Cardiorespiratory Fitness Predicts Greater Vagal Autonomic Activity in Drug Users Under Stress

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ABSTRACT: While drug use has been shown to impair cardiac autonomic regulation, exercise might overcome some of the damage. Herein, we describe how individuals with substance use disorder (SUD) have their heart rate variability (HRV) and drug-related behaviors negatively affected in response to a stressor. However, we show how cardiorespiratory fitness may attenuate those impairments in autonomic control. Fifteen individuals with SUD were matched with 15 non-SUD individuals by age, weight, height, and fitness level, and had their HRV responses under stress induced by the Cold Pressor Test (CPT). The SUD group had lower mean of R-R intervals before and after the CPT when compared with the non-SUD group. In addition, in individuals with SUD, higher cardiorespiratory fitness level predicted greater vagal activity before, during, and after CPT. Moreover, for individuals with SUD, days of abstinence predicted greater mean of R-R intervals during recovery from the CPT. Finally, years of drug use negatively predicted mean of R-R intervals during recovery. Thus, our results suggest that chronic drug use impairs cardiac autonomic regulation at rest and after a physical stress. However, cardiorespiratory fitness might attenuate these impairments by increasing vagal autonomic activity.

KEYWORDS: Autonomic nervous system, cardiorespiratory fitness, exercise, substance abuse treatment centers

Introduction

Substance use disorder (SUD) is described as a major worldwide issue[1,2] and is defined by the *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.) of the American Psychiatric Association (*DSM-5*, 2013) as a set of psychosocial criteria related to chronic drug intake.[3] SUD has been proven to be related to several problems, including psychiatric, physiological, and cognitive disorders,[4–6] as well as social and financial issues.[7,8] Despite different drugs having different brain action mechanisms, they do share common features, such as activating reward system areas by dopamine and also prompting addictive behaviors, such as tolerance, cravings, abstinence, and drug-seeking impulses.[9] Moreover, in the cardiovascular system, drug use has been shown to increase adrenergic activity and decrease vagal tone, leading to poorer heart rate variability (HRV), which creates a scenario that is associated with several pathologies and increased risk of death.[10–12]

HRV is the variation in the time intervals between consecutive heartbeats.[13] This physiological marker has proven to be influenced by the interaction between the brain and the heart. It is commonly used to evaluate the autonomic nervous system (ANS) regulation.[14,15] Higher HRV means greater parasympathetic activity and lower sympathetic tone.[16] This condition is related to fewer chances for developing cardiovascular and cerebrovascular events, and decreased negative emotional states.

Conversely, lower HRV means higher adrenergic activity and lower vagal tone, and is a common element of several pathologies such as cognitive decline, cardiovascular diseases, depression, and anxiety.[12,14,17–19] Importantly, HRV has several indices that, taken together, reveal information about the psychophysiological functioning of the body. For instance, the time domain index known as standard deviation of all R-R intervals (SDNN) is a measure of global HRV and vagal tone. It highly predicts cardiac risk, and mortality when measured during 24 hours.[20] Another time domain parameter is the root mean square of successive differences between R-R intervals (RMSSD), which is considered a parasympathetic index that is negatively associated with death risk[21] and with stress levels.[22,23] On the frequency domain, the high frequency (HF) index has been proven to be inversely associated with stress, panic, and anxiety.[24] Hence, HRV is a good physiological marker that gives information about different functions of the body.

Within the context of drug use, studies have shown a negative association between alcohol, cocaine, and methamphetamine use and HRV.[10,25,26] Poorer HRV is also known to indicate higher stress levels.[19,27] Notably, stress plays a major role in addiction. High levels of this emotional state may trigger relapse,[28] possibly due to the cue-reactivity responses to environmental stressors, which lead to drug-seeking and drug-taking behaviors.[29] For instance, studies have shown an association between psychosocial adversity,

negative affect, chronic distress, and traumatic exposure with increased risk of becoming addicted to a substance.[30–33] In addition, it has been demonstrated that stress, measured by ANS functionality, predicts relapse and the development of SUD.[28] Therefore, it is important to search for complementary strategies to keep low levels of stress and negative emotional states, thereby helping to avoid relapse and maintain abstinence.

Cardiorespiratory fitness (measured by the maximum oxygen uptake [VO_2max]) has been the most used parameter to describe functional capacity and human physical performance.[34] Notably, higher levels of VO_2max are associated to the enhancement of several functions of the human organism,[35] such as cardiovascular, neuromuscular, and cognitive functions.[36–38] It is also related to decreased stress and anxiety levels, as well as improved mental health.[39,40] Moreover, higher cardiorespiratory fitness promotes benefits on the ANS control by decreasing sympathetic outflow and increasing parasympathetic activity.[37] It acts as a strong and independent predictor of all-cause and disease-specific mortality.[34] Endurance exercise is the most effective method to improve cardiorespiratory fitness and has been suggested to be included as part of SUD rehabilitation programs;[41,42] however, it is unclear how these benefits may affect ANS control in patients with SUD, especially under stressful situations.

Other studies have successfully used the Cold Pressor Test (CPT) as an external stressor to verify cardiovascular impairments, such as blood pressure oscillations.[43] CPT requires the participants to submerge their hand into a container with water and ice. It is a feasible method to identify HRV responses.[44,45] CPT has also been used to identify differences in cardiovascular parameters between physically active and inactive healthy subjects.[46] In individuals with SUD, CPT was used to investigate pain tolerance in opioids, cocaine, and alcohol dependents.[47–49] Therefore, CPT is an interesting and well-controlled stressor that may be responsive to the variables herein (ANS control, cardiorespiratory fitness, stress, and SUD) and could be easily used to monitor SUD rehabilitation process. Thus, verifying ANS responses in individuals with SUD with different cardiorespiratory fitness during a simple stressor is important, because lower HRV and stress are closely linked to relapse and SUD progression.[28]

This study compared the ANS responses between individuals with SUD and a healthy control group before, during, and after the CPT. We verified possible associations between cardiorespiratory fitness, years of drug use, days of abstinence, age, and negative emotional feelings with HRV in individuals with SUD exposed to the CPT. We hypothesized that the SUD group would have poorer ANS control compared to the healthy subjects and that greater cardiorespiratory fitness will be associated with greater HRV in the presence of a cold stressor.

Methods

Study design

In this study, individuals with SUD undergoing treatment were recruited from 4 different rehabilitation community centers. First, a lecture about exercise benefits and the damage caused by drugs was given, followed by the study presentation. Those interested in participating were informed about all the procedures and signed the informed consent form. Second, the Mini-Mental State Examination (MMSE) and the Physical Activity Readiness Questionnaire (PAR-Q) were administered. To be approved in both questionnaires, participants had to answer "No" to all questions of the PAR-Q and to score >24 points on the MMSE. Once the volunteers, aged between 18 and 45 years old, were approved in both questionnaires, we characterized their drug-use background and history (number of hospitalizations, years of drug use, and days of abstinence since the last drug use) by applying the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) questionnaire[50] and *DSM-5* criteria (see Table 1 for SUD vs non-SUD). In sequence, the Depression, Anxiety and Stress Scale-21 (DASS-21) was administered, followed by the CPT. Finally, the subjects performed a fitness level test (the Léger shuttle-run test); 43 men who met the above criteria and were classified as having severe SUD by the *DSM-5* were used in the analysis. There were no exclusion criteria for a specific substance use.

A non-SUD group composed by 15 men were recruited at the university (ie, students and employees) to be used as the control group. The individuals were between 18 and 45 years old and healthy. The subjects were told about all the study procedures and signed the informed consent form. First, the PAR-Q and the MMSE were completed and, if the volunteer was approved, the CPT was conducted with continuous measures of HRV. Finally, the individuals performed the cardiorespiratory fitness level test. The present study was approved by the local ethics committee and is in accordance with the Declaration of Helsinki standards. To analyze the effects of chronic drug use on the cardiac ANS control under stress, we compared the HRV responses before, during, and after the CPT between the control group, n = 15, age: 28.40 ± 6.98, weight: 71.10 (64.50-80.19 kg), height: 1.71 (1.66-1.76 m), and the 15 individuals with SUD paired by cardiorespiratory fitness, age, weight, and height, n = 15, age: 28.60 ± 5.53, weight: 71.00 (66.67-72.86 kg), height: 1.70 (1.67-1.71 m).

In individuals with SUD, N = 43, age: 34.00 ± 8.03 years, weight: 71.00 (67.33-73.52 kg), height: 1.70 (1.67-1.71 m), we tested the role of cardiorespiratory fitness in the ANS responses before, during, and after the CPT through linear regression analyses. Possible co-variables (Age, Depression Score, Stress Score, Days of Abstinence, Years of Drug Use) were identified using bivariate correlations and later included in the regression models if they were significantly related to VO_2max. Table 2 provides the characteristics of the patients with SUD.

Table 1. General characteristics of drug users and matched and non-matched healthy subjects.

VARIABLES	NON-SUD (N = 15)	SUD (N = 15)	P VALUE	NON-MATCHED SUD (N = 28)
Weight (kg)	71.10 (64.50-80.19)	71 (66.67-72.86)	.74	71.40 (66.19-74.84)
Height (m)	1.71 (1.66-1.76)	1.70 (1.67-1.71)	.32	1.70 (1.66-1.71)
Age (years)	28.40 ± 6.98	28.60 ± 5.53	.93	36.50 ± 7.61
VO$_2$max	41.21 ± 2.41	42.64 ± 4.68	.30	38.39 ± 6.68
Days in abstinence	–	115 ± 90	–	143.64 ± 168.17
Years of drug use	–	11.75 ± 3.12	–	19.24 ± 10.11
Number of hospitalizations (a.u.)	–	0.50 ± 0.58	–	1.23 ± 1.48
ASSIST score of preferred drug (a.u.)	–	29.91 ± 4.23	–	31.36 ± 5.55
Days per week of drug use	–	6-7	–	6-7
Individuals who used alcohol only (n)	–	2	–	9
Individuals who used crack only (n)	–	5	–	6
Multiple drug users (n)	–	–	–	1
Individuals who used marijuana only (n)	–	8	–	12

Abbreviations: ASSIST, Alcohol, Smoking and Substance Involvement Screening Test; a.u., arbitrary units; SUD, substance use disorder, VO$_2$max, maximum consumption of oxygen.
P values are only for the comparison between healthy and matched SUD individuals.

Alcohol, Smoking and Substance Involvement Screening Test

Drug-use background, preferred drug, and issues related to chronic drug use (alcohol, crack, cocaine, marijuana, etc) were assessed by the ASSIST questionnaire developed by the World Health Organization.[36] The questionnaire has 7 questions and classifies the individual as "no need for treatment (<3 pts)," "needs a brief intervention (>4 pts)," or "needs immediate intervention (>27 pts)." Each participant can have different scores for different types of substances. The drug with the highest score was considered the volunteer's preferred drug.

CPT and HRV analysis

We used the CPT to induce a cold stress condition, as proposed by Hines and Brown[43] in 1936. The volunteers were instructed to submerge their dominant hand into a container with water and ice (0°C-2°C), and to remain in this condition for 2 minutes. Thereafter, the subject was told to take their hand out of the container and relax for 8 more minutes to allow the ANS recovery analysis.

HRV measurement was conducted to analyze the functionality of the ANS during rest, stress, and recovery conditions. Participants were told to relax in a sitting position without becoming drowsy or sleepy. The HRV recording was initiated only when the respiratory rate was below 20 breaths per minute. A heart rate monitor (RS800CX training computer, Polar, Finland) was used to record the R-R intervals every 5 seconds. Time domain parasympathetic indices were used to analyze ANS (mean R-R interval and RMSSD of the R-R intervals).[14,51] Those indices were chosen because of their good reliability in predicting vagal tone integrity[15,52] and sympatho-vagal balance.[53] Other indices, such as SDNN, NN50 (The number of pairs of successive RR intervals that differ by more than 50ms), pNN50 (The porcentage of number of pairs of successive RR intervals that differ by more than 50ms), HF, and low frequency (LF), have specific recommendations that would not be possible in the current study design, as explained in sequence. For instance, SDNN is a good marker for cardiovascular health; however, it is recommended to be measured for 24 hours to achieve significant reliability,[16] although some studies have successfully used SDNN in 5- and 1-minute measurements.[54,55] The NN50 and pNN50 need at least a 2-minute measure.[16] The frequency domain indices, HF and LF, have to be measured over a minimum of 1- and 2-minute periods, respectively.[56,57] The ratio of LF and HF (LF/HF index) has been widely criticized as it does not represent the sympatho-vagal balance as proposed decades ago[16,58] Thus, we have specifically chosen to use RMSSD as a primary vagal index because it is a reliable measure of parasympathetic activity, and it can be used for ultra-short-term periods, such as 10[59] and 30 seconds[60] as we did in the present study. Moreover, we used the mean R-R intervals, which is a representation of overall heart rate and, consequently, HRV regulation. This index indicates a sympatho-vagal balance, in which a reduced mean R-R interval denotes a higher sympathetic activity and a greater mean R-R interval denotes a greater vagal activity.[53]

Table 2. Characteristics of the whole sample of drug users.

VARIABLES	PATIENTS WITH SUD (N = 43)
Weight (kg)	71.00 (67.33-73.52)
Height (m)	1.70 (1.67-1.71)
Age (years)	34.00 ± 8.03
VO$_2$max (mL/kg/min)	40.07 ± 6.26
Depression score (a.u.)	43.54 (28.20-51.53)
Anxiety score (a.u)	35.66 (31.40-44.81)
Stress score (a.u)	28.81 (26.49-43.08)
Days of abstinence	75.00 (77.86-171.70)
Years of drug use	14.50 (13.89-20.44)
Number of hospitalizations (a.u.)	1.00 (0.47-1.84)
ASSIST score of preferred drug (a.u.)	29.00 (28.08-32.28)
Days per week of drug use	6-7
Individuals who used alcohol only (n)	11
Individuals who used crack only (n)	11
Individuals who used marijuana only (n)	1
Multiple drug users (n)	20

Abbreviations: ASSIST, Alcohol, Smoking and Substance Involvement Screening Test; SUD, substance use disorder; VO$_2$max, maximum consumption of oxygen.

The correction of artifacts was done first on the Polar Pro Trainer 5 software (Polar). The data were exported from the Polar device to the software, which automatically applies a filter using an interpolation algorithm. This algorithm substitutes the detected errors by interpolated intervals, which corresponds to the mean of the previous and next accepted interval values. Thereafter, the data were exported to the Kubios software,[61] and the cubic spline interpolation method was applied[61–63] with a medium level, because stronger levels of correction increase the number of false detections.

HRV measure was performed 5 minutes before the CPT, during the 2 minutes with the hand submerged in the container, and 8 minutes after ending the CPT (see Figure 1). HRV analyses were divided into 4 analysis windows of 30 seconds each during the 2 minutes of CPT. The same was done with the next 2 minutes to analyze the recovery condition. The following 6 minutes were divided into 3 windows of 2 minutes. As we aimed to predominantly observe the ANS during the CPT and during the moments right after the CPT, it was not necessary to divide everything into 30-second windows. However, a 6-minute analysis was a long interval to observe ANS behavior. For this reason, 2-minute windows were preferred for these last 6 minutes. Importantly, ultra-short-term HRV measurements have been conducted in several studies and are feasible to evaluate ANS.[64–66]

Depression, anxiety, and stress assessments

The psychometric DASS-21 scale[67] was adapted to Brazilian Portuguese.[68] This questionnaire has 21 questions divided into 3 domains, which assess depression, anxiety, and stress levels, and has a score ranging from 0% to 100% in each of the 3 domains. It is important to highlight that these emotional states are associated with chronic drug intake,[4,30,69] as well as to poor HRV.[52] We measured the level of these states to use them as co-variables, thereby controlling their effects on the ANS response.

Cardiorespiratory fitness tests

To verify the cardiorespiratory fitness level of SUD subjects, we administered the multistage 20-minute shuttle run (progressive effort task) created by Leger and Gadoury.[70] This test is widely used and it is a validated measure of the cardiorespiratory fitness level in adults.[70,71] The volunteer should move from one cone to another, with a distance of 20 m between the 2 cones. After reaching the cone, the subject must return to the first cone. A standard audiologic file previously recorded provides instructions and the running pace through a speaker. The VO$_2$max was estimated through a specific formula that uses age, gender, and maximum velocity achieved during the incremental test. This test has been validated in different populations and has been widely used in practical and laboratory settings.[70,72] The non-SUD group performed the Yo-Yo recovery test, which has a very similar pattern[73] to the shuttle run described above and is also validated as a VO$_2$max estimation.[74]

Statistical analysis

The Shapiro-Wilk test was used to verify data normality. Parametric data are described as mean ± SD and nonparametric data are described as median (confidence interval [CI]). For the comparison between SUD (N = 15) × non-SUD (N = 15), unpaired *t* tests or Mann-Whitney *U* tests were conducted to verify differences between groups in height, weight, VO$_2$max, and age. Furthermore, mean R-R interval and RMSSD indices were log-transformed and checked for normality after the log transformation. A 2-way repeated measures analysis of variance (ANOVA) was used to verify group (SUD × non-SUD) and time (before, during, and after the CPT) interactions.

In individuals with SUD (N = 43), Pearson or Spearman correlations were conducted to check associations between independent variables (VO$_2$max, Age, Depression Score, Stress Score, Days of Abstinence, Years of Drug Use, and Weight) and the log-transformed HRV indices before (5 minutes), during the CPT (4 windows of 30 seconds), during the short-term recovery (4 windows of 30 seconds), and during the long-term recovery (3 windows of 2 minutes). If correlations were found between these variables, regression analyses were administered using VO$_2$max as the independent variable and the RMSSD
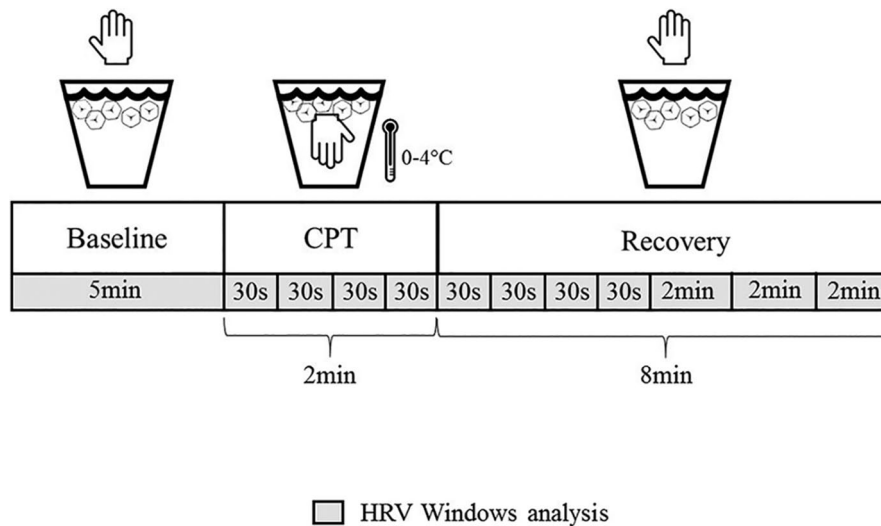
Figure 1. The CPT experiment and heart rate variability analysis before, during, and after the test. CPT indicates Cold Pressor Test; HRV, heart rate variability.

and mean R-R indices before, during and after the CPT as dependent variables controlled by possible co-variables. Nonparametric variables were log-transformed to match normality assumption. Assumptions of equality of variance, independence, linearity, and normality were verified. Multicolinearity was not observed throughout independent variables.

Results

SUD vs non–SUD

The groups presented no differences between their general characteristics, as described in Table 1. In the SUD sample, 5 were individuals who used crack only, 2 were individuals who used alcohol only, and 8 were multiple drug users (7 crack and alcohol, and 1 crack and marijuana). All 15 subjects reported using their preferred drug between 6 and 7 days a week during years of drug use. In Table 1, the data from the remaining 28 non-matched individuals with SUD are reported, thus permitting better data visualization across all 3 groups.

HRV responses before, during, and after the CPT (SUD × non–SUD)

Figure 2 (Panel A) shows the mean R-R interval behavior before, during, and after the CPT for the SUD group (N = 15) vs non-SUD group (N = 15). ANOVA showed a significant main group effect, $F_{(1,28)} = 4.29$, $P = .04$, $\eta_p^2 = .13$. Bonferroni post hoc test revealed that the non-SUD group compared with the SUD group had greater mean R-R interval at baseline (911.98 ± 122.85 ms vs 808.33 ± 95.10 ms, $P = .02$) and in the recovery condition at minutes 6 (933.92 ± 129.11 ms vs 817.66 ± 111.25 ms, $P = .01$), 8 (964.98 ± 150.41 ms vs 812.21 ± 107.97 ms, $P < .01$), and 10 (946.54 ± 149.20 ms vs 848.39 ± 100.11 ms, $P = .04$).

In addition, ANOVA showed a significant main effect of time, $F_{(11,308)} = 7.46$, $P < .01$, $\eta_p^2 = .21$, and interaction between group and time, $F_{(11,308)} = 2.03$, $P = .02$, $\eta_p^2 = .06$. Bonferroni post

hoc test showed that the mean R-R interval at baseline condition (911.98 ± 122.85 ms) was higher for the non-SUD group compared with the following conditions: 30 seconds (817.37 ± 91 ms, $P = .02$), 1 minute (820.56 ± 124.49 ms, $P = .02$), and 1:30 minutes (820.56 ± 124.49 ms, $P < .01$), as well as 2:30 minutes (840.36 ± 101.71 ms, $P < .01$) and the 8th minute in the recovery condition (964.98 ± 150.41 ms, $P < .01$). The 1st minute of CPT (817.33 ± 125.91 ms) was lower than the 6th (933.62 ± 150.41 ms, $P = .01$) and 8th (964.98 ± 150.41 ms, $P < .01$) minutes in the recovery condition. Furthermore, 1:30 minute of CPT (835.02 ± 102.93 ms) was lower than the 6th (933.62 ± 150.41 ms, $P < .01$) and 8th (964.98 ± 150.41 ms, $P < .01$) minutes in recovery condition. Finally, the 2nd minute of CPT was lower than the 8th minute (964.98 ± 150.41 ms, $P < .01$) in recovery condition. For the SUD group, Bonferroni post hoc analysis revealed that the 1st minute of CPT (770.18 ± 85.17 ms) was lower than 2:30 minutes (856.97 ± 99.83 ms, $P = .04$). Moreover, Figure 2 (Panel B) illustrates the responses of the RMSSD index before, during, and after the CPT. Analysis of variance showed no significant group effect, $F_{(1,28)} = 2.64$, $P = .11$, $\eta_p^2 = .08$; no significant time effect, $F_{(11,308)} = 3.99$, $P = .07$, $\eta_p^2 = .12$; and no interaction between group and time, $F_{(11,308)} = 1.45$, $P = .14$, $\eta_p^2 = .04$, on the RMSSD index.

General characteristics of individuals with SUD

As illustrated by Table 2, in the sample of individuals with SUD, N = 43, age: 34.0 ± 8.0 years, weight: 71.0 (67.3-73.5), height: 1.70 (1.67-1.71), we found that 11 were individuals who used alcohol only, 11 were individuals who used crack only, 1 was an individual who used marijuana only, and 20 were multiple drug users. The sample scored 29.00 (28.08-32.28) on the ASSIST questionnaire and had 1.00 (0.47-1.84) hospitalizations. Moreover, the subjects had 14.50 (13.89-20.44) years of drug use and practiced 75.00 (77.86-171.70) days of abstinence.
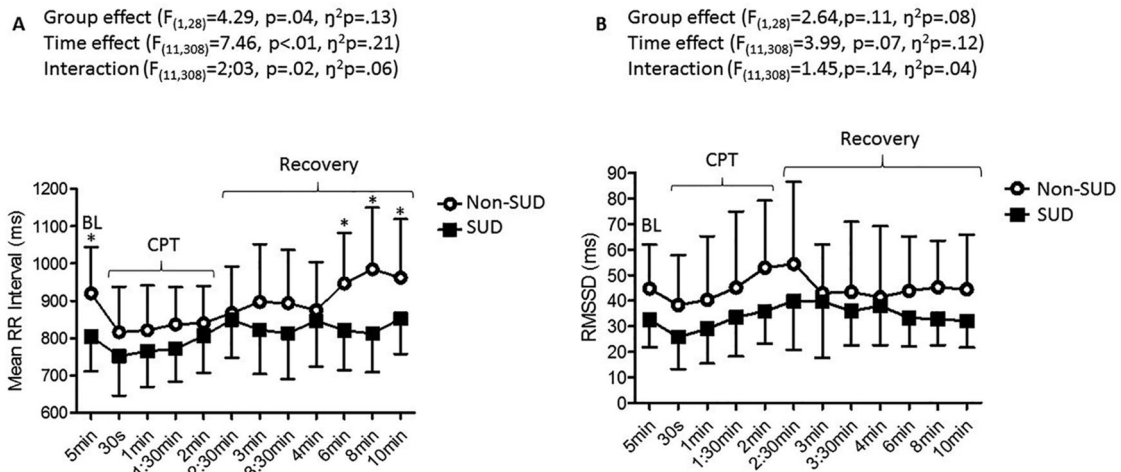
Figure 2. R-R interval and RMSSD of SUD (N = 15) and matched control (N = 15) subjects before, during, and after the CPT. Panel A: Mean R-R intervals before, during, and after the CPT of individuals with SUD and matched control healthy subjects. Panel B: RMSSD index before, during, and after the CPT of individuals with SUD and matched control healthy subjects. BL indicates baseline; CPT, Cold Pressor Test; RMSSD, root mean square of successive differences; SUD, substance use disorder. *Difference between groups.

Regression analyses results

Initially, correlations were tested between independent variables and HRV indices. No correlations were found between VO_2max and stress, depression anxiety, age, years of drug use, days of abstinence, and number of hospitalizations ($P > .05$). However, VO_2max was correlated with RMSSD index during resting condition, during the CPT (with exception of the first 30-second window), and during the short- and long-term recovery as shown in Table 3.

In addition, Table 3 shows that mean R-R intervals correlated with days of abstinence during short-term recovery at the fourth 30-second window of the short-term recovery ($r = 0.31$, $P = .04$) and at the first 30-second window of the long-term recovery ($r = 0.32$, $P = .03$). It was also revealed that mean R-R intervals negatively correlated with years of drug use at the second ($r = -0.35$, $P = .01$), third ($r = 0.41$, $P = .01$), and fourth ($r = -0.32$, $P = .02$) 30-second windows of the short-term recovery and at the first two 2-minute windows of the long-term recovery ($r = -.37$, $P = 0.02$, and $r = -0.32$, $P = .02$, respectively). Correlations between mean R-R intervals with VO_2max, age, depression, stress, anxiety, and number of hospitalizations were not found ($P > .05$).

Therefore, we conducted linear regression analyses using VO_2max as the independent variable and RMSSD before, during (with exception of the first 30-second window), and after the CPT as dependent variables. As Figure 3 demonstrates, it was found that greater VO_{2max} predicted greater RMSSD at baseline, $F_{(1,41)} = 6.86$, $P = .01$, $R^2 = 0.12$, $B = 0.013$; at CPT, second 30-second window: ($F_{(1,41)} = 4.66$, $P = .03$, $R^2 = 0.08$, $B = 0.012$), third 30-second window: ($F_{(1,41)} = 5.50$, $P = .02$, $R^2 = 0.09$, $B = 0.012$), and fourth 30-second window: ($F_{(1,41)} = 10.70$, $P < .01$, $R^2 = 0.18$, $B = 0.018$); at short-term recovery, first 30-second window: ($F_{(1,41)} = 7.29$, $P = .01$, $R^2 = 0.15$,

$B = 0.015$), second 30-second window: ($F_{(1,41)} = 6.48$, $P = .01$, $R^2 = 0.13$, $B = 0.014$), third 30-second window: ($F_{(1,41)} = 6.25$, $P = .01$, $R^2 = 0.13$, $B = 0.012$), and fourth 30-second window: ($F_{(1,41)} = 4.97$, $P = .03$, $R^2 = 0.10$, $B = 0.012$); and at long-term recovery, first 2-minute window: ($F_{(1,41)} = 7.54$, $P < .01$, $R^2 = 0.15$, $B = 0.012$), second 2-minute window: ($F_{(1,41)} = 10.34$, $P < .01$, $R^2 = 0.20$, $B = 0.015$), and third 2-minute window: ($F_{(1,41)} = 8.55$, $P < .01$, $R^2 = 0.17$, $B = 0.013$).

Thereafter, we conducted linear regression analyses using days of abstinence as the independent variable and mean R-R intervals (at the fourth CPT's 30-second window and at the first 30-second window of the short-term recovery) as dependent variables. We found that more days of abstinence predicted greater mean R-R intervals at the fourth 30-second window of the CPT, $F_{(1,41)} = 4.48$, $P = .04$, $R^2 = 0.10$, $B = 0.03$, and at the first 30-second window of the short-term recovery, $F_{(1,41)} = 4.59$, $P = .04$, $R^2 = 0.10$, $B = 0.02$.

Years of drug use also predicted mean R-R intervals at the short-term recovery (second 30-second window: $F_{(1,41)} = 5.69$, $P = .02$, $R^2 = 0.10$, $B = -0.05$; third 30-second window: $F_{(1,41)} = 8.41$, $P < .01$, $R^2 = 0.17$, $B = -0.07$; fourth 30-second window: $F_{(1,41)} = 4.69$, $P = .03$, $R^2 = 0.10$, $B = -0.05$) and at the long-term recovery (first 2-minute window: $F_{(1,41)} = 6.40$, $P = .01$, $R^2 = 0.13$, $B = -0.05$; second 2-minute window: $F_{(1,41)} = 4.64$, $P = .03$, $R^2 = 0.10$, $B = -0.04$).

Discussion

The main findings of the present study are that individuals with SUD had impaired cardiac autonomic responses before and after a stress test compared with healthy individuals. Also, greater fitness level predicted greater vagal activity (RMSSD index) before, during, and after the CPT. However, mean R-R intervals were not related to cardiorespiratory fitness and were

Table 3. Correlations between VO$_2$max, days of abstinence, and years of drug use with HRV indices (RMSSD and mean R-R intervals) before, during, and after the CPT.

		BASELINE	CPT				SHORT-TERM RECOVERY				LONG-TERM RECOVERY		
		5 MINUTES	30 SECONDS	30 SECONDS	30 SECONDS	30 SECONDS	30 SECONDS	30 SECONDS	30 SECONDS	30 SECONDS	2 MINUTES	2 MINUTES	2 MINUTES
RMSSD vs VO$_2$max	r	**0.37**	**0.26**	**0.32**	**0.34**	**0.45**	**0.38**	**0.37**	**0.36**	**0.32**	**0.39**	**0.44**	**0.41**
	P	**.01**	.08	**.03**	**.02**	**<.01**	**.01**	**.01**	**.01**	**.03**	**<.01***	**<.01***	**<.01***
RMSSD vs days in abstinence	r	0.05	−0.03	0.02	0.01	0.00	−0.02	0.00	0.01	0.06	0.16	0.00	0.03
	P	.73	.83	.89	.94	.99	.87	.98	.94	.67	.31	.99	.91
RMSSD vs years of drug use	r	−0.03	−0.06	−0.13	−0.16	−0.14	−0.11	0.02	−0.09	−0.01	0.16	0.07	0.03
	P	.80	.66	.40	.29	.34	.45	.88	.56	.95	.30	.64	.80
R-R vs VO$_2$max	r	0.17	0.04	0.29	0.23	0.28	0.14	0.01	0.04	0.19	0.09	0.14	0.12
	P	.27	.79	.06	.12	.06	.35	.92	.78	.20	.56	.36	.43
R-R vs days in abstinence	r	0.01	0.07	0.16	0.11	0.11	0.28	0.21	0.18	**0.31**	**0.32***	0.25	0.19
	P	.50	.64	.29	.47	.48	.06	.18	.23	**.04**	**.03**	.09	.22
R-R vs years of drug use	r	0.14	−0.26	−0.28	−0.12	−0.06	−0.23	**−0.35**	**−0.41**	**−0.32**	**−0.37**	**−0.32**	−0.02
	P	.37	.06	.46	.90	.82	.14	**.01**	**.01**	**.03**	**.02***	**.02***	.08

Abbreviations: CPT, Cold Pressor Test; HRV, heart rate variability; RMSSD, root mean square of successive differences; R-R, mean of R-R intervals; VO$_2$max, Maximum consumption of oxygen.
Bold values are sitnificant values (p<.05).

only predicted by years of drug use and days of abstinence during CPT's recovery.

It is well established that drug users have impaired ANS regulation.[5] Several studies have reported poorer ANS control in individuals with dependence on cocaine, alcohol, and marijuana in a resting condition.[10,25,75] This is in accordance with our results, as it was shown that the non-SUD group had a greater mean R-R interval during 5-minute rest before the CPT and in the final minutes of the recovery condition (Figure 2). This might be related to increases of sympathetic activity in the sinoatrial node promoted by drug use, thus decreasing vagal tone.[5] In addition, chronic drug use has been related to decreased activity in regions of the brain[76] that play a key role in ANS control, such as the prefrontal cortex.[77] When the prefrontal cortex is activated, it inhibits the amygdala, which may restrain sympathetic neurons and disinhibit vagal neurons on the brainstem, an area that projects axons to organs such as the heart, thereby increasing parasympathetic activity.[77] However, we did not find differences between SUD and non-SUD groups in the HRV index that represents parasympathetic activity (ie, RMSSD). Thus, our data support the argument that individuals with SUD have an impaired ANS balance as shown by the mean of R-R intervals at resting and recovery conditions (Figure 2). Future studies need to be conducted to better understand how sympathetic or parasympathetic autonomic regulation is specifically affected by drug misuse.

Despite literature being clear regarding the HRV in SUD during rest condition, no studies have reported the ANS response during a physical stress condition in individuals with SUD. This is an important issue, because higher levels of stress are closely associated with greater chances of relapse and SUD's progression.[33] Our results showed that the SUD group had poorer autonomic regulation responses after the stress condition compared with the control group. This might be due to the constant adrenergic activity and lower HRV caused by the drug use over the years. We speculate that the chronic sympathetic activation induced by drug use did not allow greater HRV oscillations.

Interestingly, we found that days of abstinence predicted greater R-R intervals during the last 30-second window of the short-term recovery and the first 30-second window of the long-term recovery. Previous findings support this relationship. For instance, individuals who used alcohol had their HRV measured at the first day of abstinence and again 5, 12, and 24 weeks later. It was found that HRV increased with prolonged abstinence of at least 6 months.[78] Similarly, individuals who used alcohol were shown to improve vagal activity, measured by the HF index, and heart rate during sleep after 4 months of abstinence.[79] In our results, days of abstinence predicted greater mean R-R intervals only at CPT's recovery, but not at baseline or during the stressor. Compared with the studies mentioned above, our sample was abstinent for a shorter period (median: 75 days). Moreover, while our participants were heterogeneous

regarding the type of drug use, most studies reported ANS recovery after abstinence in individuals who used alcohol only. Therefore, further research may focus on follow-up ANS modulation throughout the abstinence period, starting on the first day. This will help to better understand when the autonomic regulation will recover from drug misuse.

It is well known that ANS regulation is strongly associated with greater cardiorespiratory fitness.[80] Our study showed that greater cardiorespiratory capacity predicted greater parasympathetic activity before, during, and after a stressor in individuals with SUD (Figure 3). Despite cardiorespiratory being influenced by factors, such as body composition[81,82] and genetic profile,[83] physical exercise has arguably been shown to be an effective tool to improve cardiorespiratory fitness. Importantly, exercise improves ANS control by increasing parasympathetic activity, thus enhancing HRV.[37,84] One physiological explanation is that chronic exercise promotes angiotensin II reduction, with consequent disinhibition of cardiac vagal function and increased nitric oxide availability, leading to vasodilatation and decreased sympathetic activation.[85] Another possible explanation is that exercise is shown to improve prefrontal cortex oxygenation and activity,[41] an area that plays a key role in ANS functionality and vagal modulation.[77,86] Furthermore, aerobic exercise has shown to decrease negative emotional states. This might be related to producing molecules such as endorphins, endocannabinoid, and dopamine during exercise.[87] Therefore, we emphasize the greater contribution of physical exercise to parasympathetic tone while having a stressful condition.

Individuals with SUD usually have higher levels of stress.[30] It might be due to an abstinence crisis (period where the individual may develop anxiety, sleep disturbance, nausea, tremors, and sweating due to substance withdrawal) or social problems caused by chronic drug use.[28] A wide range of studies in humans provide evidence that trauma, adversity, and chronic stress alter the activity and structure of cerebral networks involved in controlling emotions, stress, reward, and higher cognitive or executive control functions,[30] which include inhibitory control, shifting attention, working memory, and decision making. Therefore, strategies that monitor stress levels might be interesting for rehabilitation in SUD patients. In addition, it has been suggested that alterations in HRV might serve as a candidate for prognostic biomarkers of treatment course, severity of illness, and relapse risk in SUD.[28] In this context, the CPT might be a simple tool not only to monitor HRV but also to check the ANS response to a stressor. It can also be a way to identify possible relapses during treatment. Moreover, in addition to the conventional treatment, physical exercise, which induces greater cardiorespiratory capacity, might be administered during rehabilitation process, because it has been shown to improve cardiorespiratory capacity, as well as brain areas, such as the prefrontal cortex, important for cognition, mental health, vagal tone maintenance,[42,88] and well-being.[89]
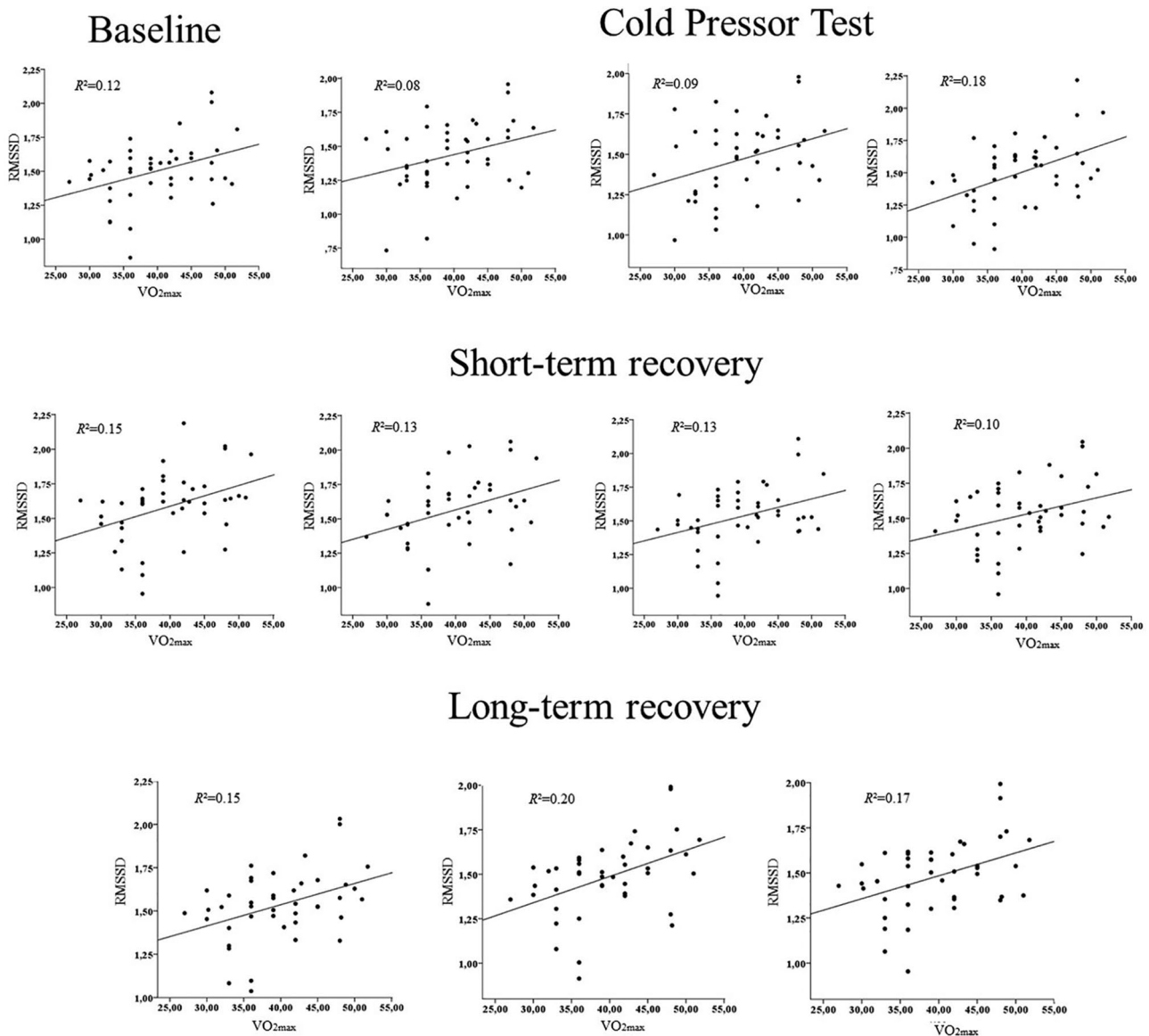
Figure 3. Linear regression plots of the association between cardiorespiratory capacity and vagal activity (RMSSD) of all individuals with SUD (N = 43) before, during, and after the CPT. CPT indicates Cold Pressor Test; RMSSD, root mean square of successive differences; SUD, substance use disorder.

It was also found that having more years of drug use significantly predicted lower mean R-R intervals during the recovery condition. Curiously, the amount of drugs ingested throughout the years did not predict lower mean R-R intervals during baseline and during the CPT. Thus, having baseline HRV in good standing does not mean that the patients have the ANS fully recovered. We suggest that the chronic effects of years of drug use on the autonomic modulation might be related to the difficulty to control addictive behaviors after a stressor stimulus. For example, studies have shown that vagal activity during alcohol cues predicts relapse in individuals who used alcohol[90] and that stress-induced smoking images decrease parasympathetic tone in nicotine users, as well as decrease the ability to resist smoking.[91] According to our data, a stressor is an important tool to identify alterations in the autonomic modulation. Therefore, the relationship between HRV, stress, and addiction seems to be important to monitor treatment and avoid relapse in patients with SUD.

We do acknowledge some limitations. The cardiorespiratory fitness tests used to estimate the VO₂max differ between groups. The Yo-Yo test was completed by the healthy group and the Leger test by the individuals with SUD. In fact, this issue may be a limitation of our results; however, the tests have similar methodological procedures,[73] and both are validated and reliable to predict VO₂max. In addition, both tests are simple to use in clinical settings and could be easily applied to monitor the cardiorespiratory fitness in individuals with SUD. Additionally, the tests involve a significant motivational component, and it is not possible to ensure that the individuals gave their maximal effort to complete the test. However, during the test, the evaluators provided strong verbal encouragement to all of the individuals to guarantee that they reached their maximum effort, and the tests used have shown a good reproducibility in laboratory-based tests of VO₂max.[70] Moreover, we have chosen to perform a physical stress test rather than a psychological one, as many other studies did previously. Therefore, future research could explore

psychological stress tests in this population while measuring HRV, to investigate ANS alterations caused by psychological stress. Furthermore, our sample was composed only by men. Despite physical exercise showing similar effects in HRV in men and women, the influence of CPT in female users of drug needs to be further explored. We also acknowledge that, despite emotional states of the healthy group not being reported, they did not have any neuropsychological concerns on the screening questionnaire. Finally, as any other cross-sectional study, our findings should be interpreted with caution. We recommend that future studies comparing the effects of different exercise programs should be performed to describe their potential benefits on cardiorespiratory fitness and ANS control in individuals with SUD under a stressful situation. The possibility to vary among different exercise activities, such as team sports, running, and resistance training, while improving ANS regulation can be important to increase motivational aspects and maintain adherence to exercise programs, thereby enhancing the benefits in the rehabilitation of individuals with SUD.

Conclusions

This is the first study to show that cardiac autonomic regulation is impaired in individuals with SUD exposed to a stressor, while cardiorespiratory fitness may attenuate those impairments by increasing vagal modulation. Thus, we suggest that regular endurance exercise, which has widely shown to increase cardiorespiratory fitness, should be included in drug-related rehabilitation programs because it might decrease the chances of individuals with SUD to relapse after experiencing stressful conditions. Also, the CPT is a simple method that may help to monitor cardiac autonomic regulation in response to a stress condition in individuals with SUD. Future clinical trials could focus on performing the CPT at the beginning of SUD treatment, and repeat it during the rehabilitation process to monitor the ANS responses and validate it as a tool to monitor autonomic recovery.

Author Contributions

DAC, KGC and EBF created the study design. DAC, VDT, HKF and MLR did data collection. DAC and KGC did data analyses. DAC wrote the manuscript and all authors significantly contributed to it. The final version was approved by EBF and submitted.

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