg) and HR ( $\Delta 14\pm 7$  bpm) ( $P<0.05$ ). These responses were associated with parasympathetic modulation withdrawal (RMSSD: baseline:  $29.8\pm 18$  vs. AMS:  $21.5\pm 14$  ms; High-frequency: baseline:  $5.2\pm 1.4$  vs. AMS:  $4.5\pm 1.3$  Ln  $\text{ms}^2$ ;  $P<0.05$ ) and decreased in the Up gain of the baroreflex (baseline:  $8.9\pm 5.1$  vs. AMS:  $6.3\pm 3.0$  mmHg/ms;  $P<0.05$ ). Groups divided by HR reactivity peak showed parasympathetic modulation withdrawal only in firefighters with lower adiposity (RMSSD: baseline:  $27.8\pm 17.6$  vs. AMS:  $14.4\pm 9.2$  ms; High-Frequency: baseline:  $5.3\pm 1.2$  vs. AMS:  $3.8\pm 1.4$  Ln  $\text{ms}^2$ ;  $P<0.05$ ). Fat percentage ( $\beta = -0.499$ ), BRS ( $\beta = 0.486$ ), and sympathetic/parasympathetic balance ( $\beta = -0.351$ ) were predictors of HR reactivity ( $P<0.05$ ).

**Conclusion:** Our results demonstrated that HR reactivity to AMS modulated by cardiac vagal withdrawal seems to be influenced by body composition in this group of firefighters.

**Keywords:** Body Composition; Heart Rate; Psychological Stress; Firefighters.

## Introduction

Work-related and frequent exposures to acute mental stress (AMS) are both associated with an increased risk of developing cardiovascular disease (CVD).<sup>1</sup> Firefighters are regularly exposed to occupational stress in nature and experience an alarmingly high rate of CVD within the profession.<sup>2</sup> Specifically, it was demonstrated that over one-third of all deaths of US firefighters from 1994 to 2004 died from coronary heart disease during or shortly after episodes of AMS, including fire suppression

and alarm response.<sup>3</sup> Increased adiposity is also a risk factor for CVD,<sup>4</sup> with firefighters regularly experiencing weight gain with an accompanied elevated risk for CVD.<sup>5</sup> However, whether or not adiposity modulates the relationship between AMS and cardiovascular health in firefighters is unknown.

AMS causes the activation of the hypothalamic-pituitary-adrenal axis (HPA) and favors the sympathetic arm of the autonomic nervous system.<sup>6</sup> Assessment of the influence of autonomic activity on HR and blood pressure

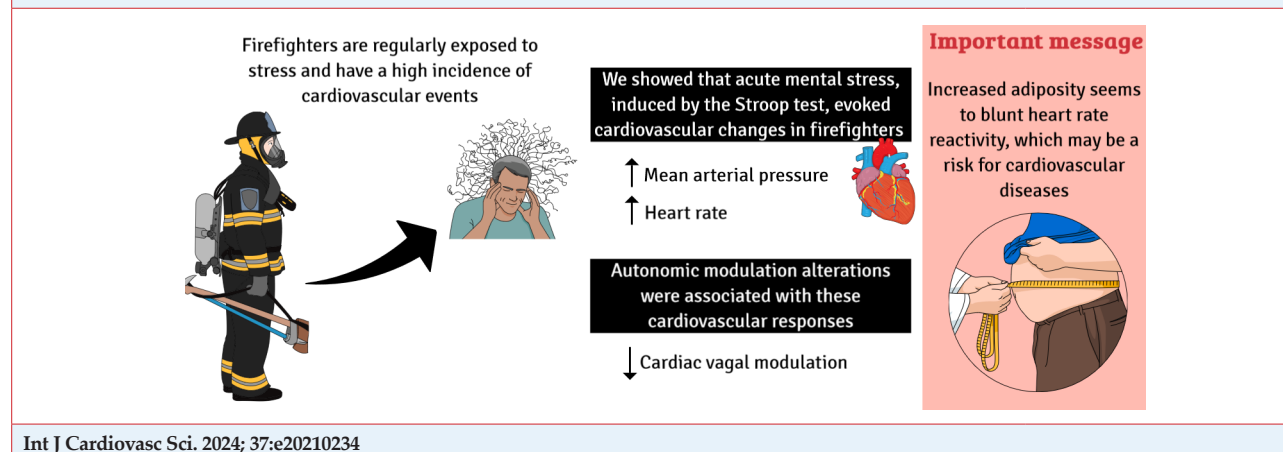
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**Central Illustration:** Heart Rate Reactivity to Acute Mental Stress is Associated With Parasympathetic Withdrawal and Adiposity in Firefighters

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can be tested non-invasively via HR variability (HRV)<sup>7</sup> and baroreflex sensitivity (BRS),<sup>8</sup> respectively. Importantly, AMS intensifies cardiovascular and autonomic changes, allowing for the detection of disturbances not identified at rest in different populations.<sup>9–11</sup> Indeed, previous research has demonstrated that during episodes of AMS, sympathetic dominance is present in firefighters as measured by HRV while undertaking simulated fire suppression tasks.<sup>12</sup>

Considering the high prevalence of CVD in firefighters and its association with AMS in the workplace, investigating cardiovascular and autonomic reactivity to AMS may contribute to explaining the increased cardiovascular risk in these professionals. Given that body fat leads to alterations in autonomic modulation and BRS,<sup>13,14</sup> we evaluated adiposity to draw potential relationships. As such, the purpose of this study was to evaluate cardiovascular and autonomic reactivity to AMS in firefighters while considering adiposity. We hypothesized that the cardiovascular and autonomic reactivity to AMS would be negatively associated with adiposity in firefighters. We also presumed that autonomic modulation and BRS would be involved in this response.

## Methods

### Participants and Study Design

A convenient sample of twenty-five male firefighters from the Military Fire Brigade of Santa Catarina, a state in the southern region of Brazil, was used in this cross-

sectional study. Firefighters were eligible to participate in the study if they were free of diagnosed chronic diseases, did not smoke, and had at least three years of service. The experimental design and protocols conformed to the Declaration of Helsinki and were approved by the Human Research Ethics Committee of the Federal University of Santa Catarina (No. 87655018.0.0000.0121).

### Experimental Design

Participants visited the laboratory once. The pre-test recommendations were not to eat or drink within three hours, not to perform the moderate or vigorous physical activity within 12 hours, not to consume alcohol within 48 hours, and not to take diuretics for at least the last seven days. After completion of the informed consent form, the Depression, Anxiety, and Stress Scale (DASS-21) was answered, and anthropometry and body composition were assessed. After 10-min of rest, the participants performed the Stroop color-word test to induce AMS. Blood pressure, HR, and cardiac interval (iRR) were continuously recorded for 5-min before (baseline), during, and 5-min after (recovery) the AMS in order to evaluate the cardiovascular reactivity. The autonomic reactivity (sympathetic and parasympathetic modulation of the heart and BRS) was calculated using iRR and blood pressure. Details of data collection and analysis are described below.

### AMS

AMS was induced using an adapted version of the Stroop color-word test.<sup>15</sup> Each participant performed

the task individually over a 3-min period. Briefly, a sequence of slides was presented on a monitor placed in front of the participant. Each slide lasted 1-sec with color names (i.e., “blue”, “yellow”, and “red”) displayed in colors unmatched by what the color name represents (e.g., “blue” appearing on the screen in yellow). A continuous auditory conflict was also provided during the test (a recorded voice saying different color names in a headphone). Participants were instructed to report the color of the word as quickly as possible, ignoring the confounding factors. Immediately after the test, the participants reported their perceived stress using the following scores: 0 = not stressful, 1 = slightly stressful, 2 = stressful, 3 = very stressful, and 4 = extremely stressful. The efficiency was calculated as the percentage of correct answers during the test.

### Cardiovascular measurements

Beat-by-beat blood pressure was continuously measured from the right index or middle finger, using photoplethysmography (Finometer Finapres Medical System, Enschede, Netherlands) at 200 Hz. Mean arterial pressure (MAP) was calculated using the formula:  $MAP = [(2 \times \text{diastolic arterial pressure}) + \text{systolic arterial pressure}] / 3$ . HR and cardiac intervals (iRR) were recorded using an HR monitor (RS800 CX, Polar Electro, Kempele, Finland) at 1,000 Hz rate.

### Cardiac autonomic modulation

The cardiac autonomic modulation was analyzed via CardioSeries (v2.4, Brazil) using iRR at time and frequency domains. We analyzed 3-minute recordings at three different time points: baseline, during AMS, and recovery. Before the cardiac autonomic modulation analysis, iRR series were examined, and artifacts and ectopic beats (i.e., non-sinusal beats) were removed manually.<sup>16</sup> For the time domain analysis, the root mean square of the successive differences (RMSSD) was evaluated. For frequency domain analysis, time series were transformed to evenly spaced series by cubic spline interpolation (4 Hz) and were distributed into half-overlapping sets of 512 points (Welch periodogram). A Hanning window was also used to attenuate artifacts and ectopic beats, and the interpolated time series presented the spectra calculated by the Fast Fourier Transform algorithm. The spectra were integrated into low-frequency (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15–0.40 Hz) bands. The participants were allowed to breathe spontaneously, and

their breathing rate was observed. All the participants showed a respiratory rate above 10, which prevented the intersection of the HF and LF bands.<sup>16</sup> The data are presented in the logarithm of absolute values ( $\ln \text{ms}^2$ ) and normalized units (nu). The LF/HF ratio was analyzed to assess the sympathovagal balance.<sup>7,17</sup>

### BRS

Spontaneous cardiovagal BRS was evaluated via the sequential method, using the software CardioSeries v2.4, as previously described.<sup>18</sup> Briefly, this method calculates the slope between changes in iRR and the corresponding changes in systolic blood pressure (SBP) as the index of BRS. Sequences of three or more consecutive heartbeats in which SBP and iRR experience a uniform change (i.e., both increase/decrease) were identified. We fixed minimal changes in blood pressure and iRR to validate a sequence (up to 1 mmHg between 2 blood pressure values and 5 ms for iRR interval). A minimum number of sequences ( $n_{\min} = 5$ ) were used to validate a BRS estimate. Ramps (up or down) with three beats were used to validate sequences. Results are presented in mmHg/ms for Up Gain (slope between increases in SBP and increases in iRR), Down Gain (slope between decreases in SBP and decreases in iRR), and Total Gain (average of Up and Down gains).

### Anthropometry and body composition

Body mass, height, and waist circumference were assessed using a digital scale (Omron HN-289, Osaka, Japan), a stadiometer on a millimeter-scale (Micheletti, SP, Brazil), and a tape measure (Sanny, SP, Brazil), respectively. Body composition (fat-free mass and fat mass) was evaluated by bioelectrical impedance using a tetrapolar and monofrequency (50 Hz) system (Biodynamics Corp. BIA 450, Seattle, WA, USA).<sup>19</sup>

### Mental Health Questionnaire

The validated Brazilian version of DASS-21 was performed to assess depression, anxiety, and stress levels the week before the test. The instrument consisted of three subscales scored on a 4-point Likert Scale, ranging from 0 (“Strongly Disagree”) to 3 (“Totally Agree”). Each subscale of the DASS-21 consists of seven items that evaluate the emotional state. The final score for depression, anxiety, and stress was calculated as the sum of the items on each of the subscales.<sup>20</sup>

## Statistical analysis

The Shapiro-Wilk test was used to test the normality of the data. Nonparametric data were log-transformed and confirmed to be normal. One-way analysis of variance (ANOVA) with repeated measurements, followed by Tukey's post hoc test, was used to compare cardiovascular parameters throughout the experiment.

We used categorized and continuous data approaches to assess the effects of adiposity on cardiovascular reactivity to AMS. For the categorized analysis, the participants were stratified into groups according to the relative HR or MAP peak during the AMS, using quartiles as follows: Low (lower quartile;  $\leq 25\%$ ), medium (intermediate quartiles;  $>25$  and  $<75\%$ ), and high (upper quartile;  $\geq 75\%$ ) relative reactivity. One-way (age, anthropometry, and adiposity) or Two-way ANOVA, with repeated measurements (cardiovascular parameters), followed by Tukey's post hoc test, was used to compare the groups. For continuous analysis, multiple linear regression was used to assess independent variables (predictors) for cardiovascular (MAP and HR) peak reactivity. Forward procedures distributed all variables into the models and removed non-significant variables ( $P > 0.20$ ). All assumptions of multiple linear regression were checked. Akaike information criterion (AIC) and Bayesian information criterion (BIC), as well as the variance inflation factor (VIF) and dispersion were used for post-estimations of the model's goodness-of-fit as multicollinearity. The Breusch-Pagan/Cook-Weisberg test was used to check the heteroscedasticity.

All analyses were conducted using STATA 13.0 (Stata Corporation1, College Station, TX, USA) and GraphPad Prism 6.0 (GraphPad1 Software, Inc., San Diego, CA, USA). Data were expressed as mean  $\pm$  standard deviation (SD). The significance level of  $P < 0.05$  was adopted.

## Results

### Characteristics of all evaluated firefighters

The participants' characteristics are shown in Table 1.

### Cardiovascular reactivity to AMS in all evaluated firefighters

Participants increased HR and MAP during the AMS (at peak) when compared to baseline (five min before

**Table 1 – Characteristics of all evaluated firefighters**

	Mean (SD)
Age (years)	38.6(8.8)
Time working at MFB (years)	14.7(10.2)
<b>Anthropometry</b>	
Height (m)	1.7(0.1)
Body mass (Kg)	82.8(13.2)
BMI (Kg/m <sup>2</sup> )	27.4(3.9)
WC (cm)	94.7(9.0)
WHtR (cm/m)	54.5(5.0)
<b>Body Composition</b>	
Body fat (%)	22.0(4.8)
Free fat mass (Kg)	64.2(8.7)
Fat mass (Kg)	18.6(6.5)
<b>Baseline cardiovascular parameters</b>	
SAP (mmHg)	135.0(11.1)
DAP (mmHg)	77.6(8.7)
MAP (mmHg)	97.2(8.3)
HR (bpm)	70.9(14.7)
DASS-21	
Depression	5.7(9.2)
Anxiety	5.0(6.2)
Stress	10.4(9.0)
<b>Perceived stress and efficiency during AMS</b>	
Perceived stress	1.6(0.9)
Efficiency (%)	79(21)

BMI: body mass index; DAP: diastolic arterial pressure; DASS-21: depression, anxiety, and stress scale; HR: heart rate; MAP: mean arterial pressure; MFB: military fire brigade; SAP: systolic arterial pressure; WC: waist circumference; WHtR: waist to height ratio; SD: standard deviation; AMS: acute mental stress.  $n = 25$ .

AMS;  $P < 0.0001$ ) and returned to baseline levels at recovery (five min after AMS;  $P > 0.05$ ) (Figure 1).

HRV decreased during AMS (Table 2). Specifically, when compared to baseline, RMSSD, LF, and HF decreased ( $P < 0.001$ ). All variables returned to baseline levels upon recovery ( $P < 0.001$ ).

Similarly, BRS decreased during AMS. Specifically, up sequences decreased when compared to baseline and recovery ( $P < 0.05$ ; Table 2).

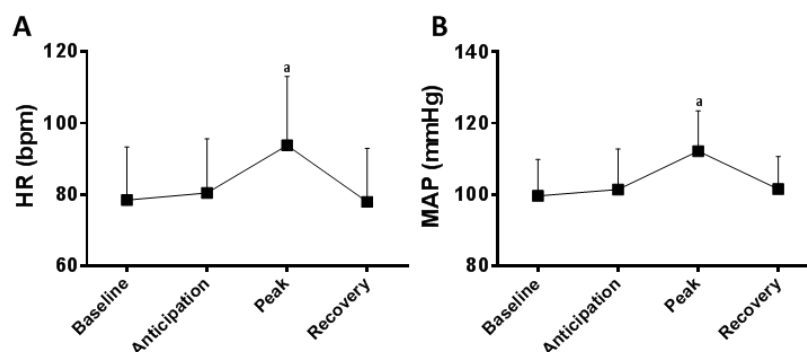


Figure 1 – A) Heart rate (HR) and B) mean arterial pressure (MAP) reactivity to acute mental stress in firefighters. ANOVA with repeated measurements followed by Tukey's post-test; a significant vs. Baseline;  $P < 0.05$ .

Table 2 – Autonomic modulation and BRS reactivity to AMS in all evaluated firefighters

	Baseline	AMS	Recovery	F-value	P-value
<b>Autonomic modulation</b>					
RMSSD (ms)	29.8(18)	21.5(14) <sup>a</sup>	31.9(21) <sup>d</sup>	17.5	<0.001
LF (Ln ms <sup>2</sup> )	7.0(0.9)	5.9(1.2) <sup>a</sup>	7.2(1.0) <sup>d</sup>	40.0	<0.001
HF (Ln ms <sup>2</sup> )	5.2(1.4)	4.5(1.3) <sup>a</sup>	5.3(1.3) <sup>d</sup>	10.3	0.001
LF (nu)	79.7(10.9)	77.9(9.5)	82.6(8.5)	2.4	0.10
HF (nu)	20.2(10.9)	22.0(9.5)	17.4(8.4)	2.4	0.10
LF/HF	6.9(3.8)	5.7(3.1)	8.2(4.6)	3.0	0.06
<b>BRS</b>					
Up Gain (mmHg/ms)	8.9(5.1)	6.3(3.0) <sup>a</sup>	8.9(5.5) <sup>d</sup>	5.5	0.01
Down Gain (mmHg/ms)	9.0(5.6)	7.5(3.1)	8.4(5.9)	1.0	0.34
Total Gain (mmHg/ms)	9.0(5.3)	6.9(2.5)	8.7(5.4)	3.0	0.08

The results are presented as the means  $\pm$  SD. One-way Anova with repeated measurements, followed by Tukey's post-test; <sup>a</sup> significant vs. Baseline; <sup>d</sup> significant vs. AMS;  $P < 0.05$ . Significant comparisons are highlighted in bold.  $n=25$ . HF, high-frequency; LF, low-frequency; RMSSD, square root of the successive differences of the RR interval; BRS: baroreflex sensitivity; AMS: acute mental stress.

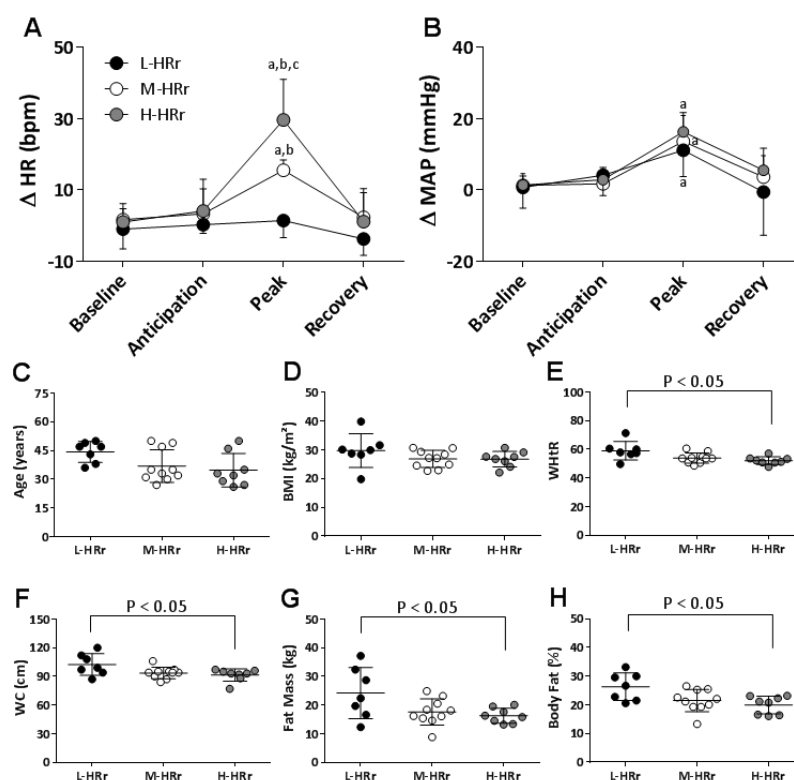
### Cardiovascular reactivity to AMS in groups stratified by peak hemodynamic response

The groups were subdivided into three groups according to the  $\Delta$ HR peak reactivity to AMS: Low (L-HRr), Medium (M-HRr), and High (H-HRr)  $\Delta$ HR peak. The analysis of the HR reactivity revealed a time and magnitude of the relative HR change effects and a time  $\times$  magnitude of the HR change interaction ( $P < 0.0001$ ). Multiple comparison analysis showed increases in HR at peak in the H-HRr and M-HRr groups when compared

to baseline ( $P < 0.0001$ ; Figure 2A). Peak HR was higher in the H-HRr group when compared to the M-HRr and L-HRr groups ( $P < 0.0001$ ). The M-HRr group also showed a greater peak HR when compared to the L-HRr group ( $P < 0.001$ ). The analysis of the MAP reactivity revealed a time effect ( $P < 0.0001$ ). Increases in MAP at the peak were observed in the H-HRr, M-HRr, and L-HRr ( $P < 0.01$ ) groups when compared to baseline (Figure 2B).

Waist to height ratio (WHtR), waist circumference (WC), absolute fat mass, and body fat percentage were





**Figure 2 – A)** heart rate (HR), and **B)** mean arterial pressure (MAP) reactivity to acute mental stress; **C)** Age; **D)** body mass index (BMI); **E)** waist to height ratio (WHtR); **F)** waist circumference (WC); **G)** fat mass; and **H)** body fat in firefighters stratified by the  $\Delta$ HR peak reactivity. Two-way ANOVA with repeated measurements followed by Tukey's post-test for panels A-B; One-way ANOVA followed by Tukey's post-test for panels C-H; \* significant vs. Baseline; <sup>a</sup> significant vs. L-HRr group; <sup>b</sup> significant vs. M-HRr group; <sup>c</sup> significant vs. H-HRr group; P < 0.05. The results are presented as the means  $\pm$  SEM for Panels A-B and as the means  $\pm$  SD for panels C-H. Low  $\Delta$ HR peak reactivity group (L-HRr; n=7), Medium  $\Delta$ HR peak reactivity group (M-HRr; n=10), High  $\Delta$ HR peak reactivity group (H-HRr; n=8).

higher in the L-HRr group when compared to the H-HRr group. No differences among groups were observed in age and body mass index (BMI —  $P > 0.05$ ; Figure 2C-H).

The time-domain HRV analysis demonstrated a time effect and a time  $\times$  magnitude of the HR change interaction ( $P < 0.01$ ) for RMSSD. Multiple comparison analysis showed decreases in the RMSSD during the AMS in the H-HRr group when compared to baseline and increases when compared to AMS ( $P < 0.0001$ ; Figure 3A).

The frequency-domain HRV analysis showed a time effect and a time  $\times$  magnitude of the HR change interaction ( $P < 0.01$ ) for LF ( $\ln \text{ms}^2$ ). Multiple comparison analysis showed decreases in the LF ( $\ln \text{ms}^2$ ) during the AMS in all groups when compared to baseline ( $P < 0.05$ ) and increases in the LF ( $\ln \text{ms}^2$ ) in all groups when compared to AMS ( $P < 0.0001$ ). A time effect and a time  $\times$  magnitude of the HR change interaction ( $P < 0.0001$ ) was also observed for HF ( $\ln \text{ms}^2$ ). Multiple comparison

analysis showed decreases in the HF ( $\ln \text{ms}^2$ ) during the AMS in the H-HRr group when compared to baseline and increases in the HF ( $\ln \text{ms}^2$ ) when compared to AMS ( $P < 0.0001$ ). The H-HRr group showed higher HF ( $\ln \text{ms}^2$ ) at recovery when compared to the M-HRr and L-HRr ( $P < 0.05$ ) groups. A time effect ( $P < 0.05$ ) was demonstrated for LF/HF ratio (Figure 3B-F). The BRS analysis showed a time effect for the Up Gain ( $P < 0.05$ ; Figure 3G-I).

No differences among groups were found in the perceived stress and efficiency during AMS and DASS-21 scores (Table 3).

The participants were also divided into three groups according to the  $\Delta$ MAP peak reactivity to AMS. However, no differences among groups were observed in age, BMI, and adiposity ( $P > 0.05$ ). In addition, no differences among groups were observed in all variables of autonomic modulation, BRS, and DASS-21 ( $P > 0.05$ ; data not shown).

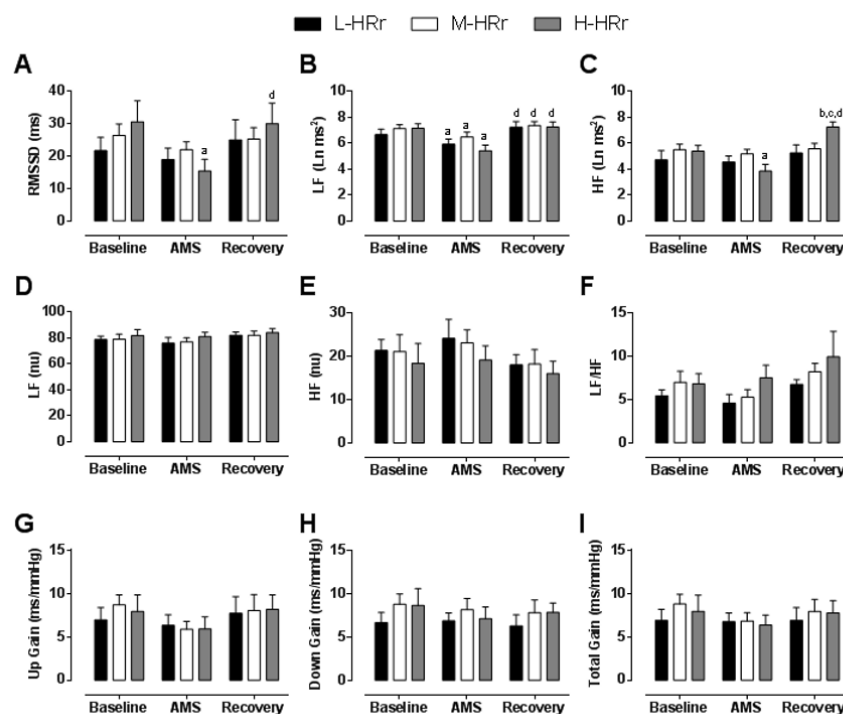


Figure 3 – A) square root of the successive differences of the cardiac intervals (RMSSD); B) absolute low frequency (LF) on a logarithm scale; C) absolute high frequency (HF) on a logarithm scale; D) normalized LF; E) normalized HF; and F) LF/HF ratio; G) up; H) down; and I) total gain of the baroreflex sensitivity in firefighters stratified by the  $\Delta$ HR peak reactivity. Two-way ANOVA with repeated measurements followed by Tukey's post-test. <sup>a</sup> significant vs. Baseline; <sup>b</sup> significant vs. L-HRr group; <sup>c</sup> significant vs. M-HRr group; <sup>d</sup> significant vs. AMS. The results are presented as the means  $\pm$  SD. Low  $\Delta$ HR peak reactivity group (L-HRr; n=7), Medium  $\Delta$ HR peak reactivity group (M-HRr; n=10), High  $\Delta$ HR peak reactivity group (H-HRr; n=8).

Table 3 – DASS-21 scores and perceived stress and efficiency during AMS in firefighters stratified by the HR peak

	L-HRr	M-HRr	H-HRr	F-value	P-value
<b>DASS-21</b>					
Depression	12.8(15.1)	3.8(5.4)	2.2(2.2)	3.1	0.06
Anxiety	9.4(10.5)	4.2(3.5)	2.5(2.0)	2.6	0.09
Stress	15.4(13.5)	9.2(5.4)	7.0(6.2)	1.9	0.17
<b>Perceived stress and efficiency during AMS</b>					
Perceived stress	1.3(0.5)	1.8(1.0)	1.8(1.0)	0.6	0.52
Efficiency (%)	78(27)	74(21)	87(12)	0.8	0.43

The results are presented as the means  $\pm$  SD. One-way Anova;  $P < 0.05$ . Low (L-HRr; n=7), Medium (M-HRr; n=10), and High (H-HRr; n=8)  $\Delta$ HR peak reactivity groups. DASS-21, Depression, anxiety, and stress scale.

### Multiple linear regression models analysis

The relative HR peak reactivity was negatively correlated with body fat percentage, BRS, and LF/HF ratio. The final

model explained 48% of the variability of the relative HR reactivity peak ( $P < 0.01$ ; Table 4). The relative MAP peak reactivity was negatively correlated with baseline MAP, and

the model explained 29% of the variability of the relative MAP reactivity peak ( $P < 0.05$ ; Table 4).

## Discussion

The purpose of the present study was to evaluate cardiovascular and autonomic reactivity to AMS in firefighters while considering adiposity parameters. The main findings were as follows: I) the Stroop color-word test successfully induced AMS in firefighters, illustrated by resultant increases in MAP and HR. These responses were associated with cardiac parasympathetic withdrawal and a decrease in the Up gain of the cardiovagal BRS; II) stratifying the participants by HRr peak, the L-HRr group showed higher waist circumference, body fat (%), absolute fat mass, and WHtR when compared to the H-HRr group; and III) body fat (%), BRS, and sympathetic/parasympathetic balance were independent predictors for HR reactivity.

Firefighters are frequently exposed to mental stressors, including responding to an alarm, fire prevention and fighting, search, rescue, and public assistance, challenging their coping skills.<sup>3</sup> Therefore, critical physiological functions (e.g., changes in HR and ANS) are impacted

during firefighters' activities, which may increase CVD risk.<sup>21</sup> In fact, one study reviewed summaries of 1,144 firefighter deaths on duty in the US over a 10-year period. The results revealed that 45% of these deaths were due to CVD, mainly due to coronary heart disease, and more frequently in firefighters with higher risk factors for CVD.<sup>3</sup> Thus, a better understanding of the cardiovascular reactivity during AMS in this population and the possible mechanisms is needed.

The Stroop color-word test's effectiveness in evoking cardiovascular changes has been observed in prior studies.<sup>15</sup> However, to the best of our knowledge, the present study is the first to demonstrate it in firefighters. Our data showed increases in MAP and HR in the group of firefighters during AMS. The multiple linear regression analysis showed a negative correlation between the HR peak during the AMS and adiposity, pointing out fat percentage as an independent predictor for HR reactivity. Corroborating with this data, dividing the participants into three groups according to the magnitude of maximal HR reactivity, we found that the H-HRr group displayed lower adiposity when compared to the L-HRr group. Our research group and

**Table 4 – Multiple linear regression analysis for independent predictors of relative HR and MAP peak reactivity in firefighters.**

<b>ΔHR peak* (dependent variable)</b> model R <sup>2</sup> = 0.48			
<b>Independent Predictors</b>	<b>β (CI 95%)</b>	<b>β<sub>standardized</sub></b>	<b>P-value</b>
Age (years)	-0.041(-0.085; 0.003)	-0.35	0.06
BRS (ms/mmHg)	0.08(0.14; 0.02)	0.48	0.01
Body fat (%)	-0.09(-0.16; -0.02)	-0.49	0.01
LF/HF ratio	-0.027(-0.054; -0.005)	-0.35	0.04
<b>ΔMAP peak (dependent variable)</b> model R <sup>2</sup> = 0.29			
<b>Independent Predictors</b>	<b>β (CI 95%)</b>	<b>β<sub>standardized</sub></b>	<b>P-value</b>
Age (years)	0.34(-0.01; 0.71)	0.42	0.06
Baseline MAP (mmHg)	-0.51(-0.93; -0.09)	-0.59	0.02
BRS (ms/mmHg)	0.47(-0.08; 1.04)	0.40	0.09
Body fat (%)	-0.41(-1.00; 0.17)	-0.30	0.16
LF/HF ratio	0.20(-0.02; 0.43)	0.36	0.08
<p>HR reactivity model: <math>P=0.003</math>; <math>F=5.95</math>; root mean square error=0.642; mean VIF= 1.20; BIC=61.5; AIC*n=55.  MAP reactivity model: <math>P=0.04</math>; <math>F=2.92</math>; root mean square error=5.07; mean VIF=1.75; BIC=168.724; AIC*n=160. The significant variables are highlighted in bold. * square root transformed variable. BRS, baroreflex sensitivity; HF, High-frequency; LF: low-frequency.</p>			



others have shown blunted cardiovascular reactivity in people with increased adiposity.<sup>11,22,23</sup> However, other studies demonstrated opposing effects.<sup>24,25</sup> The type of stressor, measurement methods of cardiovascular parameters, and participants' characteristics could contribute to these conflicting data.<sup>26</sup> Importantly, although greater cardiovascular reactivity to AMS has been linked to CVD,<sup>26</sup> a growing body of evidence supports that blunted reactivity may also predict a raised risk for other health outcomes, including obesity, depression, poor self-reported health, and compromised immunity.<sup>27</sup>

It has been demonstrated that autonomic imbalance can predict cardiometabolic diseases.<sup>28,29</sup> The available data indicate that reduced parasympathetic modulation is associated with increased morbidity and mortality.<sup>30</sup> Cardiovascular and autonomic responses induced by acute physiological or mental stress also appear to be valuable evaluations to identify healthy outcomes, adding information to those observed at rest. For instance, post-exercise HR recovery is positively correlated with the parasympathetic decline following an active postural change at rest,<sup>31</sup> and impaired autonomic response to AMS in healthy adults predicts physical and mental health and disease outcomes.<sup>9</sup> In the present study, the firefighters displayed parasympathetic withdrawal during AMS, demonstrated by decreases in the RMSSD and Ln HF. These results are in line with previous studies<sup>32,33</sup> that suggest that vagal withdrawal plays an important role in the increase in HR during stressful situations. When the firefighters were divided by peak HR reactivity, the H-HRr group showed a marked decrease in RMSSD and Ln HF, whereas the L-HRr group did not. The linear regression model showed that the sympathetic vagal balance before the AMS is an independent predictor for HR reactivity during the AMS. Since the L-HRr group presented higher adiposity, these alterations in the ANS may be related to the excess adipose tissue-induced immune alterations and can help explain, at least in part, the lower HR reactivity in the L-HR group.

Adipocyte hypertrophy, especially in the visceral adipose tissue, stimulates M1 macrophage infiltration, increasing a pro-inflammatory cytokine release.<sup>34</sup> This then leads to a systemic low-grade chronic inflammatory state, which is related to ANS alterations.<sup>14,35</sup> Pre-clinical studies have also demonstrated that increased adipose tissue leads to chronic inflammation, specifically in cardiovascular control areas.<sup>14,35</sup> Additionally,

autonomic imbalance may negatively affect the cholinergic anti-inflammatory pathway, which acts through vagus nerve regulation and controls the inflammatory response.<sup>29</sup> In fact, both RMSSD and HF are consistently associated with inflammatory markers, with HF displaying a stronger correlation.<sup>29</sup> Thus, although we did not evaluate inflammatory parameters in the present study, it may be a mechanism for lower HR reactivity in participants with higher adiposity. Future studies should test this hypothesis.

Previous studies have demonstrated an attenuation of BRS during mental<sup>36</sup> and physiological (i.e., physical exercise)<sup>37</sup> stress. Our data are in line with these studies as we observed decreases in the up gain during the AMS and a trend reduction in the total gain. Moreover, BRS was an independent predictor of HR reactivity. This is particularly relevant because associations between BRS and CVD risk and mortality have been demonstrated.<sup>8</sup> Therefore, the attenuated BRS may increase the risk of cardiac events in firefighters on duty. Increases in arterial stiffness<sup>36,37</sup> and glucocorticoid levels<sup>38</sup> seem to attenuate BRS and may be a mechanism underlying AMS-induced baroreflex changes in firefighters.<sup>36,37</sup> Future studies should evaluate these parameters to confirm this hypothesis. Truijen and colleagues<sup>39</sup> also showed higher BRS in healthy participants treated with non-selective  $\beta$ -adrenergic blockade during psychological stress, demonstrating an influence of sympathetic activation in reducing BRS. Nevertheless, in the present study, the mechanism involved in the baroreflex responses may relate to parasympathetic instead of sympathetic modulation, since we found decreases in HF and RMSSD but no increases in LF or LF/HF during AMS.

### Study limitations and strengths

This study has some key limitations. A relatively small number of participants were recruited due to the limited number of firefighters working in the Military Fire Brigade. In addition, during the assessment period, some firefighters were helping with a rescue task in another state. Only male firefighters were recruited in this study; therefore, these results should not be extended to female firefighters. Further, blood analyses for catecholamines and cortisol were not possible for this study. Our study is strengthened by capturing beat-by-beat blood pressure and iRRs, allowing for an effective analysis of hemodynamic

and autonomic reactivities to AMS. We ensured that the cardiovascular responses to AMS observed in the participants were not related to differences in the test efficiency or perceived stress. The firefighters did not suffer from anxiety, depression, or stress in the present study. This is an important control variable, since mental distress has been linked to dysregulated cardiovascular reactivity to stress.<sup>40</sup> We also applied different statistical approaches to identify factors related to cardiovascular reactivity to AMS.

## Conclusion

Our results demonstrated that HR reactivity to AMS modulated by cardiac vagal withdrawal seems to be influenced by one's body composition in this group of firefighters. Since firefighters frequently face stressful situations as part of their occupation and regularly present independent risk factors for CVD, regular assessment of body fat and planning intervention strategies to maintain it at low levels can benefit firefighters. Furthermore, identifying impaired hemodynamic reactivity and autonomic imbalance may be relevant in order to help prevent CVD in firefighters.

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## Author Contributions

Conception and design of the research and statistical analysis: Mendes H, Lima LRA, Speretta G; acquisition of data: Mendes H, Canto N; analysis and interpretation of the data: Mendes H, Canto N, Lima LRA, Speretta G; writing of the manuscript: Mendes H, Speretta G; critical revision of the manuscript for intellectual content: Canto N, Lima LRA, Speretta G.

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No potential conflict of interest relevant to this article was reported.

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## Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Ethical Committee of the Federal University of Santa Catarina under the protocol number 87655018.0.0000.0121. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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