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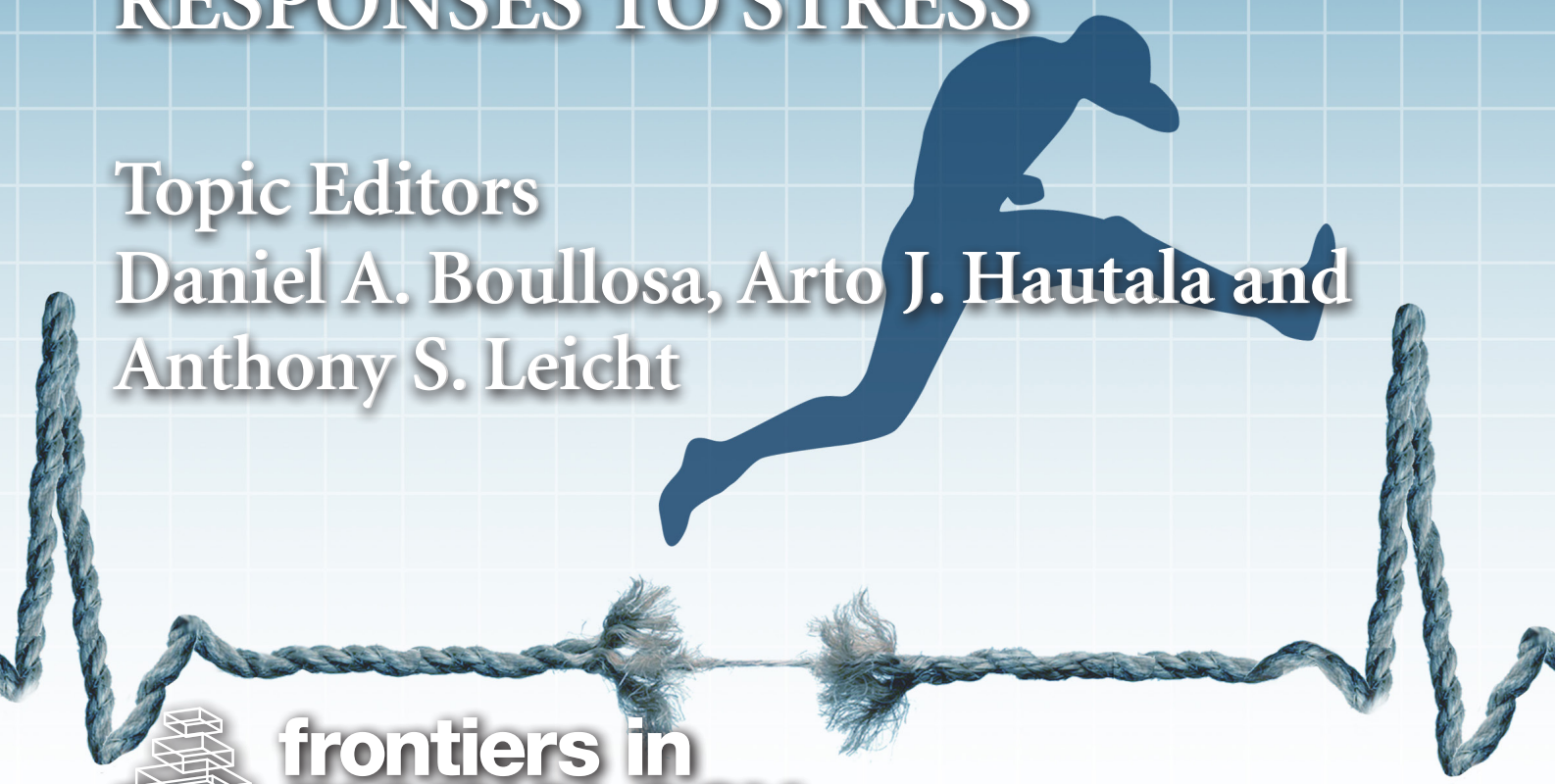
RESEARCH TOPICS

THE ROLE OF PHYSICAL FITNESS ON CARDIOVASCULAR RESPONSES TO STRESS

Topic Editors
Daniel A. Boullosa, Arto J. Hautala and Anthony S. Leicht



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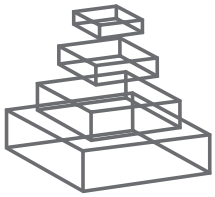

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THE ROLE OF PHYSICAL FITNESS ON CARDIOVASCULAR RESPONSES TO STRESS

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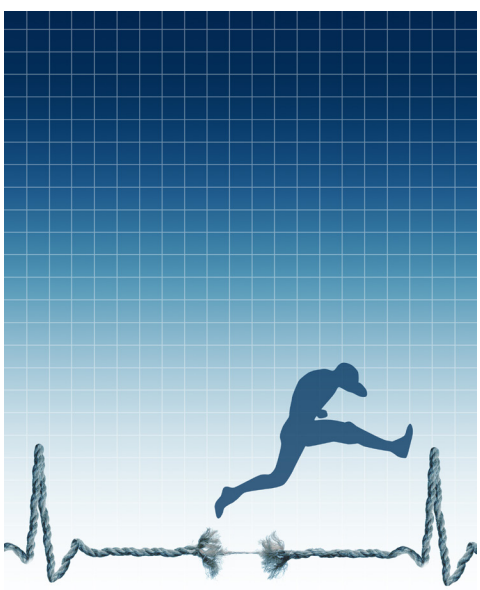

A greater fitness can help individuals to jump the hurdle of stress.
Image created by Pilar Boullosa.

Cardiovascular responses to physical and/or mental stressors has been a topic of great interest for some time. For example, significant changes of cardiovascular control and reactivity have been highlighted as important mechanisms for the protective effect of exercise as a simple and effective, non medical therapy for many pathologies. However, despite the great number of studies performed to date (e.g. >54,000 entries in Pubmed for "cardiovascular stress"), important questions of the role stress has on cardiovascular function still remain. For instance, What factors account for the different cardiovascular responses between mental and physical stressors? How do these different components of the cardiovascular system interact during stress? Which cardiovascular responses to stress are the most important for identifying normal, depressed, and enhanced cardiovascular function? Can these stress-induced responses assist with patient diagnosis and prognosis? What impact does physical fitness have on the relationship between cardiovascular function and health? The current topic examined our current understanding of cardiovascular responses to stress and the significant role that physical fitness has on these responses for improved function and health. Manuscripts focusing on heart rate variability (HRV), heart rate recovery, and other novel cardiovascular assessments were especially encouraged.

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EDITORIAL
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Introduction to the research topic: the role of physical fitness on cardiovascular responses to stress

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This e-book is the culmination of countless hours of meticulous work by global scientists. We would like to thank the researchers for their great contributions to this hot topic. The combination of these studies reflects the importance of the topic amongst researchers and practitioners and the wide interest from numerous laboratories around the world. The contributions include a variety of formats including five original investigations, three review articles, one opinion article and a hypothesis and theory article. Notably, these contributions included both human and animal models that encompassed a range of techniques from molecular mechanisms to real life interventions thus reinforcing the translational approach for the understanding of cardiovascular responses to stress.

The three review articles (Huang et al., 2013; Carnevali and Sgoifo, 2014; Tonello et al., 2014) provided a great insight into the current knowledge of cardiovascular stress and its relationship with physical activity (PA). The first review article by Huang et al. (2013) considered the big picture of the topic by combining the classical perspective–examining how different forms of induced cardiovascular stress can be attenuated in physically trained individuals, with the addition of the interrelationships between obesity, inflammation and oxidative stress with these stress responses and cardiovascular health. The subsequent review from Carnevali and Sgoifo (2014) involved animal studies that focused on a mechanistic approach with resting vagal tone leading to stress resilience, reducing the development of anxiety and depression, and the important role of PA to mediate these relationships. The mini-review from Tonello et al. (2014) extended the topic into one of the most important social stressors in modern life, the work environment. This review revealed that factors related to adverse working conditions such as excessive effort, effort-reward imbalance, over commitment, irregular shift work, and work stress were associated with reduced cardiac autonomic function. Importantly this review identified the need for further studies in this area with heart rate variability (HRV) recognized as an important tool for evaluating both work related stress including harmful physical inactivity, and adaptations to potentially important stress buffers as PA and enhanced physical fitness.

Following the review articles, two interesting papers addressed novel and important aspects for stress management therapies. The opinion article by Stults-Kolehmainen (2013) highlighted an important and frequently forgotten aspect: How does stress negatively influence PA levels in individuals with and without cardiovascular diseases? Additionally, Stults-Kolehmainen (2013) introduced the concept of "mindfulness" for reducing stress and subsequently enhancing patients PA levels. Subsequently, Demarzo et al. (2014) discussed potentially effective means to mediate the role of physical fitness on cardiovascular responses to stress using "mindfulness" interventions that may be applied to this new area of future research.

As mentioned previously, the variety of original research investigations included within this topic reinforced the importance of research translation. The cross-sectional study by Childs and de Wit (2014) reported a significant relationship between positive affect decline after a social stressor and regular exercise in healthy individuals despite no difference between exercisers and non-exercisers for post-stress heart rate, blood pressure and cortisol responses. This study effectively illustrated how regular PA enhances psychological health and strengthens emotional resilience to stressors. Likewise, the study of Hanson et al. (2013) provided additional support for the effectiveness of regular PA in attenuating physiological responses to social stressors. Interestingly, an antidepressant drug attenuated cardiovascular responses (i.e., HR and HRV) to stress only in irregular exercisers that exhibited a comparable stress-induced response similar to that of regular exercisers during placebo (Hanson et al., 2013). These two studies (Hanson et al., 2013; Childs and de Wit, 2014) highlighted the positive impact that regular PA has on mental health, especially for depressed patients, with further studies needed to elaborate on mechanisms, benefits, and potential new therapies.

Other important responses during different sources of stress were also included in this e-book. The study of Rauber et al. (2014) was the first to our knowledge that documented post-exercise hypotension (PEH) in children. This study (Rauber et al., 2014) reported an enhanced PEH after traditional games compared to active video game playing and watching TV,

and an attenuated blood pressure response during the cold pressor test following traditional games. The greater exercise intensity and metabolic demands of traditional games were highlighted as important factors for these responses with children's playing strategies fundamental for cardiovascular health.

The study of Franklin et al. (2014) documented the important contribution of other physiological responses during stress. These authors (Franklin et al., 2014) noted that endothelial function after resistance exercise (i.e., physical stressor) was impaired for obese women compared to lean women with endothelium independent-vasodilation correlated to body weight for all women. These findings provided important guidance for resistance exercise prescription of obese women to minimize cardiovascular disease risk.

Finally, the study of Sasse et al. (2013) presented a very novel hypothesis suggesting that exercise might facilitate adaptation to repeated stress via both hypothalamic-pituitary-adrenocortical axis and cardiovascular response habituation. In this study (Sasse et al., 2013), the brains of rats that performed voluntary exercise on a wheel and those that were sedentary were analyzed following control, acute and repeated noise exposures. These authors identified that unlike sedentary rats, exercising rats regulated corticotropin-releasing factor and brain derived neurotrophic factors across several brain regions. Subsequently, the hypothesis was supported with habituation to stress using exercise resulting in multiple system responses. Future studies may elaborate on the results of Sasse et al. (2013) to understand the response of different physiological systems and their interactions to stressors and applicable translation.

This e-book has taken the initial action to integrate current research findings to stimulate further discussion and research. We would like to thank the authors for their significant contributions and the many reviewers who critiqued and improved the overall topic. Future studies will clarify the importance of physical fitness, exercise and PA to regulate cardiovascular responses during stress and such benefits for cardiovascular health.

REFERENCES

Carnevali, L., and Sgoifo, A. (2014). Vagal modulation of resting heart rate in rats: the role of stress, psychosocial factors, and physical exercise. *Front. Physiol.* 5:118. doi: 10.3389/fphys.2014.00118

Childs, E., and de Wit, H. (2014). Regular exercise is associated with emotional resilience to acute stress in healthy adults. *Front. Physiol.* 5:161. doi: 10.3389/fphys.2014.00161

Demarzo, M. M. P., Montero-Marin, J., Stein, P. K., Cebolla, A., Provinciale, J. G., and García-Campayo, J. (2014). Mindfulness may both moderate and mediate the effect of physical fitness on cardiovascular responses to stress: a speculative hypothesis. *Front. Physiol.* 5:105. doi: 10.3389/fphys.2014.00105

Franklin, N. C., Ali, M., Goslawski, M., Wang, E., and Phillips, S. A. (2014). Reduced vasodilator function following acute resistance exercise in obese women. *Front. Physiol.* 5:253. doi: 10.3389/fphys.2014.00253

Hanson, C. S., Outhred, T., Brunoni, A. R., Malhi, G. S., and Kemp, A. H. (2013). The impact of escitalopram on vagally mediated cardiovascular function to stress and the moderating effects of vigorous physical activity: a randomized controlled treatment study in healthy participants. *Front. Physiol.* 4:259. doi: 10.3389/fphys.2013.00259

Huang, C.-J., Webb, H. E., Zourdos, M. C., and Acevedo, E. O. (2013). Cardiovascular reactivity, stress, and physical activity. *Front. Physiol.* 4:314. doi: 10.3389/fphys.2013.00314

Rauber, S. B., Boullosa, D. A., Carvalho, F. O., de Moraes, J. F. V. N., de Sousa, I. R. C., Simões, H. G., et al. (2014). Traditional games resulted in post-exercise hypotension and a lower cardiovascular response to the cold pressor test in healthy children. *Front. Physiol.* 5:235. doi: 10.3389/fphys.2014.00235

Sasse, S. K., Nyhuis, T. J., Masini, C. V., Day, H. E. W., and Campeau, S. (2013). Central gene expression changes associated with enhanced neuroendocrine and autonomic response habituation to repeated noise stress after voluntary wheel running in rats. *Front. Physiol.* 4:341. doi: 10.3389/fphys.2013.00341

Stults-Kolehmainen, M. A. (2013). The interplay between stress and physical activity in the prevention and treatment of cardiovascular disease. *Front. Physiol.* 4:346. doi: 10.3389/fphys.2013.00346

Tonello, L., Rodrigues, F. B., Souza, J. W. S., Campbell, C. S. G., Leicht, A. S., and Boullosa, D. A. (2014). The role of physical activity and heart rate variability for the control of work related stress. *Front. Physiol.* 5:67. doi: 10.3389/fphys.2014.00067

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Cardiovascular reactivity, stress, and physical activity

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Psychological stress has been proposed as a major contributor to the progression of cardiovascular disease (CVD). Acute mental stress can activate the sympathetic-adrenal-medullary (SAM) axis, eliciting the release of catecholamines (NE and EPI) resulting in the elevation of heart rate (HR) and blood pressure (BP). Combined stress (psychological and physical) can exacerbate these cardiovascular responses, which may partially contribute to the elevated risk of CVD and increased proportionate mortality risks experienced by some occupations (e.g., firefighting and law enforcement). Studies have supported the benefits of physical activity on physiological and psychological health, including the cardiovascular response to acute stress. Aerobically trained individuals exhibit lower sympathetic nervous system (e.g., HR) reactivity and enhanced cardiovascular efficiency (e.g., lower vascular reactivity and decreased recovery time) in response to physical and/or psychological stress. In addition, resistance training has been demonstrated to attenuate cardiovascular responses and improve mental health. This review will examine stress-induced cardiovascular reactivity and plausible explanations for how exercise training and physical fitness (aerobic and resistance exercise) can attenuate cardiovascular responses to stress. This enhanced functionality may facilitate a reduction in the incidence of stroke and myocardial infarction. Finally, this review will also address the interaction of obesity and physical activity on cardiovascular reactivity and CVD.

Keywords: psychological stress, obesity, physical activity, microvascular reactivity, inflammation, resistance exercise, oxidative stress, stress hormones

INTRODUCTION

Chronic psychological stress is a risk factor for cardiovascular disease (CVD). In addition, acute psychological stress is asssociated with factors that explain the development of atherosclerosis; endothelial dysfunction, inflammatory reactivity and oxidative stress. The American Psychological Association has provided evidence that 20% of Americans report extreme stress and 80% report that their stress levels have increased over the past year (APA, 2012). Additionally, over the past 5 years, 60% of Americans have attempted to reduce their stress, with just 7% reporting success in reducing stress (APA, 2012). Examinations of acute responses to psychological stress provide insight into the potential mechanisms that may explain the relationship of psychological stress to CVD. Greater understanding can also provide support for considering therapeutic alternatives that may alleviate the ill effects of stress.

This review will examine stress-induced cardiovascular reactivity and plausible explanations for how exercise training and physical fitness (aerobic and resistance exercise) can attenuate cardiovascular responses to stress. Important to our understanding of the development of CVD is how the benefits of physical activity in attenuating the cardiovascular stress response (enhanced functionality) may also support a reduction in the incidence of stroke and myocardial infarction. Finally, in light of the high prevalence of overweight and obesity (68.8% of US adults were categorized as overweight in 2008, and 35.7% were categorized as obese (Flegal et al., 2012) and support for the concept that obesity can be considered a chronic stressor marked by chronic inflammation, oxidative stress, and endothelial dysfunction, this review will also address the interaction of obesity and physical activity on cardiovascular reactivity and CVD (**Figure 1**).

SYMPATHOADRENAL RESPONSES TO ACUTE STRESS AND ADAPTATION TO PHYSICAL ACTIVITY

Initially, the neuroendocrine response to stress was believed to be attributable solely to the release of catecholamines from the adrenal medulla (Cannon and De La Paz, 1911; Cannon, 1914). Cannon and De La Paz (1911) research regarding sympathetic activation in response to threat or danger resulted in the introduction of the concept known as the "fight or flight" response. Furthering Cannon's work, Hans Selye (1936) conceptualized that factors such as heat or cold, forced immobilization or exercise, as well as chemical, biological, and psychological factors will elicit the exact same non-specific response of not only the catecholamines, but also corticosteroids (Cannon and De La Paz, 1911; Cannon, 1914; Selye, 1936, 1950, 1954, 1976).

The relationship between psychological stress and cardiovascular reactivity has long been suggested as an explanation for the association between psychological stress and CVD. It is known that chronic psychological stressors can lead to increased risk of arteriosclerosis, hypertension, and other metabolic disorders (Chrousos, 2000b, 2009; McCrone et al., 2001; Kyrou and Tsigos,

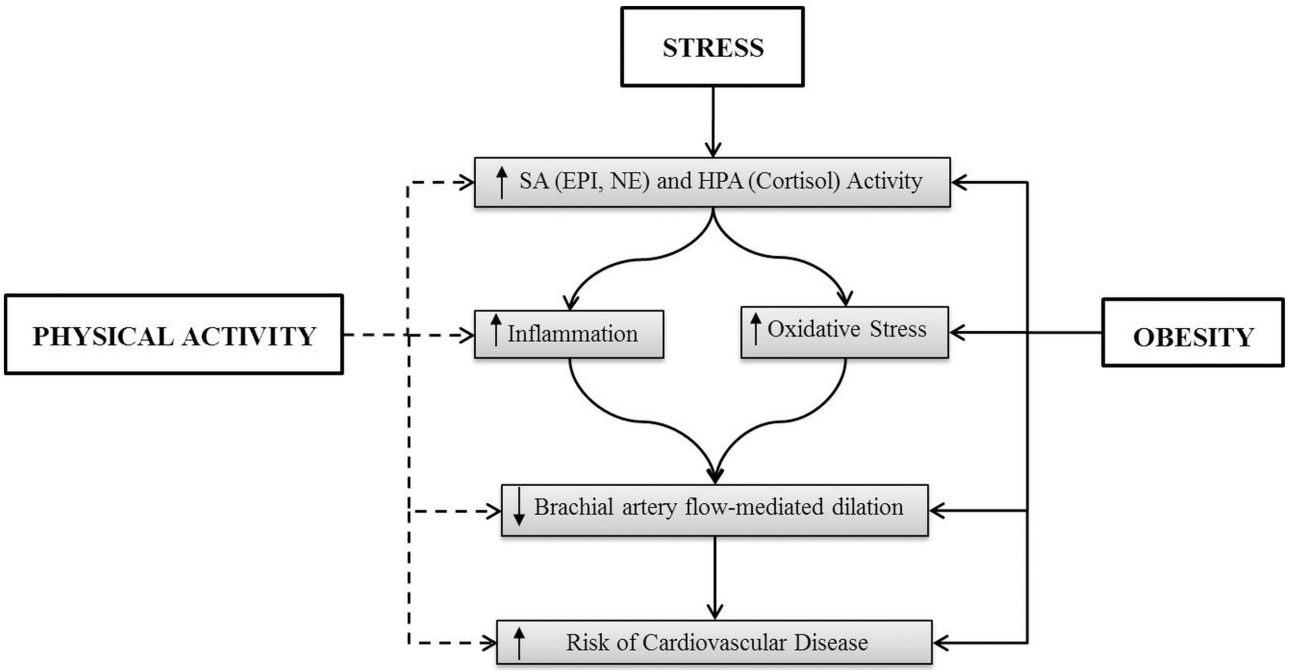

FIGURE 1 | The solid line indicates an activation of the indicated factor and the dashed line represents the suppression of the indicated factors. The effect of stress in obesity has not been delineated.

2009), while acute stressors result in acute increases in blood pressure, HR, and decreased metabolic efficiency (Crews and Landers, 1987; Hamer et al., 2006; Webb et al., 2011).

When an individual is psychologically or physiologically stressed in an acute manner, a complex chain of reactions occur, stemming from responses occurring within the sympathoadrenal (SA) and hypothalamic pituitary adrenal (HPA) axes, and the parasympathetic (PNS) and sympathetic nervous system (SNS) pathways in the body. Acute psychological stress has been shown to elicit increases in the secretion of epinephrine (EPI) and norepinephrine (NE) from the SA axis (Frankenhaeuser, 1991; Schoder et al., 2000; Gerra et al., 2001), and cortisol from the HPA axis (Frankenhaeuser, 1991; Chrousos, 2000a; Gerra et al., 2001). It has also been suggested that the increases in HR and BP response are due to a decrease in vagal tone attributed to the PNS as a result of rhythmic shifts in HR mediated by the brainstem medullary mechanisms through the vagus nerve (Hatfield et al., 1998; Spalding et al., 2000; Smeets, 2010) or an increase in afferent sympathetic neuromuscular activation (Kaufman and Hayes, 2002; Smith et al., 2006). Physical stressors, such as exercise, will elicit NE release in a curvilinear manner in response to increasing workloads, while EPI secretion increases at workloads above 60% of an individual's VO_{2max} (Hjemdahl, 1993; Rowell and Shepherd, 1996), and these increases are likely responsible for the concomitant increases in HR and BP.

While these same physiological mechanisms are experienced in response to a stressor of any type, the amount of reactivity and type of response experienced has been suggested to be impacted by multiple factors, including an individual's perception of control over a situation, the combination of multiple stressors, the level of cardiorespiratory fitness, obesity levels, and sex. The greater the amount of control an individual perceives they have over a situation results in lesser catecholamine, HR, and BP responses relative to a situation where an individual feel less control over a situation (Frankenhaeuser et al., 1976; Frankenhaeuser, 1991; Hinton et al., 1991).

While the majority of investigations of stress are often addressed from a unidirectional perspective, it has been suggested that psychological appraisals can interact with and cause alterations in peripheral physiological responses. The proposed transactional psychobiological model of cognitive appraisal during exercise suggests an individual's perception of the demands imposed by psychological and physical stressors is related to an individual's perceived ability to meet these demands Acevedo and Ekkekakis (2001). The combination of mental and physical stress has been shown to result in an exacerbated SA (Roth et al., 1990; Szabo et al., 1994; Rousselle et al., 1995; Acevedo et al., 2006; Huang et al., 2010a; Webb et al., 2010, 2008) and HPA (Webb et al., 2008, 2011, 2013) responses above that of a single stressor alone. Therefore, if pathological events are related to psychological and physical stress independently, then this combination of stressors, resulting in an exacerbated SA and HPA response, are likely be responsible for greater pathophysiological alterations in these systems.

Exercise is also believed to have an attenuating effect on an individual's reactivity level at resting levels, and research has shown this to generally be true (de Geus and van Doornen, 1993; de Geus et al., 1993; Porges, 1995; Sothmann et al., 1996; Schuler

and O'Brien, 1997), with individuals of higher fitness levels exhibiting a lesser HR response to psychological stress (Claytor, 1991; Boutcher and Nugent, 1993; Spalding et al., 2000), as well as an attenuated EPI and NE response (Boutcher and Nugent, 1993). It has also been shown that PNS responses increase after exercise and this response may assist in blunting responses that elicit increases in HR and BP (Porges, 1995; Dishman et al., 2002). Exercise may have be beneficial effects on HPA functioning because of lower stress-induced cortisol responses in physically fit compared with unfit subjects (Traustadóttir et al., 2005; Rimmele et al., 2009). However, the actual impact of cardiorespiratory fitness on cardiovascular reactivity is still a topic of debate as multiple meta-analyses and larger clinical studies investigating this topic have differed in their conclusions (Crews and Landers, 1987; Forcier et al., 2006; Hamer et al., 2006; Jackson and Dishman, 2006; Sloan et al., 2011; Alex et al., 2013), with the studies citing the differences in participant demographics, research methodologies, and measured variables adding to the difficulty in coming to definitive conclusion.

Similarly, studies investigating the relationship between adiposity and SA axis responses also lack a clear relationship, with inconsistent relationships found among obesity and catecholamine response (Macdonald, 1995), although there is support for an increase in SNS reactivity in obesity (Grassi et al., 1995; van Baak, 2001; Alvarez et al., 2002). Abdominal obesity has been associated with both exaggerated (Goldbacher et al., 2005; Steptoe and Wardle, 2005) and blunted (Hamer et al., 2007; Carroll et al., 2008; Phillips, 2011; Phillips et al., 2012) cardiovascular reactivity. Thus, these differences in findings may be related to a number of factors, including participant demographic differences and methodological issues. It can be suggested that generally, studies with appropriate statistical adjustments and stringent inclusion criteria have reported negative associations between cardiovascular reactivity and abdominal obesity (Laederach-Hofmann et al., 2000; Hamer et al., 2007; Phillips, 2011).

It is also interesting to note that while both males and females react to stress through the same psychophysiological pathways, they do so with markedly different results. Research demonstrates that males respond with greater diastolic blood pressure and total peripheral resistance changes during acute stressors (Stoney et al., 1987, 1988; Matthews and Stoney, 1988; McAdoo et al., 1990; Lai and Linden, 1992; Matthews et al., 1992; Allen et al., 1993), while females experience greater changes in HR during a psychologically stressful situation (Frankenhaeuser et al., 1976; McAdoo et al., 1990; Frankenhaeuser, 1991; Allen et al., 1993). In addition to cardiorespiratory differences, it has also been shown that when subjected to an acute psychological stress, males had a significantly greater rise in EPI levels in comparison to females', whose EPI rose slightly or not at all. A similar, but less pronounced sex difference was also found for NE, with males again registering a greater change in hormonal levels (Frankenhaeuser et al., 1978; Forsman and Lindblad, 1983; Lundberg, 1983). These differences have led to suggestions (Allen et al., 1993) that male and female responses to mental stress may be attributed to different mechanisms, with males being considered "vascular" reactors and females classified as "cardiac" reactors.

One mechanism that has been suggested to account for differences in male and female vascular response during psychological stress is a greater sensitivity in peripheral alpha- and beta-adrenergic receptors in women (Freedman et al., 1987; Girdler et al., 1990; Kajantie and Phillips, 2006). Another proposed mechanism that may account for the larger cardiac response in women may be a greater sensitivity and/or density of adrenergic receptors in the myocardium (Girdler et al., 1990; Kajantie and Phillips, 2006). Both hypotheses have been supported by research showing women respond with lower catecholamine secretions during an acute psychological stress when compared to males (Frankenhaeuser et al., 1978; Rauste-von Wright et al., 1981; Collins et al., 1985). It was also noted that differences in cortisol secretions during psychologically stressful tasks are negligible between males and females (Frankenhaeuser et al., 1978; Forsman and Lindblad, 1983; Lundberg, 1983), further suggesting that the SA axis may play a key role in reactivity differences among males and females during an acute psychological stress.

In addition, it should be noted that a number of studies have shown that men have higher BP levels than women through much of their lifetime regardless of race and ethnicity (Sandberg and Ji, 2012). Particularly, acute stress results in immediate increases in arterial blood pressure (Lutgendorf et al., 2000) to be a result of vasoconstriction prompted by enhanced SA activity (McCarty and Gold, 1996). Furthermore, chronic stress may lead to hypertension and other cardiovascular dysfunction as a result of disturbances in the SA axis and the nitric-oxide pathways (Alvarez et al., 2001; Stefano et al., 2001; Esch et al., 2002).

Interestingly, several studies demonstrate that cardiovascular responses during mental stress are better predictors of future hypertension (Wood et al., 1984; Matthews et al., 1993) than resting BP measurements. Furthermore, because an individual's BP levels respond to many factors, including daily activities and affect (Pickering, 1997), it has been hypothesized that cardiovascular responses during mental challenges may be better predictors of future resting BP levels than BP at rest. Further, it has been reported that BP levels during mental stress are more closely associated with left ventricular mass than are resting BP levels (Georgiades et al., 1996).

These acute responses to mental challenge can elicit increased cardiac output, increased systemic vascular resistance, and thus an elevation of arterial blood pressure. While sympathetic elevations that are transient in nature prepare the body for accommodating to possible physical demands that an individual may encounter, prolonged or frequently occurring elevations of the catecholamines can result in vasoconstriction in most systemic arteries and veins leading to allostatic alterations in cardiovascular responses (Gidron et al., 2002). These alterations provide conditions favorable for the development of hypertension, endothelial dysfunction, and may contribute to the development of arteriosclerosis (Chrousos and Gold, 1998; Gidron et al., 2002; Spieker et al., 2002).

Research has shown psychological stress may be attenuated by a number of factors including exercise (Crews and Landers, 1987; van Doornen and de Geus, 1989; de Geus et al., 1993; Sothmann et al., 1996; Schuler and O'Brien, 1997; Acevedo et al., 1999). Additionally, exercise has been shown to have immediate

psychological benefits comparative to those of other traditional therapeutic modalities and is a more effective anxiolytic agent than cognitive therapies when assessing anxiety (Petruzzello et al., 1991). This proposed explanation is also supported by Dienstbier's (1989) physiological toughness hypothesis, in that it is suggested that cognitively based stress reduction strategies only provide a short-term solution to anxiety reduction.

MICROVASCULAR REACTIVITY TO OXIDATIVE STRESS AND INFLAMMATION

Chronic stress has been demonstrated to be a determinant of CVD (Olinski et al., 2002). One of the earliest sub-clinical stages in the atherosclerotic process is an impairment of endothelium-dependent vasodilation, also known as endothelial dysfunction (Singhai, 2005). Acute mental stress is capable of altering physiological homeostasis such as microvascular reactivity (Huang et al., 2013; Szijgyarto et al., 2013). Laboratory-induced psychological stress has been demonstrated to induce transient endothelial dysfunction [impairment of brachial artery flow-mediated dilation (FMD)] (Ghiadoni et al., 2000; Szijgyarto et al., 2013). This stress-induced impaired FMD has been shown to be worsened in high-stress occupations (e.g., firefighting, law enforcement) and patients with stress disorders (e.g., depression) (Violanti et al., 2006; Mausbach et al., 2012; Wagner et al., 2012). Fahs et al. (2009) found increased aortic and carotid artery stiffness in firefighters. This observation has also been discovered following 3 h of firefighting activities (Fahs et al., 2011a,b). Furthermore, Joseph et al. (2010) showed a lower FMD in police officers compared to controls. This impaired FMD in police officers was associated with decreased carotid intima-media thickness, and 16 and 36% of these police officers ($N = 100$) met criteria for depression and reported posttraumatic stress disorder symptoms, respectively (Violanti et al., 2006).

A number of mechanisms have been shown to be involved in endothelial dysfunction that occurs as a result of acute mental stress. For example, Broadley et al. (2005) showed that acute mental stress-induced endothelial dysfunction (lower FMD) was prevented with an oral administration of metyrapone (an inhibitor of cortisol synthesis) in healthy individuals. Furthermore, plasma cortisol levels are higher at rest and in response to acute mental stress in firefighters and police officers compared to controls (Tomei et al., 2003; Rosati et al., 2011; Robinson et al., 2013). These reported elevations in cortisol levels have been associated with impaired FMD in police offices (Violanti et al., 2009). These findings suggest that the challenges of the HPA axis experienced by high-stress occupations may lead to an increased risk of CVD. Although the underlying mechanisms remain to be determined, elevated levels of oxidative stress and inflammation have also been implicated as contributing factors that link acute mental stress to endothelial dysfunction.

A mediator of endothelial dysfunction is shear stress (a dragging frictional force generated by blood flow in the vasculature), leading to oxidative stress (Bagi et al., 2003). Oxidative stress is an imbalance between antioxidants [e.g., nitric oxide (NO)] and reactive oxygen species (ROS) [superoxide ($O2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-)] (Sies, 1997). In healthy vascular cells, ROS is generated during the metabolism of oxygen, with the rate of ROS production being balanced by the rate of oxygen elimination (Vider et al., 2001). When ROS production is elevated, the process of cell damage occurs and can possibly facilitate the development of CVD (Ji et al., 2006). Furthermore, any increase in vascular shear stress from the cardiovascular alterations in response to a mental or physical perturbation can influence the balance of oxidative stress.

Research has previously shown that psychological stress may contribute to the development of atherosclerosis by eliciting an elevation in ROS, which can further induce oxidative DNA damage (Olinski et al., 2002). Subsequently, in a study on medical students, Sivonova et al. (2004) demonstrated greater nuclear DNA damage in lymphocytes on the day of an examination (stress condition) compared with during the time between two examination periods (non-stress condition). To further understand endothelial dysfunction by oxidative stress, Chung et al. (2010) recently examined the effects of immobilization stress (120 min/day for 14 days) in rats and found that arterial endothelial nitric oxide synthase (eNOS) mRNA and NO decreased and plasma malondialdehyde level (a marker of oxidative stress) increased along with decreased acetylcholine-induced relaxation of arteries as compared to controls. Interestingly, Huang et al. (2010a) examined oxidative stress response in healthy individuals who were exposed to acute dual challenge (physical and psychological stress). This study found that the dual challenge condition elicited greater plasma 8-isoprostane levels (a biomarker of oxidative stress) compared to exercise alone condition. This finding may provide the potential explanation that a combined physical and psychological stress often experienced chronically by occupational professionals (e.g., emergency responders, firefighters, and police officers) is a contributing factor to endothelial dysfunction, thereby increasing the risk of CVD.

In addition, vascular inflammation plays a critical role in endothelial dysfunction which can be induced by the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Ross, 1999; Esteve et al., 2007). Notably, a previous study has suggested that individuals who have a greater laboratory-induced stress response are more likely to experience higher stress in daily life (Wirtz et al., 2008), and this response is associated with increased risk for atherosclerosis (Kamarck et al., 1997). An increase in circulating TNF-a and IL-6 has been observed following acute mental stress (Steptoe et al., 2001; Heinz et al., 2003) via activation of nuclear factor-kappa B (Barnes and Karin, 1997; Bierhaus et al., 2003). Furthermore, Huang et al. (2010b) found a greater plasma IL-2 levels in firefighters who experienced a dual stress (physical and psychological stress) compared to exercise alone group. It is important to note that the magnitude and direction of inflammatory cytokine response to psychological stress vary and are dependent on the acute or chronic nature of the stimuli. For example, Kang and Fox (2001) examined chronic academic stress during examinations and found that decreased IL-2 [in both peripheral blood mononuclear cell (PBMC) and whole blood measures] and IFN-g (only PBMC) levels were observed, whereas an elevation was seen in IL-6 (in both PBMC and whole blood measures). In a recent study, Ramey et al. (2012) examined the inflammatory cytokine response in law enforcement officers and

found that the job demand (physical and psychological) assessed by the Job Content Questionnaire was positively associated with resting IL-1 beta and IL-6. This finding is further supported by Groer et al. (2010) who demonstrated a significant increase in salivary IL-6 in police officers following simulated workplace scenario (6 min of tracking a gunman through a building. It is important to note that ∼23% of law enforcement officers who reported metabolic syndrome were physically inactive (Yoo et al., 2009). In a cross-sectional study of 527 firefighters, Durand et al. (2011) demonstrated the CVD risk factors such as triglyceride and low density lipoprotein cholesterol levels are negatively associated with time and frequency of physical activity per week. These elevations in the mediators of endothelial dysfunction may be partially explained by physical inactivity. Thus, it is pivotal to understand how lifestyle changes such as exercise may help alleviate the potential for the negative health outcomes in these occupations.

Epidemiological evidence consistently shows that the benefits of physical activity and fitness on physiological and psychological health. One of the purported benefits associated with aerobic fitness is the attenuation of the cardiovascular response during psychological stress and recovery (Dienstbier, 1989; Sothmann et al., 1996; Spalding et al., 2004). For example, aerobically trained individuals have shown to attenuate reactivity and recovery of HR and blood pressure (Crews and Landers, 1987; McCubbin et al., 1992) and lower cortisol reactivity to acute mental stress (Webb et al., 2013). Furthermore, aerobic exercise training has been shown to defend against ROS-induced lipid peroxidation and to decrease the occurrence of ROS-associated diseases such as CVDs and Alzheimer disease (Mattson and Wan, 2005; Perry et al., 2005; Radak et al., 2005). A number of studies have shown that exercise training can enhance the adaptation of oxidative stress by increasing antioxidant defenses demonstrated by the up-regulation of antioxidant gene expressions such as superoxide dismutase and glutathione peroxidase (Powers and Lennon, 1999; Leeuwenburgh and Heinecke, 2001). Additionally, Nojima et al. (2008) found that an oxidative stress marker (urinary 8-OHdG level) decreased in patient with type 2 diabetes following a 12-month of aerobic exercise training. These findings suggest that regular exercise is beneficial in up-regulating the resistance against oxidative stress.

In addition, physical fitness has been shown to provide a more resilient immune defense and greater stress protection. For example, a reduction in pro-inflammatory levels (TNF-a and IL-6) was found in patients with coronary heart disease following aerobic exercise training (Goldhammer et al., 2005), and lower IL-6 concentrations have been observed in individuals who had self-reported higher physical activity levels (Pischon et al., 2003). In response to acute mental stress, physical fitness is associated with diminished pro-inflammatory cytokine responses (TNF-α and IL-6) (Hamer and Steptoe, 2007). While stress management alone has not demonstrated an improvement in FMD (Blumenthal et al., 2005), Dod et al. (2010) have shown an improvement of FMD along with decreased IL-6 levels in patients with coronary artery disease (CAD) following 12-weeks of lifestyle interventions including exercise and stress management. Therefore, the development of strategies, including exercise training, to address the negative consequences of chronic stress may help alleviate the elevated risk for CVD.

RESISTANCE EXERCISE: CARDIOVASCULAR REACTIVITY TO STRESS

Resistance exercise has long demonstrated positive adaptations in relation to skeletal muscle hypertrophy, muscular strength, and body composition (Wilmore, 1974). However, the effects of resistance training on cardiovascular reactivity and acute and long-term markers of stress are significant and perhaps overlooked. To appropriately understand these adaptations, in-depth insight must be pursued to examine the psychological effects of resistance exercise, and varying hormonal (cortisol, EPI, and NE) and hemodynamic responses (HR and BP). Additionally, knowledge related to the blood flow and inflammatory cytokine response, which may occur as a result of structured and periodized resistance training programs designed to elicit a desired training adaptation.

Pioneer research from Morgan (1969) has demonstrated a greater incidence of depression in unfit individuals compared to those who are more physically fit. Since then a plethora of data has illustrated the benefits of exercise to be associated with lower emotional distress (Steptoe and Butler, 1996) and decreased levels of depression (Steptoe et al., 1997). Additionally, longitudinal data from Paffenbarger et al. (1994) found physical activity to be inversely correlated with depression over the course of 25 years in a sample of over 10,000 men. The confounding variable, however, is that the research related to mental health has mostly examined aerobic exercise activity and is limited regarding resistance exercise. However, chronic resistance training has been shown to cause reductions in resting concentrations of the stress hormone cortisol (Hakkinen et al., 1988; Kraemer et al., 1998). This factor is of great importance as increased resting cortisol levels have been associated with impaired declarative memory in healthy adults (Kirschbaum et al., 1996). Additionally, resistance training may be an attractive strategy over the life span to not only attenuate the onset of sarcopenia (the loss of type II fibers with age), but to prevent elevations in cortisol, which have been related to memory impairments in elderly humans (Lupien et al., 1996) and to predict hippocampal dysfunction in an aging population (Lupien et al., 1998). Therefore, even though the prominent research related to exercise and mental stress focuses on endurance training, a physiological analysis of the benefits associated with resistance training suggests positive outcomes for mental stress when resistance training is included in an individual exercise routine.

Commonly, analyzed hormonal response in regards to resistance training protocols includes the anabolic hormone testosterone and the catabolic hormone cortisol, which are secreted from the HPA axis. Via the endocrine system, secretion of these hormones in response to training occurs to maintain homeostasis in the body (Galbo, 1983). Fluctuations in these hormones can occur acutely in response to exercise, or resting concentrations may be altered as an adaptation to a chronic training stress (Fry et al., 1994; Deschenes et al., 1998). As an acute response, it is typical to for significant increases in testosterone and cortisol to occur, however, smaller changes in resting levels over the long-term

may indicate a positive response to stress (Staron et al., 1994). Moreover, the literature has demonstrated that acute hormonal fluctuations are simply due to the metabolic stress response of physical activity and bear little to no impact on long-term muscle performance adaptations (Ahtiainen et al., 2005). Further, these acute changes may even lead to decreased stress and increased exercise performance over time (Hakkinen et al., 1988). Recently, investigators have demonstrated that an intense bout of resistance training (5 sets of 8 repetitions @75% of one repetitions maximum -1RM) significantly increases both the catabolic hormone cortisol and the anabolic hormone testosterone by a similar percentage (McCaulley et al., 2009). Additionally, the acute stress response seems to be augmented by total work performed or training volume (Sets X Repetitions X Weight Lifted). In response to long-term training, data has shown decreased cortisol levels to lead to greater levels of muscle performance (Staron et al., 1994; Kraemer et al., 1999). Furthermore, data exists demonstrating that long-term (2 years) resistance training may increase the testosterone to cortisol ratio (T/C ratio), which also results in an increase in muscular force development (Hakkinen et al., 1988). Therefore, a reduction in cortisol over time may be an important signal of resistance training adaptation and a decline in stress levels leading to increased exercise performance.

Another stress response to consider is the elevation and recovery paradigm of the neurohormones of the adrenal medulla: EPI and NE. A plethora of research has demonstrated that, in response to training, there are acute elevations in levels of EPI and NE similar to cortisol, signaling a stress response (Kraemer et al., 1987). This acute response shows elicited activation of the adrenal medulla, evidenced not only by increases of EPI and NE up to 15 min post-resistance training, but also a decrease in plasma peptide f (P-F) immediately following training (Bush et al., 1999). Data from Bush et al. (1999) also revealed that following the initial decrease of P-F, the hormone then reverses course and increases up to 4 h following a resistance exercise protocol of either 10 or 15 repetitions per set. This observation of elevated P-F for up to 240 min following exercise is likely demonstrating that the adrenal medulla serves a physiological function to maintain appropriate homeostasis. The prolonged increase in P-F is intriguing as other research has shown elevations only up to 15 min post-exercise (Kraemer et al., 1985a,b, 1990), but a prolonged increase may suggest that secretion of P-F from the adrenal medulla may aid in recovery via immune response. The relationship between P-F and immune response is plausible, as data has demonstrated P-F to enhance antibodies as a result of increased T-Cell function (Hiddinga et al., 1994). Ultimately, T-Cells then serve as a mediator for B-Cell stimulation and response capability (Hiddinga et al., 1994). Therefore, the prolonged increase in P-F may signal as a response to combat stress by serving as a signaling mechanism for enhanced immune system activity. Further, these authors reported that a group who performed 10 repetitions per set had greater force output and blood lactate than those who performed 15 repetitions per set, however, there was no difference in the acute hormonal stress response to exercise. This lack of difference is likely because total work was equated.

Further analysis of adrenal medulla secretions in response to resistance exercise reveals that in previously trained lifters EPI and NE seem to increase while P-F seems to decrease (Bush et al., 1999). Additionally, in trained weightlifters, there is a significant increase in P-F up to at least 240 min following exercise (Bush et al., 1999). This demonstrates an important training adaptation acting to increase a trainee's ability to combat stress. In other words, a trained lifter may fight the high physiological demands of resistance training with an increase in P-F to signal immune response leading to recovery. This training adaptation is of importance, as it is well established that the eccentric phase of resistance training leads to significant skeletal muscle myofiber damage and fatigue as evidenced by elevated plasma creatine kinase (CK) levels (Nosaka et al., 2001; Chen and Hsieh, 2005) and soreness (Nosaka et al., 2001). Concomitantly, as myofiber damage occurs an influx of neutrophils and blood monocyte secretion of IL-1 Beta into the muscle, this may last for up to 5 days (Cannon et al., 1989; Fielding et al., 1993). Therefore, elevated P-F for up to 4 h may augment the recovery process to attenuate the muscle damage response. Ultimately, total work seems to be the key factor to elicit neurohomronal response, however, training status may increase secretions of hormones from the adrenal medulla leading to an improved immune response and faster recovery.

Additionally, resistance training has frequently been used as a component of cardiac rehabilitation programs (Pollock et al., 2000) and has shown resistance training status to effect hemodynamic responses (i.e., HR and BP). It is well known that acute increases in HR and BP are significant during and following an intense bout of resistance training (Darr et al., 1988). While parasympathetic activity is responsible for the initial rise in HR during resistance training due to vagal withdrawal, it is the elevation in activity of the SNS during an intense strength training session, which is responsible for increasing HR. Interestingly, individuals with previous training experience have seen HR return to baseline levels sooner following exercise than those of a less-advanced training status (Darr et al., 1988). This response suggests that chronic adaptations to resistance training act to handle increasing levels of stress and cause a more rapid return of HR to baseline levels following an intense bout of resistance training.

Moreover, resistance training has been shown to be a safe mode of exercise in patients with CAD or congestive heart failure CHF (Karlsdottir et al., 2002), this supports the use of chronic resistance training as a means to decrease the risk of stroke and myocardial infarction. In support of this notion, Karlsdottir et al. (2002) reported that subjects with CAD and CHF increased HR and BP to similar levels as cycling at 90% ventilatory threshold when performing one set of 10 repetition at 60–70% of one-repetition maximum on either the biceps curl, leg press, or shoulder press exercises. Additionally, these authors reported the stability of left ventricular function with resistance exercise in cardiac patients. Finally, CAD and CHF patients show a similar pattern of hemodynamic responses following resistance training to that of healthy individuals. Therefore, including resistance training in a rehabilitation program for cardiac patients may be a key component and appropriate stress reducer designed to improve quality of life and increase muscle mass.

Recently, blood-flow restriction training (BFR) at rest and in conjunction with resistance exercise has become an increasingly attractive method in which to examine cardiovascular reactivity.

BFR training has been shown to increase muscle strength and hypertrophy by restricting blood flow proximal to the exercising muscle resulting in blood pooling and a restriction of venous return (Iida et al., 2007; Loenneke et al., 2012). It has been demonstrated that even without exercise, BFR training via application of a KAATSU device with a pressure of 200 mmHg has produced HR and BP responses similar to that of an orthostatic impetus (Iida et al., 2007). These intriguing results suggest that BFR may be a plausible method to be utilized as a countermeasure against orthostatic intolerance. Interestingly, recent data suggest that the introduction of BFR with resistance exercise has demonstrated greater HR and BP during exercise in young (30 years) and older (66 years) subjects (Vieira et al., 2013). However, in healthy individuals, BP and arterial compliance did not change following 6 weeks of full body resistance training in a low-intensity BFR resistance exercise group (20% of 1RM), nor did it change in moderate-intensity (70% of 1RM) or low-intensity (50% 1RM) resistance exercise groups (Fahs et al., 2011a,b). Therefore, individuals who have contraindications to performing high-intensity resistance training, such as cardiac patients, may be able to get resistance training benefits while not negatively altering arterial compliance by using blood flow restricted exercise.

In addition to the catabolic hormone cortisol discussed previously, the pro-inflammatory cytokine, IL-6, and anti-inflammatory cytokine, IL-10, can be important factors to alert of a stress response and subsequent fatigue and recovery. Because resistance exercise causes myofibrillar disruption and localized edema, an inflammatory response mediated by cytokines will occur (Izquierdo et al., 2009). It is therefore likely that the response of inflammatory cytokines in the presence of skeletal muscle damage serve as a mediator of satellite cell activation and myofiber repair to reduce stress and induce hypertrophic adaptations of the skeletal muscle (Izquierdo et al., 2009). Data has shown IL-6 to be elevated up to 48 h post-resistance training of 5 sets of 10 repetitions (Izquierdo et al., 2009). This elevation in IL-6 is consistent with research showing increased CK levels at 48 h following similar resistance protocols (Chen and Hsieh, 2005) and suggesting that IL-6 is directly correlated with the muscle damage response, further signifying the role of IL-6 in recovery and its importance in alleviating a stress response to training (Smith et al., 2000; Suzuki et al., 2002; Izquierdo et al., 2009).

The anti-inflammatory cytokine IL-10 has been increased by catecholamines (EPI and NE) following exercise (Peake et al., 2005). Further, the responsiveness of IL-10 seems to be augmented in a subsequent training bout 4 weeks later (Hirose et al., 2004). The greater response of IL-10 in a subsequent training bout may be viewed as an adaptation to stress as a result of decreased inflammation. The attenuated inflammatory response to training is a component of the repeated bout effect (RBE), which stipulates that when the same exercise (Nosaka et al., 2001) is performed in a subsequent resistance training bout, the muscle damage response is attenuated. Ultimately, IL-6 and IL-10 not only play important processes in recovery, but their blunted release secondary to chronic training can be a demonstration of the RBE and may signify a reduction in the stress response to resistance training.

Data has demonstrated the ability of resistance-trained individuals to improve adaptations to stress, including; lower cortisol levels (Hakkinen et al., 1988), a more rapid return of HR to baseline levels (Darr et al., 1988), and increases in P-F possibly leading to enhanced immune function (Bush et al., 1999). Moreover, a reduction in cortisol levels that is associated with chronic resistance training is likely an appropriate method to attenuate decreasing hippocampal function with aging. Additionally, resistance training seems to be a safe method for patients with CAF and CAD (Karlsdottir et al., 2002) and its benefits may decrease the risk of CVD. Ultimately, for an individual to reduce chronic stress it seems that resistance training is a key component to optimize this goal.

FUTURE RESEARCH DIRECTIONS: ENDOTHELIAL FUNCTION TO STRESS IN OBESITY

Obesity is associated with job-associated stress (tension and anxiety) and depression, and these stress related disorders have been found to lead to an increased risk of CVD and mortality (Nishitani and Sakakibara, 2006; Valtonen et al., 2012). Recent studies have reported ∼77% overweight and obesity rates in high-stress professionals such as young emergency responders (firefighters and ambulance recruits), police officers, and military personnel (Franke et al., 2002; Hsu et al., 2007; Tsismenakis et al., 2009; Ramey et al., 2009). Importantly, obesity has been shown to disturb cardiovascular responsivity to acute mental stress (Hamer et al., 2007), which may associate with stress-related endothelial dysfunction. Furthermore, in response to acute mental stress, Ghiadoni et al. (2000) have also shown that diabetic patients have a lower FMD compared to the control subjects. In addition, Martin et al. (2013) found that the index of adiposity (e.g., BMI, waist circumference, and waist-to-hip ratio) were negatively correlated with measures of vascular function such as hyperemic velocity time integral and hyperemic shear stress in a total of 1462 male firefighters. Thus, understanding the link between obesity and psychological stress may provide a critical contribution in determining the pathopysiological mechanisms of CVD development.

Although there is limited information investigating the impact of psychological stress on the oxidative stress in obese populations, one mediator of obesity-induced endothelial dysfunction may be the level of oxidative stress (Timimi et al., 1998; Schäfer and Bauersachs, 2008). Flint et al. (2007) has demonstrated that cortisol, norepinephrine, and epinephrine released during psychological stress can induce DNA damage within 10 min. A study by Epel et al. (2000) showed that elevated cortisol responses to acute psychological stress are associated with increased waist-to-hip ratio. This increase in oxidative stress during psychological stress could possibly be the negative effect of high circulating levels of stress hormones (e.g., cortisol).

Another potential mechanism to explain stress-induced endothelial dysfunction in obesity is the interactions of leptin with oxidative stress and inflammation. Leptin, an adipocyte-derived hormone, plays an important role in metabolism, adiposity, and vascular inflammation, and has been implicated in the development of coronary heart disease (Wannamethee et al., 2007). *In vitro* stimulation of cultured human endothelial cells with leptin has induced an increased accumulation of ROS and levels of pro-inflammatory mediator (e.g., monocyte

chemotactic protein-1) via activation of nuclear factor-kappa B (Bouloumie et al., 1999). Interestingly, recent research has shown that people who undergo acute psychological stress demonstrate increases in leptin levels, and these increases are positively correlated with waist circumference (Otsuka et al., 2006; Brydon et al., 2008). Brydon et al. (2008) also showed that a positive correlation between basal circulating leptin and IL-6 responses in response to mental stress. Taken together, these findings suggest that stress-induced leptin may partially contribute to the development of endothelial dysfunction. However, although a number of investigators have expressed interest in examining the mechanisms underlying psychological stress-induced endothelial dysfunction, the possible interaction (additive or synergistic) of obesity and psychological stress on the development of endothelial dysfunction are still not fully understood. Future investigation should attempt to expand the understanding of the mechanisms contributing endothelial dysfunction that links between obesity, psychological stress and CVD.

CONCLUSIONS

The studies that have been conducted examining the effect of physical activity on cardiovascular reactivity and CVD generally support Dienstbier's physiological toughness hypothesis (1989). More specifically, physical activity can not only reduce the immediate effects of stress but can also enhance the recovery from stressors (Crews and Landers, 1987; van Doornen and de Geus, 1989; de Geus et al., 1993; Sothmann et al., 1996; Schuler and O'Brien, 1997; Acevedo et al., 1999). Additionally, exercise has been shown to have immediate psychological benefits relative to other therapeutic treatments and can likely serve as a very effective adjuvant therapy (Petruzzello et al., 1991).

Furthermore, aerobic exercise training has been shown to defend against ROS-induced lipid peroxidation and to decrease the occurrence of ROS-associated diseases such as CVDs and Alzheimer disease (Mattson and Wan, 2005; Perry et al., 2005; Radak et al., 2005). These findings suggest that regular exercise is beneficial in up-regulating the resistance against oxidative stress. The immune system has also demonstrated positive adaptations to physical activity. Physical fitness has been shown to elicit a more resilient immune defense and greater stress protection. In response to acute mental stress, physical fitness is associated with diminished pro-inflammatory cytokine responses (TNF-α and IL-6) (Hamer and Steptoe, 2007). This effect is not only seen in aerobic activity, but also with resistance exercise. In particular, the attenuated inflammatory response to training is a component of the repeated bout effect, which stipulates that when the same exercise (Nosaka et al., 2001) is performed in a subsequent resistance training bout, the muscle damage response is attenuated. Ultimately, IL-6 and IL-10 are important in recovery, and their blunted release following chronic training can be a demonstration of the repeated bout effect that signifies a reduction in the stress response to resistance training. Further investigation into the benefits of resistance exercise and stress is warranted, although initial reports are promising and parallel the benefits of aerobic exercise.

Finally, the interaction of obesity and psychological stress on the development of CVD is not fully understood. Future examination of the mechanisms contributing to endothelial dysfunction and the links between obesity, psychological stress and CVD will undoubtedly lead to the development of more specific and effective strategies, such as physical activity training, to address the ill effects of stress on CVD.

REFERENCES

Acevedo, E., and Ekkekakis, P. (2001). The transactional psychobiological nature of cognitive appraisal during exercise in environmentally stressful conditions. *Psychology of Sport and Exercise* 2, 47–67. doi: 10.1016/S1469-0292(00)00013-3

Acevedo, E. O., Dzewaltowski, D. A., Kubitz, K. A., and Kraemer, R. R. (1999). Effects of a proposed challenge on effort sense and cardiorespiratory responses during exercise. *Med. Sci. Sports Exerc.* 31, 1460–1465. doi: 10.1097/00005768-199910000-00016

Acevedo, E. O., Webb, H. E., Weldy, M. L., Fabianke, E. C., Orndorff, G. R., and Starks, M. A. (2006). Cardiorespiratory responses of Hi Fit and Low Fit subjects to mental challenge during exercise. *Int. J. Sports Med.* 27, 1013–1022. doi: 10.1055/s-2006-923902

Ahtiainen, J. P., Pakarinen, A., Alen, M., Kraemer, W. J., and Hakkinen, K. (2005). Short vs. long rest period between the sets in hypertrophic resistance training: influence on muscle strength, size, and hormonal adaptations in trained men. *J. Strength Cond. Res.* 19, 572–582. doi: 10.1519/00124278-200508000-00015

Alex, C., Lindgren, M., Shapiro, P. A., McKinley, P. S., Brondolo, E. N., Myers, M. M., et al. (2013). Aerobic exercise and strength training effects on cardiovascular sympathetic function in healthy adults: a randomized controlled trial. *Psychosom. Med.* 75, 375–381. doi: 10.1097/PSY.0b013e3182906810

Allen, M. T., Stoney, C. M., Owens, J. F., and Matthews, K. A. (1993). Hemodynamic adjustments to laboratory stress: the influence of gender and personality. *Psychosom. Med.* 55, 505–517.

Alvarez, A., Piqueras, L., Bello, R., Canet, A., Moreno, L., Kubes, P., et al. (2001). Angiotensin II is involved in nitric oxide synthase and cyclooxygenase inhibition-induced leukocyte-endothelial cell interactions *in vivo*. *Br. J. Pharmacol.* 132, 677–684. doi: 10.1038/sj.bjp.0703867

Alvarez, G. E., Beske, S. D., Ballard, T. P., and Davy, K. P. (2002). Sympathetic neural activation in visceral obesity. *Circulation* 106, 2533–2536. doi: 10.1161/01.CIR.0000041244.79165.25

American Psychological Association. (2013). *Stress in America Press Room.* Available online at: http://www.apa.org/news/press/releases/stress/

Bagi, Z., Cseko, C., Toth, E., and Koller, A. (2003). Oxidative stress-induced dysregulation of arteriolar wall shear stress and blood pressure in hyperhomocysteinemia is prevented by chronic vitamin C treatment. *Am. J. Physiol. Heart Circ. Physiol.* 285, H2277–H2283.

Barnes, P. J., and Karin, M. (1997). Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N. Eng. J. Med.* 226, 1066–1071.

Bierhaus, A., Wolf, J., Andrassy, M., Rohleder, N., Humpert, P. M., Petrov, D., et al. (2003). A mechanism converting psychosocial stress into mononuclear cell activation. *Proc. Natl. Acad. Sci. U.S.A.* 100, 1920–1925. doi: 10.1073/pnas.0438019100

Blumenthal, J. A., Sherwood, A., Babyak, M. A., Watkins, L. L., Waugh, R., Georgiades, A., et al. (2005). Effects of exercise and stress management training on markers of cardiovascular risk in patients with ischemic heart disease: a randomized controlled trial. *JAMA* 293, 1626–1634. doi: 10.1001/jama.293.13.1626

Bouloumie, A., Marumo, T., Lafontan, M., and Busse, R. (1999). Leptin induces oxidative stress in human endothelial cells. *FASEB J.* 13, 1231–1238.

Boutcher, S. H., and Nugent, F. W. (1993). Cardiac response of trained and untrained males to a repeated psychological stressor. *Behav. Med.* 19, 21–27. doi: 10.1080/08964289.1993.9937561

Broadley, A. J., Korszun, A., Abdelaal, E., Moskvina, V., Jones, C. J., Nash, G. B., et al. (2005). Inhibition of cortisol production with metyrapone prevents mental stress-induced endothelial dysfunction and baroreflex impairment. *J. Am. Col. Cardiol.* 46, 344–350. doi: 10.1016/j.jacc.2005.03.068

Brydon, L., Wright, C. E., O'Donnell, K., Zachary, I., Wardle, J., and Steptoe, A. (2008). Stress-induced cytokine responses and central adiposity in young women. *Int. J. Obes. (Lond)* 32, 443–450. doi: 10.1038/sj.ijo.0803767

Bush, J. A., Kraemer, W. J., Mastro, A. M., Triplett-McBride, N. T., Volek, J. S., Putukian, M., et al. (1999). Exercise and recovery responses of adrenal medullary neurohormones to heavy resistance exercise. *Med. Sci. Sports Exerc.* 31, 554–559. doi: 10.1097/00005768-199904000-00010

Cannon, J. G., Fielding, R. A., Fiatarone, M. A., Orencole, S. F., Dinarello, C. A., and Evans, W. J. (1989). Increased interleukin 1 beta in human skeletal muscle after exercise. *Am. J. Physiol.* 257, R451–R455.

Cannon, W. (1914). The emergency function of the adrenal medulla in pain and the major emotions. *Am. J. Physiol. Legacy Content*. 33, 356–372.

Cannon, W., and De La Paz, D. (1911). Emotional stimulation of adrenal secretion. *J. Am. Med. Assoc*. 28, 64–70.

Carroll, D., Phillips, A. C., and Der, G. (2008). Body mass index, abdominal adiposity, obesity, and cardiovascular reactions to psychological stress in a large community sample. *Psychosom. Med*. 70, 653–660. doi: 10.1097/PSY.0b013e31817b9382

Chen, T. C., and Hsieh, S. S. (2005). Effects of 7−Day eccentric training period on muscle damage and inflammation. *Med. Sci. Sports Exerc*. 33, 1732–1738. doi: 10.1097/00005768-200110000-00018

Chrousos, G. P. (2000a). The HPA axis and the stress response. *Endocr. Res*. 26, 513–514. doi: 10.3109/07435800009048562

Chrousos, G. P. (2000b). The role of stress and the hypothalamic-pituitary-adrenal axis in the pathogenesis of the metabolic syndrome: neuro-endocrine and target tissue-related causes. *Int. J. Obes. Relat. Metab. Disord*. 24(Suppl. 2), S50–S55. doi: 10.1038/sj.ijo.0801278

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Endocrinology* 5, 374–381. doi: 10.1038/nrendo.2009.106

Chrousos, G. P., and Gold, P. W. (1998). A healthy body in a healthy mind—and vice versa—the damaging power of "uncontrollable" stress. *J. Clin. Endocrinol. Metab.* 83, 1842–1845. doi: 10.1210/jc.83.6.1842

Chung, I. M., Kim, Y. M., Yoo, M. H., Shin, M. K., Kim, C. K., and Suh, S. H. (2010). Immobilization stress induces endothelial dysfunction by oxidative stress via the activation of the angiotensin II/its type I receptor pathway. *Atherosclerosis* 213, 109–114. doi: 10.1016/j.atherosclerosis.2010.08.052

Claytor, R. P. (1991). Stress reactivity: hemodynamic adjustments in trained and untrained humans. *Med. Sci. Sports Exerc*. 23, 873–881. doi: 10.1249/00005768-199107000-00017

Collins, A., Eneroth, P., and Landgren, B. M. (1985). Psychoneuroendocrine stress responses and mood as related to the menstrual cycle. *Psychosom. Med*. 47, 512–527.

Crews, D. J., and Landers, D. M. (1987). A meta-analytic review of aerobic fitness and reactivity to psychosocial stressors. *Med. Sci. Sports Exerc*. 9, S114–120.

Darr, K. C., Bassett, D. R., Morgan, B. J., and Thomas, D. P. (1988). Effects of age and training status on heart rate recovery after peak exercise. *Am. J. Physiol*. 254, H340–H343.

de Geus, E. J., and van Doornen, L. J. (1993). The effects of fitness training on the physiological stress response. *Work Stress* 7, 141–159. doi: 10.1080/02678379308257057

de Geus, E. J., van Doornen, L. J., and Orlebeke, J. F. (1993). Regular exercise and aerobic fitness in relation to psychological make-up and physiological stress reactivity. *Psychosom. Med.* 55, 347–363.

Deschenes, M. R., Kraemer, W. J., Bush, J. A., Doughty, T. A., Kim, D., Mullen, K. M., et al. (1998). Biorhythmic influences on functional capacity of human muscle and physiological responses. *Med. Sci. Sports Exerc.* 30, 1399–1407. doi: 10.1097/00005768-199809000-00008

Dienstbier, R. A. (1989). Arousal and physiological toughness: implications for mental and physical health. *Psychol. Rev*. 96, 84–100. doi: 10.1037/0033-295X.96.1.84

Dishman, R. K., Jackson, E. M., and Nakamura, Y. (2002). Influence of fitness and gender on blood pressure responses during active or passive stress. *Psychophysiology* 39, 568–576. doi: 10.1111/1469-8986.3950568

Dod, H. S., Bhardwaj, R., Sajja, V., Weidner, G., Hobbs, G. R., Konat, G. W., et al. (2010). Effect of intensive lifestyle changes on endothelial function and on inflammatory markers of atherosclerosis. *Am. J. Cardiol*. 105, 362–367. doi: 10.1016/j.amjcard.2009.09.038

Durand, G., Tsismenakis, A. J., Jahnke, S. A., Baur, D. M., Christophi, C. A., and Kales, S. N. (2011). Firefighters' physical activity: relation to fitness and cardiovascular disease risk. *Med. Sci. Sports Exerc.* 43, 1752–1759. doi: 10.1249/MSS.0b013e318215cf25

Epel, E. S., McEwen, B., Seeman, T., Matthews, K., Castellazzo, G., Brownell, K. D., et al. (2000). Stress and body shape: stress-induced cortisol secretion is consistently greater among women with central fat. *Psychosom. Med*. 62, 623–632.

Esch, T., Stefano, G. B., Fricchione, G. L., and Benson, H. (2002). Stress in cardiovascular diseases. *Med. Sci. Monit*. 8, RA93–RA101.

Esteve, E., Castro, A., López-Bermejo, A., Vendrell, J., Ricart, W., and Fernández-Real, J. M. (2007). Serum interleukin-6 correlates with endothelial dysfunction in healthy men independently of insulin sensitivity. *Diabetes Care* 30, 939–945. doi: 10.2337/dc06-1793

Fahs, C. A., Rossow, L. M., Seo, D.-I., Loenneke, J. P., Sherk, V. D., Kim, E., et al. (2011a). Effects of different types of resistance exercise on arterial compliance and calf blood flow. *Eur. J. Appl. Physiol.* 111, 2969–2975. doi: 10.1007/s00421-011-1927-y

Fahs, C. A., Yan, H., Ranadive, S., Rossow, L. M., Agiovlasitis, S., Echols, G., et al. (2011b). Acute effects of firefighting on arterial stiffness and blood flow. *Vasc. Med*. 16, 113–118. doi: 10.1177/1358863X11404940

Fahs, C. A., Smith, D. L., Horn, G. P., Agiovlasitis, S., Rossow, L. M., Echols, G., et al. (2009). Impact of excess body weight on arterial structure, function, and blood pressure in firefighters. *Am. J. Cardiol*. 104, 1441–1445. doi: 10.1016/j.amjcard.2009.07.009

Fielding, R. A., Manfredi, T. J., Ding, W., Fiatarone, M. A., Evans, W. J., and Cannon, J. G. (1993). Acute phase response in exercise. III. neutrophil and IL-1 beta accumulation in skeletal muscle. *Am. J. Physiol*. 265, R166–R172.

Flegal, K. M., Carroll, M. D., Kit, B. K., and Ogden, C. L. (2012). Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA* 307, 491–497. doi: 10.1001/jama.2012.39

Flint, M. S., Baum, A., Chambers, W. H., and Jenkins, F. J. (2007). Induction of DNA damage, alteration of DNA repair and transcriptional activation by stress hormones. *Psychoneuroendocrinology* 2, 470–479. doi: 10.1016/j.psyneuen.2007.02.013

Forcier, K., Stroud, L. R., Papandonatos, G. D., Hitsman, B., Reiches, M., Krishnamoorthy, J., et al. (2006). Links between physical fitness and cardiovascular reactivity and recovery to psychological stressors: a meta-analysis. *Health Psychol*. 25, 723–739. doi: 10.1037/0278-6133.25.6.723

Forsman, L., and Lindblad, L. E. (1983). Effect of mental stress on baroreceptor-mediated changes in blood pressure and heart rate and on plasma catecholamines and subjective responses in healthy men and women. *Psychosom. Med*. 45, 435–445.

Franke, W. D., Ramey, S. L., and Shelley, M. C. 2nd. (2002). Relationship between cardiovascular disease morbidity, risk factors, and stress in a law enforcement cohort. *J. Occup. Environ. Med.* 44, 1182–1189. doi: 10.1097/00043764-200212000-00014

Frankenhaeuser, M. (1991). The psychophysiology of workload, stress, and health: comparison between the sexes. *Ann. Behav. Med*. 13, 197–204.

Frankenhaeuser, M., Dunne, E., and Lundberg, U. (1976). Sex differences in sympathetic-adrenal medullary reactions induced by different stressors. *Psychopharmacology (Berl.)* 47, 1–5. doi: 10.1007/BF00428693

Frankenhaeuser, M., von Wright, M. R., Collins, A., von Wright, J., Sedvall, G., and Swahn, C. G. (1978). Sex differences in psychoneuroendocrine reactions to examination stress. *Psychosom. Med*. 40, 334–343.

Freedman, R. R., Sabharwal, S. C., and Desai, N. (1987). Sex differences in peripheral vascular adrenergic receptors. *Circ. Res*. 61, 581–585. doi: 10.1161/01.RES.61.4.581

Fry, A. C., Kraemer, W. J., Stone, M. H., Warren, B. J., Fleck, S. J., Kearney, J. T., et al. (1994). Endocrine responses to over−reaching before and after 1 year of weightlifting training. *Can. J. Appl. Physiol.* 19, 400–410. doi: 10.1139/h94-032

Galbo, J. (1983). *Hormonal and Metabolic Adaptation to Exercise*. Stuttgart: Georg Thieme Verlay.

Gerra, G., Zaimovic, A., Mascetti, G. G., Gardini, S., Zambelli, U., Timpano, M., et al. (2001). Neuroendocrine responses to experimentally-induced psychological stress in healthy humans. *Psychoneuroendocrinology* 26, 91–107. doi: 10.1016/S0306-4530(00)00046-9

Georgiades, A., Lemne, C., de Faire, U., Lindvall, K., and Fredrikson, M. (1996). Stress-induced laboratory blood pressure in relation to ambulatory blood pressure and left ventricular mass among borderline hypertensive and normotensive individuals. *Hypertension* 28, 641–646. doi: 10.1161/01.HYP.28.4.641

Ghiadoni, L., Donald, A. E., Cropley, M., Mullen, M. J., Oakley, G., Taylor, M., et al. (2000). Mental stress induces transient endothelial dysfunction in humans. *Circulation* 102, 2473–2478. doi: 10.1161/01.CIR.102.20.2473

Gidron, Y., Gilutz, H., Berger, R., and Huleihel, M. (2002). Molecular and cellular interface between behavior and acute coronary syndromes. *Cardiovasc. Res.* 56, 15–21. doi: 10.1016/S0008-6363(02)00537-0

Girdler, S. S., Turner, J. R., Sherwood, A., and Light, K. C. (1990). Gender differences in blood pressure control during a variety of behavioral stressors. *Psychosom. Med*. 52, 571–591.

Goldbacher, E. M., Matthews, K. A., and Salomon, K. (2005). Central adiposity is associated with cardiovascular reactivity to stress in adolescents. *Health Psychol*. 24, 375–384. doi: 10.1037/0278-6133.24.4.375

Goldhammer, E., Tanchilevitch, A., Maor, I., Beniamini, Y., Rosenschein, U., and Sagiv, M. (2005). Exercise training modulates cytokines activity in coronary heart disease patients. *Int. J. Cardiol*. 100, 93–99. doi: 10.1016/j.ijcard.2004.08.073

Grassi, G., Seravalle, G., Cattaneo, B. M., Bolla, G. B., Lanfranchi, A., Colombo, M., et al. (1995). Sympathetic activation in obese normotensive subjects. *Hypertension* 25, 560–563. doi: 10.1161/01.HYP.25.4.560

Groer, M., Murphy, R., Bunnell, W., Salomon, K., Van Eepoel, J., Rankin, B., et al. (2010). Salivary measures of stress and immunity in police officers engaged in simulated critical incident scenarios. *J. Occup. Environ. Med*. 52, 595–602. doi: 10.1097/JOM.0b013e3181e129da

Hakkinen, K., Pakarinen, A., Alen, M., Kauhanen, H., and Komi, P. V. (1988). Neuromuscular and hormonal adaptations in athletes to strength training in two years. *J. Appl. Physiol*. 65, 2406–2412.

Hamer, M., Boutcher, Y. N., and Boutcher, S. H. (2007). Fatness is related to blunted vascular stress responsivity, independent of cardiorespiratory fitness in normal and overweight men. *Int. J. Psychophysiol*. 63, 251–257. doi: 10.1016/j.ijpsycho.2006.11.002

Hamer, M., and Steptoe, A. (2007). Association between physical fitness, parasympathetic control, and proinflammatory responses to mental stress. *Psychosom. Med*. 69, 660–666. doi: 10.1097/PSY.0b013e318148c4c0

Hamer, M., Taylor, A., and Steptoe, A. (2006). The effect of acute aerobic exercise on stress related blood pressure responses: a systematic review and meta-analysis. *Biol. Psychol*. 71, 183–190. doi: 10.1016/j.biopsycho.2005.04.004

Hatfield, B. D., Spalding, T. W., Santa Maria, D. L., Porges, S. W., Potts, J. T., Byrne, E. A., et al. (1998). Respiratory sinus arrhythmia during exercise in aerobically trained and untrained men. *Med. Sci. Sports Exerc*. 30, 206–214. doi: 10.1097/00005768-199802000-00006

Heinz, A., Hermann, D., Smolka, M. N., Rieks, M., Gräf, K. J., Pöhlau, D., et al. (2003). Effects of acute psychological stress on adhesion molecules, interleukins and sex hormones: implications for coronary heart disease. *Psychopharmacology (Berl.)* 165, 111–117. doi: 10.1007/s00213-002-1244-6

Hiddinga, H. J., Isaak, D. D., and Lewis, R. V. (1994). Enkephalin-containing peptides processed from proenkaphalin significantly enhance the antibody-forming cell responses to antigens. *J. Immunol*. 152, 3748–3759.

Hinton, J. W., Rotheiler, E. A., Gemmell, M., and Shewan, D. (1991). Heart rate, anxiety, anger, and arousal reactions to enforced time-wasting: dependence on reactive uncontrol, work involvement, and dominance factors of 'type A.' *Int. J. Psychophysiol*. 11, 115–124. doi: 10.1016/0167-8760(91)90003-G

Hirose, L., Nosaka, K., Newton, M., Laveder, A., Kano, M., Peake, J., et al. (2004). Changes in inflammatory mediators following eccentric exercise of the elbow flexors. *Exerc. Immunol. Rev*. 10, 75–90.

Hjemdahl, P. (1993). Plasma catecholamines—analytical challenges and physiological limitations. *Baillieres Clin. Endocrinol. Metab*. 7, 307–353. doi: 10.1016/S0950-351X(05)80179-X

Hsu, L. L., Nevin, R. L., Tobler, S. K., and Rubertone, M. V. (2007). Trends in overweight and obesity among 18 year-old applicants to the United States military, 1993–2006. *J. Adolesc. Health*. 41, 610–612. doi: 10.1016/j.jadohealth.2007.07.012

Huang, C. J., Franco, R. L., Evans, R. K., Mari, D. C., and Acevedo, E. O. (2013). Stress-induced Microvascular Reactivity in Normal-weight and Obese Individuals. *Appl. Physiol. Nutr. Metab*. doi: 10.1139/apnm-2013-0094

Huang, C. J., Webb, H. E., Evans, R. K., McCleod, K. A., Tangsilsat, S. E., Kamimori, G. H., et al. (2010a). Psychological stress during exercise: immunoendocrine and oxidative responses. *Exp. Biol. Med. (Maywood)* 235, 1498–1504. doi: 10.1258/ebm.2010.010176

Huang, C. J., Webb, H. E., Garten, R. S., Kamimori, G. H., Evans, R. K., and Acevedo, E. O. (2010b). Stress hormones and immunological responses to a dual challenge in professional firefighters. *Int. J. Psychophysiol*. 75, 312–318. doi: 10.1016/j.ijpsycho.2009.12.013

Iida, H., Kurano, M., Takano, H., Kubota, N., Morita, T., Meguro, K., et al. (2007). Hemodynamic and neurohumeroral responses to the restriction of femoral blood flow by KAATSU in healthy subjects. *Eur. J. Appl. Physiol*. 100, 275–285. doi: 10.1007/s00421-007-0430-y

Izquierdo, M., Ibamez, J. Calbet, J. A. L., Navarro-Amezqueta, I. Gonzalez-Izal, M., Idoate, F., et al. (2009). Cytokine and hormone responses to resistance training. *Eur. J. Appl. Physiol*. 107, 397–409. doi: 10.1007/s00421-009-1139-x

Jackson, E. M., and Dishman, R. K. (2006). Cardiorespiratory fitness and laboratory stress: a meta-regression analysis. *Psychophysiology* 43, 57–72. doi: 10.1111/j.1469-8986.2006.00373.x

Ji, L. L., Gomez-Cabrera, M. C., and Vina, J. (2006). Exercise and hormesis. *Ann. N.Y. Acad. Sci*. 1067, 425–435. doi: 10.1196/annals.1354.061

Joseph, P. N., Violanti, J. M., Donahue, R., Andrew, M. E., Trevisan, M., Burchfiel, C. M., et al. (2010). Endothelial function, a biomarker of subclinical cardiovascular disease, in urban police officers. *J. Occup. Environ. Med*. 52, 1004–1008. doi: 10.1097/JOM.0b013e3181f4385c

Kajantie, E., and Phillips, D. I. W. (2006). The effects of sex and hormonal status on the physiological response to acute psychosocial stress. *Psychoneuroendocrinology* 31, 151–178. doi: 10.1016/j.psyneuen.2005.07.002

Kaufman, M. P., and Hayes, S. G. (2002). The exercise pressor reflex. *Clin. Auton Res*. 12, 429–439. doi: 10.1007/s10286-002-0059-1

Kamarck, T. W., Everson, S. A., Kaplan, G. A., Manuck, S. B., Jennings, J. R., Salonen, R., et al. (1997). Exaggerated blood pressure responses during mental stress are associated with enhanced carotid atherosclerosis in middle-aged Finnish men: findings from the Kuopio Ischemic Heart Disease Study. *Circulation* 96, 3842–3848. doi: 10.1161/01.CIR.96.11.3842

Kang, D. H., and Fox, C. (2001). Th1 and Th2 cytokine responses to academic stress. *Res. Nurs. Health* 24, 245–257. doi: 10.1002/nur.1027

Karlsdottir, A. E., Foster, C., Porcari, J. P., Palmer-McLean, K., White-Kube, R., and Backes, R. C. (2002). Hemodynamic responses during aerobic and resistance exercise. *J. Cardio. Rehab*. 22, 170–177. doi: 10.1097/00008483-200205000-00008

Kirschbaum, C., Wolf, O. T., May, M., Wippich, W., and Hellhammer, D. H. (1996). Stress- and treatment-induced elevations of cortisol levels associated with impaired declarative memory in healthy adults. *Life Sci.* 58, 1475–1483. doi: 10.1016/0024-3205(96)00118-X

Kraemer, W. J., Diazdos, J. E., Gordon, S. E., Marchitelli, L. J., Fry, A. C., and Reynolds, K. L. (1990). The effects of graded exercise on plasma proenkephalin peptide F and catecholamine responses at sea level. *Eur. J. Appl. Physiol*. 61, 214–217. doi: 10.1007/BF00357602

Kraemer, W. J., Hakkinen, K., Newton, R. U., Nindl, B. C., Volek, J. S., McCormick, et al. (1999). Effects of heavy-resistance training on hormonal response patterns in younger vs. older men. *J. Appl. Physiol*. 87, 982–992.

Kraemer, W. J., Noble, B., Robertson, K., and Lewis, R. V. (1985a). Response of plasma proenkephalin peptide F to exercise. *Peptides* 6, 167–169. doi: 10.1016/0196-9781(85)90150-0

Kraemer, W. J., Noble, B., Culver, B., and Lewis, R. V. (1985b). Changes in plasma proenkephalin peptide F and catecholamine levels during graded exercise in men. *Proc. Natl. Acad. Sci. U.S.*A. 82, 6349–6351. doi: 10.1073/pnas.82.18.6349

Kraemer, W. J., Noble, B. J., Clark, M. J., and Culver, B. W. (1987). Physiologic responses to heavy-resistance exercise with very short rest periods. *Int. J. Sport Med*. 8, 247–252. doi: 10.1055/s-2008-1025663

Kraemer, W. J., Staron, R. S., Hagerman, F. C., Hikida, R. S., Fry, A. C., Gordon, S. E., et al. (1998). The effects of short-term resistance training on endocrine function in men and women. *Eur. J. Appl. Physiol*. 78, 69–76. doi: 10.1007/s004210050389

Kyrou, I., and Tsigos, C. (2009). Stress hormones: physiological stress and regulation of metabolism. *Curr. Opin. Pharmacol.* 9, 787–793. doi: 10.1016/j.coph.2009.08.007

Laederach-Hofmann, K., Mussgay, L., and Ruddel, H. (2000). Autonomic cardiovascular regulation in obesity. *J. Endocrinol*. 164, 59–66. doi: 10.1677/joe.0.1640059

Lai, J. Y., and Linden, W. (1992). Gender, anger expression style, and opportunity for anger release determine cardiovascular reaction to and recovery from anger provocation. *Psychosom. Med*. 54, 297–310.

Leeuwenburgh, C., and Heinecke, J. W. (2001). Oxidative stress and antioxidants in exercise. *Curr. Med. Chem.* 8, 829–838. doi: 10.2174/0929867013372896

Loenneke, J. P., Wilson, J. M., Marin, P. J., Zourdos, M. C., and Bemben, M. G. (2012). Low intensity blood flow restriction training: a meta-analysis. *Eur. J. Appl. Physiol*. 112, 1849–1859. doi: 10.1007/s00421-011-2167-x

Lundberg, U. (1983). Sex differences in behaviour pattern and catecholamine and cortisol excretion in 3-6 year old day-care children. *Biol. Psychol*. 16, 109–117. doi: 10.1016/0301-0511(83)90057-1

Lupien, S., Lecours, A. R., Schwartz, G., Sharma, S., Hauger, R. L., Meaney, M. J., et al. (1996). Longitudinal study of basal cortisol levels in healthy elderly subjects: evidence for subgroups. *Neurobiol. Aging* 17, 95–105. doi: 10.1016/0197-4580(95)02005-5

Lupien, S. J., de Leon, M., de Santi, S., Convit, A., Tarshish, C., Nair, N. P. V., et al. (1998). Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat. Neurosci.* 1, 69–73. doi: 10.1038/271

Lutgendorf, S., Logan, H., Kirchner, H. L., Rothrock, N., Svengalis, S., Iverson, K., et al. (2000). Effects of relaxation and stress on the capsaicin-induced local inflammatory response. *Psychosom. Med.* 62, 524–534.

McCarty, R., and Gold, P. E. (1996). Catecholamines, stress, and disease: a psychobiological perspective. *Psychosom. Med.* 58, 590–597.

Macdonald, I. A. (1995). Advances in our understanding of the role of the sympathetic nervous system in obesity. *Int. J. Obes. Relat. Metab. Disord*. 19(Suppl. 7), S2–S7.

Martin, B. J., Verma, S., Charbonneau, F., Title, L. M., Lonn, E. M., and Anderson, T. J. (2013). The relationship between anthropometric indexes of adiposity and vascular function in the FATE cohort. *Obesity (Silver Spring)* 21, 266–273. doi: 10.1002/oby.20266

Matthews, K. A., Owens, J. F., Allen, M. T., and Stoney, C. M. (1992). Do cardiovascular responses to laboratory stress relate to ambulatory blood pressure levels? Yes, in some of the people, some of the time. *Psychosom. Med*. 54, 686–697.

Matthews, K. A., and Stoney, C. M. (1988). Influences of sex and age on cardiovascular responses during stress. *Psychosom. Med*. 50, 46–56.

Matthews, K. A., Woodall, K. L., and Allen, M. T. (1993). Cardiovascular reactivity to stress predicts future blood pressure status. *Hypertension* 22, 479–485. doi: 10.1161/01.HYP.22.4.479

Mattson, M. P., and Wan, R. (2005). Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. *J. Nutr. Biochem*. 16, 129–137. doi: 10.1016/j.jnutbio.2004.12.007

Mausbach, B. T., Chattillion, E., Roepke, S. K., Ziegler, M. G., Milic, M., von Känel, R., et al. (2012). A longitudinal analysis of the relations among stress, depressive symptoms, leisure satisfaction, and endothelial function in caregivers. *Health Psychol.* 31, 433–440. doi: 10.1037/a0027783

McAdoo, W. G., Weinberger, M. H., Miller, J. Z., Fineberg, N. S., and Grim, C. E. (1990). Race and gender influence hemodynamic responses to psychological and physical stimuli. *J. Hypertens*. 8, 961–967. doi: 10.1097/00004872-199010000-00012

McCaulley, G. O., McBride, J. M., Cormie, P., Hudson, M. B., Nuzzo, J. L., Qundry, J. C., et al. (2009). Acute hormonal and neuromuscular responses to hypertrophy, strength, and power type resistance exercise. *Eur. J. Appl. Physiol*. 105, 695–704. doi: 10.1007/s00421-008-0951-z

McCrone, S. H., Brendle, D., and Barton, K. (2001). A multibehavioral intervention to decrease cardiovascular disease risk factors in older men. *AACN Clin. Issues* 12, 5–16. doi: 10.1097/00044067-200102000-00003

McCubbin, J. A., Cheung, R., Montgomery, T. B., Bulbulian, R., and Wilson, J. F. (1992). Aerobic fitness and opioidergic inhibition of cardiovascular stress reactivity. *Psychophysiology* 29, 687–697. doi: 10.1111/j.1469-8986.1992.tb02047.x

Morgan, W. P. (1969). A pilot investigation of physical working capacity in depressed and nondepressed psychiatric males. *Res. Q.* 40, 859–861.

Nishitani, N., and Sakakibara, H. (2006). Relationship of obesity to job stress and eating behavior in male Japanese workers. *Int. J. Obes. (Lond)* 30, 528–533. doi: 10.1038/sj.ijo.0803153

Nojima, H., Watanabe, H., Yamane, K., Kitahara, Y., Sekikawa, K., Yamamoto, H., et al. (2008). Effect of aerobic exercise training on oxidative stress in patients with type 2 diabetes mellitus. *Metabolism* 57, 170–176. doi: 10.1016/j.metabol.2007.08.021

Nosaka, K., Sakamoto, K., Newton, M., and Sacco, P. (2001). The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. *Eur. J. Appl. Physiol*. 85, 34–40. doi: 10.1007/s004210100430

Olinski, R., Gackowski, D., Foksinski, M., Rozalski, R., Roszkowski, K., and Jaruga, P. (2002). Oxidative DNA damage: assessment of the role in carcinogenesis, atherosclerosis, and acquired immunodeficiency syndrome. *Free Radic. Biol. Med.* 33, 192–200. doi: 10.1016/S0891-5849(02)00878-X

Otsuka, R., Yatsuya, H., Tamakoshi, K., Matsushita, K., Wada, K., and Toyoshima, H. (2006). Perceived psychological stress and serum leptin concentrations in Japanese men. *Obesity (Silver Spring)* 14, 1832–1838. doi: 10.1038/oby.2006.211

Paffenbarger, R. S. Jr., Lee, I.-M., and Leung, R. (1994). Physical activity and personal characteristics associ- ated with depression and suicide in American college men. *Acta Psychiatr. Scand.* 377, 16–22. doi: 10.1111/j.1600-0447.1994.tb05796.x

Peake, J. M., Suzuki, K., Hordern, M., Wilson, G., Nosaka, K., and Coombes, J. S. (2005). Plasma cytokine changes in relation to exercise intensity and muscle damage. *Eur. J. Appl. Physiol*. 95, 514–521. doi: 10.1007/s00421-005-0035-2

Perry, G., Friedland, R. P., Petot, G. J., Nunomura, A., Castellani, R. J., Kubat, Z., et al. (2005). "Alzheimer as a disease of metabolic demand: benefits of physical and brain exercise," in Exercise and Diseases, ed Z. Radak (Oxford: Meyer Meyer Sport), 7–16.

Petruzzello, S. J., Landers, D. M., Hatfield, B. D., Kubitz, K. A., and Salazar, W. (1991). A meta-analysis on the anxiety-reducing effects of acute and chronic exercise. Outcomes and mechanisms. *Sports Med*. 11, 143–182. doi: 10.2165/00007256-199111030-00002

Phillips, A. C. (2011). Blunted cardiovascular reactivity relates to depression, obesity, and self-reported health. *Biol. Psychol*. 86, 106–113. doi: 10.1016/j.biopsycho.2010.03.016

Phillips, A. C., Roseboom, T. J., Carroll, D., and de Rooij, S. R. (2012). Cardiovascular and cortisol reactions to acute psychological stress and adiposity: cross-sectional and prospective associations in the Dutch Famine Birth Cohort Study. *Psychosom. Med*. 74, 699–710. doi: 10.1097/PSY.0b013e31825e3b91

Pickering, T. G. (1997). The effects of environmental and lifestyle factors on blood pressure and the intermediary role of the sympathetic nervous system. *J. Hum. Hypertens.* 11(Suppl. 1), S9–S18.

Pischon, T., Hankinson, S. E., Hotamisligil, G. S., Rifai, N., and Rimm, E. B. (2003). Leisure-time physical activity and reduced plasma levels of obesity-related inflammatory markers. *Obes. Res*. 11, 1055–1064. doi: 10.1038/oby.2003.145

Pollock, M. L., Franklin, B. A., Balady, G. J., Chaitman, B. L., Fleg, J. L., Fletcher, B., et al. (2000). Resistance exercise in individuals with and without cardiovascular disease: benefits, rationale, safety, and prescription. An advisory from the committee on exercise, rehabilitation, and prevention, council on clinical cardiology, American heart association. *Circulation* 101, 828–833. doi: 10.1161/01.CIR.101.7.828

Porges, S. W. (1995). Cardiac vagal tone: a physiological index of stress. *Neurosci. Biobehav. Rev*. 19, 225–233. doi: 10.1016/0149-7634(94)00066-A

Powers, S. K., and Lennon, S. L. (1999). Analysis of cellular responses to free radicals: focus on exercise and skeletal muscle. *Proc. Nutr. Soc*. 58, 1025–1033. doi: 10.1017/S0029665199001342

Radak, Z., Chung, H. Y., and Goto, S. (2005). Exercise and hormesis: oxidative stress-related adaptation for successful aging. *Biogerontology* 6, 71–75. doi: 10.1007/s10522-004-7386-7

Ramey, S. L., Downing, N. R., and Franke, W. D. (2009). Milwaukee police department retirees: cardiovascular disease risk and morbidity among aging law enforcement officers. *AAOHN J*. 57, 448–453. doi: 10.3928/08910162-20091019-02

Ramey, S. L., Downing, N. R., Franke, W. D., Perkhounkova, Y., and Alasagheirin, M. H. (2012). Relationships among stress measures, risk factors, and inflammatory biomarkers in law enforcement officers. *Biol. Res. Nurs*. 14, 16–26. doi: 10.1177/1099800410396356

Rauste-von Wright, M., von Wright, J., and Frankenhaeuser, M. (1981). Relationships between sex-related psychological characteristics during adolescence and catecholamine excretion during achievement stress. *Psychophysiology* 18, 362–370. doi: 10.1111/j.1469-8986.1981.tb02467.x

Rimmele, U., Seiler, R., Marti, B., Wirtz, P. H., Ehlert, U., and Heinrichs, M. (2009). The level of physical activity affects adrenal and cardiovascular reactivity to psychosocial stress. *Psychoneuroendocrinology* 34, 190–198. doi: 10.1016/j.psyneuen.2008.08.023

Robinson, S. J., Leach, J., Owen-Lynch, P. J., and Sünram-Lea, S. I. (2013). Stress reactivity and cognitive performance in a simulated firefighting emergency. *Aviat. Space Environ. Med*. 84, 592–599. doi: 10.3357/ASEM.3391.2013

Rosati, M. V., Sancini, A., Tomei, F., Andreozzi, G., Scimitto, L., Schifano, M. P., et al. (2011). Plasma cortisol concentrations and lifestyle in a population of outdoor workers. *Int. J. Environ. Health Res.* 21, 62–71. doi: 10.1080/09603123.2010.506675

Ross, R. (1999). Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* 340, 115–126. doi: 10.1056/NEJM199901143400207

Roth, D. L., Bachtler, S. D., and Fillingim, R. B. (1990). Acute emotional and cardiovascular effects of stressful mental work during aerobic exercise. *Psychophysiology* 27, 694–701. doi: 10.1111/j.1469-8986.1990.tb03196.x

Rousselle, J. G., Blascovich, J., and Kelsey, R. M. (1995). Cardiorespiratory response under combined psychological and exercise stress. *Int. J. Psychophysiology* 20, 49–58. doi: 10.1016/0167-8760(95)00026-O

Rowell, L. B., and Shepherd, J. T. (1996). *American Physiological Society (1887-), Exercise: Regulation and Integration of Multiple Systems*. New York: Published for the American Physiological Society by Oxford University Press.

Sandberg, K., and Ji, H. (2012). Sex differences in primary hypertension. *Biol. Sex Differ.* 3, 7. doi: 10.1186/2042-6410-3-7

Schäfer, A., and Bauersachs, J. (2008). Endothelial dysfunction, impaired endogenous platelet inhibition and platelet activation in diabetes and atherosclerosis. *Curr. Vasc. Pharmacol.* 6, 52–60. doi: 10.2174/157016108783331295

Schoder, H., Silverman, D. H., Campisi, R., Sayre, J. W., Phelps, M. E., Schelbert, H. R., et al. (2000). Regulation of myocardial blood flow response to mental stress in healthy individuals. *Am. J. Physiol Heart Circ. Physiol.* 278, H360–H366.

Schuler, J. L., and O'Brien, W. H. (1997). Cardiovascular recovery from stress and hypertension risk factors: a meta-analytic review. *Psychophysiology* 34, 649–659. doi: 10.1111/j.1469-8986.1997.tb02141.x

Selye, H. (1936). A syndrome produced by diverse nocuous agents. *Nature* 138. doi: 10.1038/138032a0

Selye, H. (1950). Stress and the general adaptation syndrome. *Br. Med. J.* 1, 1383–1392. doi: 10.1136/bmj.1.4667.1383

Selye, H. (1954). Interactions between systemic and local stress. *Br. Med. J.* 1, 1167–1170. doi: 10.1136/bmj.1.4872.1167

Selye, H. (1976). Forty years of stress research: principal remaining problems and misconceptions. *Can. Med. Assoc. J.* 115, 53–56.

Sies, H. (1997). Oxidative stress: oxidants and anitoxidants. *Exp. Physiol.* 82, 291–295.

Singhai, A. (2005). Endothelial dysfunction: role in obesity-related disorders and the early original of CVD. *Proc. Nutr. Soc.* 64, 15–22. doi: 10.1079/PNS2004404

Sivonova, M., Zitnanova, I., Hlincikova, L., Skodacek, I., Trebaticka, J., and Durackova, Z. (2004). Oxidative stress in university students during examinations. *Stress* 7, 183–188. doi: 10.1080/10253890400012685

Sloan, R. P., Shapiro, P. A., DeMeersman, R. E., Bagiella, E., Brondolo, E. N., McKinley, P. S., et al. (2011). Impact of aerobic training on cardiovascular reactivity to and recovery from challenge. *Psychosom. Med.* 73, 134–141. doi: 10.1097/PSY.0b013e31820a1174

Smeets, T. (2010). Autonomic and hypothalamic-pituitary-adrenal stress resilience: impact of cardiac vagal tone. *Biol. Psychol.* 84, 290–295. doi: 10.1016/j.biopsycho.2010.02.015

Smith, L. L., Anwar, A., Fragen, M., Rananto, C., Johnson, R., and Holbert, D. (2000). Cytokines and cell adhesion molecules associated with high-intensity eccentric exercise. *Eur. J. Appl. Physiol.* 82, 61–67. doi: 10.1007/s004210050652

Smith, S. A., Mitchell, J. H., and Garry, M. G. (2006). The mammalian exercise pressor reflex in health and disease. *Exp. Physiol.* 91, 89–102. doi: 10.1113/expphysiol.2005.032367

Sothmann, M. S., Buckworth, J., Claytor, R. P., Cox, R. H., White-Welkley, J. E., and Dishman, R. K. (1996). Exercise training and the cross-stressor adaptation hypothesis. *Exerc. Sport Sci. Rev.* 24, 267–287. doi: 10.1249/00003677-199600240-00011

Spalding, T. W., Jeffers, L. S., Porges, S. W., and Hatfield, B. D. (2000). Vagal and cardiac reactivity to psychological stressors in trained and untrained men. *Med. Sci. Sports Exerc.* 32, 581–591. doi: 10.1097/00005768-200003000-00006

Spalding, T. W., Lyon, L. A., Steel, D. H., and Hatfield, B. D. (2004). Aerobic exercise training and cardiovascular reactivity to psychological stress in sedentary young normotensive men and women. *Psychophysiology* 41, 552–562. doi: 10.1111/j.1469-8986.2004.00184.x

Spieker, L. E., Hurlimann, D., Ruschitzka, F., Corti, R., Enseleit, F., Shaw, S., et al. (2002). Mental stress induces prolonged endothelial dysfunction via endothelin-a receptors. *Circulation* 105, 2817–2820. doi: 10.1161/01.CIR.0000021598.15895.34

Staron, R. S., Karapondo, D. L., Kraemer, W. J., Fry, A. C., Gordon, S. E., Falkel, J. E., et al. (1994). Skeletal muscle adaptations during early phase of heavy-resistance training in men and women. *J. Appl. Physiol.* 76, 1247–1255.

Stefano, G. B., Murga, J., Benson, H., Zhu, W., Bilfinger, T. V., and Magazine, H. I. (2001). Nitric oxide inhibits norepinephrine stimulated contraction of human internal thoracic artery and rat aorta. *Pharmacol. Res.* 43, 199–203. doi: 10.1006/phrs.2000.0765

Steptoe, A., and Butler, N. (1996). Sports participation and emotional well-being in adolescents. *Lancet* 347, 1789–1792. doi: 10.1016/S0140-6736(96)91616-5

Steptoe, A., Wardle, J., Fuller, R., Holte, A., Justo, J., Sanderman, R., et al. (1997). Leisure-time physical exercise: prevalence, attitudinal correlates, and behavioral correlates among young Europeans from 21 countries. *Prev. Med.* 26, 845–854. doi: 10.1006/pmed.1997.0224

Steptoe, A., and Wardle, J. (2005). Cardiovascular stress responsivity, body mass and abdominal adiposity. *Int. J. Obes. (Lond)* 29, 1329–1337. doi: 10.1038/sj.ijo.0803011

Steptoe, A., Willemsen, G., Owen, N., Flower, L., and Mohamed-Ali, V. (2001). Acute mental stress elicits delayed increases in circulating inflammatory cytokine levels. *Clin. Sci. (Lond)* 101, 185–192. doi: 10.1042/CS20010038

Stoney, C. M., Davis, M. C., and Matthews, K. A. (1987). Sex differences in physiological responses to stress and in coronary heart disease: a causal link? *Psychophysiology* 24, 127–131. doi: 10.1111/j.1469-8986.1987.tb00264.x

Stoney, C. M., Matthews, K. A., McDonald, R. H., and Johnson, C. A. (1988). Sex differences in lipid, lipoprotein, cardiovascular, and neuroendocrine responses to acute stress. *Psychophysiology* 25, 645–656. doi: 10.1111/j.1469-8986.1988.tb01902.x

Suzuki, K., Nakaji, S., Yamada, M., Totsuka, M., Sato, K., and Sugawara, K. (2002). Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exerc. Immunol. Rev.* 8, 6–48.

Szabo, A., Péronnet, F., Gauvin, L., and Furedy, J. J. (1994). Mental challenge elicits "additional" increases in heart rate during low and moderate intensity cycling. *Int. J. Psychophysiol.* 17, 197–204. doi: 10.1016/0167-8760(94)90063-9

Szijgyarto, I. C., King, T. J., Ku, J., Poitras, V. J., Gurd, B. J., and Pyke, K. E. (2013). The impact of acute mental stress on brachial artery flow-mediated dilation differs when shear stress is elevated by reactive hyperemia versus handgrip exercise. *Appl. Physiol. Nutr. Metab.* 38, 498–506. doi: 10.1139/apnm-2012-0328

Timimi, F. K., Ting, H. H., Haley, E. A., Roddy, M. A., Ganz, P., and Creager, M. A. (1998). Vitamin C improves endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *J. Am. Coll. Cardiol.* 31, 552–557. doi: 10.1016/S0735-1097(97)00536-6

Tomei, F., Rosati, M. V., Ciarrocca, M., Baccolo, T. P., Gaballo, M., Caciari, T., et al. (2003). Plasma cortisol levels and workers exposed to urban pollutants. *Ind. Health* 41, 320–326. doi: 10.2486/indhealth.41.320

Traustadóttir, T., Bosch, P. R., and Matt, K. S. (2005). The HPA axis response to stress in women: effects of aging and fitness. *Psychoneuroendocrinology* 30, 392–402. doi: 10.1016/j.psyneuen.2004.11.002

Tsismenakis, A. J., Christophi, C. A., Burress, J. W., Kinney, A. M., Kim, M., and Kales, S. N. (2009). The obesity epidemic and future emergency responders. *Obesity (Silver Spring)* 17, 1648–1650. doi: 10.1038/oby.2009.63

Valtonen, M. K., Laaksonen, D. E., Laukkanen, J. A., Tolmunen, T., Viinamäki, H., Lakka, H. M., et al. (2012). Low-grade inflammation and depressive symptoms as predictors of abdominal obesity. *Scand. J. Public Health* 40, 674–680. doi: 10.1177/1403494812461730

van Baak, M. A. (2001). The peripheral sympathetic nervous system in human obesity. *Obes. Rev.* 2, 3–14. doi: 10.1046/j.1467-789x.2001.00010.x

van Doornen, L. J., and de Geus, E. J. (1989). Aerobic fitness and the cardiovascular response to stress. *Psychophysiology* 26, 17–28. doi: 10.1111/j.1469-8986.1989.tb03127.x

Vider, J., Laaksonen, D. E., Kilk, A., Atalay, M., Lehtmaa, J., Zilmer, M., et al. (2001). Physical exercise induces activation of NF-kB in human peripheral blood lymphocytes. *Antioxid. Redox Signal* 3, 1131–1137. doi: 10.1089/152308601317203639

Vieira, P. J., Chiappa, G. R., Umpierre, D., Stein, R., and Ribeiro, J. P. (2013). Hemodynamic responses to resistance exercise with restricted blood flow in young and older men. *J. Strength Cond. Res.* 27, 2288–2294. doi: 10.1519/JSC.0b013e318278f21f

Violanti, J. M., Burchfiel, C. M., Fekedulegn, D., Andrew, M. E., Dorn, J., Hartley, T. A., et al. (2009). Cortisol patterns and brachial artery reactivity in a high stress environment. *Psychiatry Res.* 169, 75–81. doi: 10.1016/j.psychres.2008.06.012

Violanti, J. M., Burchfiel, C. M., Miller, D. B., Andrew, M. E., Dorn, J., Wactawski-Wende, J., et al. (2006). The Buffalo Cardio-Metabolic Occupational Police

Stress (BCOPS) pilot study: methods and participant characteristics. *Ann. Epidemiol*. 16, 148–156. doi: 10.1016/j.annepidem.2005.07.054

Wagner, J. A., Tennen, H., Finan, P. H., White, W. B., Burg, M. M., and Ghuman, N. (2012). Lifetime history of depression, type 2 diabetes, and endothelial reactivity to acute stress in postmenopausal women. *Int. J. Behav. Med*. 19, 503–511. doi: 10.1007/s12529-011-9190-5

Wannamethee, S. G., Tchernova, J., Whincup, P., Lowe, G. D., Kelly, A., Rumley, A., et al. (2007). Plasma leptin: associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease. *Atherosclerosis* 191, 418–426. doi: 10.1016/j.atherosclerosis.2006.04.012

Webb, H. E., Garten, R. S., McMinn, D. R., Beckman, J. L., Kamimori, G. H., and Acevedo, E. O. (2011). Stress hormones and vascular function in firefighters during concurrent challenges. *Biol. Psychol*. 87, 152–160. doi: 10.1016/j.biopsycho.2011.02.024

Webb, H. E., McMinn, D. R., Garten, R. S., Beckman, J. L., Kamimori, G. H., and Acevedo, E. O. (2010). Cardiorespiratory responses of firefighters to a computerized fire strategies and tactics drill during physical activity. *Appl. Ergon*. 41, 376–381. doi: 10.1016/j.apergo.2009.08.003

Webb, H. E., Rosalky, D. S., Tangsilsat, S. E., McLeod, K. A., Acevedo, E. O., and Wax, B. (2013). Aerobic fitness affects cortisol responses to concurrent challenges. *Med. Sci. Sports Exerc*. 45, 379–386. doi: 10.1249/MSS.0b013e318270b381

Webb, H. E., Weldy, M. L., Fabianke-Kadue, E. C., Orndorff, G. R., Kamimori, G. H., and Acevedo, E. O. (2008). Psychological stress during exercise: cardiorespiratory and hormonal responses. *Eur. J. Appl. Physiol*. 104, 973–981. doi: 10.1007/s00421-008-0852-1

Wilmore, J. H. (1974). Alterations in strength, body composition, and anthropometric measurements consequent to a 10-week weight training program. *Med. Sci. Sports* 6, 133–138. doi: 10.1249/00005768-197400620-00025

Wirtz, P. H., Ehlert, U., Emini, L., and Suter, T. (2008). Higher body mass index (BMI) is associated with reduced glucocorticoid inhibition of inflammatory cytokine production following acute psychological stress in men. *Psychoneuroendocrinology* 33, 1102–1110. doi: 10.1016/j.psyneuen.2008.05.002

Wood, D. L., Sheps, S. G., Elveback, L. R., and Schirger, A. (1984). Cold pressor test as a predictor of hypertension. *Hypertension* 6, 301–306. doi: 10.1161/01.HYP.6.3.301

Yoo, H., Eisenmann, J., and Franke, W. (2009). Independent and combined influence of physical activity and perceived stress on the metabolic syndrome in male law enforcement officers. *J. Occup. Environ. Med.* 51, 46–53. doi: 10.1097/JOM.0b013e31817f9e43

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Vagal modulation of resting heart rate in rats: the role of stress, psychosocial factors, and physical exercise

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In humans, there are large individual differences in the levels of vagal modulation of resting heart rate (HR). High levels are a recognized index of cardiac health, whereas low levels are considered an important risk factor for cardiovascular morbidity and mortality. Several factors are thought to contribute significantly to this inter-individual variability. While regular physical exercise seems to induce an increase in resting vagal tone, chronic life stress, and psychosocial factors such as negative moods and personality traits appear associated with vagal withdrawal. Preclinical research has been attempting to clarify such relationships and to provide insights into the neurobiological mechanisms underlying vagal tone impairment/enhancement. This paper focuses on rat studies that have explored the effects of stress, psychosocial factors and physical exercise on vagal modulation of resting HR. Results are discussed with regard to: (i) individual differences in resting vagal tone, cardiac stress reactivity and arrhythmia vulnerability; (ii) elucidation of the neurobiological determinants of resting vagal tone.

Keywords: autonomic nervous system, heart rate variability, arrhythmias, stress, anxiety, depression, exercise, rat

INTRODUCTION

The sinoatrial node has an intrinsic rate of spontaneous automaticity that sets the basic rhythm of the heartbeat (Jose and Collison, 1970). The dynamic balance between the sympathetic and parasympathetic (vagal) influences on sinoatrial node activity primarily determines the actual heart rate (HR) of a given physiological state. In healthy individuals, vagal modulation (or "tone") prevails under resting conditions. The role of resting vagal tone in healthy cardiac function has been increasingly recognized over the past 20 years (Levy and Schwartz, 1994). This was predominantly due to the rise of research that applied heart rate variability (HRV) analysis as a window into cardiac autonomic control. Mounting evidence indicates that those individuals showing higher vagal tone than average at rest tend to be more resilient to stress, adapting well across a number of different situations (El-Sheikh et al., 2001; Kok and Fredrickson, 2010; Smeets et al., 2010; Souza et al., 2013). On this regard, the beneficial effects of regular physical exercise on cardiac health appear to be mediated by an increase in resting vagal outflow (Smith et al., 1989; Rosenwinkel et al., 2001; Rennie et al., 2003; Soares-Miranda et al., 2009; Fu and Levine, 2013). On the contrary, low levels of vagal modulation may not adequately counteract sympathetic stimulation, leaving the heart vulnerable to ventricular tachyarrhythmias and sudden cardiac death (Schwartz et al., 1988; Esler, 1992; Volders, 2010). Several factors, including chronic life stress, negative personality traits, anxiety and mood states have been associated with vagal withdrawal and sympathetic predominance (Sloan et al., 1994; Friedman and Thayer, 1998; Rozanski et al., 1999; Gorman and Sloan, 2000; Lucini et al., 2005). Moreover, depression of vagal modulation characterizes cardiovascular pathology (e.g., heart failure) (Sabbah et al., 2011) and strongly predicts cardiac mortality after myocardial infarction (La Rovere et al., 1998). Interventional therapies for restoring the autonomic balance in such psychological and cardiac conditions include pharmacological, biobehavioral, and exercise strategies (Bryniarski et al., 1997; Iellamo et al., 2000; Tsai et al., 2006; Blumenthal et al., 2007; Nolan et al., 2008). Traditionally, pharmacological approaches have mainly been directed at reducing cardiac sympathetic outflow, while overlooking the possibility of enhancing vagal tone. This was presumably due to a lack of a comprehensive understanding of the central neurobiological determinants of resting vagal tone. Preclinical research is now attempting to fill this knowledge gap.

This paper focuses on current and past HRV research in rats that can potentially increase our understanding of the neurobiological mechanisms underlying vagal tone impairment/enhancement. It describes (i) how resting vagal tone is regulated and measured, (ii) the relations among individual differences in resting vagal tone, cardiac stress reactivity, and arrhythmia vulnerability, and (iii) the role of stress, psychosocial factors and physical exercise on the neural determination of resting vagal tone.

Abbreviations: DMH, dorsomedial hypothalamus; HA, high-aggressive; HAB, high-anxiety behavior; HF, high frequency; HR, heart rate; HRV, heart rate variability; LAB, low-anxiety behavior; LF, low frequency; NA, non-aggressive; NAmb, nucleus ambiguus; NTS, nucleus tractus solitarii; RMSSD, root mean square of successive R-R interval differences; RSA, respiratory sinus arrhythmia; VLF, very low frequency; 5-HT, serotonin.

ORIGIN, COMPONENTS, AND MEASUREMENTS OF RESTING VAGAL TONE

Cardiac vagal activity is generated by cardiac vagal pre-ganglionic neurones mainly located in the nucleus ambiguous (NAmb) of the lower brainstem (**Figure 1**). Their axons innervate

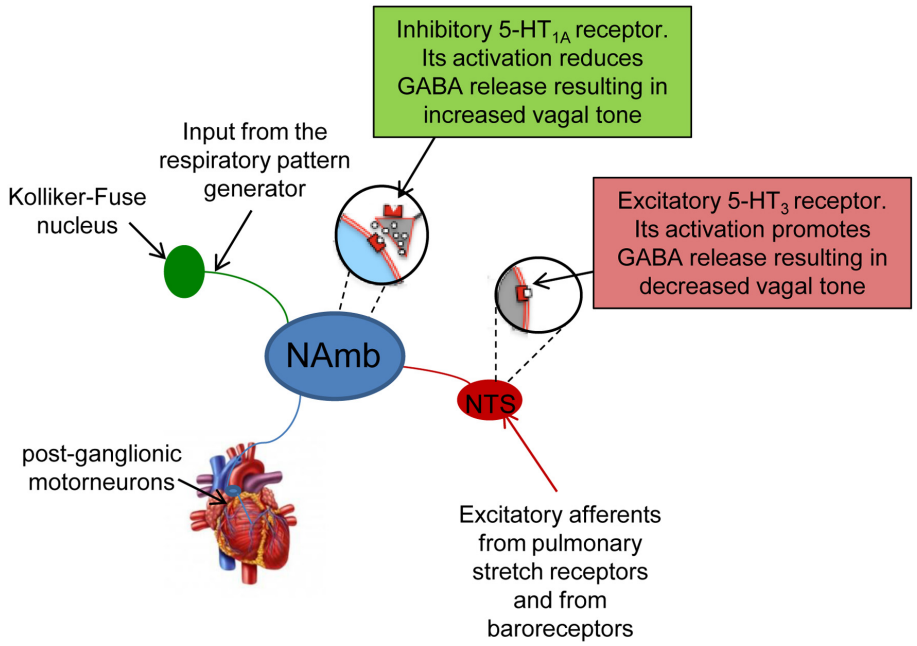

FIGURE 1 | Schematic representation of the origin and components of cardiac vagal pathway. Vagal activity is generated by preganglionic vagal neurons in the nucleus ambiguus (NAmb), which are tonically active. They receive phasic respiratory-related inhibitory influences from the central respiratory pattern generator and from the nucleus tractii solitary (NTS). Their axons innervate post-ganglionic motoneurones located in a ganglionic plexus on the heart itself.

post-ganglionic motoneurones located in a ganglionic plexus on the heart itself (Cheng et al., 1999; Cheng and Powley, 2000) (**Figure 1**). Under resting conditions, this vagal pathway fires a rapid and continuous signal (or "tonus") to the sinoatrial node, slowing HR via acetylcholine release from efferent vagal nerve discharge. Vagally-released acetylcholine counteracts sympathetic effects both post-synaptically (via cAMP) and pre-synaptically (by reducing noradrenaline release from the sympathetic terminals) (Levy, 1971).

Cardiac vagal activity is dependent on excitatory and inhibitory synaptic inputs (Mendelowitz, 1996), including baroreflex [mediated by the nucleus tractus solitarii (NTS)] and respiratory input (Rentero et al., 2002) (**Figure 1**). Breathing modulates cardiac vagal activity through two mechanisms: (i) directly by projections from the central respiratory pattern generator, and (ii) indirectly by ascending afferents from lung stretch receptors that are activated during inspiration (**Figure 1**). These ascending afferents activate GABA-ergic neurons in the NTS that inhibit cardiac vagal motorneurons in the NAmb (**Figure 1**). Collectively, these mechanisms produce respiratory sinus arrhythmia (RSA)—rhythmic oscillations of HR around its mean value—with increases in HR during inspiration as vagal influence is momentarily suppressed, and decreases in HR during the early expiration phase as vagal influence resumes. Although moment-to-moment HR fluctuations can also be generated in response to, for example, physical movement and blood pressure and thermoregulatory changes, respiration is reliably periodic. Therefore, the strength of vagal influence can be estimated by measuring the rhythmic oscillation in the intervals between consecutive heart beats that are due to RSA—this is called HRV (Task Force, 1996).

HRV analysis is an established tool to estimate cardiac autonomic regulation in humans and animal models. Although the suitability of using HRV parameters to estimate sympathetic modulation is highly debated (Reyes Del Paso et al., 2013), this approach produces reliable measures of vagal tone (Berntson et al., 1997; Reyes Del Paso et al., 2013). Traditional HRV methods fall under the broader description of being either "time-domain" or "frequency domain" analyses. Among time domain measures of HRV, which are assessed with calculations based on statistical operations on R-R intervals (Kleiger et al., 1992), the root mean square of successive R-R interval differences (RMSSD) detects high frequency oscillations of HR, and therefore estimates parasympathetic nervous system activity (Stein et al., 1994). Frequency domain measures are based on spectral analysis of a sequence of R-R intervals and provide information on how power (variance) is distributed as a function of frequency. Usually, three oscillatory components are distinguished in the spectral profile: the very low (VLF), the low (LF), and the high (HF) frequency band. The power of HF band includes respiration-linked oscillations of HR and therefore reflects the modulation of vagus nerve discharge caused by respiration (Berntson et al., 1997), whereas the LF and VLF bands are related to a more gradual interplay between sympathetic and parasympathetic influences (Reyes Del Paso et al., 2013). The power of LF and HF bands is often reported in normalized (relative or fractional) units, which correspond to the relative value of each power in proportion to the total power (usually minus the VLF component). In particular, LF to HF

ratio is taken as a synthetic measure of sympatho-vagal balance as it estimates the fractional distribution of power (Task Force, 1996).

It is worth mentioning for completeness sake that algorithms based on the chaos theory and nonlinear dynamics have been developed in order to evaluate in greater detail the intrinsic complexity of HRV (for an historical overview of the evolution of the concept of HRV and its application see Billman, 2011). Nonlinear methods are based on the assumption that the mechanisms involved in HR regulation interact with each other in a nonlinear way. The basic concept of nonlinear HRV methods is to try to capture the non-periodic behavior and complexity that exist inside the R-R interval dynamics. Various nonlinear methods have been tested in several sets of R-R interval data (Bigger et al., 1996; Lombardi et al., 1996; Makikallio et al., 1997, 1999; Huikuri et al., 1998, 2000), providing additional prognostic information and complementing traditional time- and frequency-domain analyses.

INDIVIDUAL DIFFERENCES IN RESTING VAGAL TONE: PHYSIOLOGICAL SIGNIFICANCE

Porges' polyvagal theory (Porges, 1995b) introduces a new perspective relating vagal tone during steady states (i.e., resting vagal tone) and vagal reactivity in response to environmental demands. According to this theory, measures of resting vagal tone are informative of an organism's ability to maintain homeostasis and the potential responsiveness of that organism. In particular, high levels of resting vagal tone can be considered a sign of autonomic flexibility, the capability of the parasympathetic nervous system to generate adequate responses to environmental challenges by modifying HR, respiration and arousal (Porges, 1995a,b, 2007; Beauchaine, 2001). On the contrary, depression of vagal modulation may predict mismatches between environmental demands and cardiac (re)-activity, thus increasing vulnerability to cardiac arrhythmias and sudden cardiac death (Schwartz et al., 1988; Esler, 1992; Volders, 2010).

A deeper insight into the complex relations among individual differences in resting vagal tone, cardiac stress reactivity and arrhythmia vulnerability might be facilitated by HRV studies in rats. Conventional methods for HRV research in rats rely on conscious state electrocardiogram (ECG) recorded by telemetry (Sgoifo et al., 1996). By applying this approach, we obtained a detailed characterization of autonomic regulation of resting HR in two strains of rats, Wild-type and Wistar (**Figure 2**). Results indicate that Wild-type rats were characterized by higher HR than Wistar counterparts both during the dark/active and light/inactive phases of the daily cycle (**Figure 2**). HRV analysis allowed unveiling the autonomic determinants underlying the differences in resting HR between these two rat strains. As suggested by the vagal indexes RMSSD and HF power, Wild-type rats were clearly characterized by lower levels of vagal modulation compared to Wistar rats (**Figure 2**). Consequently,

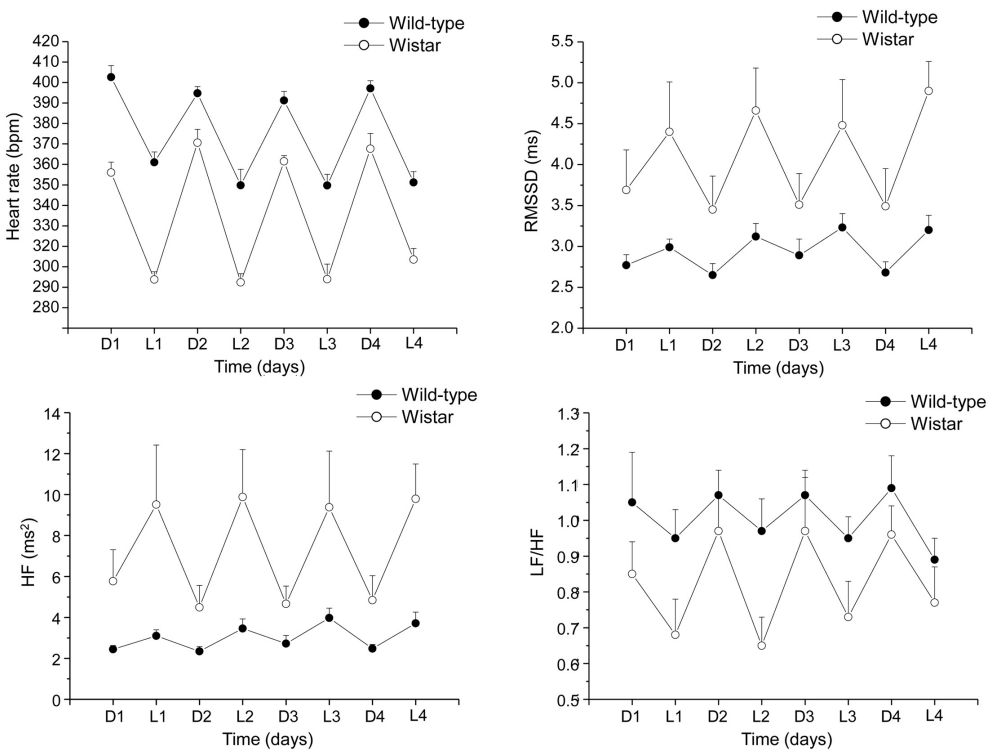

FIGURE 2 | Daily rhythms of heart rate, RMSSD, spectral power in HF band (0.75–2.5 Hz) and LF (0.2–0.75 Hz) to HF ratio in 4-month-old Wild-type ($n = 10$) and Wistar ($n = 10$) rats. These values (reported as means ± s.e.m.) were obtained by averaging multiple 2-min segments acquired every hour during the 12 hours-light and 12 hours-dark phases, as previously described in detail (Carnevali et al., 2013a). Results of Two-Way ANOVA for repeated measures: group difference for heart rate ($F = 103.7$, $p < 0.01$), RMSSD ($F = 6.8$, $p < 0.05$) HF ($F = 7.4$, $p < 0.5$), and LF to HF ratio ($F = 4.4$, $p < 0.05$) values.

in Wild-type rats the sympatho-vagal balance (indexed by the LF to HF ratio) was shifted toward a sympathetic dominance (**Figure 2**). These results indicate that these two rat strains may be viewed as extremes, in terms of cardiac autonomic modulation, within the range of the natural variation of this species, and therefore may represent a valid model for investigating the neurobiological determinants underlying individual differences in resting vagal tone. As discussed before, this inter-individual variability may influence the individual's response to subsequent challenges. Addressing this issue, the two strains were tested under stress conditions (i.e., restraint test). Even though the peak HR reached in response to restraint stress was higher in Wild-type rats, these animals showed reduced overall HR stress responsiveness compared to Wistar rats, as suggested by the values of the area under the response curve above baseline (AUC) (**Figure 3**). In Wild-type rats, this phenomenon appeared to be related to a reduced vagal flexibility, namely a smaller vagal withdrawal in response to stress, and was coupled with a larger, although modest, vulnerability to arrhythmias compared to Wistar counterparts (**Figure 3**). Interestingly, a previous study demonstrated that arrhythmia vulnerability was remarkably larger in Wild-Type than Wistar rats in response to a social stressor (Sgoifo et al., 1998). Collectively, these findings support the view that low levels of vagal modulation of resting HR may be associated with an inability to flexibly generate adequate cardiac responses to stress, even in the absence of evident cardiovascular pathologies.

EFFECTS OF STRESS AND PSYCHOSOCIAL FACTORS ON VAGAL MODULATION OF RESTING HR

Several humans studies provide clear and convincing evidence that chronic life stress, negative personality traits such us aggressiveness, anger, and hostility, anxiety and mood states contribute significantly to the pathogenesis and progression of cardiac disorders (Sloan et al., 1994; Friedman and Thayer, 1998; Rozanski et al., 1999; Gorman and Sloan, 2000; Lucini et al., 2005; Albus, 2010). Putative underlying mechanisms may include a disruption of the sympatho-vagal balance, through an increase in sympathetic activity and/or a decrease in vagal tone. Rat studies on the integration of stress, psychosocial factors, and cardiac autonomic neural control have just started investigating the underlying mechanistic links.

STRESS

Several rat studies have investigated the short- and long-term effects of repeated stress exposure on cardiac autonomic regulation. In a study conducted by Trombini et al. (2012), rats

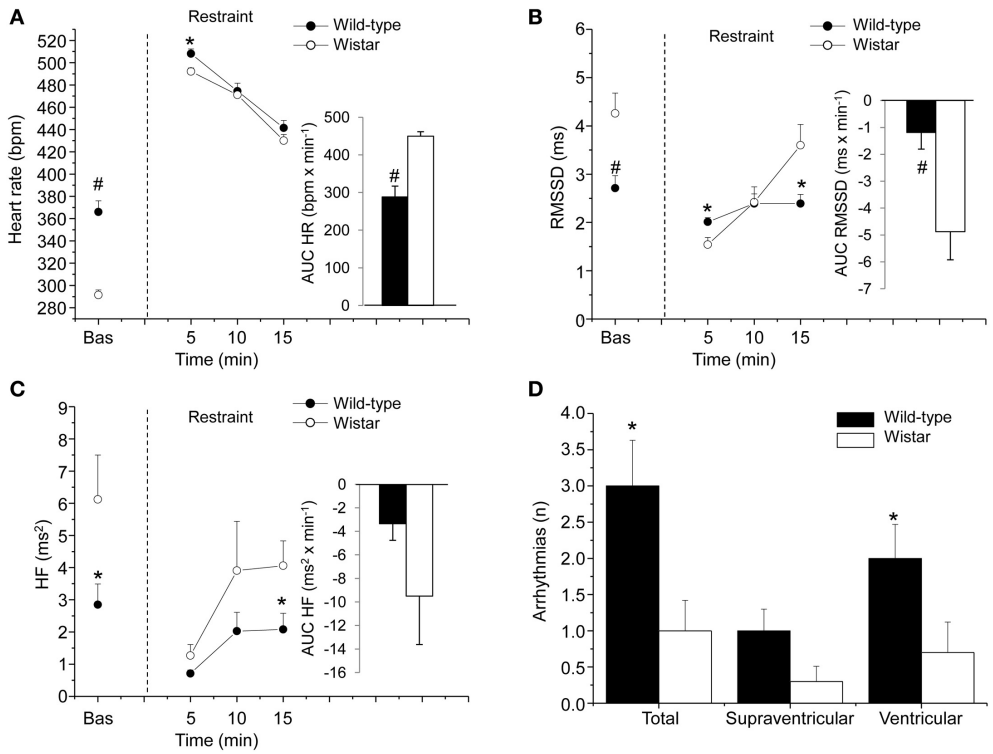

FIGURE 3 | Cardiac response to a restraint stress in 4-month-old Wild-type ($n = 10$) and Wistar ($n = 10$) rats. Panels **(A–C)** show the time course of changes in heart rate, RMSSD values, and high-frequency (HF, 0.75–2.5 Hz) spectral power, respectively, in baseline conditions (bas, 30 min) and during the restraint test (15 min). Each point during the restraint phase is the mean of 5-min intervals. Inner graphs in **(A–C)** represent the area under the response time curve above baseline (AUC) of heart rate, RMSSD, and HF values during the restraint phase. Panel **(D)** illustrates the incidence of cardiac arrhythmias during the restraint phase. Data analysis was conducted as described previously in detail (Carnevali et al., 2013a). Values are reported as means ± s.e.m. * and # indicate a significant difference between HAB and LAB rats ($p < 0.05$ and $p < 0.01$, respectively; Student's *t*-test).

submitted to intermittent episodes of restraint stress showed a prolonged increase in resting vagal drive in the days that followed the end of the stress period, as indicated by the increase in the vagal index RMSSD. Likewise, in another study, sub-chronically stressed rats (5 days of footsocks) exhibited a substantial increase in resting vagal tone that lasted well beyond the duration of the stressor (Carnevali et al., 2011). This peculiar phenomenon might be called "enduring vagal rebound" or "persistent vagal rebound" (Carnevali et al., 2011). In the literature, the term "vagal rebound" usually refers to a short-term vagal hyperactivity following a stressor, a sympathetic overdrive or reperfusion following acute myocardial infarction (Chiladakis et al., 2001; Mezzacappa et al., 2001). In this study (Carnevali et al., 2011), it was a relatively persistent, long-term consequence of repeated stress exposure, whose underlying neurobiological determinants have yet to be determined. However, it appears that this enduring vagal rebound is corticosterone- and serotonin-independent, as it was not prevented by metyrapone (inhibitor of corticosteroid synthesis) or fluoxetine (serotonin-selective reuptake inhibitor) treatments (Carnevali et al., 2011). Such sustained vagal activation after repeated stress exposure has been interpreted as a sign of adaptation, a transient compensatory phenomenon that initially overcomes the commonly observed stress-induced sympathetic hyperactivity (Carnevali et al., 2011). This adaptation might fail after a more prolonged exposure to stress and/or exposure to a more severe stressor, leading the organism to a maladaptive phase of vagal withdrawal and sympathetic dominance. Indeed, like humans, the most pervasive stressors in rats fall within the social domain (Bjorkqvist, 2001; Sgoifo et al., 2014). On this regard, the resident-intruder stress paradigm (Miczek, 1979) is regarded as an ethologically relevant model of psychosocial stress in rats, which involves subjecting a male intruder rat to aggressive threats and overt attacks by an unfamiliar male rat (resident) in the resident's home cage. This stress paradigm has recently been applied to study the shared pathophysiology that links stress-related psychological disorders such as anxiety and depression with cardiovascular disease (Carnevali et al., 2012b, 2013b; Wood et al., 2012; Sevoz-Couche et al., 2013). This raises another important issue that will be discussed in the next sections: the interplay between stress and related psychological disorders in the modulation of resting vagal tone.

ANXIETY

Cardiac autonomic function in rats displaying symptoms of stress-evoked anxiety was investigated in a study conducted by Sevoz-Couche et al. (2013). In this study, HRV analysis was conducted in rats submitted to intermittent daily episodes of social defeat, following a classical resident-intruder paradigm. Five days after the last defeat, stressed rats showed symptoms of an anxiety-like behavior that were accompanied by signs of reduced vagal modulation of resting HR, as suggested by the decrease in the vagal indexes RMSSD and HF power (Sevoz-Couche et al., 2013). Interestingly, such reduction in cardiac vagal tone was prevented by inhibition of the dorsomedial hypothalamus (DMH) with muscimol and the blockade of the NTS 5-HT_3 receptors with granisetron. During acute stress, the DMH acts on the rostral cuneiform nucleus that, in turn, activates the rostral periacqueductal gray (Netzer et al., 2011). Downstream to this structure, serotonin (5-HT) is released to activate 5-HT_3 receptors that are localized presynaptically on NTS vagal afferents (Leslie et al., 1990) (**Figure 1**). When activated, these receptors trigger glutamatergic activation of local GABAergic interneurons that project to the NAmb on pre-ganglionic vagal neurons, thereby inhibiting vagal activity (Loewy, 1990) (**Figure 1**). Therefore, it is reasonable to hypothesize that chronic activation of 5-HT_3 receptors in the NTS due to overactivity of 5-HT in this region and/or hypersensitivity of 5-HT_3 receptors is at the origin of vagal tone impairment in repeatedly stressed animals. These findings highlight the role of DMH and NTS in vagal tone impairment following repeated social challenge.

The alterations in cardiac vagal tone that have been described in this animal model of stress-evoked anxiety may also characterize other forms of anxiety. Addressing this issue, we have recently investigated cardiac autonomic function in a rat model of trait anxiety, namely Wistar rats selectively bred for either high (HAB) or low (LAB) anxiety-related behavior (Carnevali et al., 2014). The HAB/LAB rats have been proved to display robust and consistent differences in their level of baseline anxiety (Landgraf and Wigger, 2002) and therefore represent a valid and reliable model for investigating the autonomic correlates of extremes in anxiety-related behavior. HAB rats clearly displayed a lower vagal modulation of resting HR (indexed by RMSSD and HF values) compared to LAB rats during both the light/inactive and dark/active phases of the daily cycle (**Figure 4**). One of the most puzzling findings was that, despite this evident difference in resting vagal tone, HAB and LAB rats had similar HR values (**Figure 4**). This is indicative of the fact that simple measurements of HR do not necessarily provide accurate insights into the functional regulatory characteristics of the autonomic nervous system. Indeed, mean HR values are indicative of the net effects of sympathetic and vagal influences on cardiac activity, which often result from a combination of concurrent changes in activity within both branches (Berntson et al., 1991). Therefore, it is reasonable to hypothesize that the reduced cardiac vagal tone observed in HAB rats was coupled with a decreased cardiac sympathetic influence on the sinoatrial node compared to LAB rats. Supporting this view, HAB and LAB rats showed similar LF to HF ratio (index of sympatho-vagal balance), suggesting that vagal and sympathetic influences on cardiac pacemaker activity were equally balanced in the two groups, leading to similar HR values (**Figure 4**). When tested under stress conditions (i.e., restraint test), HAB rats showed reduced HR stress responsiveness compared to LAB counterparts, as suggested by the values of the area under the response curve above baseline (AUC) (**Figure 5**). In HAB rats, this phenomenon appeared to be related to a reduced vagal flexibility, namely a smaller vagal withdrawal in response to stress, and was coupled with a tendentially larger vulnerability to ventricular arrhythmias compared to LAB counterparts (**Figure 5**). These findings support the view that a low tonic vagal modulation of HR in HAB rats may have determined an inability to flexibly generate adequate cardiac responses to environmental demands. Furthermore, these findings provide a strong basis for future mechanistic investigation aimed at defining, using this rat model, the central neural determinants of vagal

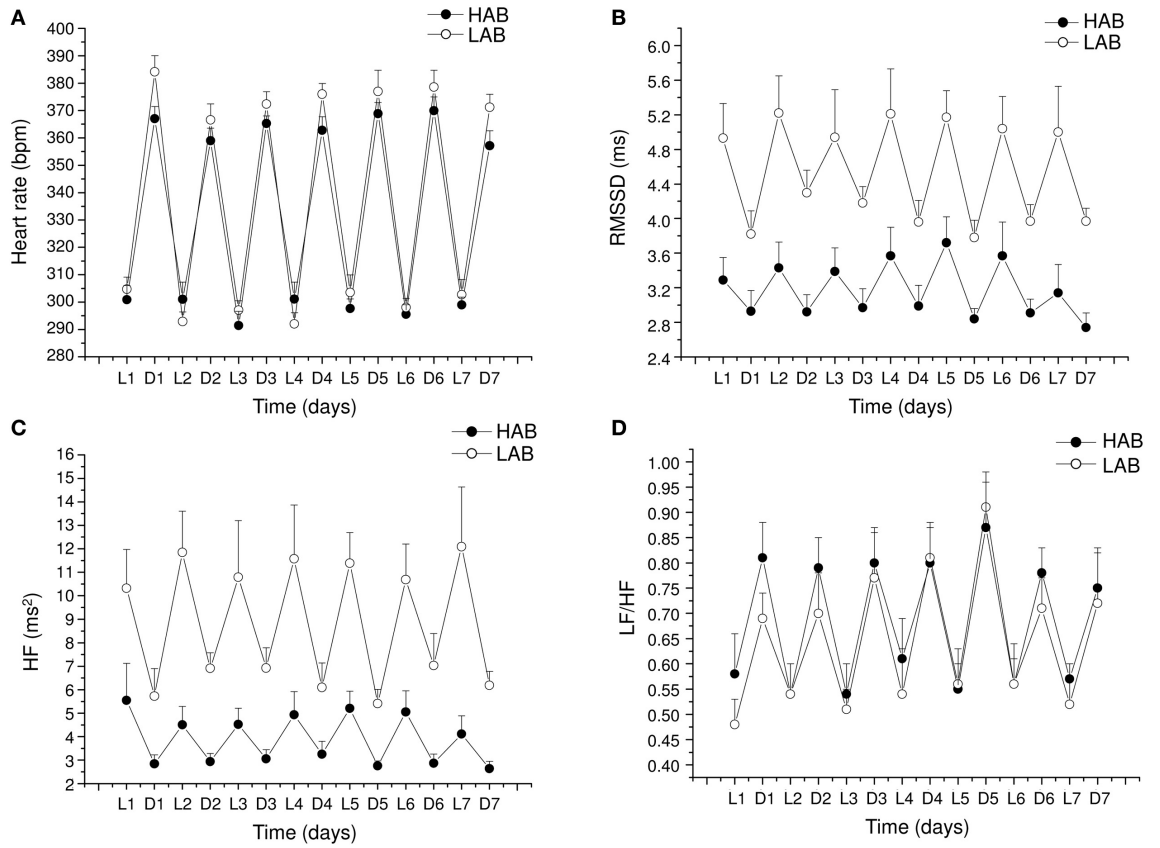

FIGURE 4 | Daily rhythms of (A) heart rate, (B) RMSSD, (C) spectral power in HF band (0.75–2.5 Hz) and (D) LF (0.2–0.75 Hz) to HF ratio in 4-month-old Wistar rats selectively bred for either high (HAB, $n = 10$) or low (LAB, $n = 10$) anxiety-related behavior. These values (reported as means ± SEM) were obtained by averaging multiple 2-min segments acquired every hour during the 12 h-light and 12 h-dark phases, as previously described in detail (Carnevali et al., 2014). Results of two-way ANOVA for repeated measures: group difference for RMSSD ($F = 16.4$, $p < 0.01$) and HF ($F = 17.48$, $p < 0.01$) values. Modified from Carnevali et al. (2014).

control impairment in individuals with high levels of baseline anxiety.

DEPRESSION

The complex interplay among social stress, depression and autonomic neural modulation of HR was investigated in a rat model of social stress by Wood et al. (2012). Rats exposed to 7 consecutive days of social defeat displayed symptoms of a depressive-like state. Forty eight hours after the final social defeat stress, depressed rats showed reduced HRV and signs of sympathetic predominance (increased LF to HF ratio). It was not determined whether such autonomic imbalance was due to a decrease in vagal tone and/or an increase in sympathetic tone. Similar changes in cardiac autonomic neural outflow were observed in rats submitted to a chronic mild stress model of depression (Grippo et al., 2004, 2006). Importantly, such abnormal modulation of resting HR in depressed rats was partially abolished by fluoxetine treatment (Grippo et al., 2006), suggesting a potential involvement of 5-HT neurotransmission in mediating autonomic changes in depressed individuals. Supporting this view, a study conducted by Hildreth et al. (2008) provided evidence of resting vagal tone impairment in a genetic rat model of depression (i.e., the Flinders-Sensitive Line rat) that was related to abnormal serotonergic control of vagal modulation of HR. Human studies indicate that brain 5-HT levels (Mann and Stoff, 1997) and 5-HT receptor function, particularly the 5-HT_{1A} receptors (Parsey et al., 2006), are abnormal in depression. Importantly, it has been demonstrated that chronic social defeat downregulates the 5-HT_{1A} receptor in the rat brain (Kieran et al., 2010). The 5-HT_{1A} receptors play an important role in inhibiting sympathetic neurons located in the medullary raphe area that activates the heart during stress (Nalivaiko, 2006; Nalivaiko and Sgoifo, 2009). In addition to sympathoinhibition, activation of 5-HT_{1A} receptors may have vagomimetic effects (Ngampramuan et al., 2008; Dutschmann et al., 2009; Carnevali et al., 2012a). Indeed, given that vagal preganglionic neurons in the NAmb are under tonic inhibition by GABA-ergic interneurons that express 5-HT_{1A} receptors, activation of such inhibitory 5-HT_{1A} receptors may lead to disinhibition of cardiomotor vagal neurons and consequently increase cardiac vagal tone (Jordan, 2005) (**Figure 1**). Therefore, a decreased density/function of 5-HT_{1A} receptors in these areas may be at the origin of the imbalance in the autonomic neural modulation of resting HR in chronically stressed and depressed individuals. Further work is required in order to determine whether serotonergic mechanisms

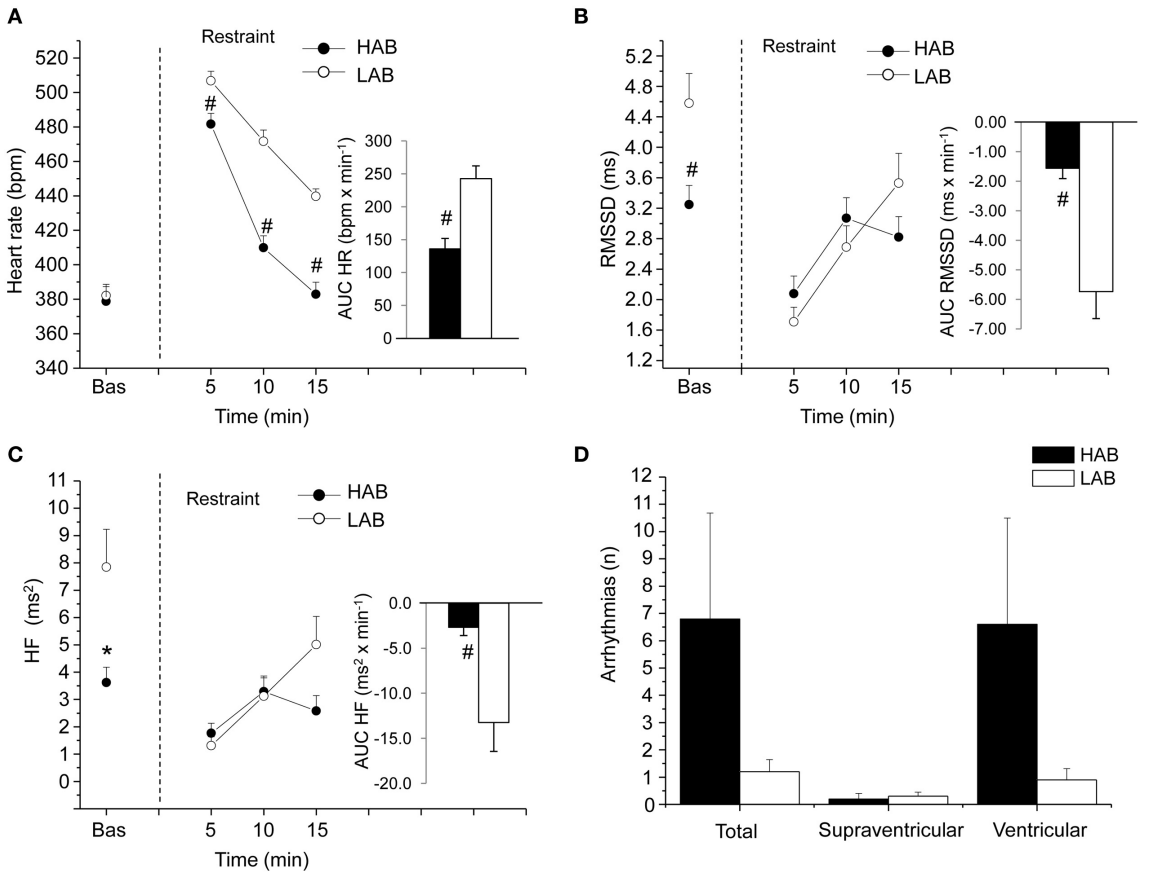

FIGURE 5 | Cardiac response to a restraint stress test in 4-mont-old Wistar rats selectively bred for either high (HAB, $n = 10$) or low (LAB, $n = 10$) anxiety-related behavior. Panels **(A–C)** show the time course of changes in heart rate, RMSSD values and high-frequency (HF, 0.75–2.5 Hz) spectral power in baseline conditions (bas, 30 min) and during the restraint test (15 min), respectively. Each point during the restraint phase is the mean of 5-min intervals. Inner graphs in **(A–C)** represent the area under the response time curve above baseline (AUC) of heart rate, RMSSD and HF values during the restraint phase. Panel **(D)** illustrates the incidence of cardiac arrhythmias during the restraint phase. Values are reported as means ± s.e.m. * and # indicate a significant difference between HAB and LAB rats ($p < 0.05$ and $p < 0.01$, respectively; Student's *t*-test). Modified with permission from Carnevali et al. (2014).

do contribute to driving the changes in autonomic modulation of resting HR in rat models of depression.

AGGRESSIVENESS

The association between personality traits (i.e., aggressiveness) and autonomic neural control of resting HR was the focus of one of our recent investigations (Carnevali et al., 2013a). In this study, high-aggressive (HA) and non-aggressive (NA) rats were selected from a population of adult male Wild-type rats on the basis of their latency time to attack a male intruder in a classical resident-intruder test performed on 3 consecutive days (HA: mean attack latency <90 s; NA: no overt aggression during the three tests, each lasting 600 s) (Koolhaas et al., 2013). As suggested by RMSSD and HF power indexes, HA rats exhibited lower levels of vagal modulation of resting HR during both the light/inactive and dark/active active phases of the daily cycle (**Figure 6**). However, despite different levels of resting vagal tone, HA and NA rats had similar HR values (**Figure 6**). Similarly to what has been argued above, it has been hypothesized that the reduced cardiac vagal tone observed in HA rats was coupled with a decreased sympathetic influence on sinoatrial node activity compared to NA rats. Consequently, vagal and sympathetic influences were equally balanced in the two groups (LF to HF ratio), leading to similar HR values (**Figure 6**) (Carnevali et al., 2013a). When tested under stress conditions (i.e., restraint and psychosocial stress test), HA and NA rats showed similar tachycardic responses. However, under these conditions HA rats displayed lower vagal antagonism and larger incidence of tachyarrhythmias compared to NA rats (**Figure 7**) (Carnevali et al., 2013a). In addition, injection of beta adrenergic agonist isoproterenol provoked a much larger incidence of ventricular tachyarrhythmias in HA rats compared to NA counterparts (Carnevali et al., 2013a) (**Figure 7**). The results of this study support the view that low levels of vagal modulation of resting HR are associated with arrhythmia susceptibility that may predict vulnerability to cardiac morbidity and mortality. Future mechanistic experiments using this rat model are needed in order to determine the neural determinants of vagal control impairment in aggressive individuals.

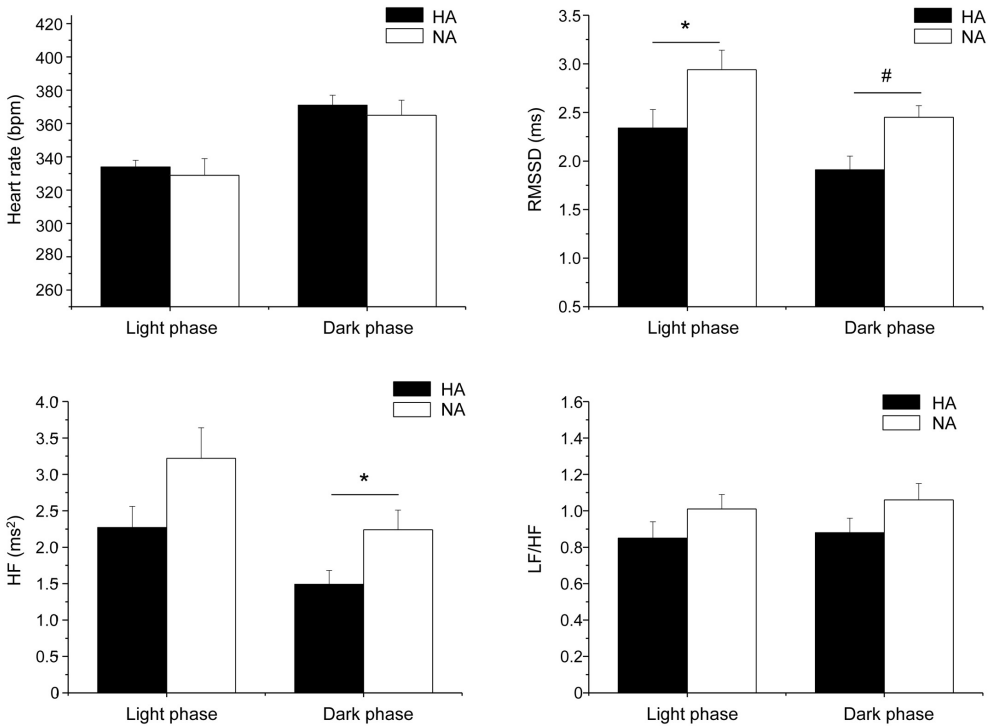

FIGURE 6 | Daily rhythms of heart rate, RMSSD, spectral power in HF band (0.75–2.5 Hz) and LF (0.2–0.75 Hz) to HF ratio in high-aggressive (HA, $n = 10$) and non-aggressive (NA, $n = 10$) Wild-type rats. For the 12 hours-light and 12 hours-dark phases, values are reported as means ± s.e.m. of data obtained by averaging multiple 2-min segments acquired every hour over a period of 6 days. * and # indicate a significant difference between HAB and LAB rats (Student's *t*-test, $p < 0.05$ and $p < 0.01$, respectively). Modified with permission from Carnevali et al. (2013a).

EFFECTS OF PHYSICAL EXERCISE ON VAGAL MODULATION OF RESTING HR

Human studies provide evidence that regular physical exercise increases life expectancy in healthy individuals and reduces cardiac-related events in patients with coronary heart disease and heart failure (Rosenwinkel et al., 2001; Buch et al., 2002). The cardioprotective effects of physical training may be mediated, at least in part, by an increase in vagal modulation of resting HR (Crimi et al., 2009). However, central neural mechanisms that underlie these exercise-induced effects on vagal tone are not well understood. Cardiac autonomic adaptation induced by physical training has been subject of research in several carefully designed and rigorously conducted rat studies. These studies reported a clear decrease in resting HR in rats submitted to training with aerobic exercises (i.e., swimming, running) (Medeiros et al., 2004; Rossi et al., 2009; Souza et al., 2009; Sant'Ana et al., 2011; Neto et al., 2013). The effect of the autonomic nervous system in mediating training-induced resting bardycardia was investigated by means of (i) pharmacological approaches (after cardiac muscarinic and adrenergic blockade) and (ii) HRV analysis. Resting bradycardia after exercise training was mainly explained by a vagal effect (Medeiros et al., 2004) or by a reduction in intrinsic HR accompanied by a moderate increase in vagal modulation (indexed by HF spectral power values) (Rossi et al., 2009; Souza et al., 2009; Sant'Ana et al., 2011; Neto et al., 2013).

One possible limitation of these studies is that they applied protocols of forced exercise (treadmill or swimming). In doing so, the effects of physical exercise *per se* on cardiac autonomic function might have been confounded by the effects of accompanying psychological stress (the effects of repeated stress exposure on vagal modulation have been discussed before). On this regard, an elegant study conducted by Beig et al. (2011) investigated the effects of voluntary running on cardiac autonomic function. This study addressed another important issue: that is, whether the effects of voluntary exercise on autonomic function were long-lasting. This question is of major relevance, since loss of exercise-induced cardioprotection has been described soon after exercise cessation (Lin and Horvath, 1972). Rats submitted to a protocol of voluntary exercise exhibited resting bradycardia associated with increased vagal modulation (indexed by HF spectral power values) (Beig et al., 2011). Importantly, these effects persisted 10–12 days after termination of the training protocol, suggesting that voluntary exercise has an enduring effect on resting vagal tone.

Interestingly, voluntary training did not affect stress-induced tachycardia, but augmented resistance to cardiac arrhythmias, as evidenced by the higher doses of the proarrhythmic drug aconitine needed to provoke arrhythmic effects in trained rats (Beig et al., 2011). Such antiarrhythmic effect of exercise training seems to reflect electrophysiological changes (i.e., prolongation of the effective refractory period) induced by long-lasting increase

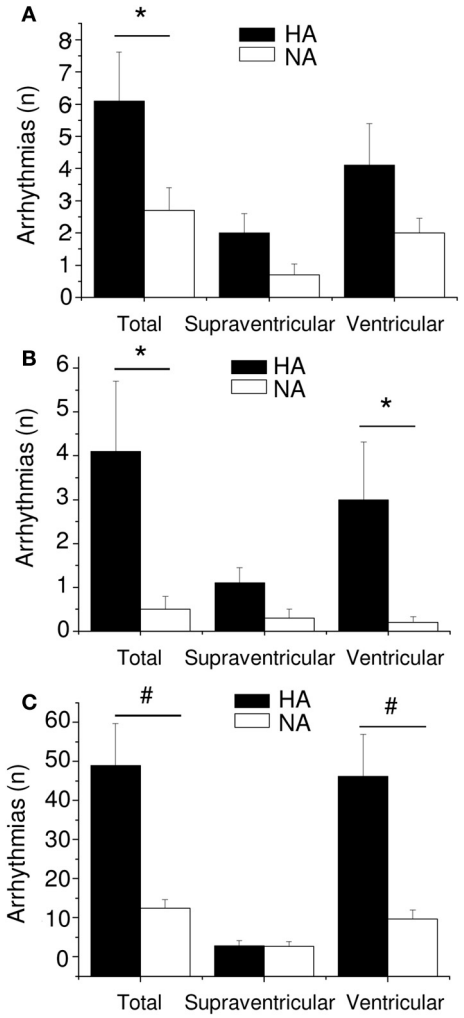

FIGURE 7 | Incidence of arrhythmias in high-aggressive (HA, $n = 10$) and non-aggressive (NA, $n = 10$) Wild-type rats during a restraint test (panel A), a psychosocial stress test (panel B) and following β-adrenoceptor pharmacological stimulation with isoproterenol (panel C). Values are reported as mean ± s.e.m. of number of events (n) per 15-min recording period. * and # indicate a significant difference between HA and NA rats ($p < 0.05$ and $p < 0.01$, respectively). Reproduced with permission from Carnevali et al. (2013a).

in vagal outflow in trained rats (Beig et al., 2011). This suggests that the benefits of increased resting vagal tone induced by exercise training may be attributed to (i) an HR-lowering effect and (ii) other parasympathetic effects on the electrophysiological properties of the myocardium. However, the mechanistic links whereby physical training provokes changes in resting vagal outflow are currently unknown. One hypothesis is that exercise might reduce tonic $GABA_A$ergic inhibition of neurons in the NTS involved in HR control (**Figure 1**), therefore increasing vagal influences on cardiac peacemaker activity (Mueller and Hasser, 2006). Given that physical exercise increases central 5-HT synthesis (Blomstrand et al., 1989), and that central 5-HT increases vagal modulation in conscious rats (Ngampramuan et al., 2008), it is tempting to speculate that activation of inhibitory $5\text{-}HT_{1A}$ receptors located on GABA-ergic interneurons in the Namb might mediate disinhibition of cardiomotor vagal neurons in that area (**Figure 1**). Further work is required in order to (i) evaluate potential correlations between duration/intensity of voluntary exercise and effects on resting vagal tone and (ii) provide a more comprehensive understanding of central neural mechanisms underlying exercise-induced vagal tone enhancement.

CONCLUDING REMARK

The influence of vagal tone on resting HR is highly variable among individuals. Several psychosocial and physiological conditions are thought to contribute significantly to this large inter-individual variability. From the data summarized in this paper, it is quite evident that rat studies can be extremely useful for investigating (i) the effects of stress, psychosocial factors, and physical exercise on vagal modulation of resting HR, and (ii) the consequences of such effects on cardiac stress reactivity and arrhythmia vulnerability. Moreover, the results of these studies have provided preliminary insights into the central neural mechanisms underlying vagal tone impairment/enhancement, which may be exploited by future experiments aimed at developing pharmacological approaches for enhancing vagal activity and cardioprotection.

REFERENCES

Albus, C. (2010). Psychological and social factors in coronary heart disease. *Ann. Med.* 42, 487–494. doi: 10.3109/07853890.2010.515605

Beauchaine, T. (2001). Vagal tone, development, and Gray's motivational theory: toward an integrated model of autonomic nervous system functioning in psychopathology. *Dev. Psychopathol.* 13, 183–214. doi: 10.1017/S0954579401002012

Beig, M. I., Callister, R., Saint, D. A., Bondarenko, E., Walker, F. R., Day, T. A., et al. (2011). Voluntary exercise does not affect stress-induced tachycardia, but improves resistance to cardiac arrhythmias in rats. *Clin. Exp. Pharmacol. Physiol.* 38, 19–26. doi: 10.1111/j.1440-1681.2010.05456.x

Berntson, G. G., Bigger, J. T. Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Berntson, G. G., Cacioppo, J. T., and Quigley, K. S. (1991). Autonomic determinism: the modes of autonomic control, the doctrine of autonomic space, and the laws of autonomic constraint. *Psychol. Rev.* 98, 459–487. doi: 10.1037/0033-295X.98.4.459

Bigger, J. T. Jr., Steinman, R. C., Rolnitzky, L. M., Fleiss, J. L., Albrecht, P., and Cohen, R. J. (1996). Power law behavior of RR-interval variability in healthy middle-aged persons, patients with recent acute myocardial infarction, and patients with heart transplants. *Circulation* 93, 2142–2151. doi: 10.1161/01.CIR.93.12.2142

Billman, G. E. (2011). Heart rate variability - a historical perspective. *Front. Physiol.* 2:86. doi: 10.3389/fphys.2011.00086

Bjorkqvist, K. (2001). Social defeat as a stressor in humans. *Physiol. Behav.* 73, 435–442. doi: 10.1016/S0031-9384(01)00490-5

Blomstrand, E., Perrett, D., Parry-Billings, M., and Newsholme, E. A. (1989). Effect of sustained exercise on plasma amino acid concentrations and on 5-hydroxytryptamine metabolism in six different brain regions in the rat. *Acta Physiol. Scand.* 136, 473–481. doi: 10.1111/j.1748-1716.1989.tb08689.x

Blumenthal, J. A., Sherwood, A., Rogers, S. D., Babyak, M. A., Doraiswamy, P. M., Watkins, L., et al. (2007). Understanding prognostic benefits of exercise and antidepressant therapy for persons with depression and heart disease: the UPBEAT study–rationale, design, and methodological issues. *Clin. Trials* 4, 548–559. doi: 10.1177/1740774507083388

Bryniarski, L., Kawecka-Jaszcz, K., Bacior, B., Grodecki, J., and Rajzer, M. (1997). Effect of exercise rehabilitation on heart rate variability in hypertensives after myocardial infarction. *J. Hypertens.* 15, 1739–1743. doi: 10.1097/00004872-199715120-00082

Buch, A. N., Coote, J. H., and Townend, J. N. (2002). Mortality, cardiac vagal control and physical training–what's the link? *Exp. Physiol.* 87, 423–435. doi: 10.1111/j.1469-445X.2002.tb00055.x

Carnevali, L., Bondarenko, E., Sgoifo, A., Walker, F. R., Head, G. A., Lukoshkova, E. V., et al. (2011). Metyrapone and fluoxetine suppress enduring behavioral but not cardiac effects of subchronic stress in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 301, R1123–1131. doi: 10.1152/ajpregu.00273.2011

Carnevali, L., Mastorci, F., Audero, E., Graiani, G., Rossi, S., Macchi, E., et al. (2012a). Stress-induced susceptibility to sudden cardiac death in mice with altered serotonin homeostasis. *PLoS ONE* 7:e41184. doi: 10.1371/journal.pone.0041184

Carnevali, L., Mastorci, F., Graiani, G., Razzoli, M., Trombini, M., Pico-Alfonso, M. A., et al. (2012b). Social defeat and isolation induce clear signs of a depression-like state, but modest cardiac alterations in wild-type rats. *Physiol. Behav.* 106, 142–150. doi: 10.1016/j.physbeh.2012.01.022

Carnevali, L., Trombini, M., Graiani, G., Madeddu, D., Quaini, F., Landgraf, R., et al. (2014). Low vagally-mediated heart rate variability and increased susceptibility to ventricular arrhythmias in rats bred for high anxiety. *Physiol. Behav.* 128C, 16–25. doi: 10.1016/j.physbeh.2014.01.033

Carnevali, L., Trombini, M., Porta, A., Montano, N., De Boer, S. F., and Sgoifo, A. (2013a). Vagal withdrawal and susceptibility to cardiac arrhythmias in rats with high trait aggressiveness. *PLoS ONE* 8:e68316. doi: 10.1371/journal.pone.0068316

Carnevali, L., Trombini, M., Rossi, S., Graiani, G., Manghi, M., Koolhaas, J. M., et al. (2013b). Structural and electrical myocardial remodeling in a rodent model of depression. *Psychosom. Med.* 75, 42–51. doi: 10.1097/PSY.0b013e318276cb0d

Cheng, Z., and Powley, T. L. (2000). Nucleus ambiguus projections to cardiac ganglia of rat atria: an anterograde tracing study. *J. Comp. Neurol.* 424, 588–606. doi: 10.1002/1096-9861(20000904)424:4<588::AID-CNE3>3.0.CO;2-7

Cheng, Z., Powley, T. L., Schwaber, J. S., and Doyle, F. J. 3rd. (1999). Projections of the dorsal motor nucleus of the vagus to cardiac ganglia of rat atria: an anterograde tracing study. *J. Comp. Neurol.* 410, 320–341.

Chiladakis, J. A., Pashalis, A., Patsouras, N., and Manolis, A. S. (2001). Autonomic patterns preceding and following accelerated idioventricular rhythm in acute myocardial infarction. *Cardiology* 96, 24–31. doi: 10.1159/000047382

Crimi, E., Ignarro, L. J., Cacciatore, F., and Napoli, C. (2009). Mechanisms by which exercise training benefits patients with heart failure. *Nat. Rev. Cardiol.* 6, 292–300. doi: 10.1038/nrcardio.2009.8

Dutschmann, M., Waki, H., Manzke, T., Simms, A. E., Pickering, A. E., Richter, D. W., et al. (2009). The potency of different serotonergic agonists in counteracting opioid evoked cardiorespiratory disturbances. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 2611–2623. doi: 10.1098/rstb.2009.0076

El-Sheikh, M., Harger, J., and Whitson, S. M. (2001). Exposure to interparental conflict and children's adjustment and physical health: the moderating role of vagal tone. *Child Dev.* 72, 1617–1636. doi: 10.1111/1467-8624.00369

Esler, M. (1992). The autonomic nervous system and cardiac arrhythmias. *Clin. Auton. Res.* 2, 133–135. doi: 10.1007/BF01819669

Friedman, B. H., and Thayer, J. F. (1998). Anxiety and autonomic flexibility: a cardiovascular approach. *Biol. Psychol.* 47, 243–263. doi: 10.1016/S0301-0511(97)00027-6

Fu, Q., and Levine, B. D. (2013). Exercise and the autonomic nervous system. *Handb. Clin. Neurol.* 117, 147–160. doi: 10.1016/B978-0-444-53491-0.00013-4

Gorman, J. M., and Sloan, R. P. (2000). Heart rate variability in depressive and anxiety disorders. *Am. Heart J.* 140, 77–83. doi: 10.1067/mhj.2000.109981

Grippo, A. J., Beltz, T. G., Weiss, R. M., and Johnson, A. K. (2006). The effects of chronic fluoxetine treatment on chronic mild stress-induced cardiovascular changes and anhedonia. *Biol. Psychiatry* 59, 309–316. doi: 10.1016/j.biopsych.2005.07.010

Grippo, A. J., Santos, C. M., Johnson, R. F., Beltz, T. G., Martins, J. B., Felder, R. B., et al. (2004). Increased susceptibility to ventricular arrhythmias in a rodent model of experimental depression. *Am. J. Physiol. Heart Circ. Physiol.* 286, H619–H626. doi: 10.1152/ajpheart.00450.2003

Hildreth, C. M., Padley, J. R., Pilowsky, P. M., and Goodchild, A. K. (2008). Impaired serotonergic regulation of heart rate may underlie reduced baroreflex sensitivity in an animal model of depression. *Am. J. Physiol. Heart Circ. Physiol.* 294, H474–H480. doi: 10.1152/ajpheart.01009.2007

Huikuri, H. V., Makikallio, T. H., Airaksinen, K. E., Seppanen, T., Puukka, P., Raiha, I. J., et al. (1998). Power-law relationship of heart rate variability as a predictor of mortality in the elderly. *Circulation* 97, 2031–2036. doi: 10.1161/01.CIR.97.20.2031

Huikuri, H. V., Makikallio, T. H., Peng, C. K., Goldberger, A. L., Hintze, U., and Moller, M. (2000). Fractal correlation properties of R-R interval dynamics and mortality in patients with depressed left ventricular function after an acute myocardial infarction. *Circulation* 101, 47–53. doi: 10.1161/01.CIR.101.1.47

Iellamo, F., Legramante, J. M., Massaro, M., Raimondi, G., and Galante, A. (2000). Effects of a residential exercise training on baroreflex sensitivity and heart rate variability in patients with coronary artery disease: a randomized, controlled study. *Circulation* 102, 2588–2592. doi: 10.1161/01.CIR.102.21.2588

Jordan, D. (2005). Vagal control of the heart: central serotonergic (5-HT) mechanisms. *Exp. Physiol.* 90, 175–181. doi: 10.1113/expphysiol.2004.029058

Jose, A. D., and Collison, D. (1970). The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc. Res.* 4, 160–167. doi: 10.1093/cvr/4.2.160

Kieran, N., Ou, X. M., and Iyo, A. H. (2010). Chronic social defeat downregulates the 5-HT1A receptor but not Freud-1 or NUDR in the rat prefrontal cortex. *Neurosci. Lett.* 469, 380–384. doi: 10.1016/j.neulet.2009.12.032

Kleiger, R. E., Stein, P. K., Bosner, M. S., and Rottman, J. N. (1992). Time domain measurements of heart rate variability. *Cardiol. Clin.* 10, 487–498.

Kok, B. E., and Fredrickson, B. L. (2010). Upward spirals of the heart: autonomic flexibility, as indexed by vagal tone, reciprocally and prospectively predicts positive emotions and social connectedness. *Biol. Psychol.* 85, 432–436. doi: 10.1016/j.biopsycho.2010.09.005

Koolhaas, J. M., Coppens, C. M., De Boer, S. F., Buwalda, B., Meerlo, P., and Timmermans, P. J. (2013). The resident-intruder paradigm: a standardized test for aggression, violence and social stress. *J. Vis. Exp.* 4:e4367. doi: 10.3791/4367

Landgraf, R., and Wigger, A. (2002). High vs low anxiety-related behavior rats: an animal model of extremes in trait anxiety. *Behav. Genet.* 32, 301–314. doi: 10.1023/A:1020258104318

La Rovere, M. T., Bigger, J. T. Jr., Marcus, F. I., Mortara, A., and Schwartz, P. J. (1998). Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet* 351, 478–484. doi: 10.1016/S0140-6736(97)11144-8

Leslie, R. A., Reynolds, D. J., Andrews, P. L., Grahame-Smith, D. G., Davis, C. J., and Harvey, J. M. (1990). Evidence for presynaptic 5-hydroxytryptamine3 recognition sites on vagal afferent terminals in the brainstem of the ferret. *Neuroscience* 38, 667–673. doi: 10.1016/0306-4522(90)90060-H

Levy, M. N. (1971). Sympathetic-parasympathetic interactions in the heart. *Circ. Res.* 29, 437–445. doi: 10.1161/01.RES.29.5.437

Levy, M. N., and Schwartz, P. J. (1994). *Vagal Control of the Heart: Experimental Basis and Clinical Implications.* Armonk, NY: Futura Publishing Co.

Lin, Y. C., and Horvath, S. M. (1972). Autonomic nervous control of cardiac frequency in the exercise-trained rat. *J. Appl. Physiol.* 33, 796–799.

Loewy, A. D. (1990). "Anatomy of the autonomic nervous system: an overview," in *Central Regulation of the Autonomic Function,* eds A. D. Loewy and K. M. Spyer (New York, NY: Oxford University Press), 1–16.

Lombardi, F., Sandrone, G., Mortara, A., Torzillo, D., La Rovere, M. T., Signorini, M. G., et al. (1996). Linear and nonlinear dynamics of heart rate variability after acute myocardial infarction with normal and reduced left ventricular ejection fraction. *Am. J. Cardiol.* 77, 1283–1288. doi: 10.1016/S0002-9149(96)00193-2

Lucini, D., Di Fede, G., Parati, G., and Pagani, M. (2005). Impact of chronic psychosocial stress on autonomic cardiovascular regulation in otherwise healthy subjects. *Hypertension* 46, 1201–1206. doi: 10.1161/01.HYP.0000185147.32385.4b

Makikallio, T. H., Hoiber, S., Kober, L., Torp-Pedersen, C., Peng, C. K., Goldberger, A. L., et al. (1999). Fractal analysis of heart rate dynamics as a predictor of mortality in patients with depressed left ventricular function after acute myocardial infarction. TRACE Investigators. TRAndolapril Cardiac Evaluation. *Am. J. Cardiol.* 83, 836–839. doi: 10.1016/S0002-9149(98)01076-5

Makikallio, T. H., Seppanen, T., Airaksinen, K. E., Koistinen, J., Tulppo, M. P., Peng, C. K., et al. (1997). Dynamic analysis of heart rate may predict subsequent ventricular tachycardia after myocardial infarction. *Am. J. Cardiol.* 80, 779–783. doi: 10.1016/S0002-9149(97)00516-X

Mann, J. J., and Stoff, D. M. (1997). A synthesis of current findings regarding neurobiological correlates and treatment of suicidal behavior. *Ann. N.Y. Acad. Sci.* 836, 352–363. doi: 10.1111/j.1749-6632.1997.tb52370.x

Medeiros, A., Oliveira, E. M., Gianolla, R., Casarini, D. E., Negrao, C. E., and Brum, P. C. (2004). Swimming training increases cardiac vagal activity and induces cardiac hypertrophy in rats. *Braz. J. Med. Biol. Res.* 37, 1909–1917. doi: 10.1590/S0100-879X2004001200018

Mendelowitz, D. (1996). Firing properties of identified parasympathetic cardiac neurons in nucleus ambiguus. *Am. J. Physiol.* 271, H2609–H2614.

Mezzacappa, E. S., Kelsey, R. M., Katkin, E. S., and Sloan, R. P. (2001). Vagal rebound and recovery from psychological stress. *Psychosom. Med.* 63, 650–657.

Miczek, K. A. (1979). A new test for aggression in rats without aversive stimulation: differential effects of d-amphetamine and cocaine. *Psychopharmacology* 60, 253–259. doi: 10.1007/BF00426664

Mueller, P. J., and Hasser, E. M. (2006). Putative role of the NTS in alterations in neural control of the circulation following exercise training in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290, R383–R392. doi: 10.1152/ajpregu.00455.2005

Nalivaiko, E. (2006). 5-HT(1A) receptors in stress-induced cardiac changes: a possible link between mental and cardiac disorders. *Clin. Exp. Pharmacol. Physiol.* 33, 1259–1264. doi: 10.1111/j.1440-1681.2006.04521.x

Nalivaiko, E., and Sgoifo, A. (2009). Central 5-HT receptors in cardiovascular control during stress. *Neurosci. Biobehav. Rev.* 33, 95–106. doi: 10.1016/j.neubiorev.2008.05.026

Neto, O. B., Abate, D. T., Junior, M. M., Mota, G. R., Orsatti, F. L., Silva, R. C., et al. (2013). Exercise training improves cardiovascular autonomic activity and attenuates renal damage in spontaneously hypertensive rats. *J. Sports Sci. Med.* 12, 52–59.

Netzer, F., Bernard, J. F., Verberne, A. J., Hamon, M., Camus, F., Benoliel, J. J., et al. (2011). Brain circuits mediating baroreflex bradycardia inhibition in rats: an anatomical and functional link between the cuneiform nucleus and the periaqueductal grey. *J. Physiol.* 589, 2079–2091. doi: 10.1113/jphysiol.2010.203737

Ngampramuan, S., Baumert, M., Beig, M. I., Kotchabhakdi, N., and Nalivaiko, E. (2008). Activation of 5-HT(1A) receptors attenuates tachycardia induced by restraint stress in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294, R132–R141. doi: 10.1152/ajpregu.00464.2007

Nolan, R. P., Jong, P., Barry-Bianchi, S. M., Tanaka, T. H., and Floras, J. S. (2008). Effects of drug, biobehavioral and exercise therapies on heart rate variability in coronary artery disease: a systematic review. *Eur. J. Cardiovasc. Prev. Rehabil.* 15, 386–396. doi: 10.1097/HJR.0b013e3283030a97

Parsey, R. V., Oquendo, M. A., Ogden, R. T., Olvet, D. M., Simpson, N., Huang, Y. Y., et al. (2006). Altered serotonin 1A binding in major depression: a [carbonyl-C-11]WAY100635 positron emission tomography study. *Biol. Psychiatry* 59, 106–113. doi: 10.1016/j.biopsych.2005.06.016

Porges, S. W. (1995a). Cardiac vagal tone: a physiological index of stress. *Neurosci. Biobehav. Rev.* 19, 225–233. doi: 10.1016/0149-7634(94)00066-A

Porges, S. W. (1995b). Orienting in a defensive world: mammalian modifications of our evolutionary heritage. A Polyvagal Theory. *Psychophysiology* 32, 301–318. doi: 10.1111/j.1469-8986.1995.tb01213.x

Porges, S. W. (2007). The polyvagal perspective. *Biol. Psychol.* 74, 116–143. doi: 10.1016/j.biopsycho.2006.06.009

Rennie, K. L., Hemingway, H., Kumari, M., Brunner, E., Malik, M., and Marmot, M. (2003). Effects of moderate and vigorous physical activity on heart rate variability in a British study of civil servants. *Am. J. Epidemiol.* 158, 135–143. doi: 10.1093/aje/kwg120

Rentero, N., Cividjian, A., Trevaks, D., Pequignot, J. M., Quintin, L., and McAllen, R. M. (2002). Activity patterns of cardiac vagal motoneurons in rat nucleus ambiguus. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 283, R1327–R1334. doi: 10.1152/ajpregu.00271.2002

Reyes Del Paso, G. A., Langewitz, W., Mulder, L. J., Van Roon, A., and Duschek, S. (2013). The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology* 50, 477–487. doi: 10.1111/psyp.12027

Rosenwinkel, E. T., Bloomfield, D. M., Arwady, M. A., and Goldsmith, R. L. (2001). Exercise and autonomic function in health and cardiovascular disease. *Cardiol. Clin.* 19, 369–387. doi: 10.1016/S0733-8651(05)70223-X

Rossi, B. R., Mazer, D., Silveira, L. C., Jacinto, C. P., Di Sacco, T. H., Blanco, J. H., et al. (2009). Physical exercise attenuates the cardiac autonomic deficit induced by nitric oxide synthesis blockade. *Arq. Bras. Cardiol.* 92, 31–38. doi: 10.1590/S0066-782X2009000100006

Rozanski, A., Blumenthal, J. A., and Kaplan, J. (1999). Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation* 99, 2192–2217. doi: 10.1161/01.CIR.99.16.2192

Sabbah, H. N., Ilsar, I., Zaretsky, A., Rastogi, S., Wang, M., and Gupta, R. C. (2011). Vagus nerve stimulation in experimental heart failure. *Heart Fail. Rev.* 16, 171–178. doi: 10.1007/s10741-010-9209-z

Sant'Ana, J. E., Pereira, M. G., Dias Da Silva, V. J., Dambros, C., Costa-Neto, C. M., and Souza, H. C. (2011). Effect of the duration of daily aerobic physical training on cardiac autonomic adaptations. *Auton. Neurosci.* 159, 32–37. doi: 10.1016/j.autneu.2010.07.006

Schwartz, P. J., Vanoli, E., Stramba-Badiale, M., De Ferrari, G. M., Billman, G. E., and Foreman, R. D. (1988). Autonomic mechanisms and sudden death. New insights from analysis of baroreceptor reflexes in conscious dogs with and without a myocardial infarction. *Circulation* 78, 969–979. doi: 10.1161/01.CIR.78.4.969

Sevoz-Couche, C., Brouillard, C., Camus, F., Laude, D., De Boer, S. F., Becker, C., et al. (2013). Involvement of the dorsomedial hypothalamus and the nucleus tractus solitarii in chronic cardiovascular changes associated with anxiety in rats. *J. Physiol.* 591, 1871–1887. doi: 10.1113/jphysiol.2012.247791

Sgoifo, A., Carnevali, L., and Grippo, A. J. (2014). The socially stressed heart. Insights from studies in rodents. *Neurosci. Biobehav. Rev.* 39C, 51–60. doi: 10.1016/j.neubiorev.2013.12.005

Sgoifo, A., De Boer, S. F., Buwalda, B., Korte-Bouws, G., Tuma, J., Bohus, B., et al. (1998). Vulnerability to arrhythmias during social stress in rats with different sympathovagal balance. *Am. J. Physiol.* 275, H460–H466.

Sgoifo, A., Stilli, D., Medici, D., Gallo, P., Aimi, B., and Musso, E. (1996). Electrode positioning for reliable telemetry ECG recordings during social stress in unrestrained rats. *Physiol. Behav.* 60, 1397–1401. doi: 10.1016/S0031-9384(96)00228-4

Sloan, R. P., Shapiro, P. A., Bigger, J. T. Jr., Bagiella, E., Steinman, R. C., and Gorman, J. M. (1994). Cardiac autonomic control and hostility in healthy subjects. *Am. J. Cardiol.* 74, 298–300. doi: 10.1016/0002-9149(94)90382-4

Smeets, T., Giesbrecht, T., Raymaekers, L., Shaw, J., and Merckelbach, H. (2010). Autobiographical integration of trauma memories and repressive coping predict post-traumatic stress symptoms in undergraduate students. *Clin. Psychol. Psychother.* 17, 211–218. doi: 10.1002/cpp.644

Smith, M. L., Hudson, D. L., Graitzer, H. M., and Raven, P. B. (1989). Exercise training bradycardia: the role of autonomic balance. *Med. Sci. Sports Exerc.* 21, 40–44. doi: 10.1249/00005768-198902000-00008

Soares-Miranda, L., Sandercock, G., Valente, H., Vale, S., Santos, R., and Mota, J. (2009). Vigorous physical activity and vagal modulation in young adults. *Eur. J. Cardiovasc. Prev. Rehabil.* 16, 705–711. doi: 10.1097/HJR.0b013e3283316cd1

Souza, G. G., Magalhaes, L. N., Cruz, T. A., Mendonca-De-Souza, A. C., Duarte, A. F., Fischer, N. L., et al. (2013). Resting vagal control and resilience as predictors of cardiovascular allostasis in peacekeepers. *Stress* 16, 377–383. doi: 10.3109/10253890.2013.767326

Souza, H. C., De Araujo, J. E., Martins-Pinge, M. C., Cozza, I. C., and Martins-Dias, D. P. (2009). Nitric oxide synthesis blockade reduced the baroreflex sensitivity in trained rats. *Auton. Neurosci.* 150, 38–44. doi: 10.1016/j.autneu.2009.04.007

Stein, P. K., Bosner, M. S., Kleiger, R. E., and Conger, B. M. (1994). Heart rate variability: a measure of cardiac autonomic tone. *Am. Heart J.* 127, 1376–1381. doi: 10.1016/0002-8703(94)90059-0

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circ. Res.* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Trombini, M., Hulshof, H. J., Graiani, G., Carnevali, L., Meerlo, P., Quaini, F., et al. (2012). Early maternal separation has mild effects on cardiac autonomic balance and heart structure in adult male rats. *Stress* 15, 457–470. doi: 10.3109/10253890.2011.639414

Tsai, M. W., Chie, W. C., Kuo, T. B., Chen, M. F., Liu, J. P., Chen, T. T., et al. (2006). Effects of exercise training on heart rate variability after coronary angioplasty. *Phys. Ther.* 86, 626–635.

Volders, P. G. (2010). Novel insights into the role of the sympathetic nervous system in cardiac arrhythmogenesis. *Heart Rhythm* 7, 1900–1906. doi: 10.1016/j.hrthm.2010.06.003

Wood, S. K., McFadden, K. V., Grigoriadis, D., Bhatnagar, S., and Valentino, R. J. (2012). Depressive and cardiovascular disease comorbidity in a rat model of social stress: a putative role for corticotropin-releasing factor. *Psychopharmacology* 222, 325–336. doi: 10.1007/s00213-012-2648-6

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The role of physical activity and heart rate variability for the control of work related stress

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Physical activity (PA) and exercise are often used as tools to reduce stress and therefore the risk for developing cardiovascular diseases (CVD). Meanwhile, heart rate variability (HRV) has been utilized to assess both stress and PA or exercise influences. The objective of the present review was to examine the current literature in regards to workplace stress, PA/exercise and HRV to encourage further studies. We considered original articles from known databases (PubMed, ISI Web of Knowledge) over the last 10 years that examined these important factors. A total of seven studies were identified with workplace stress strongly associated with reduced HRV in workers. Longitudinal workplace PA interventions may provide a means to improve worker stress levels and potentially cardiovascular risk with mechanisms still to be clarified. Future studies are recommended to identify the impact of PA, exercise, and fitness on stress levels and HRV in workers and their subsequent influence on cardiovascular health.

Keywords: autonomic nervous system, physical fitness, exercise, allostatic load, employees

INTRODUCTION

The adaptive process by which an organism maintains homeostasis is known as allostasis with variable allostatic loads commonly experienced by humans (McEwen and Seeman, 1999; Frodl and O'Keane, 2012). When exposure to chronic stress becomes excessive, the allostatic load experienced may promote important alterations in stress sensitive systems that are intimately linked to the pathophysiology of many diseases (Juster et al., 2010). The most studied of all stress related disorders is cardiovascular disease (CVD), which has been highlighted as the leading cause of mortality worldwide (World Health Organization, 2011). Previously, stress experienced by a person at their place of employment or work has been suggested to substantially increase their CVD risk (Yarnell, 2008; Thayer et al., 2010) with the risk of coronary heart disease being increased by 50% among workers (Kivimaki et al., 2006). As individuals spend most of their daily time at work (e.g., 8–10 h per day), a greater examination of the impact of interventions focusing on managing work-related stress as an important component of allostatic loads is warranted to reduce CVD risk and promote life-long resiliency against abnormal allostatic loading for workers (Juster et al., 2010). Development of healthy workplaces and practices may provide important environments that combat chronic stress and its consequent adverse contribution to the increasing work-related development of disease (Taylor et al., 1997).

Physical activity (PA) and exercise have been extensively recognized as important influences on the relationship between psychosocial stress and CVD (Hamer, 2012) probably because of its influence on physical fitness. Thus, it would be expected that more active individuals, who conversely possess higher physical fitness, would be more resilient to mental stresses (Hamer, 2012). In this regard, interventions that involve PA in conjunction with other beneficial practices (e.g., social support) in the workplace may be very effective for the control of allostatic load at an individual level (Juster et al., 2010). However, the expected greater stress resilience in those individuals with a greater physical fitness has been questioned as the stress-buffering effect of physical fitness has not always been demonstrated (Jackson and Dishman, 2006). This lack of demonstration may be related to methodological constraints of previous studies (Hamer, 2012) with further studies warranted to elucidate the important role that PA and exercise could have on workers cardiovascular health, potentially as an important stress-buffer.

Heart rate variability (HRV) is an easy and non-invasive tool for the assessment of variations in beat-to-beat intervals and autonomic nervous system activity with HRV obtained by linear methods within the domains of time and frequency analyses, and nonlinear methods (Task Force, 1996). HRV has been studied extensively in regards to CVD (Vrijkotte et al., 2000; Kivimaki et al., 2006; Yarnell, 2008; Thayer et al., 2010; Frodl and O'Keane, 2012), exercise (Proper et al., 2002; Kiviniemi et al., 2007; Buchheit et al., 2010; Boullosa et al., 2012), and stress (Hjortskov et al., 2004; Collins et al., 2005; Yarnell, 2008; Loerbroks et al., 2010; Uusitalo et al., 2011). The use of HRV as a practical monitoring tool for allostatic load though has been scarce and may provide a simple instrument for workers in the workplace. Greater HRV has been related to lower cardiovascular risk (Kiviniemi et al., 2010), greater physical fitness and responsiveness to aerobic training (Hautala et al., 2010), greater PA levels in workers (Rennie et al., 2003), and lower

work related stress in workers (Uusitalo et al., 2011). Collectively, these and other previous studies (Orsila et al., 2008; Thayer and Lane, 2009; Hynynen et al., 2011) emphasize the importance of HRV for the assessment of cardiovascular stress in the workplace with PA and fitness potentially enhancing HRV control. Therefore, the aim of this mini-review was to review the current literature regarding HRV, PA, fitness, and workplace stress to better delineate the current understanding and potential for future studies.

MATERIALS AND METHODS

An extensive search of relevant studies listed within the National Library of Medicine (PubMed) and ISI Web of Knowledge databases over the past 10 years (2003–2013) was conducted. The following combination of terms was utilized during the search: (exercise OR PA OR physical fitness) AND (workers OR occupational OR work OR job) AND cardiovascular stress (**Figure 1**). Inclusion criteria included: original articles; written in English; study population consisted of workers; included assessments of PA and/or exercise; assessment of work-related stress, and cardiac autonomic control monitored via HRV. The review, evaluation and selection of articles based upon inclusion criteria were carried out independently by three of the authors.

RESULTS

After initial searches based on the combination of specific terms were performed, there were 228 and 383 articles identified within PubMed and ISI Web of Knowledge databases, respectively (**Figure 1**). The titles and abstracts of these 611 articles were reviewed with 593 excluded as not meeting the inclusion criteria. Subsequently, 18 articles were obtained in full and examined further for details according to the inclusion criteria (**Figure 1**). Based upon these criteria, only seven articles were included in this study (see **Table 1**). These studies examined autonomic function via HRV for job stress evaluation, however studies included a variety of analyses, different scheduling of R-R recordings, and various populations that make comparisons difficult. Nevertheless, several studies (Chandola et al., 2008; Clays et al., 2011; Uusitalo et al., 2011) reported a reduced HRV in workers reporting work-related stress. Interestingly, only Chandola et al. (2008) reported a positive association between work stress and CVD possibly as a consequence of the negative influence of stress on health behaviors (i.e., reduced PA and poor diet). Meanwhile, others (Eller et al., 2011b; Lindholm et al., 2012) did not find any direct relationship between stress and HRV indices although a reduced HRV was exhibited during an imbalance between effort and reward, and in those workers experiencing irregular shiftwork, important sources of work-related stress. Likewise Melville et al. (2012) and Cheema et al. (2013) reported no relationship between HRV and stress with reductions in stress and anxiety following workplace yoga interventions accompanied by no changes in HRV.

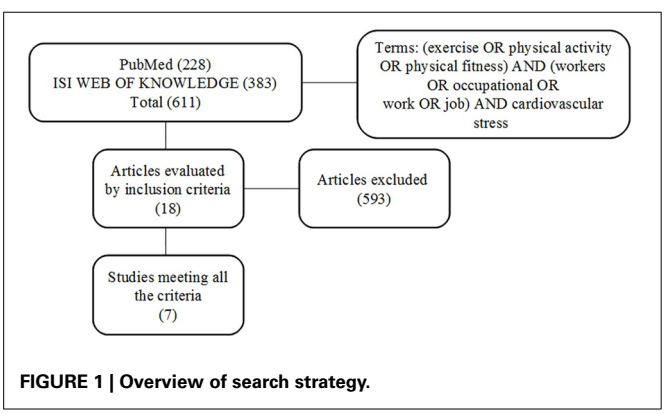

FIGURE 1 | Overview of search strategy.

All included studies assessed the level of PA or exercise with most simply reporting the level in a descriptive function. Only one study (Cheema et al., 2013) examined PA or exercise as an independent variable in a longitudinal design of work related stress and autonomic control of HR. Surprisingly, this recent study (Cheema et al., 2013) reported a worsening of autonomic control of HR (i.e., decrements in HRV indices) for the yoga intervention group vs. the control group. Paradoxically, the participants of this study exhibited improvements in some fitness characteristics and a reduced anxiety state after the yoga intervention suggesting an effective intervention but with an unexpected reduced HRV (Cheema et al., 2013). Previously, Melville et al. (2012) examined the acute effects of both yoga and meditation interventions on perceived stress and HRV with no significant changes in HRV during the acute post-intervention period (i.e., 15 min), despite significant reductions in perceived stress after both interventions.

DISCUSSION

This review suggests that autonomic function could be a simple and effective measure to identify workplace stress. Additionally, a single study with longitudinal workplace PA interventions reported improved worker stress levels without significant positive changes in cardiac autonomic activity, a mechanism known to be cardioprotective. Based upon the current few studies of PA and stress, and equivocal relationships between stress and cardiovascular health in workers, we encourage more studies involving varying PA and exercise interventions in the workplace to better examine the benefits of PA and exercise on both stress and HRV.

Overall, the current review provides further support of the applicability of cardiac autonomic function monitoring for work related stress. Factors related to adverse working conditions such as excessive effort (Vrijkotte et al., 2004), effort-reward imbalance (Eller et al., 2011a; Uusitalo et al., 2011), over commitment (Vrijkotte et al., 2004; Lindholm et al., 2012), irregular shift work (Lindholm et al., 2012), and work stress (Chandola et al., 2008) were significantly related to reduced cardiac autonomic function. Therefore, HRV monitoring may provide a simple and non-invasive assessment of stress and allostatic load in working environments that employers could utilize in the efficient management of employees. The recent systematic review of Jarczok et al. (2013) provides further support for HRV use in employee management with adverse psychosocial work conditions reported to be negatively associated with autonomic nervous system function as indexed by HRV.

It should be pointed out that within the current review, a variety of different HRV analyses were employed that limited comparisons between studies. The selection of HRV methodology is an important issue as tool sensitivity may compromise

Table 1 | Summary of studies involving HRV, work stress, and physical activity/exercise in the last 10 years.

Authors	Characteristics of participants	Assessment of stress	Assessment of physical activity/exercise	Evaluation method of HRV	Results
Chandola et al., 2008	A total of 10,308 London-based male and female civil servants aged 35–55 years	Self-reported work stress was measured by the job-strain questionnaire	Physical activity was measured by self-reported frequency of moderate activities (e.g., three times a week, at least once a week, at least once a month, never)	HRV—5 min of RR interval data were collected	There was an association between work stress and low HRV for participants at all ages. Greater reports of work stress were associated with lower HRV (LF, HF, and TP). Around 32% of the effect of work stress on Coronary Heart Disease (CHD) was explained by the effect of work stress on health behaviors (i.e., low physical activity and poor diet in particular) and the metabolic syndrome. The association between work stress and CHD was stronger among employees younger than 50 and those still in employment
Uusitalo et al., 2011	19 adults (18 women and 1 man), average 42 years (range 24–57 years)	Effort-reward imbalance (ERI) questionnaire: Effort refers to the demanding aspects of the work environment (six items, rating scale from 1 to 5): Reward refers to esteem, career opportunities and job security (11 items, rating scale from 1 to 5) A score of imbalance was obtained by calculating the ratio between Effort and Reward	*Actiwatch activity monitoring system* (Neurotechnology Ltd., Cambridge, UK).	Two 36-h, measurements were recorded on two different work days—36–84-h. Data were measured with Polar RR- recorder during work and at home	No significant differences were identified between daytime and work time physical activity scores. Daytime HRV (i.e., RMSSD) correlated significantly and negatively with daytime stress feelings on both days. Work time irritation correlated negatively with night time HRV (i.e., SDNN, RMSSD, and LF) on both days. The relationship between worker physical activity level and HRV, and stress at work, was not examined
Clays et al., 2011	653 healthy male workers. (40–55 years)	Job Stress Questionnaire (JSQ) contained 27 questions, were reduced and treated as 18 separate items for a Total JSQ score. Additionally, an adapted scale based on only five items: general satisfactions at work, responsibilities at work, imposed work pace, difficult professional relations and complaints about physical work conditions were examined as a Work Stressor Index	The Minnesota Leisure Time Physical Activity questionnaire: assessment of mean energy expenditure during leisure time physical activity in the past 12 months	Mean 24-h of ambulatory ECG recordings. HRV assessed during regular activities on a working day	Leisure time physical activity score median (IQR) = 70.05 (37.75–1.14). The relationship between worker physical activity level and HRV, and stress at work, was not examined. Both the JSQ scale and the adapted Work Stressor Index were positively and significantly related to mean HR and HRV (i.e., LF/HF). A significant negative correlation was reported between the Work Stressor Index and HRV (i.e., pNN50 and HF)

(Continued)

Table 1 | Continued

Authors	Characteristics of participants	Assessment of stress	Assessment of physical activity/exercise	Evaluation method of HRV	Results
Eller et al., 2011a	231 public sector workers (Mean age of 49.3 ± 8.8 for females, 51.2 ± 9.7 for males)	Effort-reward imbalance (ERI) questionnaire. Effort was evaluated by four questions and reward was evaluated by seven questions; answers were provided using a five point scale. Score of imbalance was obtained by calculating the effort/(reward × 4/7)	The degree of physical activity was measured via questionnaire, using four levels from very low activity: 1 = almost physical passive to high activity: 4 = intensive physical activity for more than 4 h/week	Ambulatory electrocardiograms (∼18 h) Commencing between 9:00–12:00 at the work place and ending the next morning	45.5% of women and 34.4% of men were engaged in physical activity for 2–4-h per week. Consistent associations between the psychosocial work environment (i.e., ERI-model) and HRV. The ranges of the ERI were higher for women (3.5) when compared to men (2.5). Analyses including ERI, were adjusted for sex, year, age, and time of measurement showed that women had significantly higher lnHR and lower ln(LF/HF) compared with men
Lindholm et al., 2012	66 workers with irregular shift work (ISW) and 66 workers with normal daytime work (RDW) (age 41.3 ± 10.3)	Questionnaire with several items: demographics, general health experience, physical health status, sleep, and insomnia symptoms, psychosocial status, stress, work satisfaction, and performance (Ahlberg et al., 2008)	Questionnaire with several items including leisure time exercise	Measured by ambulatory long-term ECG (24 h)	Regular weekly physical activity (leisure time exercise, walking/cycling to work) was reported by 84% of workers. Approximately 69% of ISW workers and 60% of RDW workers undertook less than three sessions of physical activity per week. The ISW group exhibited lower values of RMSSD in the late evening and first hours of sleep with insufficient recovery from daytime sleepiness.
Melville et al., 2012	20 sedentary workers aged 39.6 ± 9.5 years (8 women)	Means of a 100 mm visual analog scale in which participants rated their state of stress/relaxation at that particular moment, ranging from "extreme relaxation" (0 mm) to "extreme stress" (100 mm)	Three acute sessions were examined: 15 min of yoga, 15 min of meditation, and 15 min of no exercise (control) with each session separated by ≥24-h. Each session was followed by 15 min of recovery	Short-term recordings of heart rate and HRV continuously using a telemetric monitor. Data were grouped into seven 5-min phases including 1 × 5 min recording at baseline, 3 × 5 min recordings during and following each session	Compared to the control session, HR was significantly greater during yoga (6.5%) and significantly lower during meditation (3.9%) with HR during the yoga and meditation sessions significantly different. HRV (SDNN and TP) were significantly reduced during the yoga and meditation sessions compared to control. Perceived stress was significantly decreased immediately after the yoga ($p < 0.003$) and meditation ($p < 0.000$) sessions vs. control, and these effects were maintained for 15 min during recovery.

(Continued)

Table 1 | Continued

Authors	Characteristics of participants	Assessment of stress	Assessment of physical activity/exercise	Evaluation method of HRV	Results
Cheema et al., 2013	Academic and general staff of a university divided into yoga ($n = 18$; 10 weeks hatha yoga sessions, three times a week, during lunch break, 50 min per session), and control ($n = 19$) groups	Job Descriptive Index (JDI); Job in General (JIG) scale	Upper-body muscular endurance was evaluated using a standardized push-up test. Low-back and abdominal endurance was evaluated by means of an isometric, side-bridge test. Low-back and hip flexibility was evaluated via standardized sit-and-reach test	10 min ECG recordings before (Week 0) and after the intervention (Week 10, at least 48 h following the final yoga session in those randomized to the experimental group)	Log HF was not significantly improved in the yoga group vs. the control group over time ($p = 0.48$). The yoga group significantly reduced pNN50 ($p = 0.04$) and increased log LF/HF ($p = 0.04$) vs. the control group. The yoga intervention significantly increased low-back and hip flexibility ($p < 0.001$). *Post-hoc* analysis comparing participants who completed ≥70% of yoga sessions ($n = 11$) to control ($n = 19$) yielded the same findings, except that the high adherers also reduced state anxiety ($p = 0.02$) and RMSSD ($p = 0.05$), and tended to improve the push-up test ($p = 0.07$) vs. control.

Abbreviations: RMSSD, root mean squared differences of successive NN intervals; pNN50, % differences between adjacent RR intervals N50 ms; SDNN, standard deviation of normal-to-normal (NN) intervals; CHD, Coronary Heart Disease; LF, Low Frequency; LF/HF, Low Frequency to High Frequency Ratio; HR, Heart Rate; HF, High Frequency; JSQ, Job Stress Questionnaire; ISW, Irregular shift work; RDW, Regular daytime work; ERI, Effort-reward imbalance; IQR, interquartile range. HRV, heart rate variability; ECG, electro cardiogram; lnHR, normalized heart rate; ln(LF/HF), normalized Low Frequency to High Frequency Ratio; TP, total power; JDI, Job Descriptive Index; JIG, Job in General scale.

the detection of cardiac autonomic responses (Hynynen et al., 2011). Aspects such as variations in HRV measures examined (e.g., linear, non-linear, etc.), data analysis (e.g., supine, seated, Fast Fourier Transform, Autoregression, etc.) as well as the quality of data (e.g., degree of ectopy/artifact, sampling rate, recording length, etc.) make comparisons between studies challenging (Jarczok et al., 2013). For instance, Hynynen et al. (2011) reported that HRV during an orthostatic test upon wakening may be more useful for the analysis of stress in real life compared to night time recordings. In contrast, HRV measures recorded during both work and at night were reported to be also sensitive markers of mental stress alterations at work (Hjortskov et al., 2004; Yarnell, 2008). Further studies should determine the optimal HRV methods for detecting cardiac autonomic stress related adaptations. Factors such as the HRV measure (i.e., time or frequency domain, nonlinear, etc.), time, and duration of recordings and body posture (Young and Leicht, 2011; Boullosa et al., 2012) may play an important role that requires further clarification. Given the possible influence of factors external to work related stressors during long recordings, the use of laboratory controlled recordings should be included in further studies for a better evaluation of autonomic control of HR (Lombardi and Stein, 2011). Additionally, other cardiac autonomic indices (i.e., heart rate recovery; HRR, post-exercise) could also be employed as a delayed HRR was reported in individuals with high levels of stress and depression (Gordon et al., 2012). Consequently, the simple assessment of HRV and HRR may provide a comprehensive evaluation of the cardiac autonomic health of workers and prognosis for work-related stress and responsiveness to exercise training (Huovinen et al., 2011).

Another important restriction of studies within the current review was the different assessment tools for stress levels or psychosocial characteristics of work. For instance, the following tools were utilized in the current review studies: effort reward imbalance model (ERI-model) (Eller et al., 2011a; Uusitalo et al., 2011), Job Descriptive Index (JDI); Job in General (JIG) scale (Cheema et al., 2013), job stress questionnaire (Clays et al., 2011), stress questionnaire (Lindholm et al., 2012), job-strain questionnaire (Chandola et al., 2008), and a 100 mm visual analog scale (Melville et al., 2012). While each of the aforementioned tools has been used to assess stress at work, differences in specificity, reliability, and validity of these tools and their relationship with cardiac autonomic activity made comparisons difficult. Standardized use of stress assessment tools may help clarify the relationship between stress and cardiac autonomic activity. For example, Jarczok et al. (2013) highlighted that measurements of stress could be grouped when evaluated by ERI and JDC questionnaires with these questionnaires demonstrating a significant association between high strain/stress work and decreased vagal tone. Identification of the ultimate assessment tool may help to better understand the relationship between cardiovascular stress and workers health. Further, this tool may also overcome the confounding variables that affect the measurement of cardiac autonomic control such as age, sex, disease, caffeine intake, PA, smoking, and alcohol consumption (Jarczok et al., 2013).

One disappointing aspect of the current review was the low incidence of exercise and PA with only two studies involving exercise interventions (i.e., yoga or yoga and meditation) (Melville et al., 2012; Cheema et al., 2013) in the workplace. Further, these recent studies (Melville et al., 2012; Cheema et al., 2013) revealed contradictory results regarding the relationship between exercise interventions, stress, and HRV. The remaining studies (Vrijkotte et al., 2004; Clays et al., 2011; Eller et al., 2011a; Uusitalo et al., 2011; Lindholm et al., 2012) simply recorded PA as a factor of consideration in their analyses. Moreover, only one study objectively recorded the levels of PA via accelerometry (Uusitalo et al., 2011) with the remaining studies utilizing questionnaires for this purpose. Further, very few examined physical fitness with those studies assessing physical fitness (Clays et al., 2011; Uusitalo et al., 2011; Cheema et al., 2013) and not the potential role of this factor on stress responses and HRV. Therefore, the results of our review confirmed the variety of factors utilized in past studies with future studies recommended to examine objective measures of exercise and PA as these have been previously associated with HRV levels (Rennie et al., 2003; Hautala et al., 2010; Takada et al., 2010).

Previous studies have observed a weak and inverse association between job strain and leisure time PA indicating the potentially important role of PA for managing work related stress (Loerbroks et al., 2010). Previously, Choi et al. (2010) reported that having on-the-job learning opportunities and decision authority about their tasks may be conducive to increasing active leisure time PA in middle-aged US workers. While these previous studies have analyzed the impact of work conditions on leisure time PA, other studies have analyzed the impact of complex interventions including exercise in the workplace. For instance, Eriksen et al. (2002) previously demonstrated the greater effectiveness of complex interventions including PA than more focused non-PA interventions (e.g., stress management alone). Subsequently, Tveito and Eriksen (2009) reported in a randomized controlled study that a program consisting of physical exercise, stress management training, health information and an examination of the participants' workplace, promoted improvements in health, physical fitness, muscle pain, stress management, maintenance of health, and work situation. Overall, these previous studies highlighted the appropriateness of complex, exercise or PA based interventions at work for stress management and other health related outcomes. Paradoxically, in our focused review we found only two studies with workplace exercise interventions. Melville et al. (2012) evaluated the impact of a brief intervention (e.g., 15 min of yoga) at work on the acute cardiovascular responses (i.e., HR, HRV, and blood pressure) and perceived stress. Interestingly, this study reported a higher HRV during yoga and meditation interventions that returned to baseline immediately after the session with a reduction of perceived stress for 15 min post-intervention (Melville et al., 2012). Although the results of this study were promising, these results, within a small time of evaluation, should be considered with caution, especially due to the low intensity and duration of yoga practice. However, such an exercise intervention could be promising for the control of acute work-related stress responses (Hamer, 2012). Another study (Cheema et al., 2013) also evaluated the effectiveness of a workplace program based of hatha yoga (10 weeks, 50 min at lunch time, 3

times per week) on physiological stress (HRV) and associated health-related outcomes in a cohort of office workers. Despite the reduction of anxiety and fitness improvements among those with high adherence rates, the decrease in HRV after the intervention period raised concerns about the effectiveness of this exercise modality for the cardiac health of workers. Further studies with other exercise and PA interventions, which are different concepts (Caspersen et al., 1985), may clarify the most appropriate modality and dose-response for the control of stress and associated CVD in workers.

In the current review, most studies were cross-sectional with only two involving exercise interventions (yoga and meditation). Based on this review, there is a notable lack of studies comparing longitudinal interventions and the cause-effect relationships between physical fitness, exercise, and PA, and cardiovascular stress (Hamer, 2012). Moreover, identification of barriers and facilitators for PA or exercise at work (Renton et al., 2011; Leicht et al., 2013) may assist with the development of the most appropriate interventions to assist workers based on their employment demands. For instance, emergency physicians with different shifts (14 or 24-h) exhibited significant differences in stress levels (Dutheil et al., 2012). Such factors may assist in developing the best interventions and any potential changes in working environments (Conn et al., 2009). Although the use of complex interventions including exercise, psychological and nutritional habits have been previously recommended (Carson et al., 2010; Strijk et al., 2012), the inclusion of sole exercise and/or PA interventions may determine the appropriate dose-response (Vanhees et al., 2012). This would be very important as the levels of physical fitness that protect against CVD when considering specific job demands remain undefined (Huang and Acevedo, 2011). Moreover, CVD risk factors and stress exhibit a bidirectional relationship as stress promotes changes in behavior and pathophysiological parameters and vice versa (Huang and Acevedo, 2011) that should be considered in both prevention and treatment interventions (Stults-Kolehmainen, 2013). Thus, stressed workers may be less inclined to perform PA contributing to a greater deterioration of their health and progression of pathophysiological conditions (Azevedo et al., 2012; Silva and Barreto, 2012). Identification of barriers and motivators within workers would be an important aspect for designing effective and individualized intervention programs that also considers PA levels and sitting time derived from specific job activities (Leicht et al., 2013).

In summary, the results of the current review suggested a relationship between work related stress and autonomic control of HR. However, the extent of the positive benefits of both PA and exercise on both stress and HRV remains to be elucidated. Thus, future research, especially longitudinal studies, with different work categories and samples are needed to better understand how different levels of physical fitness, energy expenditure and exercise modes prevent or minimize the allostatic load and subsequent stress on workers and their impact on cardiovascular health.

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REFERENCES

Ahlberg, K., Jahkola, A., Savolainen, A., Kononen, M., Partinen, M., Hublin, C., et al. (2008). Associations of reported bruxism with insomnia and insufficient sleep symptoms among media personnel with or without irregular shift work. *Head Face Med.* 28, 4. doi: 10.1186/1746-160X-4-4

Azevedo, S. M., Singh-Manoux, A., Brunner, E. J., Kaffashian, S., Shipley M. J., and Kimimaki M. (2012). Bidirectional association between physical activity and symptoms of anxiety and depression: the whitehall II study. *Eur. J. Epidemiol.* 27, 537–546. doi: 10.1007/s10654-012-9692-8

Boullosa, D. A., Abreu, L., Tuimil, J. L., and Leicht, A. S., (2012). Impact of a soccer match on the cardiac autonomic control of referees. *Eur. J. Appl. Physiol.* 112, 2233–2242. doi: 10.1007/s00421-011-2202-y

Buchheit, M., Mendez-Villanueva, A., Quoud, M. J., Poulos, N., and Bourdon, P. (2010). Determinants of the variability of heart rate measures during a competitive period in young soccer players. *Eur. J. Appl. Physiol.* 109, 869–878. doi: 10.1007/s00421-010-1422-x

Carson, R. L., Baumgartner, J. J., Matthews, R. A., and Tsouloupas, C. N. (2010). Emotional exhaustion, absenteeism, and turnover intentions in childcare teachers: examining the impact of physical activity behaviors. *J. Health Psychol.* 15, 905–914. doi: 10.1177/1359105309360697

Caspersen, C. J., Powell, K. E., and Christenson, G. M. (1985). Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Rep.* 100, 126–131.

Chandola, T., Britton. A., Brunner, E., Hemingway, H., Malik, M., Kumari, M., et al. (2008). Work stress and coronary heart disease: what are the mechanisms? *Eur. Heart J.* 29, 640–648. doi: 10.1093/eurheartj/ehm584

Cheema, B. S., Houridis, A., Busch, L., Raschke-Cheema, V., Melville, G. W., Marshall, P. W., et al. (2013). Effect of an office worksite-based yoga program on heart rate variability: outcomes of a randomized controlled trial. *BMC Complement. Altern. Med.* 13:82. doi: 10.1186/1472-6882

Choi, B., Schnall, P. L., Yang. H., Dobson, M., Landsbergis, P., Israel, L., et al. (2010). Psychosocial working conditions and active leisure-time physical activity in middle-aged us workers. *Int. J. Occup. Med. Environ. Health* 23, 239–253. doi: 10.2478/v10001-010-0029-0

Clays, E., Bacquer, D. D., Crasset, V., Kittel, F., Smet, P., Kornitzer, M., et al. (2011). The perception of work stressors is related to reduced parasympathetic activity. *Int. Arch. Occup. Environ. Health* 84, 185–191. doi: 10.1007/s00420-010-0537-z.

Collins, S. M., Karasek, R. A., and Costas, K. (2005). Job strain and autonomic indices of cardiovascular disease risk. *Am. J. Ind. Med.* 48, 182–193. doi: 10.1002/ajim.20204

Conn, V. S., Hafdahl, A. R., Cooper, P. S., Brown, L. M., and Lusk, S. L. (2009). Meta-analysis of workplace physical activity interventions. *Am. J. Prev. Med.* 37, 330–339. doi: 10.1016/j.amepre.2009.06.008

Dutheil, F., Boudet, G., Lac, G., Ouchchane, L., Chamous, A., Duclos, M., et al. (2012). Jobstress study: comparison of heart rate variability in emergency physicians working a 24-hour shift or a 14-hour night shift—a randomized trial. *Int. J. Cardiol.* 158, 322–325. doi: 10.1016/j.ijcard.2012.04.141

Eller, N. H., Blod, M., Nielsen, M., Kristiansen, J., and Netterstrom, B. (2011a). Effort reward imbalance is associated with vagal withdrawal in danish public sector employees. *Int. J. Psychophysiol.* 81, 218–224. doi: 10.1016/j.ijpsycho.2011.06.014

Eller, N. H., Kristiansen, J., and Hansen, A. M. (2011b). Long-term effects of psychosocial factors of home and work on biomarkers of stress. *Int. J. Psychophysiol.* 79, 195–202. doi: 10.1016/j.ijpsycho.2010.10.009

Eriksen, H. R., Ihlebaek, C., Mikkelsen, A., Grønningsaeter, H., Sandal, G. M., and Ursin, H. (2002). Improving subjective health at the worksite: a randomized controlled trial of stress management training, physical exercise and an integrated health programme. *Occup. Med.* 52, 383–391. doi: 10.1093/occmed/52.7.383

Frodl, T., and O'Keane, V. (2012). How does the brain deal with cumulative stress? *a* review with focus on developmental stress, HPA axis function and hippocampal structure in humans. *Neurobiol. Dis.* 52, 24–37. doi: 10.1016/j.nbd.2012.03.012

Gordon, J. L., Ditto, B., and Antono, B. D. (2012). Cognitive depressive symptoms associated with delayed heart rate recovery following interpersonal stress in healthy men and women. *Psychophysiology* 49, 1082–1089. doi: 10.1111/j.1469-8986.2012.01397.x

Hamer, M. (2012). Psychosocial stress and cardiovascular disease risk: the role of physical activity. *Psychosom. Med.* 74, 896–903. doi: 10.1097/PSY.0b013e31827457f4

Hautala, A. J., Karjalainen, J., Kiviniemi, A. M., Kinnunen, H.,Mäkikallio, T. H.,Huikuri, H. V.,et al. (2010). Physical activity and heart rate variability measured simultaneously during waking hours. *Am. J. Physiol. Heart Circ. Physiol.* 298, 874–880. doi: 10.1152/ajpheart.00856.2009

Hjortskov, N., Rissén, D., Blangsted, A. K., Fallentin, N., Lundberg, U., and Sogaard, K. (2004). The effect of mental stress on heart rate variability and blood pressure during computer work. *Eur. J. Appl. Physiol.* 92, 84–89. doi: 10.1007/s00421-004-1055-z

Huang, C. J., and Acevedo, E. O. (2011). Occupational Stress: the influence of obesity and physical activity/fitness on immune function. *Occup. Stress.* 5, 486–493. doi: 10.1177/1559827611418168

Huovinen, J., Kyrolainen, H., Linnamo, V., Tanskanen, M., Kinnunen, H., Hakkinen, K., et al. (2011). Cardiac autonomic function reveals adaptation to military training. *Eur. J. Sport Sci.* 11, 231–240. doi: 10.1080/17461391.2010.506659.

Hynynen, E., Konttinen, N., Kinnunen, U., Kyrolainen, H., and Rusko, H. (2011). The incidence of stress symptoms and heart rate variability during sleep and orthostatic test. *Eur. J. Appl. Physiol.* 111, 733–741. doi: 10.1007/s00421-010-1698-x

Jackson, E. M., and Dishman, R. K. (2006) Cardiorespiratory fitness and laboratory stress: a meta-regression analysis. *Psychophysiology* 43, 57–72. doi: 10.1111/j.1469-8986.2006.00373.x

Jarczok, M. N., Jarczok. M., Mauss, D., Koenig, J., Li, J., Herr, R. M., et al. (2013). Autonomic nervous system activity and workplace stressors - a systematic review. *Neurosci. Biobehav. Rev.* 37, 1810–1823. doi: 10.1016/j.neubiorev.2013.07.004

Juster, R. P., McEwen, B. S., and Lupien, S. J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neurosci. Biobehav. Rev.* 35, 2–16. doi: 10.1016/j.neubiorev.2009.10.002

Kivimaki, M., Virtanen, M., Elovainio, M., Kouvonen, A., Vaananen, A. J., and Vahtera, J. (2006). Work stress in the etiology of coronary heart disease - A meta-analysis. *Scand. J. Work Environ. Health.* 32, 431–442. doi: 10.5271/sjweh.1049

Kiviniemi, A. M., Hautala, A. J., Kinnunen, H., Nissila, J., Virtanen, P., Karjalainen, J., et al. (2010). Daily exercise prescription on the basis of HR variability among men and women. *Med. Sci. Sports Exerc.* 42, 1355–1363. doi: 10.1249/MSS.0b013e3181cd5f39

Kiviniemi, A. M., Hautala, A. J., Kinnunen, H., and Tulppo, M. P. (2007). Endurance training guided individually by daily heart rate variability measurements. *Eur. J. Appl. Physiol.* 101, 743–751. doi: 10.1007/s00421-007-0552-2

Leicht, A. S., Sealey, R. M., and Devine, S. (2013). Relationship between employment category and gender on quality of life, physical activity and their barriers and motivators, for full-time University staff. *Int. J. Workplace Health Manage.* 6, 160–173. doi: 10.1108/IJWHM-04-2012-0008

Lindholm, H., Sinisalo, J., Ahlberg, J., Hirvonen, A., Hublin, C., Partinen, M., et al. (2012). Attenuation of vagal recovery during sleep and reduction of cortisol/melatonin ratio in late afternoon associate with prolonged daytime sleepiness among media workers with irregular shift work. *Am. J. Ind. Med.* 55, 643–649. doi: 10.1002/ajim.22042

Loerbroks, A., Schilling, O., Haxsen, V., Jarczok, M. N., Thayer, J. F., and Fischer, J. E. (2010). The fruits of ones labor: Effort-reward imbalance but not job strain is related to heart rate variability across the day in 35-44-year-old workers. *J. Psychosom. Res.* 69, 151–159. doi: 10.1016/j.jpsychores.2010.03.004

Lombardi, F., and Stein, P. K. (2011) Origin of heart rate variability and turbulence: an appraisal of autonomic modulation of cardiovascular function. *Front. Physiol.* 2:95. doi: 10.3389/fphys.2011.00095

McEwen, B. S., and Seeman, T. (1999). Protective and damaging effects of mediators of stress. elaborating and testing the concepts of allostasis and allostatic load. *Ann. N.Y Acad. Sci.* 896, 30–47. doi: 10.1111/j.1749-6632.1999.tb08103.x

Melville, G. W., Chang, D., Colagiuri, B., Marshall, P. W., and Cheema,B. S. (2012). Fifteen minutes of chair-based yoga postures or guided meditation performed in the office can elicit a relaxation response. *Evid. Based Complement. Alternat. Med.* 2012, 501986. doi: 10.1155/2012/501986

Orsila, R., Virtanen, M., Luukkaala, T., Tarvainon, M., Karjalainen, P., Viik, J., et al. (2008). Perceived mental stress and reactions in heart rate variability – a pilot study among employees of an electronics company. *Int. J. Occup. Saf. Ergon.* 14, 275–283. Available online at: http://www.ciop.pl/27071

Proper, K. I., Staal, B. J., Hildebrandt, V. H., Van Der Beek, A. J., and Van Mechelen, W. (2002). Effectiveness of physical activity programs at worksites with respect to work-related outcomes. *Scand. J. Work Environ. Health.* 28, 75–84. doi: 10.5271/sjweh.651

Rennie, K. L., Hemingway, H., Kumari, M., Brunner, E., Malik, M., and Marmot, M. (2003). Effects of moderate and vigorous physical activity on heart rate variability in a British study of civil servants. *Am. J. Epidemiol.* 158, 135–143. doi: 10.1093/aje/kwg120

Renton, S. J., Lightfoot, N. E., and Maar, M. A. (2011). Physical activity promotion in call centres: employers' perspectives. *Health Educ. Res.* 26, 1050–1059. doi: 10.1093/her/cyr055

Silva, L. S., and Barreto, S. M. (2012). Averse phycosocial working conditions and poor quality of life among financial servisse employees in Brazil. *J. Occup. Health.* 54, 88–95. doi: 10.1539/joh.11-0072-OA

Strijk, J. E., Proper, K. I., van der Beek, A. J., and van Mechelen, W. (2012). A worksite vitality intervention to improve older workers' lifestyle and vitality – related out comes: results of a randomized controlled trial. *J. Epidemiol. Community Health.* 66, 1071–1078. doi: 10.1136/jech-2011-200626

Stults-Kolehmainen, M. A. (2013). The interplay between stress and physical activity in the prevention and treatment of cardiovascular disease. *Front. Physiol.* 4:346. doi: 10.3389/fphys.2013.00346

Takada, M., Ebara, T., and Kamijima, M. (2010). Heart rate variability assessment in Japanese workers recovered from depressive disorders resulting from job stress: measurements in the workplace. *Int. Arch. Occup. Environ. Health.* 83, 521–529. doi: 10.1007/s00420-009-0499-1

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Taylor, S. E., Repetti, R. L., and Seeman, T. (1997). Health psychology: what is an unhealthy environment and how does it get under the skin? *Annu. Rev. Psychol.* 48, 411–447. doi: 10.1146/annurev.psych.48.1.411

Thayer, J. F., and Lane, R. D. (2009). Claude Bernard and the heart brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Thayer, J. F., Yamamoto, S. S., and Brosschot, J. F. (2010). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int. J. Cardiol.* 141, 122–131. doi: 10.1016/j.ijcard.2009.09.543

Tveito, T. H., and Eriksen, H. R. (2009). Integrated health programme: a workplace randomized controlled trial. *J. Adv. Nurs.* 65, 110–119. doi: 10.1111/j.1365-2648.2008.04846.x

Uusitalo, M. T., Martinmaki, K., Mauno, S., Kinnunen, U., and Rusko, H. (2011). Heart rate variability related to effort at work. *Appl. Ergon.* 42, 830–838. doi: 10.1016/j.apergo.2011.01.005

Vanhees, L., Geladas, N., Hansen, D., Kouidi, E., Niebauer, J., Reiner, Z., et al. (2012). Importance of characteristics and modalities of physical activity and exercise in the management of cardiovascular health in individuals with cardiovascular risk factors: recommendations from the EACPR. Part II. *Eur. J. Prev. Cardiol.* 19, 1005–1033. doi: 10.1177/1741826711430926

Vrijkotte, T. G., Van Doornen, L. J., and De Geus, E. J. (2004). Overcommitment to work is associated with changes in cardiac sympathetic regulation. *Psychosom. Med.* 66, 656–663. doi: 10.1097/01.psy.0000138283.65547.78

Vrijkotte, T. G. M., van Doornen, L. J. P., and de Geus, E. J. C. (2000). Effects of work stress on ambulatory blood pressure, heart rate, and

heart rate variability. *Hypertension* 35, 880–886. doi: 10.1161/01.HYP.35.4.880

World Health Organization. (2011). *Global Status Report on Noncommunicable Diseases 2010*. Geneva.

Yarnell, J. (2008). Stress at work–an independent risk factor for coronary heart disease? *Eur. Heart J.* 29, 579–80. doi: 10.1093/eurheartj/ehm641

Young, F. L., and Leicht, A. S. (2011). Short-term stability of resting heart rate variability: influence of position and gender. *Appl. Physiol. Nutr. Metab.* 36, 210–218. doi: 10.1139/h10-103.

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The interplay between stress and physical activity in the prevention and treatment of cardiovascular disease

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Cardiovascular disease (CVD) continues to menace developed and developing nations alike. However, in the United States alone, 40.5% of the population is projected to have CVD or a closely related disorder by 2030 (Heidenreich et al., 2011). Within the same time frame, costs associated with CVD are expected to balloon up to $818 billion (Heidenreich et al., 2011). These societal strains are increasing parallel to rising mental health disorders and psychosocial stress (Centers for Disease Control, 2011). In this context, stress refers to both physical and emotional challenges, some of which may be transient and rather innocuous (e.g., final examinations, strenuous exercise) with potential positive adaptations. Other stress may be chronic and uncontrollable (e.g., caregiving for a loved one with a terminal illness), which may produce excessive wear and tear, resulting in lasting and harmful insults to one's physical and mental health (McEwen, 2007). The American Psychological Association (APA) reports that 72% of Americans perceived that their stress increased or held steady from 2007 to 2011. Fourth fifths of this population reported an increase from 2010 to 2011 (American Psychological Association, 2012).

Unsurprisingly, there are well-established connections between stress and CVD. Indeed, stress provides an independent contribution to CVD outcomes (Vitaliano et al., 2002). Stress is linked to the pathogenesis of coronary heart disease (CHD) (Rozanski et al., 1999), incidence of acute myocardial infarctions (Rosengren et al., 2004) and worse survival from cardiac events (Kivimaki et al., 2002; Milani and Lavie, 2009). In one eye opening investigation, Milani and Lavie (2009) found that patients with high psychosocial stress in cardiac rehabilitation were almost 4 times as likely to die as those with low stress (22 vs. 5%). The experience of distress, the emotional outcome of stress, also exacerbates the morbidity associated with CVD (Centers for Disease Control, 2011). Those who are objectively stressed, such as caretakers of those with chronic conditions, have higher resting heart rates, blood pressure, and greater incidence of metabolic syndrome (Vitaliano et al., 2002). Individuals reporting higher levels of stressful life events have higher scores on risk factors for CVD, such as smoking (Ansell et al., 2012), systemic inflammation (Puustinen et al., 2011) and obesity (Sinha and Jastreboff, 2013). They are nearly twice as likely to start using anti-hypertensive medication over time compared to less stressed individuals (Rod et al., 2009). Furthermore, those with CVD are often stressed by their condition—adjusting to a life with a long-term hardship (Bodenheimer et al., 2002). This demonstrates that stress both contributes toward disease and, reciprocally, emanates from the experience of disease, which leaves little doubt that mental health interventions are needed throughout the progression and treatment of CVD.

Given these findings, an effective prevention and/or treatment plan for CVD would also target stress, but would minimize additional side effects and healthcare costs. To this end, exercise interventions have proved effective. In terms of CVD pathology, exercise improves the odds of recuperating from stroke, reduces hypertension and diminishes symptoms of heart failure and CHD—in each case with few serious side effects (Kujala, 2009). Recent evidence finds that exercise is just as effective as or even more effective than medications. For instance, Naci and Ioannidis (2013) recently found that exercise had a stronger effect than anticoagulants and antiplatelets in the treatment of stroke. Moreover, there is convincing evidence that those who exercise are much less likely to develop CVD over time (Lee et al., 2012; Matheson et al., 2013). The same is true for depression and post-traumatic stress disorder (PTSD) (Leardmann et al., 2011). In a large military cohort, those who exercised at least 20 min of vigorous activity two times a week were >40% less likely to develop new onset PTSD over >3 year period (Leardmann et al., 2011). Among those experiencing difficulties with stress, aerobic and resistance exercise has been effective in reducing stress-induced cardiac reactivity (King et al., 2002; Faulk and Bartholomew, 2012) and perceived stress (Wilcox et al., 2008), particularly when paired with healthy dietary changes (Imayama et al., 2011) and behavioral modification programming (Atlantis et al., 2004). Given the strong association between stress and CVD, is it possible that part of the health-enhancing power of exercise stems from its ability to mitigate the effects of stress (Milani and Lavie, 2009)? Extensive data supports the thought that physical activity (PA) buffers the relationship between stress and physical health problems (Gerber and Pühse, 2009; Emeny et al., 2012; Hamer, 2012; Rueggeberg et al., 2012). In other words, at high levels of stress, greater PA is associated with better health (Rueggeberg et al., 2012). Additionally, those who are physically fit are more resilient to the effects of stress, such as high work demands, resulting in less heart disease and associated mortality (Holtermann et al., 2010).

Data from our own laboratory show that higher levels of moderate to vigorous exercise are associated with fewer complaints of cardiovascular problems—particularly when stress is low (Stults-Kolehmainen et al., accepted). Under high levels of stress, however, higher levels of exercise were not related to better health, contradicting previous research (Rueggeberg et al., 2012). While PA—and more specifically, exercise—certainly have a salubrious effect on both psychosocial stress and CVD, and lifestyle interventions increasingly emphasize all forms of PA, I was left to wonder about the possible limitations of these health behaviors. One possible explanation for these discrepancies is that various forms of PA may have differential influence on the stress and CVD relationship (Fredman et al., 2006). PA, by definition, is any movement that results in energy expenditure, including occupational and spontaneous forms of locomotion (Garber et al., 2011). This is important because stressed populations, particularly laborers, may engage in moderate to high levels of occupational activity. Exercise, however, is typically performed with the intent of increasing physical fitness. Aside from athletes, military personnel and physical education students, it is usually considered recreational and is completed during one's leisure time. Therefore, it is possible that quantifications of exercise simply capture the luxury of having more time for rest and relaxation (Iwasaki et al., 2001). A preponderance of evidence would support the observations that those with less leisure are more frequently stressed, but also that those who are stressed typically have less leisure time (Fredman et al., 2006; Lutz et al., 2007).

Consequently, in the pursuit of better health, stress may have the upper hand over one's ability to engage in healthful levels of PA (Salmon, 2001). In fact, Rafer Lutz established that stress has a stronger effect on PA than the reverse order of influence (Lutz et al., 2007). This was the first and only prospective design with the specific aim of untangling these effects. Lutz, John Bartholomew and I later published data that suggested that those who are in earlier stages-of-change for exercise (i.e., pre-contemplators, contemplators) are most vulnerable to this effect (Lutz et al., 2010). This association does not appear to be limited to non-habituated exercisers, however. In our recent systematic review, Rajita Sinha and I found that >85% of prospective studies examining the association of stress and PA/exercise reported an inverse relationship between these two constructs. In other words, the experience of high mental stress predicted less PA. Several studies used sophisticated designs to objectively capture periods of greater stress and examine future levels of PA or exercise (Griffin et al., 1993; Steptoe et al., 1996; Vitaliano et al., 1998; Roemmich et al., 2003; Oaten and Cheng, 2005; Smith et al., 2005; Sherman et al., 2009). However, only one investigation employed a true experimental design and manipulated an acute mental stressor (Roemmich et al., 2003). In this study, children participated in two conditions, the order of which was randomly selected. In the stress condition, these subjects were required to give a prepared talk on a social topic. This task induced a moderate to strong stress response, which was followed by the opportunity to be either physically active (riding a bicycle) or remain sedentary. Behavioral responses were compared to changes observed within a passive reading control condition. While responses subjective stress were not related to changes in exercise ($r = -0.19$), children were less likely to expend energy after the stress condition compared to the control (a 21% difference in total minutes of activity). Total energy expenditure was also lower in the stress condition. Also examining this outcome, Smith et al. (2005) found approximately a 1000 kcal difference of weekly activity in a cohort of parents with a child who recently received a cancer diagnosis vs. parents of a healthy control child. Effects sizes for this association were large: 1.71 (Cohen's d) at diagnosis and 1.13 at a 3-month follow up. Such differences were due to less PA and more sedentary behavior, such as TV viewing. Given the long waits in doctors' offices to which these parents are subject, this is not surprising. In another longitudinal case-control study, caregivers had more stress and less frequent exercise bouts over two time points compared to matched controls (Vitaliano et al., 1998).

Following students before and during a final examinations period is a highly convenient method to observe potential influences of stress on health behaviors (Griffin et al., 1993; Steptoe et al., 1996; Oaten and Cheng, 2005; Sherman et al., 2009). In two cases, control groups have been utilized to determine whether other factors could explain changes in PA, such as the weather (Steptoe et al., 1996; Oaten and Cheng, 2005). These studies have identified significant declines in PA compared to controls during examinations, and such changes are due to less frequency, duration, and perceived ease of exercise. In an interesting permutation on this design, Sherman (Sherman et al., 2009) found that PA declined across a 14-day period that terminated with students' most stressful examination—a medium effect ($p = 0.03$, $d = 0.62$; $\eta^2 = 0.26$). Unfortunately, none of these studies looked at changes in PA after the completion of a stressful examination period or over multiple cycles of examinations, which would provide an additional insight on the dynamic effects of stress on exercise behavior. A rebound in PA after a stressful frame of time would complement existing data. Nevertheless, when viewed collectively, the published data substantiate the notion that both acute and transient or chronic life stressors have an impact on facets of PA.

These findings have direct implications for clinicians interested in improving CVD outcomes. Exercise is effective for treating both CVD and stress, but the patient would benefit from a treatment plan that goes beyond a prescription of exercise and "usual care." Navigating the complexities of a more active lifestyle is stressful in itself, particularly for the uninitiated (Stults-Kolehmainen and Sinha, 2013). Second, those who are chronically stressed experience both poor recovery from exercise and derive less affective benefits, placing them at risk for dropout early after initiation of an exercise routine (Stults-Kolehmainen and Bartholomew, 2012). Exercise cannot help those who do not adhere to a structured regimen, and stressed populations are at risk for drop out and poor compliance with their doctor's prescriptions. For instance, depressed cardiac rehabilitation patients are less likely to adhere to an exercise program; coming

to fewer sessions and dropping out at high rates (Glazer et al., 2002). As a metaphor, the sun-scorched plant benefits from water, but inordinate heat will also evaporate the new moisture. Only when shade is introduced can full restoration occur. In a similar light, the human organism needs relief from stress to effectively take advantage of exercise. The combination of PA and stress management would likely have a synergistic effect. In a randomized controlled trial, Blumenthal et al. (2005) demonstrated that exercise training and stress management groups significantly improved flow-mediated arterial dilation and other clinical outcomes compared to a third group receiving only usual care. They did not test the utility of both exercise and stress management combined, which has been successfully tested by Ornish et al. (1998). Their lifestyle intervention resulted in 50% reductions in the re-occurrence of cardiac events. Given this progression, a logical follow-up study should fully intertwine stress management procedures, such as mindfulness-based stress reduction (MBSR) (Ludwig and Kabat-Zinn, 2008), as part of the exercise regimen. Mindfulness practices can be conducted during movement (i.e., controlled breathing and contractions with proper alignment), during rest periods (e.g., focusing on changing internal states) and directly following a workout (e.g., sensing stillness and the process of unwinding). Such a combination may enhance physical recovery (Stults-Kolehmainen and Bartholomew, 2012), boost affective valence (increase positive emotions), and reinforce exercise behavior resulting in more effective treatment with time and cost savings.

Stress is a common denominator underlying CVD and the lack of PA. It independently contributes to pathologies of the cardiovascular system, exacerbates distress associated with these problems and impedes one's progress toward a healthier lifestyle. As this is a complicated set of relationships, I would argue that novel therapeutic regimens are needed to address the manifestation of stress at multiple levels. Fully melding exercise interventions with proven stress management techniques, such as MBSR, would be a useful strategy.

REFERENCES

American Psychological Association. (2012). *Stress in America* [Online]. Available online at: www.stressinamerica.org (Accessed November 7, 2013).

Ansell, E. B., Gu, P. H., Tuit, K., and Sinha, R. (2012). Effects of cumulative stress and impulsivity on smoking status. *Hum. Psychopharmacol.* 27, 200–208. doi: 10.1002/hup.1269

Atlantis, E., Chow, C. M., Kirby, A., and Singh, M. F. (2004). An effective exercise-based intervention for improving mental health and quality of life measures: a randomized controlled trial. *Prev. Med.* 39, 424–434. doi: 10.1016/j.ypmed.2004.02.007

Blumenthal, J. A., Sherwood, A., Babyak, M. A., Watkins, L. L., Waugh, R., Georgiades, A., et al. (2005). Effects of exercise and stress management training on markers of cardiovascular risk in patients with ischemic heart disease - A randomized controlled trial. *JAMA* 293, 1626–1634. doi: 10.1001/jama.293.13.1626

Bodenheimer, T., Lorig, K., Holman, H., and Grumbach, K. (2002). Patient self-management of chronic disease in primary care. *JAMA* 288, 2469–2475. doi: 10.1001/jama.288.19.2469

Centers for Disease Control. (2011). Mental illness surveillance among adults in the united states. *MMWR Surveill. Summ.* 60, 1–30. Available online at: http://origin.glb.cdc.gov/mmWR/preview/mmwrhtml/su6003a1.htm

Emeny, R. T., Lacruz, M. E., Baumert, J., Zierer, A., Von Eisenhart, R. A., Autenrieth, C. S., et al. (2012). Job strain associated CRP is mediated by leisure time physical activity: results from the MONICA/KORA study. *Brain Behav. Immun.* 26, 1077–1084. doi: 10.1016/j.bbi.2012.07.004

Faulk, K. E., and Bartholomew, J. B. (2012). The moderating effect of physical activity on cardiovascular reactivity following single fat feedings. *Psychophysiology* 49, 145–149. doi: 10.1111/j.1469-8986.2011.01283.x

Fredman, L., Bertrand, R. M., Martire, L. M., Hochberg, M., and Harris, E. L. (2006). Leisure-time exercise and overall physical activity in older women caregivers and non-caregivers from the caregiver-SOF study. *Prev. Med.* 43, 226–229. doi: 10.1016/j.ypmed.2006.04.009

Garber, C. E., Blissmer, B., Deschenes, M. R., Franklin, B. A., Lamonte, M. J., Lee, I. M., et al. (2011). Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med. Sci. Sports Exerc.* 43, 1334–1359. doi: 10.1249/MSS.0b013e318213fefb

Gerber, M., and Pühse, U. (2009). Do exercise and fitness protect against stress-induced health complaints? A review of the literature. *Scand. J. Public Health* 37, 801–819. doi: 10.1177/1403494809350522

Glazer, K. M., Emery, C. F., Frid, D. J., and Banyasz, R. E. (2002). Psychological predictors of adherence and outcomes among patients in cardiac rehabilitation. *J. Cardiopulm. Rehabil.* 22, 40–46. doi: 10.1097/00008483-200201000-00006

Griffin, K. W., Friend, R., Eitel, P., and Lobel, M. (1993). Effects of environmental demands, stress, and mood on health practices. *J. Behav. Med.* 16, 643–661. doi: 10.1007/BF00844724

Hamer, M. (2012). Psychosocial stress and cardiovascular disease risk: the role of physical activity. *Psychosom. Med.* 74, 896–903. doi: 10.1097/PSY.0b013e31827457f4

Heidenreich, P. A., Trogdon, J. G., Khavjou, O. A., Butler, J., Dracup, K., Ezekowitz, M. D., et al. (2011). Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* 123, 933–944. doi: 10.1161/CIR.0b013e31820a55f5

Holtermann, A., Mortensen, O. S., Burr, H., Sogaard, K., Gyntelberg, F., and Suadicani, P. (2010). Physical demands at work, physical fitness, and 30-year ischaemic heart disease and all-cause mortality in the Copenhagen Male Study. *Scand. J. Work Environ. Health* 36, 357–365. doi: 10.5271/sjweh.2913

Imayama, I., Alfano, C. M., Kong, A., Foster-Schubert, K. E., Bain, C. E., Xiao, L. R., et al. (2011). Dietary weight loss and exercise interventions effects on quality of life in overweight/obese postmenopausal women: a randomized controlled trial. *Int. J. Behav. Nutr. Phys. Act.* 8, 1–12. doi: 10.1186/1479-5868-8-118

Iwasaki, Y., Zuzanek, J., and Mannell, R. C. (2001). The effects of physically active leisure on stress-health relationships. *Can. J. Public Health* 92, 214–218.

King, A. C., Baumann, K., O'Sullivan, P., Wilcox, S., and Castro, C. (2002). Effects of moderate-intensity exercise on physiological, behavioral, and emotional responses to family caregiving: a randomized controlled trial. *J. Gerontol. A Biol. Sci. Med. Sci.* 57, M26–M36. doi: 10.1093/gerona/57.1.M26

Kivimaki, M., Leino-Arjas, P., Luukkonen, R., Riihimaki, H., Vahtera, J., and Kirjonen, J. (2002). Work stress and risk of cardiovascular mortality: prospective cohort study of industrial employees. *Br. Med. J.* 325, 857–860. doi: 10.1136/bmj.325.7369.857

Kujala, U. M. (2009). Evidence on the effects of exercise therapy in the treatment of chronic disease. *Br. J. Sports Med.* 43, 550–555. doi: 10.1136/bjsm.2009.059808

Leardmann, C. A., Kelton, M. L., Smith, B., Littman, A. J., Boyko, E. J., Wells, T. S., et al. (2011). Prospectively assessed posttraumatic stress disorder and associated physical activity. *Public Health Rep.* 126, 371–383.

Lee, I. M., Shiroma, E. J., Lobelo, F., Puska, P., Blair, S. N., Katzmarzyk, P. T., et al. (2012). Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet* 380, 219–229. doi: 10.1016/S0140-6736(12)61031-9

Ludwig, D. S., and Kabat-Zinn, J. (2008). Mindfulness in medicine. *JAMA* 300, 1350–1352. doi: 10.1001/jama.300.11.1350

Lutz, R. S., Lochbaum, M. R., Lanning, B., Stinson, L. G., and Brewer, R. (2007). Cross-lagged relationships among leisure-time exercise and perceived stress in blue-collar workers. *J. Sport Exerc. Psychol.* 29, 687–705.

Lutz, R. S., Stults-Kolehmainen, M. A., and Bartholomew, J. B. (2010). Exercise caution when

stressed: stages of change and the stress-exercise participation relationship. *Psychol. Sport Exerc.* 11, 560–567. doi: 10.1016/j.psychsport.2010.06.005

Matheson, G., Klügl, M., Engebretsen, L., Bendiksen, F., Blair, S., Börjesson, M., et al. (2013). Prevention and management of non-communicable disease: the IOC consensus statement, Lausanne 2013. *Sports Med.* 43, 1075–1088. doi: 10.1007/s40279-013-0104-3

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* 87, 873–904. doi: 10.1152/physrev.00041.2006

Milani, R. V., and Lavie, C. J. (2009). Reducing psychosocial stress: a novel mechanism of improving survival from exercise training. *Am. J. Med.* 122, 931–938. doi: 10.1016/j.amjmed.2009.03.028

Naci, H., and Ioannidis, J. P. A. (2013). Comparative effectiveness of exercise and drug interventions on mortality outcomes: meta-epidemiological study. *BMJ* 347. doi: 10.1136/bmj.f5577. [Epub ahead of print].

Oaten, M., and Cheng, K. (2005). Academic examination stress impairs self-control. *J. Soc. Clin. Psychol.* 24, 254–279. doi: 10.1521/jscp.24.2.254.62276

Ornish, D., Scherwitz, L. W., Billings, J. H., Gould, K. L., Merritt, T. A., Sparler, S., et al. (1998). Intensive lifestyle changes for reversal of coronary heart disease. *JAMA* 280, 2001–2007. doi: 10.1001/jama.280.23.2001

Puustinen, P. J., Koponen, H., Kautiainen, H., Mantyselka, P., and Vanhala, M. (2011). Psychological distress and C-reactive protein: do health behaviours and pathophysiological factors modify the association? *Eur. Arch. Psychiatry Clin. Neurosci.* 261, 277–284. doi: 10.1007/s00406-010-0134-x

Rod, N. H., Gronbaek, M., Schnohr, P., Prescott, E., and Kristensen, T. S. (2009). Perceived stress as a risk factor for changes in health behaviour and cardiac risk profile: a longitudinal study. *J. Intern. Med.* 266, 467–475. doi: 10.1111/j.1365-2796.2009.02124.x

Roemmich, J. N., Gurgol, C. M., and Epstein, L. H. (2003). Influence of an interpersonal laboratory stressor on youths' choice to be physically active. *Obes. Res.* 11, 1080–1087. doi: 10.1038/oby.2003.148

Rosengren, A., Hawken, S., Ounpuu, S., Sliwa, K., Zubaid, M., Almahmeed, W. A., et al. (2004). Association of psychosocial risk factors with risk of acute myocardial infarction in 11,119 cases and 13,648 controls from 52 countries (the INTERHEART study): case-control study. *Lancet* 364, 953–962. doi: 10.1016/S0140-6736(04)17019-0

Rozanski, A., Blumenthal, J. A., and Kaplan, J. (1999). Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation* 99, 2192–2217. doi: 10.1161/01.CIR.99.16.2192

Rueggeberg, R., Wrosch, C., and Miller, G. E. (2012). The different roles of perceived stress in the association between older adults' physical activity and physical health. *Health Psychol.* 31, 164–171. doi: 10.1037/a0025242

Salmon, P. (2001). Effects of physical exercise on anxiety, depression, and sensitivity to stress: a unifying theory. *Clin. Psychol. Rev.* 21, 33–61. doi: 10.1016/S0272-7358(99)00032-X

Sherman, D. K., Bunyan, D. P., Creswell, J. D., and Jaremka, L. M. (2009). Psychological vulnerability and stress: the effects of self-affirmation on sympathetic nervous system responses to naturalistic stressors. *Health Psychol.* 28, 554–562. doi: 10.1037/a0014663

Sinha, R., and Jastreboff, A. M. (2013). Stress as a common risk factor for obesity and addiction. *Biol. Psychiatry* 73, 827–835. doi: 10.1016/j.biopsych.2013.01.032

Smith, A. W., Baum, A., and Wing, R. R. (2005). Stress and weight gain in parents of cancer patients. *Int. J. Obes.* 29, 244–250. doi: 10.1038/sj.ijo.0802835

Steptoe, A., Wardle, J., Pollard, T. M., Canaan, L., and Davies, G. J. (1996). Stress, social support and health-related behavior: a study of smoking, alcohol consumption and physical exercise. *J. Psychosom. Res.* 41, 171–180. doi: 10.1016/0022-3999(96)00095-5

Stults-Kolehmainen, M. A., and Bartholomew, J. B. (2012). Psychological stress impairs short-term muscular recovery from resistance exercise. *Med. Sci. Sports Exerc.* 44, 2220–2227. doi: 10.1249/MSS.0b013e31825f67a0

Stults-Kolehmainen, M. A., and Sinha, R. (2013). The effects of stress on physical activity and exercise: a systematic review. *Sports Med.* 41. doi: 10.1007/s40279-013-0090-5. [Epub ahead of print].

Stults-Kolehmainen, M. A., Sinha, R., and Tuit, K. (accepted). Lower cumulative stress is associated with better health for physically active individuals in the community. *Stress*.

Vitaliano, P. P., Scanlan, J. M., Ochs, H. D., Syrjala, K., Siegler, I. C., and Snyder, E. A. (1998). Psychosocial stress moderates the relationship of cancer history with natural killer cell activity. *Ann. Behav. Med.* 20, 199–208. doi: 10.1007/BF02884961

Vitaliano, P. P., Scanlan, J. M., Zhang, J., Savage, M. V., Hirsch, I. B., and Siegler, I. C. (2002). A path model of chronic stress, the metabolic syndrome, and coronary heart disease. *Psychosom. Med.* 64, 418–435.

Wilcox, S., Dowda, M., Leviton, L. C., Bartlett-Prescott, J., Bazzarre, T., Campbell-Voytal, K., et al. (2008). Active for life - final results from the translation of two physical activity programs. *Am. J. Prev. Med.* 35, 340–351. doi: 10.1016/j.amepre.2008.07.001

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HYPOTHESIS AND THEORY ARTICLE
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Mindfulness may both moderate and mediate the effect of physical fitness on cardiovascular responses to stress: a speculative hypothesis

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The psychological construct of mindfulness refers to an awareness that emerges by intentionally paying attention to the present experience in a non-judgmental or evaluative way. This particular quality of awareness has been associated to several indicators of physical and psychological health, and can be developed using mindfulness-based interventions (MBIs), and therefore MBIs have been successfully applied as preventive and complementary interventions and therapies in medicine and psychology. Together with quiet sitting and lying meditation practices, mindful physical exercises such as "mindful walking" and "mindful movement" are key elements in MBIs and couple muscular activity with an internally directed focus, improving interoceptive attention to bodily sensations. In addition, MBIs seem to share similar mechanisms with physical fitness (PF) by which they may influence cardiovascular responses to stress. Based on these facts, it is feasible to raise the question of whether physical training itself may induce the development of that particular quality of awareness associated with mindfulness, or if one's dispositional mindfulness (DM) (the tendency to be more mindful in daily life) could moderate the effects of exercise on cardiovascular response to stress. The role of mindfulness as a mediator or moderator of the effect of exercise training on cardiovascular responses to stress has barely been studied. In this study, we have hypothesized pathways (moderation and mediation) by which mindfulness could significantly influence the effects of PF on cardiovascular responses to stress and discussed potential practical ways to test these hypotheses.

Keywords: mindfulness, moderator effects, mediational model, physical fitness, cardiovascular system, psychological stress, mindful exercise

MINDFULNESS IN MEDICINE AND PSYCHOLOGY

Interest in mindfulness has increased exponentially in recent decades in academic and clinical contexts (Dimidjian and Kleiber, 2013). The psychological construct of mindfulness refers to an awareness that emerges by intentionally paying attention to the present experience in a non-judgmental way (Kabat-Zinn, 2003). In other words, a "mindful" mind brings together attentional and attitudinal features at the same time, self-regulating attention toward present-moment direct experiences and attitude in a non-judgmental tone toward internal and external phenomena (physical, affective and behavioral).

This particular quality of non-evaluative awareness can improve one's physical and psychological health status, and therefore several approaches generally called "mindfulness-based interventions" (MBIs) have been developed and tested in the last 40 years (Cullen, 2011). Evidence about their efficacy and effectiveness in improving mindfulness and health is rapidly accumulating (Khoury et al., 2013).

MBIs, e.g., "Mindfulness-based Stress Reduction" (MBSR) and "Mindfulness-based Cognitive Therapy" (MBCT), are generally 8-weeks group-based interventions that mix mindfulness practices (mostly derived from ancient eastern meditative techniques) with contemporary cognitive and behavioral approaches to improve one's mindfulness levels and capabilities to adaptively manage stressful life events (Cullen, 2011). Moreover, MBIs have been successfully applied as preventive and adjuvant therapies in patients with depression, anxiety, chronic pain, cardiovascular disorders, cancer, and other non-communicable diseases (Bonadonna, 2003; Edelman et al., 2006; Kuyken et al., 2008; Fortney and Taylor, 2010; Khoury et al., 2013). In addition, MBIs have been adapted to special targeted population (Carson et al., 2006; Duncan and Bardacke, 2010; Cullen, 2011), including

the sports psychology field, in this case aiming to improve athletes' psychological well-being and to enhance their performance (Gardner and Moore, 2012).

It has been suggested that MBIs result in improvements in autonomic and central nervous systems regulation, attention control, emotional and behavioral self-awareness and regulation, self-compassion, resilience, dispositional flow, and body awareness, and these mechanisms may account for their efficacy (for reviews about mindfulness mechanisms see Hölzel et al., 2011; Gardner and Moore, 2012).

MINDFULNESS: TRAIT AND STATE

Several questionnaires and scales to measure mindfulness trait or state have been developed in the last two decades (Park et al., 2013), which allow systematic investigation in this field. It has been observed that to some degree the particular type of awareness associated to mindfulness may be innate (Garland et al., 2013).

Trait or dispositional mindfulness (DM), the tendency to be more mindful in daily life (Garland et al., 2013), seems to be a sum of individual genetics and life experiences, and DM is not necessarily related to having participated in an MBI or practicing meditative exercises. DM can be measured by scales addressing mindfulness trait, e.g., the Mindful Attention Awareness Scale (MAAS) (Brown and Ryan, 2003) and the Five Facets Mindfulness Questionnaire (FFMQ) (Bohlmeijer et al., 2011), and it has been significantly related to several indicators of psychological and physical health including: higher levels of positive affect, improvement in personal stress management skills, and in adaptive emotional regulation (Hayes-Skelton and Graham, 2013; Khoury et al., 2013; Garcia-Campayo et al., 2014).

As expected, DM can be also modulated by participating in MBIs and practicing specific mindfulness techniques such as several types of meditative practices (Garland et al., 2010; Park et al., 2013). These interventions can induce transient increases in the mindfulness state, and may promote long-term improvements in DM if applied in a regular basis (Soler et al., 2014). The "mindfulness state," i.e., mindfulness addressed as a statelike mental behavior, which is context-dependent, transient and variable, is also measureable, and few scales have been developed for this purpose (Tanay and Bernstein, 2013).

MINDFULNESS AND CARDIOVASCULAR RESPONSES TO STRESS

Chronic stress is a well-known risk factor for cardiovascular diseases (CVD). Physical and psychological stressors can provoke non-adaptive stress-induced cardiovascular responses marked by dysfunctional cortisol and catecholamine releasing, systemic inflammation, oxidative stress, and unbalanced autonomic nervous system, leading to endothelial dysfunction, increased blood pressure, among others factors, which chronically may induce hypertension and CVD (Koolhaas et al., 2011; Huang et al., 2013; Stoner et al., 2013). Current evidence suggests that MBIs may buffer responses to stressors by mechanisms direct or indirectly related to these biomarkers of non-adaptive cardiovascular responses to stress.

A recent study showed that an MBSR program (the original program that have influenced subsequent types of MBIs) had a favorable influence both on biomarkers of stress regulation, such as cortisol secretion and sleep (Brand et al., 2012). In this regard, stress-sleep connection may be an important mechanism influencing responses to stress (Demarzo and Stein, 2012). In the same direction, another study showed that an MBI resulted in increases in both objectively- and subjectively-measured sleep continuity in patients using anti-depressant medication (Britton et al., 2012).

MBIs and other behavioral interventions designed to reduce emotional reactivity may be of therapeutic benefit in chronic inflammatory conditions (Rosenkranz et al., 2013). Even a brief mindfulness intervention in the workplace may be an effective and probably cost-effective way to reduce systemic inflammation (Malarkey et al., 2013). MBSR has also shown promise as a novel treatment approach for reducing loneliness-related pro-inflammatory gene expression in older adults (Creswell et al., 2012). Moreover, analysis of oxidative stress levels in people who meditate indicated that meditation correlates with lower oxidative stress and higher melatonin levels, a well-known antioxidant agent (Martarelli et al., 2011).

Another mechanism that may link mindfulness to cardiovascular responses to stress is influencing on the autonomic nervous system, the body's most primitive and automatic regulator of the stress responses (Thayer et al., 2010). One way to measure the functioning of the autonomic nervous system is to analyze heart rate variability (HRV). It is well-known that depressed HRV, suggesting a lack of flexibility of autonomic control, has been linked to stress factors (Thayer et al., 2010). Recent studies have shown the potential of mindfulness practices to improve HRV, e.g., increasing the parasympathetic tone (Peressutti et al., 2011; Libby et al., 2012; Krygier et al., 2013).

Furthermore, it was observed in a recent study that MBSR helped reduce blood pressure levels and blood pressure reactivity to stress among healthy community-dwelling individuals reporting elevated stress levels (Nyklíček et al., 2013a). In addition, Prakhinkit et al. (2013) observed in a randomized controlled study that both walking meditation (a key mindfulness practice in MBIs) and traditional walking exercise may improve endothelium-dependent vasodilation in elderly with depressive symptoms (Prakhinkit et al., 2013).

Moreover, MBIs may improve both the cardiovascular response to stressors and provide a complementary approach for the prevention and treatment of CVD. A large study showed that a multidimensional intervention based on mindfulness and other integrative medicine principles reduced the 10-year risk of coronary heart disease (CHD) for outpatients with 1 or more known cardiovascular risk factors when compared to usual care, although the effect of the specific components of the intervention were not tested separately (Edelman et al., 2006). Furthermore, a MBSR program may reduce characteristics of the distressed personality ('Type D), and this effect seems to be mediated by increase in self-reported mindfulness (Nyklíček et al., 2013b). The "Type D" personality is characterized by a combination of negative affectivity and social inhibition, and has been associated with adverse health outcomes, including CVD (Nyklíček et al., 2013b).

HYPOTHESIS RATIONALE

Together with sitting and lying meditation practices, mindfulness exercises such as "mindful walking" and "mindful movements" are key elements in MBIs and couple muscular activity with an internally directed focus (Kabat-Zinn, 2005), improving interoceptive attention to bodily sensations (Ospina et al., 2007; Posadzki and Jacques, 2009; Farb et al., 2013; Gryffin and Chen, 2013; Hagins et al., 2013). This may suggest that a regular physical activity made with internal focus may also improve one's mindfulness levels (Tsang et al., 2008).

In addition, as shown in the previous section, MBIs may buffer cardiovascular responses to stress probably sharing similar mechanisms by which physical fitness (PF) influence these responses (Huang et al., 2013; Stults-Kolehmainen, 2013). **Figure 1** schematically represents potential physiological mechanisms shared by physical activity and mindfulness by which they may attenuate cardiovascular responses to stress (Huang et al., 2013).

Based on these facts, it is feasible to raise the question of whether physical training itself may induce the development of that particular quality of awareness associated with mindfulness (mindfulness as a mediator variable), or if one's DM could moderate the effects of exercise on cardiovascular response to stress. Until now, there has been a lack of information in scientific literature regarding the role of mindfulness as a mediator or moderator of effects of exercise training on stress and health in general, and on cardiovascular responses to stress in particular. Thus, in this study we aimed to develop the hypothesis that mindfulness influences the effects of PF on cardiovascular responses to stress by both moderating or mediating these effects, and discussed potential practical ways to test these hypotheses.

MINDFULNESS AS A MODERATOR OF THE EFFECTS OF EXERCISE ON CARDIOVASCULAR RESPONSE TO STRESS

As represented in the **Figure 2** (line "C"), our first hypothesis is that DM acts as a moderator in the association between PF (independent variable) and cardiovascular responses to stress (dependent variable), by altering the strength of the association. By considering both PF and DM as continuous variables (Baron and Kenny, 1986), probably the moderation occurs in a linear direction (the more DM, the more improvement on dysfunctional stress-induced cardiovascular reactivity caused by PF). Thus, we may theorize that exercise training affects differently people with higher or lower DM.

Although there is no published data about this potential moderator role of DM, it is feasible to speculate on it as mindfulness may be seen as an "independent variable" (see **Figure 2**, line "B") in the relationship with the "dependent variable" cardiovascular response to stress, an important pre-requisite to be considered as a moderator factor (Baron and Kenny, 1986). In addition, it has been suggested that sex, obesity levels, and individual's perception of control over a situation may be moderators of the effects of PF (Huang et al., 2013), and so DM could be another factor

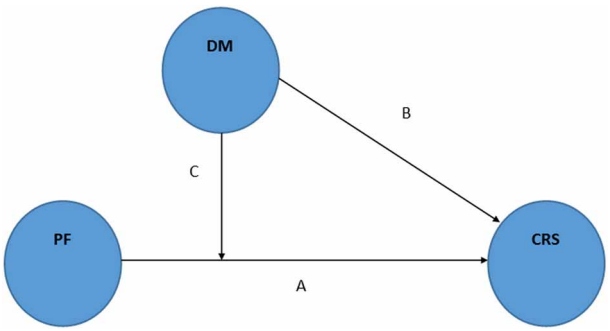

FIGURE 2 | Schematic representation of the moderator hypothesis: mindfulness as moderator of the physical fitness effects on cardiovascular responses to stress. Adapted from Baron and Kenny (1986). PF: physical fitness. DM: dispositional mindfulness. CRS: cardiovascular responses to stress.

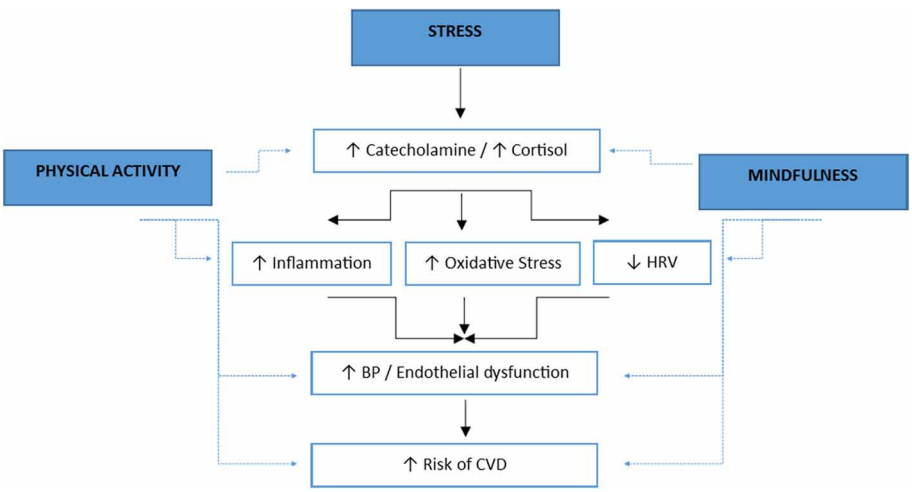

FIGURE 1 | Potential physiological mechanisms shared by physical activity and mindfulness by which they may attenuate cardiovascular responses to stress (the solid line indicates an activation of the indicated factor and the dashed line represents the attenuation of the indicated factors). Adapted from Huang et al. (2013). HRV: heart rate variability. BP: blood pressure. CVD: cardiovascular disease.

to be taken in account in future studies. Moreover, it is interesting to note that some authors have discussed the idea that only less stressed people could benefit from exercise effects (Stults-Kolehmainen, 2013), and so DM could be a potential confounder factor in this phenomena, as it is well-known that DM is related to less perceived stress and stress-induced biomarkers (Ciesla et al., 2012; Murphy et al., 2012).

In addition, recent findings have identified variables potentially associated with DM that may be confounding variables for the effects of PF on cardiovascular responses to stress. Lower or higher DM may mean lower or higher levels of psychological well-being, healthy behaviors, adherence to an exercise program, emotional reactivity, social anxiety, and co-morbidity, and so may alter the strength of the association between exercise training and cardiovascular responses to stress (Ulmer et al., 2010; Bränström et al., 2011; Salmoirago-Blotcher et al., 2011; Ciesla et al., 2012; Brown et al., 2013; Garcia et al., 2014). It matters mainly if investigators are not using a randomized controlled design to study that association. Even in this case, stratified randomization for DM may be of interest in order to prevent this potential bias.

In order to test the hypothesis that DM is a moderator of the effects of PF on cardiovascular responses to stress, an investigator should address this fundamental research question: does an exercise-training program produce more benefits for people with higher levels of DM but fewer for those with lower levels? If it was the case, it would be expected to find an interaction as hypothesized in **Figure 3**, where is shown potential additive or synergistic effects between PF and DM. To test such a question, the investigator should pre-specify DM as a moderator variable of interest before the intervention is delivered, and should observe whether DM influences the strength of the association between the exercise training intervention and outcomes related to cardiovascular responses to stress. In other words, to test whether the causal relation between PF and cardiovascular responses to stress changes as a function of DM (Baron and Kenny, 1986). In statistical terms, the slope of the interaction between DM and the exercise-training program improving PF, in a multivariate regression model, should be significant.

FIGURE 3 | Dispositional mindfulness (DM) as moderator of the physical fitness effects on cardiovascular responses to stress.

MINDFULNESS AS A MEDIATOR OF THE EFFECTS OF EXERCISE ON CARDIOVASCULAR RESPONSE TO STRESS

Our second hypothesis is that mindfulness may be useful to understand the processes through which PF influences changes in cardiovascular responses to stress (i.e., mindfulness as a mediator mechanism of PF effects). In this case, rather than hypothesizing a direct causal relationship between the exercise training and outcomes, our mediational model hypothesizes that exercise training causes changes on mindfulness levels, which in turn helps to account for outcomes related to cardiovascular responses to stress (**Figure 4**, lines "B" plus "C").

Two recent studies give support to this mediational hypothesis. Goldin and colleagues (Goldin et al., 2012) using a randomized controlled design observed, among other findings, that both MBI and an 8-week aerobic exercise (AE) program were with reductions in self-reported negative emotional reactivity to negative self-beliefs. Although it was not their primary outcome, interestingly, they also observed that AE increased participants' mindfulness levels measured by the Kentucky Inventory of Mindfulness Skills (Baum et al., 2010). This increase was statistically significant but less intense than that induced by the MBI, although there were no significant differences between groups in pre-to-post change (Goldin et al., 2012). Another study, led by Zgierska et al. (2013), interestingly showed that increases in DM measured by MAAS seems to be a better predictor than exercise intensity in preventing cold illness severity when comparing a MBI to an AE program to prevent acute respiratory infection, using a randomized controlled design (Zgierska et al., 2013). Together, these findings suggest that a "classical" AE program also has the potential to promote mindful awareness, and the hypothesis that mindfulness is a mediator of the exercise training on cardiovascular responses to stress may be feasible.

A potential mechanism that could explain this finding is that during and just after (recovery period) a physical exercise, mainly of moderate to vigorous intensity, it is common the awareness of our present moment body sensations such as our breathing rhythm, body temperature, or some kind of body transient momentary discomfort, or even some changing in our internal affective state. Such a combination may enhance one's acute

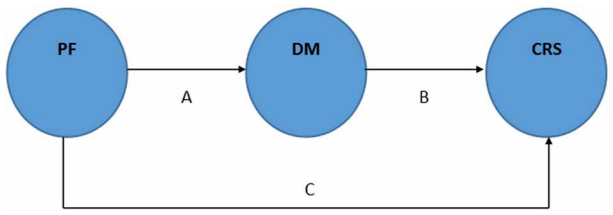

FIGURE 4 | Schematic representation of the mediator hypothesis: mindfulness as mediator (A + B) of the physical fitness effects on cardiovascular responses to stress. Adapted from Baron and Kenny (1986). PF: physical fitness. DM: dispositional mindfulness. CRS: cardiovascular responses to stress.

mindfulness state, and chronically one's DM. This has resemblance with the cross-stressor adaptation hypothesis that postulates that there is not only a direct adaptation to the exercise load but also a transfer to everyday life stress situations (Sothmann et al., 1996).

To confirm the mediational hypothesis presented herein, an investigator should test whether an exercise program produces the observed outcomes in cardiovascular responses to stress partially via changes in mindfulness levels (mediator variable). In general, to test mediation hypotheses, it is common to use statistical methods such as regression analysis in order to verify whether both the intervention (exercise program) and the outcome (cardiovascular responses to stress) covary as expected with the hypothesized mediating variable (mindfulness levels), and whether controlling for mindfulness levels explains part of the exercise program effects (Baron and Kenny, 1986). In other words, it is necessary to demonstrate that the relationship between the exercise program and the cardiovascular responses to stress is significantly lower when the mindfulness level is included in the equation. Using the representation in **Figure 4**, when paths "A" and "B" are controlled, a previously significant relation between the independent (PF) and dependent variable (cardiovascular responses to stress) is less or no longer significant (Baron and Kenny, 1986).

MINDFULNESS AS BOTH A MODERATOR AND MEDIATOR OF THE EFFECTS OF EXERCISE ON CARDIOVASCULAR RESPONSE TO STRESS

Our third hypothesis is a combined model with mindfulness having both mediator and moderator status (**Figure 5**). Baron and Kenny coined the term "mediated moderation" for this potential kind of relation among variables (Baron and Kenny, 1986). In **Figure 5**, mediated moderation would be indicated by "PF × DM1" affecting "CRS" in line "D," and "PF × DM1" affecting "DM2" and "DM2" affecting "CRS" in lines "D" plus "B". So it is possible for "DM2" (post-intervention DM) to mediate both the effect of PF on cardiovascular responses to stress, and the effect of "PF × DM1" (pre-intervention DM moderating PF) on that outcome.

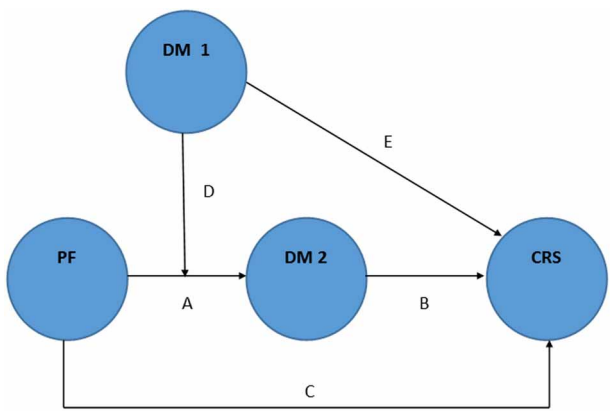

FIGURE 5 | Schematic representation of the general hypothesis: mindfulness as both moderator and mediator of the physical fitness effects on cardiovascular responses to stress ("D" represents "mediated moderation"). Adapted from Baron and Kenny (1986). PF: physical fitness. DM: dispositional mindfulness. CRS: cardiovascular responses to stress. DM 1: DM pre-intervention. DM 2: DM post-intervention.

It may be explained by the fact that pre-intervention DM levels (DM1) may differentially affect post-intervention DM (DM2) induced by PF ("mediated moderation"). In other words, one's pre-intervention DM moderates the effects of PF on post-intervention DM (DM2) levels, and then DM2 mediates the effects of PF on cardiovascular responses to stress (dependent variable). Once more, there is no direct evidence about this hypothesis, but it has been suggested that individuals with higher pre-intervention DM have greater benefits (e.g., higher post-intervention DM) from participating in a MBI (Shapiro et al., 2011), and the same process may occur regarding the effects of an exercise intervention. To test this hypothetical model, researchers might use modeling of covariance structures.

IMPLICATIONS FOR FUTURE STUDIES

Based on our hypotheses, some further studies could be performed. One possibility would be to compare results from AE programs done with additional mindfulness training to usual training (non-mindful component) using the mediational hypothesis analysis. Another study, maybe the most innovative type, would be to compare an exercise session performed with additional instruction to enhance mindfulness, i.e., with full attention to movements and bodily sensations, to a usual training session without this kind of instruction. This last possibility was also suggested by Stults-Kolehmainen (2013, p. 3). In this same study, another possibility for control group would be individuals that intentionally do not stay aware of the exercise, as it has been suggested that multitasking during exercise results in less beneficial stress adaptations (Breus and O'Connor, 1998).

With regard to this last potential study, there are few well-designed studies that have already compared mindful modes of exercise to non-mindful ones but not specifically related to cardiovascular responses to stress (Netz and Lidor, 2003; Tsang et al., 2008). Although an initial study had ambiguous results (Brown et al., 1995), one interesting study examined the effect of a single session of mindful exercise on mood. The intervention compared four physical exercise modes: yoga, the Feldenkrais technique (awareness through movement), aerobic dance, and swimming, with a cognitive exercise delivered by computer lessons serving as control. Measures of mood improved following Feldenkrais, swimming, and yoga, but did not improve following aerobic dance and computer lessons (Netz and Lidor, 2003), supporting the idea that mindful exercise may have additional benefits compared to non-mindful modes of exercises.

In addition, future studies should examine some competing theories as alternatives to our hypotheses. One possibility is that higher scores in DM could help persons to better perform or tolerate exercise training (e.g., through better training psychological and physiological adaptation), consequently improving adherence and compliance to exercise protocols. Another competing argument would be that exercise training might not result in an increase in DM, but that exercise training could improve certain factors such as perceived stress (Starkweather, 2007) which are

also improved by the practice of mindfulness, and so both would have positive effects, but exercise would not necessarily lead to improvements in DM.

As expected, our theoretical model has limitations. A main limitation is that scales and questionnaires to measure mindfulness are still object of scientific debate, and some authors have argued that they lack construct validity in measuring the complexity of mindfulness (Grossman et al., 2011; Park et al., 2013). In this way, future situational measurements of mindfulness (Mitchell et al., 2013) could be more effective to support or reject our hypotheses. Another main limitation is that mindfulness seems to be a multidimensional construct (Grossman et al., 2011; Park et al., 2013) and we have not addressed this fact herein in order to simplify the model. In this direction, further research should address the differential moderator and mediator roles of these mindfulness sub-dimensions, e.g., those addressed by the FFMQ ("observing," "describing," "acting with awareness," "non-judging of inner experience," and "non-reactivity to inner experience") (Bohlmeijer et al., 2011).

CONCLUSION

In conclusion, we have developed the hypothesis that mindfulness may both moderate and mediate the effects of PF on cardiovascular response to stress. This innovative structural model may oxygenate the debate in this research field and help researchers better understand the effects of PF on cardiovascular responses to stress. In addition, general research on both fields of exercise and mindfulness may benefit from this kind of theoretical approach as well.

Conceivably, a "mindful" exercise may provide benefits that are not available in "non-mindful" regular exercise or in opposition to situations where exercise is performed in a multitasking context (Breus and O'Connor, 1998). If so, a mixture of aerobic or resistance physical training with mindfulness would have the potential to improve cardiovascular response to stress in a more effective direction.

Based on these feasible hypotheses, exercise researchers should consider mindfulness in their future research protocols in order to address questions such as whether a higher DM is associated with better health outcomes in an exercise protocol, or if a mindfulness training session mixed into an exercise program could improve health and physical performance among patients, general population, and athletes.

AUTHOR CONTRIBUTIONS

Marcelo M. P. Demarzo presented the initial concept of the hypothesis, drafted the first version, and organized subsequent versions until the final format of the manuscript. Jesús Montero-Marin, Phyllis K. Stein, Ausiàs Cebolla, Jaime G. Provinciale, and Javier García-Campayo contributed to the development and improvement of the hypothesis and manuscript until their final version.

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REFERENCES

Baron, R. M., and Kenny, D. A. (1986). The moderator–mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J. Pers. Soc. Psychol.* 51, 1173–1182. doi: 10.1037/0022-3514.51.6.1173

Baum, C., Kuyken, W., Bohus, M., Heidenreich, T., Michalak, J., and Steil, R. (2010). The psychometric properties of the Kentucky Inventory of Mindfulness Skills in clinical populations. *Assessment* 17, 220–229. doi: 10.1177/1073191109356525

Bohlmeijer, E., ten Klooster, P. M., Fledderus, M., Veehof, M., and Baer, R. (2011). Psychometric properties of the five facet mindfulness questionnaire in depressed adults and development of a short form. *Assessment* 18, 308–320. doi: 10.1177/1073191111408231

Bonadonna, R. (2003). Meditation's impact on chronic illness. *Holist. Nurs. Pract.* 17, 309–319.

Brand, S., Holsboer-Trachsler, E., Naranjo, J. R., and Schmidt, S. (2012). Influence of mindfulness practice on cortisol and sleep in long-term and short-term meditators. *Neuropsychobiology* 65, 109–118. doi: 10.1159/000330362

Bränström, R., Duncan, L. G., and Moskowitz, J. T. (2011). The association between dispositional mindfulness, psychological well-being, and perceived health in a Swedish population-based sample. *Br. J. Health Psychol.* 16(Pt 2), 300–316. doi: 10.1348/135910710X501683

Breus, M. J., and O'Connor, P. J. (1998). Exercise-induced anxiolysis: a test of the "time out" hypothesis in high anxious females. *Med. Sci. Sports and Exerc.* 30, 1107–1112. doi: 10.1097/00005768-199807000-00013

Britton, W. B., Haynes, P. L., Fridel, K. W., and Bootzin, R. R. (2012). Mindfulness-based cognitive therapy improves polysomnographic and subjective sleep profiles in antidepressant users with sleep complaints. *Psychother. Psychosom.* 81, 296–304. doi: 10.1159/000332755

Brown, D. R., Wang, Y., Ward, A., Ebbeling, C. B., Fortlage, L., Puleo, E., et al. (1995). Chronic psychological effects of exercise and exercise plus cognitive strategies. *Med. Sci. Sports Exerc.* 27, 765–775. doi: 10.1249/00005768-199505000-00021

Brown, K. W., Goodman, R. J., and Inzlicht, M. (2013). Dispositional mindfulness and the attenuation of neural responses to emotional stimuli. *Soc. Cogn. Affect. Neurosci.* 8, 93–99. doi: 10.1093/scan/nss004

Brown, K. W., and Ryan, R. M. (2003). The benefits of being present: mindfulness and its role in psychological well-being. *J. Pers. Soc. Psychol.* 84, 822–848. doi: 10.1037/0022-3514.84.4.822

Carson, J. W., Carson, K. M., Gil, K. M., and Baucom, D. H. (2006). "Mindfulness-Based Relationship Enhancement (MBRE) in couples," in *Mindfulness-Based Treatment Approaches: Clinician's Guide to Evidence Base and Applications*, ed J. W. Carson (Durham, NC: Elsevier Academic Press), 309–331. doi: 10.1016/B978-012088519-0/50015-0

Ciesla, J. A., Reilly, L. C., Dickson, K. S., Emanuel, A. S., and Updegraff, J. A. (2012). Dispositional mindfulness moderates the effects of stress among adolescents: rumination as a mediator. *J. Clin. Child Adolesc. Psychol.* 41, 760–770. doi: 10.1080/15374416.2012.698724

Creswell, J. D., Irwin, M. R., Burklund, L. J., Lieberman, M. D., Arevalo, J. M. G., Ma, J., et al. (2012). Mindfulness-based stress reduction training reduces loneliness and pro-inflammatory gene expression in older adults: a small randomized controlled trial. *Brain Behav. Immun.* 26, 1095–1101. doi: 10.1016/j.bbi.2012.07.006

Cullen, M. (2011). Mindfulness-based interventions: an emerging phenomenon. *Mindfulness* 2, 186–193. doi: 10.1007/s12671-011-0058-1

Demarzo, M. M. P., and Stein, P. K. (2012). Mental stress and exercise training response: stress-sleep connection may be involved. *Front. Physiol.* 3:178. doi: 10.3389/fphys.2012.00178

Dimidjian, S., and Kleiber, B. (2013). Being mindful about the use of mindfulness in clinical contexts. *Cogn. Behav. Pract.* 20, 57–59. doi: 10.1016/j.cbpra.2012.02.006

Duncan, L. G., and Bardacke, N. (2010). Mindfulness-based childbirth and parenting education: promoting family mindfulness during the perinatal period. *J. Child Fam. Stud.* 19, 190–202. doi: 10.1007/s10826-009-9313-7

Edelman, D., Oddone, E. Z., Liebowitz, R. S., Yancy, W. S. Jr., Olsen, M. K., Jeffreys, A. S., et al. (2006). A multidimensional integrative medicine intervention to improve cardiovascular risk. *J. Gen. Intern. Med*. 21, 728–734. doi: 10.1111/j.1525-1497.2006.00495.x

Farb, N. A. S., Segal, Z. V., and Anderson, A. K. (2013). Mindfulness meditation training alters cortical representations of interoceptive attention. *Soc. Cogn. Affect. Neurosci.* 8, 15–26. doi: 10.1093/scan/nss066

Fortney, L., and Taylor, M. (2010). Meditation in medical practice: a review of the evidence and practice. *Primary Care* 37, 81–90. doi: 10.1016/j.pop.2009.09.004

Garcia, M. C., Pompéia, S., Hachul, H., Kozasa, E. H., de Souza, A. A., Tufik, S., et al. (2014). Is mindfulness associated with insomnia after menopause? *Menopause* 21, 301–305. doi: 10.1097/GME.0b013e31829996fc

Garcia-Campayo, J., Navarro-Gil, M., Andrés, E., Montero-Marin, J., López-Artal, L., and Demarzo, M. M. P. (2014). Validation of the Spanish versions of the long (26 items) and short (12 items) forms of the Self-Compassion Scale (SCS). *Health Qual. Life Outcomes* 12:4. doi: 10.1186/1477-7525-12-4

Gardner, F. L., and Moore, Z. E. (2012). Mindfulness and acceptance models in sport psychology: A decade of basic and applied scientific advancements. *Can. Psychol.* 53, 309–318. doi: 10.1037/a0030220

Garland, E. L., Gaylord, S. A., Boettiger, C. A., and Howard, M. O. (2010). Mindfulness training modifies cognitive, affective, and physiological mechanisms implicated in alcohol dependence: results of a randomized controlled pilot trial. *J. Psychoactive Drugs* 42, 177–192. doi: 10.1080/02791072.2010.10400690

Garland, S. N., Campbell, T., Samuels, C., and Carlson, L. E. (2013). Dispositional mindfulness, insomnia, sleep quality and dysfunctional sleep beliefs in post-treatment cancer patients. *Pers. Indiv. Differ.* 55, 306–311. doi: 10.1016/j.paid.2013.03.003

Goldin, P., Ziv, M., Jazaieri, H., and Gross, J. J. (2012). Randomized controlled trial of mindfulness-based stress reduction versus aerobic exercise: effects on the self-referential brain network in social anxiety disorder. *Front. Hum. Neurosci.* 6:295. doi:10.3389/fnhum.2012.00295

Grossman, P. (2011). Defining mindfulness by how poorly I think I pay attention during everyday awareness and other intractable problems for psychology's (re)invention of mindfulness: comment on Brown et al. (2011). *Psychol. Assess.* 23, 1034–1040. doi: 10.1037/a0022713

Gryffin, P. A., and Chen, W. C. (2013). Implications of t'ai chi for smoking cessation. *J. Altern. Complement. Med.* 19, 141–145. doi: 10.1089/acm.2011.0094

Hagins, M., States, R., Selfe, T., and Innes, K. (2013). Effectiveness of yoga for hypertension: systematic review and meta-analysis. *Evid. Based Complement. Alternat. Med.* 2013:649836. doi: 10.1155/2013/649836

Hayes-Skelton, S., and Graham, J. (2013). Decentering as a common link among mindfulness, cognitive reappraisal, and social anxiety. *Behav. Cogn. Psychother.* 41, 317–328. doi: 10.1017/S1352465812000902

Hölzel, B. K., Lazar, S. W., Gard, T., Schuman-Olivier, Z., Vago, D. R., and Ott, U. (2011). How does mindfulness meditation work? Proposing mechanisms of action from a conceptual and neural perspective. *Perspect. Psychol. Sci.* 6, 537–559. doi: 10.1177/1745691611419671

Huang, C.-J., Webb, H. E., Zourdos, M. C., and Acevedo, E. O. (2013). Cardiovascular reactivity, stress, and physical activity. *Front. Physiol.* 4:314. doi: 10.3389/fphys.2013.00314

Kabat-Zinn, J. (2003). Mindfulness-based stress reduction (MBSR). *Constr. Hum. Sci.* 8, 73–107. Available online at: http://psycnet.apa.org/psycinfo/2004-19791-008

Kabat-Zinn, J. (2005). *Full Catastrophe Living: Using the Wisdom of Your Body and Mind to Face Stress, Pain, and Illness.* 15th Anniversary Edn. New York, NY: Delta Trade Paperback/Bantam Dell.

Khoury, B., Lecomte, T., Fortin, G., Masse, M., Therien, P., Bouchard, V., et al. (2013). Mindfulness-based therapy: a comprehensive meta-analysis. *Clin. Psychol. Rev.* 33, 763–771. doi: 10.1016/j.cpr.2013.05.005

Koolhaas, J. M., Bartolomucci, A., Buwalda, B., de Boer, S. F., Flügge, G., Korte, S. M., et al. (2011). Stress revisited: a critical evaluation of the stress concept. *Neurosci. Biobehav. Rev.* 35, 1291–1301. doi: 10.1016/j.neubiorev.2011.02.003

Krygier, J. R., Heathers, J. A. J., Shahrestani, S., Abbott, M., Gross, J. J., and Kemp, A. H. (2013). Mindfulness meditation, well-being, and heart rate variability: a preliminary investigation into the impact of intensive Vipassana meditation. *Int. J. Psychophysiol.* 89, 305–313. doi: 10.1016/j.ijpsycho.2013.06.017

Kuyken, W., Byford, S., Taylor, R. S., Watkins, E., Holden, E., White, K., et al. (2008). Mindfulness-based cognitive therapy to prevent relapse in recurrent depression. *J. Consult. Clin. Psychol.* 76, 966–978. doi: 10.1037/a0013786

Libby, D. J., Worhunsky, P. D., Pilver, C. E., and Brewer, J. A. (2012). Meditation-induced changes in high-frequency heart rate variability predict smoking outcomes. *Front. Hum. Neurosci.* 6:54. doi: 10.3389/fnhum.2012.00054

Malarkey, W. B., Jarjoura, D., and Klatt, M. (2013). Workplace based mindfulness practice and inflammation: a randomized trial. *Brain Behav. Immun.* 27, 145–154. doi: 10.1016/j.bbi.2012.10.009

Martarelli, D., Cocchioni, M., Scuri, S., and Pompei, P. (2011). Diaphragmatic breathing reduces exercise-induced oxidative stress. *Evid. Based Complement. Alternat. Med.* 2011:932430. doi: 10.1093/ecam/nep169

Mitchell, J. C., Bach, P. A., and Cassisi, J. E. (2013). The use of structured imagery and dispositional measurement to assess situational use of mindfulness skills. *PLoS ONE* 8:e70253. doi: 10.1371/journal.pone.0070253

Murphy, M. J., Mermelstein, L. C., Edwards, K. M., and Gidycz, C. A. (2012). The benefits of dispositional mindfulness in physical health: a longitudinal study of female college students. *J. Am. Coll. Health* 60, 341–348. doi: 10.1080/07448481.2011.629260

Netz, Y., and Lidor, R. (2003). Mood alterations in mindful versus aerobic exercise modes. *J. Psychol.* 137, 405–419. doi: 10.1080/00223980309600624

Nyklíček, I., Mommersteeg, P. M. C., Van Beugen, S., Ramakers, C., and Van Boxtel, G. J. (2013a). Mindfulness-based stress reduction and physiological activity during acute stress: a randomized controlled trial. *Health Psychol.* 32, 1110–1113. doi: 10.1037/a0032200

Nyklíček, I., van Beugen, S., and Denollet, J. (2013b). Effects of mindfulness-based stress reduction on distressed (Type D) personality traits: a randomized controlled trial. *J. Behav. Med.* 36, 361–370. doi: 10.1007/s10865-012-9431-3

Ospina, M. B., Bond, K., Karkhaneh, M., Tjosvold, L., Vandermeer, B., Liang, Y., et al. (2007). Meditation practices for health: state of the research. *Evid. Rep. Technol. Assess.* 1–263. Available online at: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L350342742

Park, T., Reilly-Spong, M., and Gross, C. R. (2013). Mindfulness: a systematic review of instruments to measure an emergent patient-reported outcome (PRO). *Qual. Life Res.* 22, 2639–2659. doi: 10.1007/s11136-013-0395-8

Peressutti, C., Martín-gonzález, J. M., and García-manso, J. M. (2011). Does mindfulness meditation shift the cardiac autonomic nervous system to a highly orderly operational state? *Int. J. Cardiol.* 154, 210–212. doi: 10.1016/j.ijcard.2011.10.054

Posadzki, P., and Jacques, S. (2009). Tai chi and meditation: a conceptual (re)synthesis? *J. Holist. Nurs.* 27, 103–114. doi: 10.1177/0898010108330807. Available online at: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L355102988

Prakhinkit, S., Suppapitiporn, S., Tanaka, H., and Suksom, D. (2013). Effects of buddhism walking meditation on depression, functional fitness, and endothelium-dependent vasodilation in depressed elderly. *J. Altern. Complement. Med.* doi: 10.1089/acm.2013.0205. [Epub ahead of print].

Rosenkranz, M. A., Davidson, R. J., Maccoon, D. G., Sheridan, J. F., Kalin, N. H., and Lutz, A. (2013). A comparison of mindfulness-based stress reduction and an active control in modulation of neurogenic inflammation. *Brain Behav. Immun.* 27, 174–184. doi: 10.1016/j.bbi.2012.10.013

Salmoirago-Blotcher, E., Crawford, S., Carmody, J., Rosenthal, L., and Ockene, I. (2011). Characteristics of dispositional mindfulness in patients with severe cardiac disease. *J. Evid. Based Complement. Altern. Med.* 16, 218–225. doi: 10.1177/2156587211405525

Shapiro, S. L., Brown, K. W., Thoresen, C., and Plante, T. G. (2011). The moderation of Mindfulness-based stress reduction effects by trait mindfulness: results from a randomized controlled trial. *J. Clin. Psychol.* 67, 267–277. doi: 10.1002/jclp.20761

Soler, J., Cebolla, A., Feliu-Soler, A., Demarzo, M. M. P., Pascual, J. C., Baños, R., et al. (2014). Relationship between meditative practice and self-reported mindfulness: the MINDSENS composite index. *PLoS ONE* 9:e86622. doi: 10.1371/journal.pone.0086622

Sothmann, M. S., Buckworth, J., Claytor, R. P., Cox, R. H., White-Welkley, J. E., and Dishman, R. K. (1996). Exercise training and the cross-stressor adaptation hypothesis. *Exerc. Sport Sci. Rev.* 24, 267–287. doi: 10.1249/00003677-199600240-00011

Starkweather, A. R. (2007). The effects of exercise on perceived stress and IL-6 levels among older adults. *Biol. Res. Nurs.* 8, 186–194. doi: 10.1177/1099800406295990

Stoner, L., Lucero, A. A., Palmer, B. R., Jones, L. M., Young, J. M., and Faulkner, J. (2013). Inflammatory biomarkers for predicting cardiovascular disease. *Clin. Biochem.* 46, 1353–1371. doi: 10.1016/j.clinbiochem.2013.05.070

Stults-Kolehmainen, M. A. (2013). The interplay between stress and physical activity in the prevention and treatment of cardiovascular disease. *Front. Physiol.* 4:346. doi: 10.3389/fphys.2013.00346

Tanay, G., and Bernstein, A. (2013). State Mindfulness Scale (SMS): development and initial validation. *Psychol. Assess.* 25, 1286–1299. doi: 10.1037/a0034044

Thayer, J. F., Yamamoto, S. S., and Brosschot, J. F. (2010). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int. J. Cardiol.* 141, 122–131. doi: 10.1016/j.ijcard.2009.09.543

Tsang, H. W. H., Chan, E. P., and Cheung, W. M. (2008). Effects of mindful and non-mindful exercises on people with depression: a systematic review. *Br. J. Clin. Psychol.* 47(Pt 3), 303–322. doi: 10.1348/014466508X279260

Ulmer, C. S., Stetson, B. A., and Salmon, P. G. (2010). Mindfulness and acceptance are associated with exercise maintenance in YMCA exercisers. *Behav. Res. Ther.* 48, 805–809. doi: 10.1016/j.brat.2010.04.009

Zgierska, A., Obasi, C. N., Brown, R., Ewers, T., Muller, D., Gassman, M., et al. (2013). Randomized controlled trial of mindfulness meditation and exercise for the prevention of acute respiratory infection: possible mechanisms of action. *Evid. Based Complement. Altern. Med.* 2013:952716. doi: 10.1155/2013/952716

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Regular exercise is associated with emotional resilience to acute stress in healthy adults

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Physical activity has long been considered beneficial to health and regular exercise is purported to relieve stress. However empirical evidence demonstrating these effects is limited. In this study, we compared psychophysiological responses to an acute psychosocial stressor between individuals who did, or did not, report regular physical exercise. Healthy men and women ($N = 111$) participated in two experimental sessions, one with the Trier Social Stress Test (TSST) and one with a non-stressful control task. We measured heart rate, blood pressure, cortisol, and self-reported mood before and at repeated times after the tasks. Individuals who reported physical exercise at least once per week exhibited lower heart rate at rest than non-exercisers, but the groups did not differ in their cardiovascular responses to the TSST. Level of habitual exercise did not influence self-reported mood before the tasks, but non-exercisers reported a greater decline in positive affect after the TSST in comparison to exercisers. These findings provide modest support for claims that regular exercise protects against the negative emotional consequences of stress, and suggest that exercise has beneficial effects in healthy individuals. These findings are limited by their correlational nature, and future prospective controlled studies on the effects of regular exercise on response to acute stress are needed.

Keywords: physical activity, stress, TSST, heart rate, blood pressure, cortisol, mood

INTRODUCTION

The Centers for Disease Control and Prevention (CDC) and the American College of Sports Medicine (ACSM) recommend that American adults exercise for at least 30 min on most days to improve their health and quality of life (Pate et al., 1995; Haskell et al., 2007). Indeed, clinical trials have shown that regular exercise is an effective treatment for disease, including physical ailments e.g., cardiovascular disease (Elrick, 1996), and psychiatric disorders e.g., depression (Dinas et al., 2011). Further, regular exercise is frequently associated with general well-being and lower rates of mood and anxiety disorders in cross-sectional studies (Dua and Hargreaves, 1992; Slaven and Lee, 1997; Goodwin, 2003) and with improved longevity and decreased mortality in prospective studies (Kujala et al., 1998; Samitz et al., 2011). However, the mechanisms underlying the benefits of exercise are not clear. One way in which exercise may promote health is via enhanced resilience to stress, since stress exposure and chronic stress burden has been associated with physical and mental illness (McEwen, 2007).

Acute stress produces a cascade of physiological and psychological effects that are coordinated by the sympathetic nervous system (SNS) and the hypothalamic pituitary adrenal axis (HPAA, López et al., 1999; Chrousos, 2009). Soon after encountering a stressor, defined as any emotional, physical or psychological threat that perturbs homeostasis, heart rate and blood pressure increase, along with mental alertness and tension, and cortisol is released into the blood from the adrenals (Habib et al., 2001). This multidimensional stress response is extremely beneficial and serves to ready the organism to deal with the imminent threat, however when it is improperly activated it can have deleterious effects and contribute to diseases such as atherosclerosis, obesity and depression (McEwen, 2006). Intense physical activity can also be considered a stressor since it activates the same systems involved in responding to an external threat (Hackney, 2006); bouts of exercise increase heart rate, blood pressure and levels of cortisol. Thus, regular activation of stress systems by physical exercise may produce beneficial adaptations such that these systems are able to respond to acute stress more effectively, for example with reduced vigor or shorter duration. This idea has been termed the cross-stressor adaptation hypothesis (Sothmann, 2006).

Previous studies have investigated psychophysiological responses to mentally challenging or psychologically stressful laboratory tasks among healthy individuals with respect to levels of physical fitness. Most of these studies have focused upon cardiovascular reactivity to psychologically stressful tasks with mixed findings. For example, more physically fit individuals have exhibited enhanced reactivity (de Geus et al., 1993), blunted reactivity (Crews and Landers, 1987) or no difference (Steptoe et al., 1990; Blumenthal et al., 1991; Summers et al., 1999; Spalding et al., 2004; Poole et al., 2011) in comparison to their less physically fit counterparts. Further, two recent meta-analyses of studies reached different conclusions regarding cardiovascular reactivity; Jackson and Dishman (2006) reported that physical fitness was associated with a greater cardiovascular response yet quicker recovery, while Forcier et al. (2006) reported

that cardiovascular responses were blunted. A limited number of studies have examined cortisol responses to psychological stress. In these studies, physically fit men, older women, and highly active children exhibited blunted cortisol responses to psychological stress in comparison to their less physically active counterparts (Traustadottir et al., 2004; Rimmele et al., 2007; Martikainen et al., 2013). Finally, few studies have reported on emotional responses to stress with respect to levels of physical exercise, and again the findings have been mixed (e.g., Sinyor et al., 1983; Choi and Salmon, 1995; Summers et al., 1999). The discrepancies in findings between studies may be due to methodological differences, including stress induction methods (e.g., speech tasks tend to produce greater psychophysiological responses than mental challenge tasks, Dickerson and Kemeny, 2004) and subject samples studied (e.g., previous studies show evidence of sex differences in responses to psychological stress, Kirschbaum et al., 1999; Kelly et al., 2008; Childs et al., 2010).

In this study we aimed to assess multidimensional responses i.e., physiological (heart rate, blood pressure, salivary cortisol) and psychological (anxiety, positive mood) to a standardized acute psychosocial stressor among young healthy men and women, and to compare stress responses between individuals who reported regular physical exercise and those who did not. To our knowledge no studies have measured multidimensional aspects of stress responses i.e., cardiovascular, hormonal and emotional, in the same individuals which is important to show the existence of relationships between the different modalities. Furthermore, very few studies have examined stress-induced changes in positive and negative mood states with respect to levels of habitual physical exercise. We hypothesized, based on previous reports, that non-exercisers participants would exhibit greater cardiovascular and emotional reactivity, and dampened cortisol responses to stress in comparison to regular exercisers.

MATERIALS AND METHODS

SUBJECTS

Participants ($n = 111$) were recruited from the University and surrounding area by flyers and advertisements. They attended the laboratory for an in-person medical screening that included a health and drug use questionnaire and an ECG. Participants were healthy adults, aged 18–32, with body mass index 19–29 kg/m^2. Exclusion criteria included a current or past year diagnosis of a Major Axis I psychiatric disorder (American Psychiatric Association, 1994), an abnormal electrocardiogram, use of prescription medications including, in women, oral contraceptives (Kirschbaum et al., 1995, 1999), or night shift work. Individuals who smoked >5 cigarettes/week were also excluded as smoking has been shown to alter responses to the TSST (Kirschbaum et al., 1993b; al'Absi et al., 2003; Childs and de Wit, 2009). Information on physical activity was obtained from a questionnaire administered at screening; participants indicated whether they exercised on a regular basis (outside of normal activities including commuting), and how many times per week they exercised. Participants were then classified as sedentary ($n = 30$) or regular exercisers (i.e., $\geq$1 occasion per week, $n = 81$). Participants were told that the study aim was to examine the effects of verbal tasks on mood and physiology. At the end of the study, they were fully debriefed about the study aims and paid for their participation.

PROCEDURE

The University of Chicago Hospital's Institutional Review Committee for the use of human subjects approved the study protocols. All participants provided informed consent at a separate orientation session conducted before the study began. Participants completed two sessions at least 48 h apart, one with a stressful task and another with a non-stressful control task, in randomized order. The stressor that we used was the Trier Social Stress Test (TSST; Kirschbaum et al., 1993a). This is a standardized and widely used psychosocial stressor that reliably induces changes in physiological and psychological dimensions (Dickerson and Kemeny, 2004). Participants also completed a non-stressful control task on a separate day to account for diurnal rhythms in mood and physiology (Childs and de Wit, 2009; Het et al., 2009; Childs et al., 2010; Lovallo et al., 2010).

All study procedures were conducted at the Human Behavioral Pharmacology Laboratory at the University of Chicago. Experimental sessions were conducted in testing rooms furnished as a comfortable living area, with an easy chair for relaxing (when participants were not completing study measures), a television and video player, and a desk with a computer for completing study questionnaires. On arrival, participants provided breath and urine samples to detect recent drug or alcohol use (no one tested positive) and then relaxed for 30 min to acclimatize to the laboratory. Baseline measures were obtained and then participants were read instructions for the task to be performed that day. They were allowed 10 min to prepare for each task, at the end of which they were escorted to an adjacent room to perform the task. The TSST consisted of a 5 min speech and 5 min mental arithmetic (serial subtraction) performed before two interviewers who were unknown to the participant and who provided no feedback. There was also a video camera present which projected the participants' image onto a television screen throughout the task. The control task was performed in the absence of a video camera, and involved the participant talking to the research assistant for 5 min about a favorite book, movie or television program, followed by playing a computer game (Solitaire) for 5 min. Before, and at repeated times after the tasks, participants rated their mood, saliva samples were obtained for cortisol analysis, and vital signs were obtained.

DEPENDENT MEASURES

Saliva samples were collected using Salivette® cotton wads (Sarstedt Inc., Newton, NC) at −30, 10, 20, and 60 min after the tasks and were analyzed by the Core Laboratory at the University of Chicago Hospitals General Clinical Research Center for levels of cortisol (Salimetrics LLC, State College, PA, sensitivity = 0.003 ug/dL). Heart rate (HR) was measured continuously throughout the experimental session (one reading per minute) using a Polar chest band and monitor (Mini-Logger, Mini Mitter/Respironics, Bend, OR). Scores were averaged over consecutive 10 min periods. Blood pressure was measured using

a monitor (Critikon Dinamap Plus Vital Signs Monitor, GE Healthcare Technologies, Waukesha, WI) at −30, 0, 10, 20, and 30 min after the tasks. Self-reported mood was measured using the Profile of Mood States questionnaire (POMS, McNair and Droppleman, 1971) at −30 and 0 min after the task.

Personality traits were assessed using the Multidimensional Personality Questionnaire Brief Form (MPQ-BF, Patrick et al., 2002). This questionnaire is an empirically-derived personality instrument with an orthogonal factor structure that yields 11 well-defined primary trait scores and three superfactors termed Positive Emotionality (PEM, extraversion), Negative Emotionality (NEM, neuroticism) and Constraint (CON, behavioral spontaneity).

Some data points were missing due to sample loss or equipment failure, thus there were minor variations in samples sizes between the separate analyses (heart rate $n = 77$; blood pressure $n = 111$; mood $n = 96$; cortisol $n = 99$; personality $n = 102$).

STATISTICAL ANALYSES

Two markers of stress reactivity were calculated for the outcome measures; (1) peak change from pre-task baseline, which provides a measure of the intensity of the response, and (2) area under the curve relative to the pre-task baseline (AUC, Altman, 1991; Pruessner et al., 2003), which provides information about response duration and recovery of homeostasis after stress exposure. We first confirmed the efficacy of the stress task by comparing responses in the outcome measures between the two tasks using one factor (Task) repeated measures analysis of variance (ANOVA). We also compared responses to the tasks between men and women using two factor (Task∗Sex) repeated measures ANOVA, since others have previously reported sex differences in responses to the TSST (Kirschbaum et al., 1999; Kelly et al., 2008; Childs et al., 2010). Sex was included as an additional factor in later analyses for any outcome measures that were significantly influenced by Sex.

We then compared demographic and personality characteristics between the groups using independent samples *t*-test (for continuous variables) and chi-squared analysis (for categorical variables). We also compared baseline measures (average of pre-task scores from each session) between the groups using independent samples *t*-test. We compared responses to the tasks between groups using two-factor repeated measures (Task∗Group) ANOVA. All analyses were conducted using SPSS v19 for windows. Finally, we assessed relationships between self-reported frequency of exercise per week and net responses to stress (i.e., response after TSST minus response after control task) using Pearson's correlation coefficient. Effect sizes are reported using partial eta squared (η_ρ^2) for analyses of variance; 0.01, 0.06, and 0.14 are considered, respectively, small, medium, and large effect sizes.

RESULTS

DEMOGRAPHICS

Most participants were of European descent (53%) and in their early twenties (22.1 ± 0.4 years, **Table 1**). The groups did not differ on any demographic or personality characteristics.

Table 1 | Demographic characteristics of study participants.

	Non-exercisers	Exercisers
N (male/female)	30 (7/23)	81 (35/46)
Exercise frequency (times/week)	0	3.5 ± 0.2
RACE (%)		
European American*	30	62
African American	33	17
Other	37	21
Age (years)	21.8 ± 0.7	22.3 ± 0.4
Body mass index (kg/m^2)	22.0 ± 0.4	22.2 ± 0.2
CURRENT DRUG USE		
Caffeine (drinks/week)	8.0 ± 1.8	5.9 ± 0.7
Alcohol (drinks/week)	2.8 ± 0.7	4.0 ± 0.4
Cigarettes (per week)	0.2 ± 0.1	1.1 ± 0.6
PERSONALITY		
Positive emotionality	75.0 ± 2.1	74.0 ± 1.7
Negative emotionality	28.2 ± 2.8	26.5 ± 1.6
Constraint	71.2 ± 3.2	70.4 ± 1.9

*Values indicate mean ± s.e.m. Asterisks indicate a significant difference between the samples (*p < 0.05, Chi-squared analysis).*

BASELINE MEASURES

Before the tasks began, heart rate was significantly lower among individuals who reported regular exercise [$t_{(80)} = 2.2$; $p < 0.05$, mean difference = 6.3 ± 2.9 bpm] and baseline heart rate was significantly negatively correlated with the frequency of exercise per week ($r = -0.24$; $p < 0.05$) in the whole group. Blood pressure, cortisol and mood did not differ significantly between the groups at baseline (**Table 2**).

STRESS REACTIVITY AND SEX DIFFERENCES

In comparison to the control task, the TSST significantly increased heart rate, blood pressure, and cortisol among all participants (**Table 3**). The TSST also significantly increased negative affect (Anxiety, Depression, Anger, Confusion) and decreased positive mood states (Friendly, Elation, Positive Mood).

Overall, men exhibited greater cortisol responses to the tasks than women [Sex effect: Peak change $F_{(1, 96)} = 16.7$; $p < 0.001$; $\eta_\rho^2 = 0.15$; AUC $F_{(1, 95)} = 18.1$; $p < 0.001$; $\eta_\rho^2 = 0.16$] and greater stress-induced increases in systolic blood pressure than women [Task∗Sex effect: $F_{(1, 106)} = 4.2$; $p < 0.05$; $\eta_\rho^2 = 0.04$]. Therefore, Sex was included as a factor in later analyses of these measures. There were no other sex differences in other cardiovascular or emotional responses to the tasks.

INFLUENCE OF EXERCISE ON STRESS REACTIVITY

Heart rate, blood pressure and cortisol reactivity to the stress procedure did not differ between the groups. Individuals who did not regularly exercise exhibited significantly greater decreases in positive affect after stress [Task∗Group effect: Elation $F_{(1, 94)} = 8.38$; $p < 0.01$; $\eta_\rho^2 = 0.08$, Positive Mood $F_{(1, 94)} = 3.06$; $p = 0.08$; $\eta_\rho^2 = 0.03$, **Figure 1**]. Overall, regular exercisers also felt more "friendly" after both tasks [Group effect: Friendliness $F_{(1, 94)} = 4.39$; $p < 0.05$; $\eta_\rho^2 = 0.05$, **Figure 1**]. Correlation analyses did not show any evidence of significant relationships between the

Table 2 | Baseline values of physiological measures.

	Non-exercisers	Exercisers
Heart rate (bpm)	79.0 ± 2.5	72.4 ± 1.3*
Systolic blood pressure (mm Hg)	108.1 ± 2.1	112.1 ± 1.5
Diastolic blood pressure (mm Hg)	65.9 ± 1.6	64.9 ± 0.9
Cortisol (ug/dL)	0.47 ± 0.08	0.38 ± 0.03

*Values indicate mean ± s.e.m. Asterisks indicate a significant difference between the samples (*p < 0.05, Independent Samples t-test).*

Table 3 | Responses to the control task and TSST among all participants.

	Control	TSST	*t*
Cortisol (ug/dL)	−0.11 ± 0.03	0.04 ± 0.04	−4.3***
Heart rate (bpm)	4.1 ± 0.9	13.0 ± 1.2	7.4***
Systolic (mm Hg)	4.1 ± 1.0	12.5 ± 1.1	6.0***
Diastolic (mm Hg)	2.3 ± 0.8	8.5 ± 0.7	5.6***
Friendliness	−0.06 ± 0.08	−0.55 ± 0.08	4.7***
Anxiety	0.01 ± 0.05	0.37 ± 0.07	−4.6***
Depression	0.05 ± 0.03	0.14 ± 0.04	−2.2*
Anger	0.01 ± 0.04	0.32 ± 0.06	−5.0***
Elation	−0.03 ± 0.08	−0.46 ± 0.08	4.3***
Confusion	0.05 ± 0.05	0.28 ± 0.06	−3.1**
Positive Mood	−0.08 ± 0.09	−0.60 ± 0.09	4.5***

*Values indicate mean ± s.e.m. Asterisks indicate a significant difference between the samples (*p < 0.05, **p < 0.01, ***p < 0.001, Independent Samples t-test).*

frequency of exercise per week and psychophysiological responses to stress.

DISCUSSION

This study aimed to compare reactivity to acute stress between healthy individuals who exercise regularly and those who do not. We examined cardiovascular, cortisol and emotional responses to a standardized psychosocial stressor in comparison to a non-stressful control task among healthy male and female participants and in relation to their self-reported levels of regular physical exercise. There were several interesting findings. Overall, heart rate was significantly lower among regular exercisers than sedentary participants, yet cardiovascular reactivity to stress or the tasks overall did not differ between the groups. The groups did not significantly differ in levels of cortisol at baseline or after stress. Also, while subjective mood states did not differ between the groups at baseline, emotional responses to the tasks did differ between the groups; participants who regularly exercised exhibited less of a decline in positive affect after stress than sedentary participants. These findings suggest that regular exercisers may be more resistant to acute stress, which may protect them against future poor health.

The finding that regular exercisers exhibited a smaller decline in positive affect during a stressful situation provides some of the first direct evidence to support that habitual physical activity is associated with stress resilience in healthy individuals. Exercise

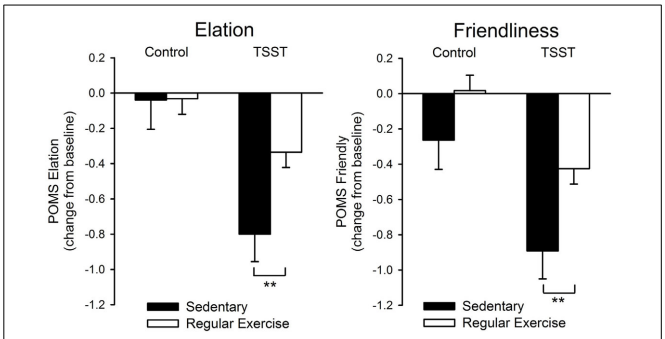

FIGURE 1 | Change in positive affect (POMS Elation, Friendliness) after the control and TSST tasks among regular exercisers and non-exercisers. Bars represent mean ± s.e.m. change from pre-task baseline. Asterisks indicate a significant difference between groups (**$p < 0.01$, Independent sample *t*-test).

has been associated with greater well-being in cross-sectional studies of healthy adults but empirical evidence of its beneficial effects is limited. Both groups showed similar levels of positive and negative affect at baseline, and the groups did not differ in personality measures of positivity or negativity. Thus, regular exercise was not associated with higher baseline levels of positive mood, but instead selectively influenced the ability of a stressful situation to diminish positive affect. Interestingly, stress-induced increases in negative affect were similar between the groups. Recent theories have begun to place more importance on the role of positive emotions during stress independent of negative affect (Folkman, 2008). Moreover, positive, but not negative affect, has been linked to a decreased risk of mortality (Moskowitz et al., 2008; Davis, 2009). Thus, an ability to maintain greater positive mood during stress exposure among regular exercisers may serve a protective function, minimizing the accumulation of stress burden with repeated exposures that is linked with the development of disease. Possible explanations for a resistance to stress-induced decreases in positive affect include that individual appraisals of the situation, self-resources, or coping strategies may be more positive among regular exercisers. For example, positive coping strategies have been linked to greater positive affect during stress (Folkman and Moskowitz, 2000, 2004; Lazarus, 2000). Thus, future studies should also look to assess primary and secondary appraisals of stressful situations and the various coping strategies utilized by exercisers and non-exercisers.

Regular exercisers did not exhibit altered reactivity in other components of responses to acute stress which echoes the findings of some previous reports (Blumenthal et al., 1991; Summers et al., 1999; Spalding et al., 2004; Poole et al., 2011). Also, in line with others' findings, heart rate was significantly lower overall among exercisers in comparison to non-exercisers (e.g., de Geus et al., 1990, 1993; Summers et al., 1999), and baseline heart rate was correlated with the frequency of self-reported exercise per week. Thus, although our analysis depended upon self-reported levels of exercise which are more unreliable than objective measures of physical activity, the significant relationship between baseline heart and

frequency of self-reported exercise reinforces the validity of our approach.

There were several limitations to the present study. First, as mentioned, we relied upon self-reports of regular physical exercise which can be unreliable. A better method for future studies would be to obtain an objective measure of physical activity, such as that provided by an activity monitor or accelerometer which participants could wear for a week before testing. A second limitation was that the groups were self-selecting, that is there may be a bias introduced when subjects who spontaneously engage in exercise are compared to non-exercising persons. Although there was no difference between regular exercisers and sedentary participants in baseline mood or the personality traits of extraversion and neuroticism, there may be an underlying factor associated with both exercise and stress resilience that is not accounted for in this study. Finally, the proportion of European Americans who reported regular exercise was significantly greater than that reported by other races, and since race has also been shown to influence stress responses (Shen et al., 2004; Chong et al., 2008; Fauvel and Ducher, 2009; Li et al., 2009; Christian et al., 2013), we cannot negate the influence of race upon our findings. Nevertheless, studies of differences in stress responses between races have mainly reported differences in physiological responses to stress, and in this study we did not find any group differences in this measure (Murphy et al., 1992; Saab et al., 1992; Kelsey et al., 2000; Wilcox et al., 2005; Kim, 2008). Thus, in order to conclusively disentangle the effects of race from physical exercise, our findings should be replicated in samples with similar distributions of European Americans.

CONCLUDING REMARKS

In conclusion, in this study we assessed multidimensional responses to acute stress in healthy participants who differed in levels of regular exercise. The stress procedure produced a smaller decline in positive mood among the regular exercisers, compared to the sedentary individuals. Responses to the psychosocial stressor used in this study may reflect the way individuals typically respond to daily stressors, suggesting that regular exercisers are more resistant to the emotional effects of acute stress, which in turn, may protect them against diseases related to chronic stress burden.

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REFERENCES

al'Absi, M., Wittmers, L. E., Erickson, J., Hatsukami, D., and Crouse, B. (2003). Attenuated adrenocortical and blood pressure responses to psychological stress in *ad libitum* and abstinent smokers. *Pharmacol. Biochem. Behav.* 74, 401–410. doi: 10.1016/S0091-3057(02)01011-0

Altman, D. G. (1991). *Practical Statistics for Medical Research, 1st Edn*. New York, NY: Chapman and Hall.

American Psychiatric Association. (1994). *Diagnostic and Statistical Manual of Psychiatry, 4th Edn*. Washinton, DC: American Psychological Association.

Blumenthal, J. A., Fredrikson, M., Matthews, K. A., Kuhn, C. M., Schniebolk, S., German, D., et al. (1991). Stress reactivity and exercise training in premenopausal and postmenopausal women. *Health Psychol.* 10, 384–391. doi: 10.1037/0278-6133.10.6.384

Childs, E., and de Wit, H. (2009). Hormonal, cardiovascular, and subjective responses to acute stress in smokers. *Psychopharmacology* 203, 1–12. doi: 10.1007/s00213-008-1359-5

Childs, E., Dlugos, A., and de Wit, H. (2010) Cardiovascular, hormonal, and emotional responses to the TSST in relation to sex and menstrual cycle phase. *Psychophysiology* 47, 550–559. doi: 10.1111/j.1469-8986.2009.00961.x

Choi, P. Y., and Salmon, P. (1995). Stress responsivity in exercisers and non-exercisers during different phases of the menstrual cycle. *Soc. Sci. Med.* 41, 769–777. doi: 10.1016/0277-9536(95)00043-7

Chong, R. Y., Uhart, M., McCaul, M. E., Johnson, E., and Wand, G. S. (2008). Whites have a more robust hypothalamic-pituitary-adrenal axis response to a psychological stressor than blacks. *Psychoneuroendocrinology* 33, 246–254. doi: 10.1016/j.psyneuen.2007.10.014

Christian, L. M., Glaser, R., Porter, K., and Iams, J. D. (2013). Stress-induced inflammatory responses in women: effects of race and pregnancy. *Psychosom. Med.* 75, 658–669. doi: 10.1097/PSY.0b013e31829bbc89

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nat. Rev. Endocrinol.* 5, 374–381. doi: 10.1038/nrendo.2009.106

Crews, D. J., and Landers, D. M. (1987). A meta-analytic review of aerobic fitness and reactivity to psychosocial stressors. *Med. Sci. Sports Exerc.* 19, S114–S120. doi: 10.1249/00005768-198710001-00004

Davis, M. C. (2009). Building emotional resilience to promote health. *Am. J. Lifestyle Med.* 3(1 Suppl.), 60S–63S. doi: 10.1177/1559827609335152

de Geus, E. J., van Doornen, L. J., de Visser, D. C., and Orlebeke, J. F. (1990). Existing and training induced differences in aerobic fitness: their relationship to physiological response patterns during different types of stress. *Psychophysiology* 27, 457–478. doi: 10.1111/j.1469-8986.1990.tb02343.x

de Geus, E. J., van Doornen, L. J., and Orlebeke, J. F. (1993). Regular exercise and aerobic fitness in relation to psychological make-up and physiological stress reactivity. *Psychosom. Med.* 55, 347–363.

Dickerson, S. S., and Kemeny, M. E. (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol. Bull.* 130, 355–391. doi: 10.1037/0033-2909.130.3.355

Dinas, P. C., Koutedakis, Y., and Flouris, A. D. (2011). Effects of exercise and physical activity on depression. *Ir. J. Med. Sci.* 180, 319–325. doi: 10.1007/s11845-010-0633-9

Dua, J., and Hargreaves, L. (1992). Effect of aerobic exercise on negative affect, positive affect, stress, and depression. *Percept. Mot. Skills* 75, 355–361. doi: 10.2466/pms.1992.75.2.355

Elrick, H. (1996). Exercise is medicine. *Phys. Sportsmed.* 24, 72–76.

Fauvel, J. P., and Ducher, M. (2009) Racial differences and cardiovascular response to psychological stress. *Am. J. Hypertens.* 22, 696. doi: 10.1038/ajh.2009.96

Folkman, S. (2008). The case for positive emotions in the stress process. *Anxiety Stress Coping* 21, 3–14. doi: 10.1080/10615800701740457

Folkman, S., and Moskowitz, J. T. (2000). Positive affect and the other side of coping. *Am. Psychol.* 55, 647–654. doi: 10.1037/0003-066X.55.6.647

Folkman, S., and Moskowitz, J. T. (2004). Coping: pitfalls and promise. *Annu. Rev. Psychol.* 55, 745–774. doi: 10.1146/annurev.psych.55.090902.141456

Forcier, K., Stroud, L. R., Papandonatos, G. D., Hitsman, B., Reiches, M., Krishnamoorthy, J., et al. (2006). Links between physical fitness and cardiovascular reactivity and recovery to psychological stressors: a meta-analysis. *Health Psychol.* 25, 723–739. doi: 10.1037/0278-6133.25.6.723

Goodwin, R. D. (2003). Association between physical activity and mental disorders among adults in the United States. *Prev. Med.* 36, 698–703. doi: 10.1016/S0091-7435(03)00042-2

Habib, K. E., Gold, P. W., and Chrousos, G. P. (2001). Neuroendocrinology of stress. *Endocrinol. Metab. Clin. North Am.* 30, 695–728. doi: 10.1016/S0889-8529(05)70208-5

Hackney, A. C. (2006). Stress and the neuroendocrine system: the role of exercise as a stressor and modifier of stress. *Expert Rev. Endocrinol. Metab.* 1, 783–792. doi: 10.1586/17446651.1.6.783

Haskell, W. L., Lee, I. M., Pate, R. R., Powell, K. E., Blair, S. N., Franklin, B. A., et al. (2007). Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Med. Sci. Sports Exerc.* 39, 1423–1434. doi: 10.1249/mss.0b013e3180616b27

Het, S., Rohleder, N., Schoofs, D., Kirschbaum, C., and Wolf, O. T. (2009). Neuroendocrine and psychometric evaluation of a placebo version of the 'Trier Social Stress Test'. *Psychoneuroendocrinology* 34, 1075–1086. doi: 10.1016/j.psyneuen.2009.02.008

Jackson, E. M., and Dishman, R. K. (2006). Cardiorespiratory fitness and laboratory stress: a meta-regression analysis. *Psychophysiology* 43, 57–72. doi: 10.1111/j.1469-8986.2006.00373.x

Kelly, M. M., Tyrka, A. R., Anderson, G. M., Price, L. H., and Carpenter, L. L. (2008). Sex differences in emotional and physiological responses to the Trier Social Stress Test. *J. Behav. Ther. Exp. Psychiatry* 39, 87–98. doi: 10.1016/j.jbtep.2007.02.003

Kelsey, R. M., Alpert, B. S., Dahmer, M. K., Krushkal, J., and Quasney, M. W. (2000). Alpha-adrenergic receptor gene polymorphisms and cardiovascular reactivity to stress in Black adolescents and young adults. *Psychophysiology* 49, 401–412. doi: 10.1111/j.1469-8986.2011.01319.x

Kim, H. S. (2008). Culture and the cognitive and neuroendocrine responses to speech. *J. Pers. Soc. Psychol.* 94, 32–47. doi: 10.1037/0022-3514.94.1.32

Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., and Hellhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom. Med.* 61, 154–162.

Kirschbaum, C., Pirke, K. M., and Hellhammer, D. H. (1993a). The 'Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 28, 76–81. doi: 10.1159/000119004

Kirschbaum, C., Pirke, K. M., and Hellhammer, D. H. (1995). Preliminary evidence for reduced cortisol responsivity to psychological stress in women using oral contraceptive medication. *Psychoneuroendocrinology* 20, 509–514. doi: 10.1016/0306-4530(94)00078-O

Kirschbaum, C., Strasburger, C. J., and Langkrar, J. (1993b). Attenuated cortisol response to psychological stress but not to CRH or ergometry in young habitual smokers. *Pharmacol. Biochem. Behav.* 44, 527–531. doi: 10.1016/0091-3057(93)90162-M

Kujala, U. M., Kaprio, J., Sarna, S., and Koskenvuo, M. (1998). Relationship of leisure-time physical activity and mortality: the Finnish twin cohort. *JAMA* 279, 440–444. doi: 10.1001/jama.279.6.440

Lazarus, R. S. (2000). Toward better research on stress and coping. *Am. Psychol.* 55, 665–673. doi: 10.1037/0003-066X.55.6.665

Li, Z., Snieder, H., Su, S., Ding, X., Thayer, J. F., Treiber, F. A., et al. (2009). A longitudinal study in youth of heart rate variability at rest and in response to stress. *Int. J. Psychophysiol.* 73, 212–217. doi: 10.1016/j.ijpsycho.2009.03.002

López, J. F., Akil, H., and Watson, S. J. (1999). Neural circuits mediating stress. *Biol. Psychiatry* 46, 1461–1471. doi: 10.1016/S0006-3223(99)00266-8

Lovallo, W. R., Farag, N. H., and Vincent, A. S. (2010). Use of a resting control day in measuring the cortisol response to mental stress: diurnal patterns, time of day, and gender effects. *Psychoneuroendocrinology* 35, 1253–1258. doi: 10.1016/j.psyneuen.2010.02.015

Martikainen, S., Pesonen, A. K., Lahti, J., Heinonen, K., Feldt, K., Pyhala, R., et al. (2013). Higher levels of physical activity are associated with lower hypothalamic-pituitary-adrenocortical axis reactivity to psychosocial stress in children. *J. Clin. Endocrinol. Metab.* 98, E619–E627. doi: 10.1210/jc.2012-3745

McEwen, B. S. (2006). Protective and damaging effects of stress mediators: central role of the brain. *Dialogues Clin. Neurosci.* 8, 367–381. doi: 10.1016/j.ejphar.2007.11.071

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* 87, 873–904. doi: 10.1152/physrev.00041.2006

McNair, D., and Droppleman, M. L. L. (eds.). (1971). *Profile of Mood States*. San Diego, CA: Educational and Industrial Testing Service.

Moskowitz, J. T., Epel, E. S., and Acree, M. (2008). Positive affect uniquely predicts lower risk of mortality in people with diabetes. *Health Psychol.* 27, S73–S82. doi: 10.1037/0278-6133.27.1.S73

Murphy, J. K., Alpert, B. S., and Walker, S. S. (1992). Ethnicity, pressor reactivity, and children's blood pressure. Five years of observations. *Hypertension* 20, 327–332. doi: 10.1161/01.HYP.20.3.327

Pate, R. R., Pratt, M., Blair, S. N., Haskell, W. L., Macera, C. A., Bouchard, C., et al. (1995). Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA* 273, 402–407. doi: 10.1001/jama.1995.03520290054029

Patrick, C. J., Curtin, J. J., and Tellegen, A. (2002). Development and validation of a brief form of the Multidimensional Personality Questionnaire. *Psychol. Assess.* 14, 150–163. doi: 10.1037/1040-3590.14.2.150

Poole, L., Steptoe, A., Wawrzyniak, A. J., Bostock, S., Mitchell, E. S., and Hamer, M. (2011). Associations of objectively measured physical activity with daily mood ratings and psychophysiological stress responses in women. *Psychophysiology* 48, 1165–1172. doi: 10.1111/j.1469-8986.2011.01184.x

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., and Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 28, 916–931. doi: 10.1016/S0306-4530(02)00108-7

Rimmele, U., Zellweger, B. C., Marti, B., Seiler, R., Mohiyeddini, C., Ehlert, U., et al. (2007). Trained men show lower cortisol, heart rate and psychological responses to psychosocial stress compared with untrained men. *Psychoneuroendocrinology* 32, 627–635. doi: 10.1016/j.psyneuen.2007.04.005

Saab, P. G., Llabre, M. M., Hurwitz, B. E., Frame, C. A., Reineke, L. J., Fins, A. I., et al. (1992). Myocardial and peripheral vascular responses to behavioral challenges and their stability in black and white Americans. *Psychophysiology* 29, 384–397. doi: 10.1111/j.1469-8986.1992.tb01712.x

Samitz, G., Egger, M., and Zwahlen, M. (2011). Domains of physical activity and all-cause mortality: systematic review and dose-response meta-analysis of cohort studies. *Int. J. Epidemiol.* 40, 1382–1400. doi: 10.1093/ije/dyr112

Shen, B. J., Stroud, L. R., and Niaura, R. (2004). Ethnic differences in cardiovascular responses to laboratory stress: a comparison between asian and white americans. *Int. J. Behav. Med.* 11, 181–186. doi: 10.1207/s15327558ijbm1103_7

Sinyor, D., Schwartz, S. G., Peronnet, F., Brisson, G., and Seraganian, P. (1983). Aerobic fitness level and reactivity to psychosocial stress: physiological, biochemical, and subjective measures. *Psychosom. Med.* 45, 205–217.

Slaven, L., and Lee, C. (1997). Mood and symptom reporting among middle-aged women: the relationship between menopausal status, hormone replacement therapy, and exercise participation. *Health Psychol.* 16, 203–208. doi: 10.1037/0278-6133.16.3.203

Sothmann, M. S. (2006). "The cross-stressor adaptation hypothesis and exercise training," in *Psychobiology of Physical Activity*, eds E. O. Acevedo and P. Ekkekakis (Champaign, IL: Human Kinetics), 149–160.

Spalding, T. W., Lyon, L. A., Steel, D. H., and Hatfield, B. D. (2004). Aerobic exercise training and cardiovascular reactivity to psychological stress in sedentary young normotensive men and women. *Psychophysiology* 41, 552–562. doi: 10.1111/j.1469-8986.2004.00184.x

Steptoe, A., Moses, J., Mathews, A., and Edwards, S. (1990). Aerobic fitness, physical activity, and psychophysiological reactions to mental tasks. *Psychophysiology* 27, 264–274. doi: 10.1111/j.1469-8986.1990.tb00381.x

Summers, H., Lustyk, M. K., Heitkemper, M., and Jarrett, M. E. (1999). Effect of aerobic fitness on the physiological stress response in women. *Biol. Res. Nurs.* 1, 48–56. doi: 10.1177/109980049900100107

Traustadottir, T., Bosch, P. R., Cantu, T., and Matt, K. S. (2004). Hypothalamic-pituitary-adrenal axis response and recovery from high-intensity exercise in women: effects of aging and fitness. *J. Clin. Endocrinol. Metab.* 89, 3248–3254. doi: 10.1210/jc.2003-031713

Wilcox, S., Bopp, M., Wilson, D. K., Fulk, L. J., and Hand, G. A. (2005). Race differences in cardiovascular and cortisol responses to an interpersonal challenge in women who are family caregivers. *Ethn. Dis.* 15, 17–24.

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The impact of escitalopram on vagally mediated cardiovascular function to stress and the moderating effects of vigorous physical activity: a randomized controlled treatment study in healthy participants

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Recent concerns over the impact of antidepressant medications, including the selective serotonin reuptake inhibitors (SSRIs), on cardiovascular function highlight the importance of research on the moderating effects of specific lifestyle factors such as physical activity. Studies in affective neuroscience have demonstrated robust acute effects of SSRIs, yet the impact of SSRIs on cardiovascular stress responses and the moderating effects of physical activity remain to be determined. This was the goal of the present study, which involved a double-blind, randomized, placebo-controlled, cross-over trial of a single-dose of escitalopram (20 mg) in 44 healthy females; outcomes were heart rate (HR) and its variability. Participants engaging in at least 30 min of vigorous physical activity at least 3 times per week (regular exercisers) showed a more resilient cardiovascular stress response than irregular vigorous exercisers, a finding associated with a moderate effect size (Cohen's $d = 0.48$). Escitalopram attenuated the cardiovascular stress response in irregular exercisers only (HR decreased: Cohen's $d = 0.80$; HR variability increased: Cohen's $d = 0.33$). HR during stress under escitalopram in the irregular exercisers was similar to that during stress under placebo in regular exercisers. These findings highlight that the effects of regular vigorous exercise during stress are comparable to the effects of an acute dose of escitalopram, highlighting the beneficial effects of this particular antidepressant in irregular exercisers. Given that antidepressant drugs alone do not seem to protect patients from cardiovascular disease (CVD), longitudinal studies are needed to evaluate the impact of exercise on cardiovascular stress responses in patients receiving long-term antidepressant treatment.

Keywords: selective serotonin reuptake inhibitors (SSRIs), escitalopram, exercise, physical activity, cardiovascular stress response, heart rate, heart rate variability, HRV

INTRODUCTION

Depression and cardiovascular disease (CVD) are leading burdens of disease and this burden is projected to worsen up to 2030 and beyond with ageing of the population and increasing prevalence of multi-morbidity (Mathers and Loncar, 2006; Langan et al., 2013). Critically, depression increases risk for the development of CVD 1.5-fold, while patients with CVD and depression have a 2- to 3-fold increased risk of future cardiac events compared to those cardiac patients who do not have depression (Rudisch and Nemeroff, 2003). Psychological stress plays a key role in the development of depression (Cohen et al., 2007), and increases risk of mortality from a number of causes including CVD, cancer and external causes over an 8 year follow-up period (Russ et al., 2012). While many biological factors, including the hypothalamic-pituitary-adrenal (HPA) axis dysfunction and altered inflammatory processes, contribute to the relationship between stress, psychiatric illness and CVD, vagally mediated cardiovascular function—indexed by increases in heart rate (HR) and decreases in heart rate variability (HRV)—may underlie a substantial part of this risk (Thayer et al., 2010a; Nemeroff and Goldschmidt-Clermont, 2012). Importantly, the inhibitory function of the vagus nerve regulates both the HPA axis and inflammatory processes (Tracey, 2002; Thayer and Sternberg, 2006; Huston and Tracey, 2010; Kemp and Quintana, 2013), leading to proposals that chronic impairment in vagal function is an early marker of future morbidity and mortality (Kemp et al., 2010; Thayer et al., 2010b; Åberg et al., 2013; Kemp and Quintana, 2013).

Acute psychological stress is associated with parasympathetic withdrawal, sympathetic activation, an increase in HR and reductions in its variability (Madden and Savard, 1995; Porges, 1995; Steptoe and Kivimäki, 2012), while chronic stress is associated with persistent cardiovascular stress responses, which may then contribute to psychiatric illness, physical ill-health and all-cause

mortality (Thayer and Brosschot, 2005; Thayer et al., 2010b; Lemogne et al., 2011; Åberg et al., 2013; Kemp and Quintana, 2013). Epidemiological research has even demonstrated that an increased resting HR increases the risk of suicide by 19–37% over a follow-up period of 9 years even after accounting for covariates including depressed mood (Lemogne et al., 2011). In another study lower cardiovascular fitness determined using the cycle ergonometric test at 18 years of age predicts an increased risk of suicide attempt and death by suicide over a follow-up period of 42 years (Åberg et al., 2013). As lower cardiovascular fitness is associated with increased HR and reduced HR variability (Rennie, 2003), these findings highlight an important role of vagally mediated cardiovascular function in regards to psychological wellbeing as well as physical health. Importantly, increased parasympathetic control is associated with positive emotions (Kok and Fredrickson, 2010), resilience (Kashdan and Rottenberg, 2010) and improved regulation over the HPA axis and inflammatory processes (Tracey, 2002; Thayer and Sternberg, 2006; Huston and Tracey, 2010; Kemp and Quintana, 2013), thereby contributing to psychological wellbeing and physical health. National guidelines (Australian Government Department of Health and Ageing: http://www.health.gov.au/internet/main/publishing.nsf/content/health-pubhlth-strateg-phys-act-guidelines) distinguish between regular moderate-intensity physical activity and regular vigorous exercise, recommending at least 30 min of vigorous exercise on top of regular activity for health and fitness benefits.

The selective serotonin reuptake inhibitors (SSRIs) are a first-line treatment option (Kemp et al., 2008) for both mood and anxiety disorders and admixtures of the two, and are also used widely in bipolar depression (Malhi, 2012). SSRIS have important acute neurophysiological effects (Kemp and Nathan, 2004; Kemp et al., 2004) providing the foundation upon which subsequent therapeutic response is based (Harmer et al., 2009). Acute effects are often studied in healthy samples to avoid confounds of illness and psychopathology (Harmer, 2010). Interestingly, acute SSRI treatment has been shown to attenuate cardiovascular responses to stress (Straneva-Meuse, 2004a; Golding et al., 2005; Jiang et al., 2013), However, longitudinal research indicates that they may also have adverse cardiovascular effects, including reductions in vagally mediated cardiovascular function (Licht et al., 2010b) and sudden cardiac death (Whang et al., 2009), highlighting the importance of research on antidepressant actions and the moderating effects of specific lifestyle factors such as physical activity. Physical activity is associated with improved psychological health (Powell et al., 2011; Åberg et al., 2013) and lower cardiovascular (and proinflammatory) responses to stress (Hamer and Steptoe, 2007) lowering the risk of future CVD (Mora et al., 2007; Hamer and Stamatakis, 2009; Hamer et al., 2009) and mortality (Leitzmann et al., 2007). The higher the parasympathetic activity at rest—indicated by lower HR and higher HRV—the more autonomic resources available to tackle subsequent stressors; a proposal known as the "autonomic resource hypothesis" (Hynynen et al., 2008; Boullosa et al., 2012). Thus, research on the impact of antidepressants and the moderating effects of physical activity has important implications for not only better understanding therapeutic response to antidepressant medications, but also for the long-term health of the cardiovascular system. Recent research in outpatients with coronary heart disease and depression (Blumenthal et al., 2012) demonstrates that exercise and sertraline (a SSRI) are equally effective at reducing depressive symptoms after 16 weeks of treatment, but that exercise led to greater improvements in HRV collected using 24-h Holter recordings compared with sertraline. However, it remains unclear, to what extent physical activity and SSRIs interact in moderating the effects of SSRIs on psychological stress.

Here we report on a proof-of-concept, laboratory-based, experimental study to examine the acute effects of a commonly prescribed SSRI, escitalopram, on HR and its variability under stress, with the aim of determining whether individual differences in levels of physical activity moderate these effects. Better understanding the impact of the SSRIs and the moderating effects that specific lifestyle factors exert has important implications for therapeutic responsiveness, psychological wellbeing, and physical health. We focus specifically on acute treatment effects in healthy female volunteers. We hypothesized that acute administration of escitalopram would attenuate increases in HR and decreases in HRV during stress, relative to placebo, and that regular vigorous exercise would facilitate this effect.

METHODS

PARTICIPANTS

Forty-four healthy female volunteers (aged 18–47, $M = 23.70$, $SD = 5.89$) completed the study, and gave written informed consent in accordance with National Health Medical Research Council guidelines. Participants were recruited using university-wide staff and student newsletters. Participants were free from medication (other than hormonal contraceptives), physical and psychiatric illness, symptoms of depression and anxiety [PHQ-9 (Kroenke et al., 2001) and GAD-7 (Spitzer et al., 2006) assessment], illicit drug use, alcoholism, smoking, brain injury, neurological disorders, and sustained loss of consciousness. Only female participants were recruited for this study as females display higher rates of mental disorders (Nolen-Hoeksema, 2001; Kessler, 2003; Slade et al., 2009); focusing on females also allowed us to avoid known gender differences in baseline HRV (Rajendra Acharya et al., 2006), HRV responses to cognitive stress (Li et al., 2009), and responsiveness to SSRI treatment (Khan et al., 2005). Finally, participants abstained from caffeine on the morning of the experiment and no participant tested positive on pregnancy tests, which were conducted at each session. Ethics approval for the trial was secured from the University of Sydney's Human Research Ethics Committee (ref. 13901) and the Northern Sydney Central Coast Area Health Service Human Research Ethics Committee (ref. 1105-178 M), and it was registered with the Australian New Zealand Clinical Trials Registry (ANZCTR; ACTRN126111000719932).

EXPERIMENTAL DESIGN

Participants were randomly assigned to receive escitalopram (20 mg) or placebo *per os*, tested in a double-blind crossover design, with two sessions per participant separated by at least 1 week to ensure a sufficient drug washout of at least 5 half-lives ($t_{1/2} = 26.7$ h; Sogaard, 2005). Forty-five percent of participants received placebo at the first session. Relative to other commonly

prescribed SSRIs, escitalopram has improved efficacy, a lower side effect profile, and greater selectivity for serotonergic transport proteins (Fernandez et al., 2007; Cipriani et al., 2009a). The maximum recommended dosage of 20 mg was used to maximize receptor occupancy (Kasper et al., 2009) and subsequent physiological effects from a single dose.

MEASUREMENT OF PHYSICAL ACTIVITY

Participants reported the frequency in which they engaged in vigorous physical activity using the International Physical Activity Questionnaire (IPAQ; Craig et al., 2003), a widely used, reliable and valid self-report assessment. Physical activity data is transformed into energy expenditure estimates of metabolic equivalent tasks (MET), such that one MET is equivalent to the energy cost of sitting quietly for an hour (1 kcal/kg/h). Participants were categorized into low or high vigorous activity groups—according to national guidelines (Australian Government Department of Health and Ageing: http://www.health.gov.au/internet/main/publishing.nsf/content/health-pubhlth-strateg-phys-act-guidelines). These guidelines recommend at least 30 min of vigorous physical activity 3 days a week on top of regular moderate-intensity activity for health and fitness benefits. Participants categorized in the low activity grouping did not meet the criteria of at least 30 min of vigorous physical activity 3 days a week, while participants in the high activity grouping met or exceeded this criterion. Groups did not differ on time spent sitting, walking or performing moderate physical activity (all $p > 0.1$). Groups were compared on resting state HR, providing a (partial) validation of questionnaire-based categorization (see participant characteristics).

STRESS MANIPULATION

The mental arithmetic task component of the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) was used to elicit stress (Jönsson et al., 2010) and associated physiological correlates, including HR, blood pressure, catecholamines and cortisol (Straneva-Meuse, 2004a; Vermetten, 2006). Participants were instructed to count backwards subtracting thirteens, beginning at either 2083 or 2027 for 5-min. Participants were given one of these two alternate versions of the arithmetic task across sessions. No feedback was given for correct responses, and the experimenter vocalized the word "error" when a mistake was made, instructing the participant to start counting from the beginning, further increasing stress and social pressure as per prior studies (Hjortskov et al., 2004).

PROCEDURE

Testing was conducted in a psychophysiology laboratory at the Clinical Assessment and Diagnostic Evaluation (CADE) Clinic (www.cadeclinic.com), Royal North Shore Hospital. Participants arrived in the early morning, having consumed breakfast, and abstained from caffeine, as caffeine increases sympathetic nervous system (SNS) activity (Sondermeijer et al., 2002). Participants completed a consent form and pregnancy test. Height and weight were measured at the first session to calculate BMI. Testing commenced 3 h after administration of either placebo or 20 mg of escitalopram, so as to coincide with the time of the expected peak plasma concentration ($T_{max} = 3.0 \pm 1.5$ h; Sogaard, 2005; Rao, 2007) During the waiting period, the participant was provided with a standardized snack and in the first session only, completed the IPAQ. The snack helped to minimize autonomic and mood changes associated with hunger. In order to determine the presence of side effects, subjective wellness was recorded during the waiting period and after psychophysiological testing. Participants reported whether they experienced side effects and were asked to guess their treatment condition in order to examine the potential impact of treatment unblinding at each session.

All participants were tested in the late morning, and at the same time on each session to control for potential changes in HRV with circadian rhythms (Kleiger et al., 2005). Participants sat in a reclined chair with their legs raised and were instructed to breathe normally, remain still and awake for 5-min of data collection in the resting state. Following this, 5-min recordings were made during the stress condition in which participants were required to perform the mental arithmetic task. The experimenter was seated in close proximity to the participant and acted in a formal manner, in order to further increase the stressful nature of the procedure through social pressure (Hjortskov et al., 2004). After testing, subjective ratings of relaxation and stress for each condition were recorded on a five-point Likert scale (from low to high) to determine the efficacy of the experimental manipulations.

Participants provided saliva samples (1 mL) for estradiol and progesterone analysis before treatment administration at each session. The hormonal saliva samples were stored frozen until assay. On the day of assay, samples were thawed for determination of salivary progesterone and estradiol using commercially available kits (Salimetrics, USA) according to the manufacturers instructions. Thawed samples were centrifuged at $1500 \times g$ for 15 min to collect clear saliva which was used without further processing for all assays. All samples were brought to room temperature before adding to assay wells and all samples were analyzed in duplicate. Hormonal menstrual phase was determined in accordance with previous research (Lu et al., 1999; Gandara et al., 2007).

DATA COLLECTION AND ANALYSIS

HR and R-R interval recordings—on which analyses of HRV were conducted—were made during two conditions, consistent with Task Force guidelines (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996): (1) a 5-min resting state and (2) a 5-min social stress task, using a Polar RS800CX training device at a sampling rate of 1000 Hz. These devices have been validated against the electrocardiogram (ECG) and have excellent reliability, especially in young, healthy individuals in a supine position and when analysis is conducted on normalized values (Gamelin et al., 2006; Weippert et al., 2010) (see also Quintana et al., 2012), as was the case in the current study. This device is wirelessly connected to an electrode strap worn just beneath the chest, that has been wetted with saline solution (0.9% NaCl) to simulate sweat and ensure conductivity. R-R intervals were extracted from text files and analyzed in Kubios HRV analysis software (version 2.0, 2008, biosignal analysis and medical imaging group, University of Kuopio, Finland, MATLAB). Each file was visually inspected for

artifacts (ectopic and missing beats), and medium automatic filter corrections were applied to each data set. Measures of HRV included HF HRV (normalized units, n.u.) (0.15–0.40 Hz) and the Root Mean Square of the Successive Differences (RMSSD).

STATISTICAL ANALYSIS

All statistical analyses were conducted using IBM SPSS Statistics (Version 20) for Windows 7. The impact of escitalopram and the moderating effects of physical activity levels on HR and heart rate variability (HRV) were examined using mixed within- and between-subjects analysis of variance (ANOVA). Within subject factors were treatment (escitalopram vs. placebo) and task (stress vs. rest) and the between subjects factor was physical activity (high vs. low). A factor of "treatment order" was not included in statistical analysis for parsimony; order effects are rare, are generally underpowered even when an appreciable effect is present, and sufficient drug washout ameliorates such concerns (Senn, 1994; Senn et al., 2004; Mills et al., 2009). Independent samples *t*-tests were conducted to determine whether low and high activity groupings differed across demographic variables (**Table 1**). ANOVA was also employed to determine whether subjective ratings of relaxation and stress differed across treatment sessions and physical activity groupings. Cohen's *d* effect size statistics were calculated for each pairwise comparison. Cohen's guidelines (Cohen, 1988a,b) identify 0.2, 0.5, and 0.8 as small, medium, and large effects, respectively. Partial eta-squared (η_p^2) was reported for ANOVA effects as an indicator of effect size (small = 0.01 medium = 0.06, large = 0.14; (Cohen, 1988a,b). The statistical threshold of 0.05 (two-tailed) was set for all analyses, except where directional effects were expected, in which one-tailed tests were employed. Manipulation checks included a focus on whether hormone or menstrual status, or treatment blinding differentially impacted on the results reported below. With respects to hormone and menstrual status, χ^2 tests were conducted to check for equal proportions of participant menstrual phase within treatment sessions and equal proportions of participants using hormonal contraceptives in each order of treatment. Regression analyses were also conducted on hormone concentrations in order to determine the relationships between hormone levels and the HR and HRV responses under each treatment and task condition. With respects to treatment blinding, χ^2 tests were performed to examine the relationship between guessing treatment condition and experiencing side effects. The effect of guessing and side effects on HR and HRV under each treatment and task condition was determined using repeated-measures ANOVAs on HR and HRV separately.

RESULTS

PARTICIPANT CHARACTERISTICS

Participant characteristics are presented in **Table 1** and flow of participants through the different stages of the study is presented in **Figure 1**. The two physical activity groupings were matched in terms of age, BMI, years of education, time spent sitting, walking or performing moderate physical activity (all $p > 0.1$). The high activity group spent more minutes in the past week in vigorous activity [$t_{(38)} = 6.93$, $p < 0.001$, $d = 2.26$], and had a higher weekly energy expenditure [$t_{(38)} = 3.50$, $p = 0.001$, $d = 1.12$] relative to the low activity group. Regardless of treatment, resting HR was significantly lower in the high activity group in comparison to the low activity group [$t_{(38)} = 1.71$, $p = 0.048$ (one-tailed), $d = 0.55$], suggesting cardiovascular adaptations in the high activity group that may be explained by the link between bradycardia, regular exercise and physical fitness. Four participants were identified as multivariate outliers on HR variables using Tabachnick and Fidell's (2007) multivariate outliers identification procedure, and were subsequently excluded from further analysis. Forty participants aged between 18 and 47 ($M = 23.70$, $SD = 5.89$) were included in final analyses.

HORMONAL RESULTS

The χ^2 tests demonstrated that participants were equally distributed between menstrual phases on placebo, $\chi^2_{(2)} = 0.950$, p = 0.622, and escitalopram, $\chi^2_{(2)} = 0.950$, $p = 0.622$, treatment sessions. There was an equal distribution of participants who took hormonal contraceptives to those who did not, $\chi^2_{(2)} = 0.100$, $p = 0.752$ and hormonal contraceptive status was equally distributed between those who received placebo or escitalopram at their first session, $\chi^2_{(1)} = 0.002$, $p = 0.962$. Regression analyses on HR and HRV data for each task and treatment condition showed no significant associations with progesterone or estradiol concentration [all $p > 0.05$ (Bonferroni corrected for multiple analyses)]. Additionally, there were no differences between those who did and did not take hormonal contraceptives on each task and treatment condition [HR, $F_{(1,\ 38)} = 0.544$, $p = 0.465$; HRV, $F_{(2,\ 37)} = 0.733$, $p = 0.487$].

TREATMENT BLINDING MANIPULATION CHECK

A greater proportion of participants (70%) were found to correctly guess treatment condition than that which was expected by chance, $\chi^2_{(1)} = 6.400$, $p = 0.011$. Side effects also occurred in 48% of the sample, although these did not occur in a greater proportion than that expected by chance, $\chi^2_{(1)} = 0.100$, $p = 0.752$. There was, however, a significant association between the subjective experience of side effects and correct treatment guessing, $\chi^2_{(1)} = 6.535$, $p = 0.011$, indicating that participants who correctly guessed treatment condition were likely to have made this correct guess based on their experience of side effects. Repeated-measures ANOVAs testing the interaction between presence of side effects and correct guessing of treatment were performed on the HR and HF HRV measures. Importantly, no significant interaction effects of correct guessing × side effects × treatment × task on HR [$F_{(1,\ 36)} = 0.428$, $p = 0.517$] or HRV [$F_{(1,\ 35)} = 0.995$, $p = 0.380$] were observed.

SUBJECTIVE RATINGS OF TASK

A significant interaction between task and physical activity groupings on subjective ratings was observed, [$F_{(1,\ 32)} = 7.17$, $p = 0.012$, $\eta_p^2 = 0.18$]. Follow-up pairwise comparisons demonstrated that participants in the high physical activity group reported significantly lower mental stress during the stress task, than the low physical activity group, $t_{(32)} = 2.84$, $p = 0.008$, d = 1.00. Therefore, the less physically active group found the mental stress task more stressful than the more physically active

Table 1 | Participant demographic information.

	Low PA ($n = 18$)		High PA ($n = 22$)		Statistics
	Mean	***SD***	**Mean**	***SD***	
Age (years)	24.06	7.78	23.41	3.91	$t_{(38)} = 0.34$, $p = 0.735$
Education (years)	16.11	2.76	16.77	2.83	$t_{(38)} = -0.74$, $p = 0.462$
Ethnicity (C/NC)	1/17		1/21		$\chi^2_{(1)} = 0.021$, $p = 0.884$
BMI (kg/m^2)	21.88	2.60	23.40	3.30	$t_{(38)} = -1.58$, $p = 0.122$
Hormonal contraceptive use (Y/N)	9/9		12/10		$\chi^2_{(1)} = 0.082$, $p = 0.775$
Menstrual phase placebo (F/M/L)	8/5/5		8/6/8		$\chi^2_{(2)} = 0.387$, $p = 0.824$
Menstrual phase drug (F/M/L)	6/5/7		10/8/4		$\chi^2_{(2)} = 2.132$, $p = 0.344$
Side effects (Y/N)	8/10		11/11		$\chi^2_{(1)} = 0.123$, $p = 0.726$
Correct treatment guess (Y/N)	14/4		14/8		$\chi^2_{(1)} = 0.943$, $p = 0.332$
AUDIT total (SD)	6.11 (4.13)		6.45 (5.32)		$t_{(38)} = -0.224$, $p = 0.824$
Smoking (Y/N)	0/18		0/22		n/a
PHQ-9 (SD)	1.61 (1.20)		1.45 (1.22)		$t_{(38)} = 0.407$, $p = 0.686$
GAD-7 (SD)	0.72 (0.895)		1.41 (1.26)		$t_{(38)} = -1.945$, $p = 0.059$
IPAQ (mins/wk)					
Vigorous intensity activity	30.28	58.37	207.05	94.30	$t_{(38)} = -6.93$, $p < 0.001$ (one-tailed), $d = 2.26$
Moderate intensity activity	206.11	230.12	278.41	222.43	$t_{(38)} = -1.01$, $p = 0.320$
Walking	461.94	297.85	457.50	284.12	$t_{(38)} = 0.05$, $p = 0.962$
Sitting	366.67	180.75	351.82	153.30	$t_{(38)} = 0.28$, $p = 0.780$
Total physical activity	698.33	428.23	942.94	408.14	$t_{(38)} = -1.85$, $p = 0.073$
Energy expenditure, METs-min/week	2620.53	1650.94	4546.11	1793.27	$t_{(38)} = -3.50$, $p = 0.001$, $d = 1.12$
Resting HR	71.78	8.56	66.14	14.10	$t_{(38)} = 1.71$, $p = 0.048$ (one-tailed), $d = 0.55$

Abbreviations: PA, physical activity; BMI, body mass index; PHQ-9, patient health questionnaire; GAD-7, generalised anxiety disorder screener; IPAQ, international physical activity questionnaire; MET, metabolic equivalent task.

group, regardless of treatment. Importantly, however, a main effect of task was also observed indicating that participants were more stressed in the arithmetic task than during the rest condition [$F_{(1, 32)} = 102.77$, $p < 0.001$, $\eta_p^2 = 0.76$], confirming the validity of the stress condition in our sample, regardless of physical activity groupings. No main effects of physical activity [$F_{(1, 32)} = 2.49$, $p = 0.124$] or treatment [$F_{(1, 32)} = 2.35$, $p = 0.135$], or interactions between treatment and task [$F_{(1, 32)} = 2.26$, $p = 0.142$] or treatment, task, and physical activity [$F_{(1, 32)} = 0.228$, $p = 0.637$] were observed on subjective ratings.

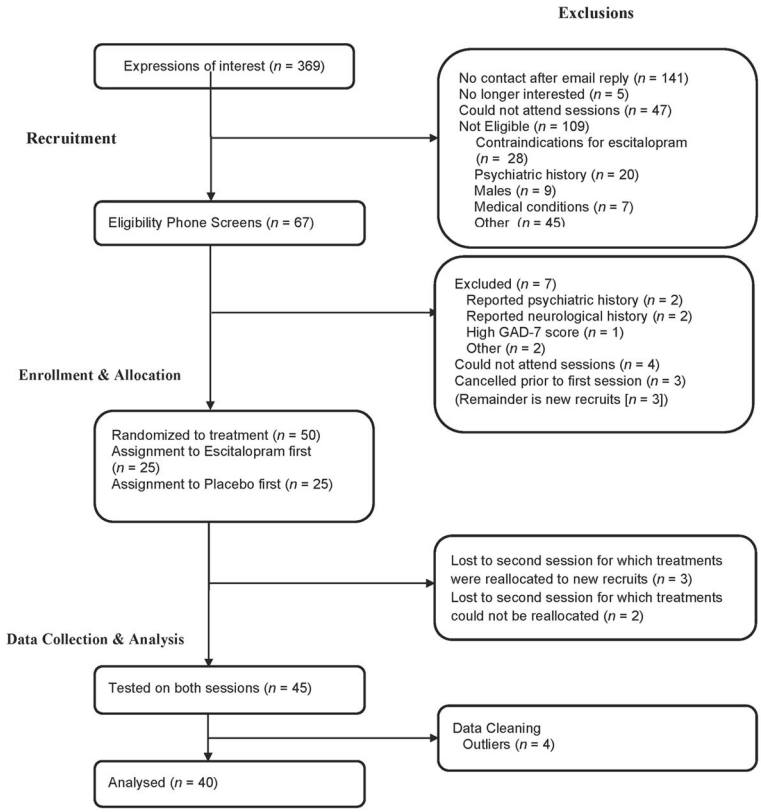

FIGURE 1 | A depiction of participants' attrition from the experiment and the reasons and stages at which they were excluded.

IMPACT OF ESCITALOPRAM AND THE MODERATING EFFECTS OF PHYSICAL ACTIVITY

Descriptive statistics for HR and HRV in high and low activity groupings for task and treatment are presented in **Figure 2** and **Table 2**.

Heart rate

A three-way interaction between treatment, task and activity level was significant, [$F_{(1,\ 38)} = 7.18$, $p = 0.011$ $\eta_p^2 = 0.16$]. Relative to placebo, escitalopram reduced HR in both the low activity [$t_{(38)} = 2.80$, $p = 0.008$, $d = 0.45$] and high activity [$t_{(38)} = 2.24$, $p = 0.031$, $d = 0.36$] groups during rest. Escitalopram also reduced HR in the low activity group [$t_{(38)} = 4.97$, $p < 0.001$, $d = 0.80$] during stress. By contrast, escitalopram did not reduce HR in the high activity group during stress (**Figure 2**). Notably, HR for the high activity group during stress under placebo, was markedly reduced relative to those in the low activity group [$t_{(38)} = 2.13$, $p = 0.040$, $d = 0.48$], and equivalent to HR for the low activity group under drug (**Figure 2**), indicating that those in the high activity group display a more resilient cardiovascular response to stress than those in the low activity group. This interpretation is further supported by the observation of an interaction between task and physical activity grouping on subjective ratings of task (reported above). These findings were observed on a background of a significant treatment × task interaction [$F_{(1,\ 38)} = 6.61, p = 0.014, \eta_p^2 = 0.15$], a main effect of treatment [$F_{(1,\ 38)} = 22.18, p < 0.001, \eta_p^2 = 0.37$], and a main effect of task [$F_{(1,\ 38)} = 210.43$, $p < 0.001$, $\eta_p^2 = 0.85$] collapsed across physical activity groupings. These findings indicate that the increase in HR under stress (relative to rest) was decreased by escitalopram (a treatment × task interaction), that HR was decreased by escitalopram (a main effect of treatment) and that HR was increased under stress (a main effect of task).

Heart rate variability

No significant three-way interaction was found for HRV [$F_{(2,\ 37)} = 0.23$, $p = 0.80$]. Univariate tests for the three-way interaction between treatment, task and vigorous activity were not significant for HF $F_{(1,\ 38)} = 0.06$, $p = 0.804$, or RMSSD, $F_{(1,\ 38)} = 0.47$, $p = 0.496$. However, a main effect of task was observed indicating that HRV was decreased by stress, relative to the rest, regardless of treatment [RMSSD, $F_{(1,\ 38)} = 35.99$, $p < 0.001$, $d = 0.96$; and HF, $F_{(1,\ 38)} = 84.60$, $p < 0.001$, $d = 1.47$]. Interestingly, *post-hoc*, pairwise comparisons indicated that escitalopram—relative to placebo—increased RMSSD (but not HF) during stress in the low activity group [$t_{(37)} = 2.07$, $p = 0.046$, $d = 0.33$], mirroring the findings of HR. No other pairwise comparisons were significant.

DISCUSSION

Recent reports on the adverse cardiovascular effects of the second-generation antidepressants (Whang et al., 2009; Licht et al.,

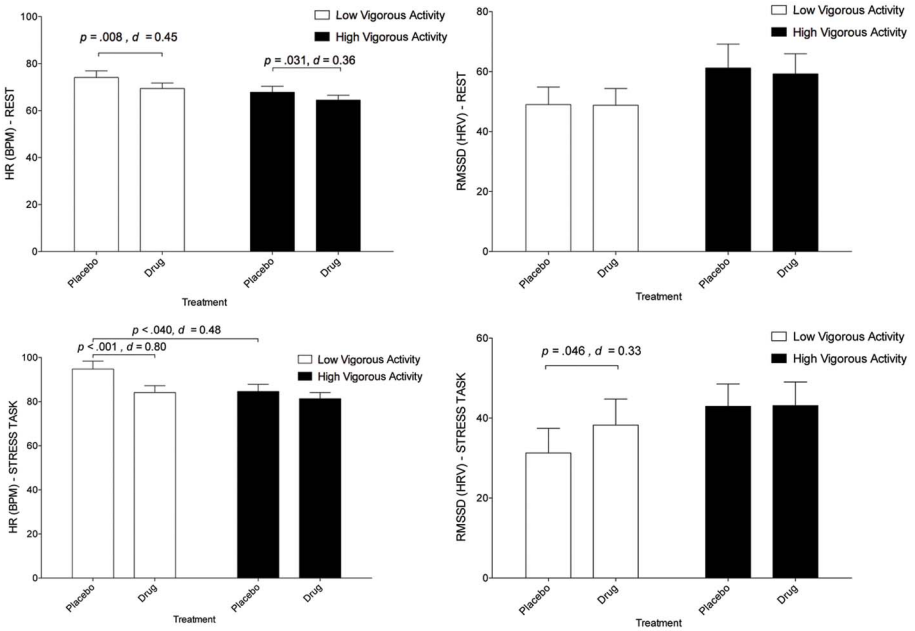

FIGURE 2 | The interaction between treatment and physical activity category on cardiovascular responses (with standard error bars). Top left graph: Effects on HR at rest. Top right graph: effects on RMSSD at rest. Lower left graph: Effects on HR during stress. Lower right graph: effects on RMSSD during stress.

Table 2 | HR and RMSSD means and standard deviations for vigorous exercise groups under each treatment for rest and stress conditions.

		Low vigorous activity		%Δ	High vigorous activity		%Δ
		Placebo *M* (*SD*)	Drug *M* (*SD*)		Placebo *M* (*SD*)	Drug *M* (*SD*)	
HR	Rest	74.13 (8.56)	69.43 (6.93)	−6.3	67.84 (14.10)	64.45 (11.83)	−5
	Stress	94.80 (10.45)	84.12 (10.03)	−11.2	84.62 (17.93)	81.34 (15.21)	−3.9
	%Δ	27.9	21.2		24.7	26.2	
RMSSD	Rest	49.03 (24.80)	48.79 (23.77)	−0.4	61.22 (37.45)	59.27 (31.41)	−3.2
	Stress	31.27 (14.18)	38.25 (18.17)	22.3	42.94 (32.81)	43.13 (33.45)	0.4
	%Δ	−36.2	−21.6		−29.9	−27.2	

2010a), highlight the importance of research on specific lifestyle factors such as physical activity. Major findings observed here were that (a) participants in the high physical activity group (regular vigorous exercisers) reported feeling less stressed than those in the low physical activity grouping (irregular exercisers) during the stress task (i.e., an interaction between task and physical activity groupings on subjective ratings on task); this finding was associated with a large effect size (Cohen's $d = 1.00$), (b) all participants reported feeling more stressed in the stress vs. rest condition (i.e., a main effect of task), a finding associated with a large effect size ($\eta_p^2 = 0.76$), providing an important validation of our task in the context of an interaction between group and physical activity grouping, (c) irregular—relative to regular—exercisers display attenuated cardiovascular responses to stress following treatment with escitalopram (i.e., a three-way interaction between treatment, task and activity for HR, also indicated in a *post-hoc* test on HRV), a finding associated with a moderate to large effect size (HR: Cohen's $d = 0.80$; RMSSD HRV: Cohen's $d = 0.33$), and (d) regular—relative to irregular—exercisers displayed a markedly lower HR under placebo (i.e., a three-way interaction between treatment, task and activity for HR), a finding associated with a moderate effect size (Cohen's $d = 0.48$).

We show here that irregular exercisers—those participants that did not engage in 30 min of vigorous physical activity at least 3 days a week—reported feeling more stressed after the stress task and following an acute dose of escitalopram treatment, they displayed improvements in vagally mediated cardiovascular function during stress. Importantly, these subjective and objective findings relating to the impact of acutely administered escitalopram during stress were also observed regardless of physical activity grouping. The interactions with physical activity simply highlight that the beneficial effects of escitalopram were greatest in irregular exercisers. This effect was most prominent for HR, but was also observed in planned comparisons for HR variability. The

impact of escitalopram on HRV under stress was not as robust as the observed findings for HR, however, this observation is understandable because under conditions of stress, parasympathetic activity, which is the primary driver of changes in HRV, is withdrawn and this reduces the capacity of other factors to moderate reductions in HRV during stress (observed as a main effect of task).

While animal and human studies (Babyak et al., 2000; Engesser-Cesar et al., 2007; Arunrut et al., 2009) have indicated that the combination of exercise and antidepressant medication may confer no advantage over either treatment alone, there is another issue at stake here. Although antidepressant medication—including the tricyclic medications, the serotonin and noradrenaline reuptake inhibitor and the selective serotonin reuptake inhibitors—may have short- to medium-term benefits including the amelioration of depressive symptoms and increased resilience to stress, research has begun to highlight the longer-term adverse cardiovascular effects of antidepressants (Whang et al., 2009; Licht et al., 2010a). Reductions in HRV have been attributable to the specific effects of antidepressants (Licht et al., 2008, 2010a) while increases in HRV are associated with their cessation (Licht et al., 2010a). An epidemiological study (Whang et al., 2009) on 63,469 women aged 30–55 years without baseline coronary heart disease (CHD) reported that while depressive symptoms were associated with fatal CHD, antidepressant use (61% of participants were using an SSRI) was specifically associated with a 3.34 increased risk for sudden cardiac death even after controlling for a variety of confounds. A more recent study has reported that reduced cardiovascular fitness is associated with an increased risk of suicide over a 42 year follow-up period (Åberg et al., 2013) highlighting the importance of cardiovascular health over the lifespan. Together, these findings highlight the importance of research on the cardiovascular effects of antidepressants and specific lifestyle factors such as physical activity. The present study makes an important contribution to this effort highlighting that the cardiovascular effects of regular vigorous exercise and of an acute dose of escitalopram during stress are comparable, i.e., both are associated with a decrease in HR and an increase in HRV.

Here we propose a simplified model for the beneficial effects of regular vigorous physical activity and SSRI treatment on vagal tone, initiating a path toward mental and physical wellbeing (see **Figure 3**). Increased vagal function is associated with increased psychophysiological flexibility (Thayer et al., 2009; Kashdan and Rottenberg, 2010) and stress resilience, which in turn promotes wellbeing (Oveis et al., 2009; Geisler et al., 2010, 2013; Kashdan and Rottenberg, 2010; Kok and Fredrickson, 2010; Thayer et al., 2010b; Kemp and Quintana, 2013). Increased vagal function also initiates downstream neurophysiological changes such as enhanced growth factors (Follesa et al., 2007), improved regulation of HPA axis function and inflammatory processes (Tracey, 2002; Thayer and Sternberg, 2006; Huston and Tracey, 2010; Pavlov and Tracey, 2012), leading to improved wellbeing (Thayer et al., 2010b; Kemp and Quintana, 2013). The model also highlights the direct links between regular vigorous physical activity and wellbeing (discussed above). However, it is noted that overtrained athletes also display a decease in HRV, which limits the autonomic resources available to respond to emotional and physical stress (Hynynen et al., 2008; Boullosa et al., 2012).

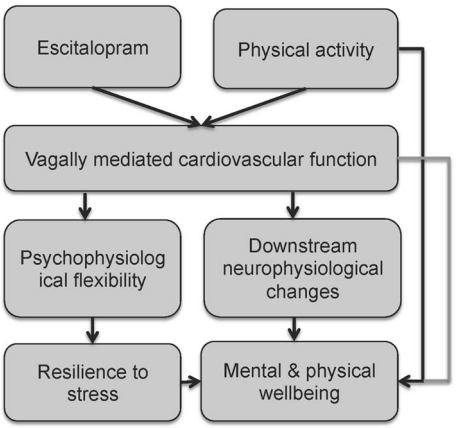

FIGURE 3 | A simplified model for the beneficial effects of regular vigorous physical activity and SSRI treatment on vagal tone, initiating a path towards mental and physical wellbeing.

In the present study we demonstrated that acute administration of escitalopram is associated with increased vagal function under both resting and task conditions; a finding that was particularly robust for HR. This finding is consistent with reported short- to medium-term beneficial effects of SSRIs on cardiovascular and neuroendocrine responses to stress in depressed patients (e.g., Straneva-Meuse, 2004b), but contrast against longer-term research outcomes (Whang et al., 2009; Licht et al., 2010a) highlighting the adverse effects of the SSRI class of antidepressants. Here, an explanation for this apparent discrepancy is the complexity of central and autonomic 5-HT effects on cardiovascular function, which include bradycardia, associated with activation of 5-HT_{1A} receptors, as well as tachycardia, associated with activation of $5HT_2$ receptors. It is possible that the effects of SSRIs shift from parasympathetic—as shown here—to sympathetic activation with increasing length of use. Further research is urgently needed on the combination of antidepressants and physical activity over longer-time frames than typical clinical trials (i.e., years rather than months).

A number of limitations are worth noting. Participants correctly guessed treatment condition beyond chance due to side effects, consistent with those typically experienced with escitalopram (Cipriani et al., 2009b). However, neither experience of side effects, nor accuracy of treatment guess impacted on HR or HRV. Experimenters were also blinded to individual participants' physical activity levels and participants were not aware that physical activity was of primary interest. It is therefore unlikely that the experience of side effects and correctly guessing treatment would have confounded the obtained findings. Another limitation is the use of a questionnaire based-measure of physical activity. It is possible that our measure reflects recent regular physical activity, rather than fitness *per se*. Future studies could consider use of objective measures of fitness such as VO_2 max to confirm that those engaging in regular activity are indeed characterized by higher levels of fitness. Future studies with clinical samples and chronic treatment are also needed to further examine the utility of combining antidepressant medication and vigorous physical activity.

In summary, results from this experimental study highlight the adverse impact of stress on vagally mediated cardiovascular function and the beneficial moderating effects of escitalopram as well as regular vigorous physical exercise. Escitalopram was observed to increase vagal function under both rest and stress conditions, and under stress, these effects are specific to irregular exercisers, but not regular exercisers. We propose a simplified model for understanding the beneficial effects of physical activity and antidepressants on cardiovascular function that may subsequently lead to improved mental and physical activity. Our study and model provide a foundation on which future research could be based. Increasing concerns about the bidirectional relationship between depression and CVD, reinforce the need for further research on the impact of antidepressants, physical activity and their combination, that is not only focused on amelioration of depressive symptoms, but also on vagally mediated cardiovascular function.

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REFERENCES

Åberg, M. A. I., Nyberg, J., Torén, K., Sörberg, A., Kuhn, H. G., and Waern, M. (2013). Cardiovascular fitness in early adulthood and future suicidal behaviour in men followed for up to 42 years. *Psychol. Med.* 1–10. doi: 10.1017/S0033291713001207

Arunrut, T., Alejandre, H., Chen, M., Cha, J., and Russo-Neustadt, A. (2009). Differential behavioral and neurochemical effects of exercise, reboxetine and citalopram with the forced swim test. *Life Sci.* 84, 584–589. doi: 10.1016/j.lfs.2009.02.005

Babyak, M., Blumenthal, J. A., Herman, S., Khatri, P., Doraiswamy, M., Moore, K., et al. (2000). Exercise treatment for major depression: maintenance of therapeutic benefit at 10 months. *Psychosom. Med.* 62, 633–638.

Blumenthal, J. A., Sherwood, A., Babyak, M. A., Watkins, L. L., Smith, P. J., Hoffman, B. M., et al. (2012). Exercise and pharmacological treatment of depressive symptoms in patients with coronary heart disease: results from the UPBEAT (Understanding the Prognostic Benefits of Exercise and Antidepressant Therapy) study. *J. Am. Coll. Cardiol.* 60, 1053–1063. doi: 10.1016/j.jacc.2012.04.040

Boullosa, D. A., Abreu, L., Tuimil, J. L., and Leicht, A. S. (2012). Impact of a soccer match on the cardiac autonomic control of referees. *Eur. J. Appl. Physiol.* 112, 2233–2242. doi: 10.1007/s00421-011-2202-y

Cipriani, A., Furukawa, T. A., Salanti, G., Geddes, J. R., Higgins, J. P., Churchill, R., et al. (2009a). Comparative efficacy and acceptability of 12 new-generation antidepressants: a multiple-treatments meta-analysis. *Lancet* 373, 746–758. doi: 10.1016/S0140-6736(09)60046-5

Cipriani, A., Santilli, C., Furukawa, T. A., Signoretti, A., Nakagawa, A., McGuire, H., et al. (2009b). Escitalopram versus other antidepressive agents for depression. *Cochrane Database Syst. Rev.* CD006532. doi: 10.1002/14651858.CD006532.pub2

Cohen, J. (1988a). Set correlation and contingency tables. *Appl. Psych. Meas.* 12, 425–434. doi: 10.1177/014662168801200410

Cohen, J. (1988b). *Statistical Power Analysis for the Behavioral Sciencies.* Hillsdale, New Jersey: Lawrence Erlbaum Associates.

Cohen, S., Janicki-Deverts, D., and Miller, G. E. (2007). Psychological stress and disease. *JAMA* 298, 1685–1687. doi: 10.1001/jama.298.14.1685

Craig, C. L., Marshall, A. L., Sjöström, M. M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., et al. (2003). International physical activity questionnaire: 12-country reliability and validity. *Medicine and science in sports and exercise* 35, 1381–1395. doi: 10.1249/01.MSS.0000078924.61453.FB

Engesser-Cesar, C., Anderson, A. J., and Cotman, C. W. (2007). Wheel running and fluoxetine antidepressant treatment have differential effects in the hippocampus and the spinal cord. *Neuroscience* 144, 1033–1044. doi: 10.1016/j.neuroscience.2006.10.016

Fernandez, A., Bang, S. E., Srivathsan, K., and Vieweg, W. V. R. (2007). Cardiovascular side effects of newer antidepressants. *Anadolu Kardiyol Derg* 7, 305–309.

Follesa, P., Biggio, F., Gorini, G., Caria, S., Talani, G., Dazzi, L., et al. (2007). Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. *Brain Res.* 1179, 28–34. doi: 10.1016/j.brainres.2007.08.045

Gamelin, F. X., Berthoin, S., and Bosquet, L. (2006). Validity of the polar S810 heart rate monitor to measure R-R intervals at rest. *Med. Sci. Sports Exerc.* 38, 887–893. doi: 10.1249/01.mss.0000218135.79476.9c

Gandara, B. K., Leresche, L., and Mancl, L. (2007). Patterns of salivary estradiol and progesterone across the menstrual cycle. *Ann. N.Y Acad. Sci.* 1098, 446–450. doi: 10.1196/annals.1384.022

Geisler, F. C. M., Kubiak, T., Siewert, K., and Weber, H. (2013). Cardiac vagal tone is associated with social engagement and self-regulation. *Biol. Psychol.* 93, 279–286. doi: 10.1016/j.biopsycho.2013.02.013

Geisler, F. C. M., Vennewald, N., Kubiak, T., and Weber, H. (2010). The impact of heart rate variability on subjective well-being is mediated by emotion regulation. *Pers. Individ. Dif.* 49, 723–728. doi: 10.1016/j.paid.2010.06.015

Golding, M., Kotlyar, M., Carson, S. W., Hoyler, S., Lazarus, C., Davidson, C., et al. (2005). Effects of paroxetine on cardiovascular response to mental stress in subjects with a history of coronary artery disease and no psychiatric diagnoses. *Psychopharmacology* 182, 321–326. doi: 10.1007/s00213-005-0075-7

Hamer, M., and Stamatakis, E. (2009). Physical activity and risk of cardiovascular disease events: inflammatory and metabolic mechanisms. *Med. Sci. Sports Exerc.* 41, 1206–1211. doi: 10.1249/MSS.0b013e3181971247

Hamer, M., Stamatakis, E., and Steptoe, A. (2009). Dose-response relationship between physical activity and mental health: the Scottish Health Survey. *Br. J. Sports Med.* 43, 1111–1114. doi: 10.1136/bjsm.2008.046243

Hamer, M., and Steptoe, A. (2007). Association between physical fitness, parasympathetic control, and proinflammatory responses to mental stress. *Psychosom. Med.* 69, 660–666. doi: 10.1097/PSY.0b013e318148c4c0

Harmer, C. J. (2010). Antidepressant drug action: a neuropsychological perspective. *Depress. Anxiety* 27, 231–233. doi: 10.1002/da.20680

Harmer, C. J., Goodwin, G. M., and Cowen, P. J. (2009). Why do antidepressants take so long to work. A cognitive neuropsychological model

of antidepressant drug action. *Br. J. Psychiatry* 195, 102–108. doi: 10.1192/bjp.bp.108.051193

Hjortskov, N., Rissén, D., Blangsted, A. K., Fallentin, N., Lundberg, U., and Søgaard, K. (2004). The effect of mental stress on heart rate variability and blood pressure during computer work. *Eur. J. Appl. Physiol.* 92, 84–89. doi: 10.1007/s00421-004-1055-z

Huston, J. M., and Tracey, K. J. (2010). The pulse of inflammation: heart rate variability, the cholinergic anti-inflammatory pathway and implications for therapy. *J. Intern. Med.* 269, 45–53. doi: 10.1111/j.1365-2796.2010.02321.x

Hynynen, E., Uusitalo, A., Konttinen, N., and Rusko, H. (2008). Cardiac autonomic responses to standing up and cognitive task in overtrained athletes. *Int. J. Sports Med.* 29, 552–558. doi: 10.1055/s-2007-989286

Jiang, W., Velazquez, E. J., Kuchibhatla, M., Samad, Z., Boyle, S. H., Kuhn, C., et al. (2013). Effect of escitalopram on mental stress–induced myocardial ischemiaresults of the REMIT trialescitalopram and stress-induced myocardial ischemia. *JAMA* 309, 2139–2149. doi: 10.1001/jama.2013.5566

Jönsson, P., Wallergård, M., Österberg, K., Hansen, A. M., Johansson, G., and Karlson, B. (2010). Cardiovascular and cortisol reactivity and habituation to a virtual reality version of the trier social stress test: a pilot study. *Psychoneuroendocrinology* 35, 1397–1403. doi: 10.1016/j.psyneuen.2010.04.003

Kashdan, T. B., and Rottenberg, J. (2010). Psychological flexibility as a fundamental aspect of health. *Clin. Psychol. Rev.* 30, 865–878. doi: 10.1016/j.cpr.2010.03.001

Kasper, S., Sacher, J., Klein, N., Mossaheb, N., Attarbaschi-Steiner, T., Lanzenberger, R., et al. (2009). Differences in the dynamics of serotonin reuptake transporter occupancy may explain superior clinical efficacy of escitalopram versus citalopram. *Int. Clin. Psychopharmacol.* 24, 119–125. doi: 10.1097/YIC.0b013e32832a8ec8

Kemp, A., Gray, M., Silberstein, R., Armstrong, S., and Nathan, P. (2004). Augmentation of serotonin enhances pleasant and suppresses unpleasant cortical electrophysiological responses to visual emotional stimuli in humans. *Neuroimage* 22, 1084–1096. doi: 10.1016/j.neuroimage.2004.03.022

Kemp, A. H., Gordon, E., Rush, A., and Willams, L. M. (2008). Improving the prediction of treatment response in depression: integration of clinical, cognitive, nsychophysiological, neuroimaging, and genetic measures. *CNS Spectr.* 13, 1066–1086.

Kemp, A. H., and Nathan, P. J. (2004). Acute augmentation of serotonin suppresses cardiovascular responses to emotional valence. *Int. J. Neuropsychopharmacol.* 7, 65–70. doi: 10.1017/S1461145703003894

Kemp, A. H., and Quintana, D. S. (2013). The relationship between mental and physical health: insights from the study of heart rate variability. *Int. J. Psychophysiol.* doi: 10.1016/j.ijpsycho.2013.06.018. [Epub ahead of print].

Kemp, A. H., Quintana, D. S., Gray, M. A., Felmingham, K. L., Brown, K., and Gatt, J. M. (2010). Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. *Biol. Psychiatry* 67, 1067–1074. doi: 10.1016/j.biopsych.2009.12.012

Kessler, R. (2003). Epidemiology of women and depression. *J. Affect. Disord.* 74, 5–13. doi: 10.1016/S0165-0327(02)00426-3

Khan, A., Brodhead, A. E., Schwartz, K. A., Kolts, R. L., and Brown, W. A. (2005). Sex differences in antidepressant response in recent antidepressant clinical trials. *J. Clin. Psychopharmacol.* 25, 318–324. doi: 10.1097/01.jcp.0000168879.03169.ce

Kirschbaum, C., Pirke, K. M., and Hellhammer, D. H. (1993). The "Trier Social Stress Test–"a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 28, 76–81. doi: 10.1159/000119004

Kleiger, R., Stein, P., and Bigger, J. Jr. (2005). Heart rate variability: measurement and clinical utility. *Ann. Noninvasive Electrocardiol.* 10, 88–101. doi: 10.1111/j.1542-474X.2005.10101.x

Kok, B. E., and Fredrickson, B. L. (2010). Upward spirals of the heart: autonomic flexibility, as indexed by vagal tone, reciprocally and prospectively predicts positive emotions and social connectedness. *Biol. Psychol.* 85, 432–436. doi: 10.1016/j.biopsycho.2010.09.005

Kroenke, K., Spitzer, R. L., and Williams, J. B. (2001). The PHQ-9: validity of a brief depression severity measure. *J. Gen. Intern. Med.* 16, 606–613. doi: 10.1046/j.1525-1497.2001.016009606.x

Langan, J., Mercer, S. W., and Smith, D. J. (2013). Multimorbidity and mental health: can psychiatry rise to the challenge. *Br. J. Psychiatry* 202, 391–393. doi: 10.1192/bjp.bp.112.123943

Leitzmann, M. F., Park, Y., Blair, A., Ballard-Barbash, R., Mouw, T., Hollenbeck, A. R., et al. (2007). Physical activity recommendations and decreased risk of mortality. *Arch. Intern. Med.* 167, 2453–2460. doi: 10.1001/archinte.167.22.2453

Lemogne, C., Thomas, F., Consoli, S. M., Pannier, B., Jégo, B., and Danchin, N. (2011). Heart rate and completed suicide: evidence from the IPC cohort study. *Psychosom. Med.* 73, 731–736. doi: 10.1097/PSY.0b013e3182365dc7

Li, Z., Snieder, H., Su, S., Ding, X., Thayer, J. F., Treiber, F. A., et al. (2009). A longitudinal study in youth of heart rate variability at rest and in response to stress. *Int. J. Psychophysiol.* 73, 212–217. doi: 10.1016/j.ijpsycho.2009.03.002

Licht, C. M. M., de Geus, E. J. C., van Dyck, R., and Penninx, B. W. J. H. (2010a). Longitudinal evidence for unfavorable effects of antidepressants on heart rate variability. *Biol. Psychiatry* 68, 861–868. doi: 10.1016/j.biopsych.2010.06.032

Licht, C. M. M., Geus, E. J. C., Dyck, R., and Penninx, B. W. J. H. (2010b). Longitudinal evidence for unfavorable effects of antidepressants on heart rate variability. *Biol. Psychiatry* 68, 1–8. doi: 10.1016/j.biopsych.2010.06.032

Licht, C. M. M., de Geus, E. J. C., Zitman, F. G., Hoogendijk, W. J. G., van Dyck, R., and Penninx, B. W. J. H. (2008). Association between major depressive disorder and heart rate variability in the Netherlands Study of Depression and Anxiety (NESDA). *Arch. Gen. Psychiatry* 65, 1358–1367. doi: 10.1001/archpsyc.65.12.1358

Lu, Y., Bentley, G. R., Gann, P. H., Hodges, K. R., and Chatterton, R. T. (1999). Salivary estradiol and progesterone levels in conception and nonconception cycles in women: evaluation of a new assay for salivary estradiol. *Fertil. Steril.* 71, 863–868. doi: 10.1016/S0015-0282(99)00093-X

Madden, K., and Savard, G. K. (1995). Effects of mental state on heart rate and blood pressure variability in men and women. *Clin. Physiol.* 15, 557–569. doi: 10.1111/j.1475-097X.1995.tb00544.x

Malhi, G. S. (2012). Bipolar antidepressant agents: shaken not stirred. *Aust. N. Z. J. Psychiatry*. 46, 289–292. doi: 10.1177/0004867412445131

Mathers, C., and Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.* 3:e442. doi: 10.1371/journal.pmed.0030442

Mills, E. J., Chan, A.-W., Wu, P., Vail, A., Guyatt, G. H., and Altman, D. G. (2009). Design, analysis, and presentation of crossover trials. *Trials* 10, 27. doi: 10.1186/1745-6215-10-27

Mora, S., Cook, N., Buring, J. E., Ridker, P. M., and Lee, I.-M. (2007). Physical activity and reduced risk of cardiovascular events. *Circulation* 116, 2110–2118. doi: 10.1161/CIRCULATIONAHA.107.729939

Nemeroff, C. B., and Goldschmidt-Clermont, P. J. (2012). Heartache and heartbreak—the link between depression and cardiovascular disease. *Nat. Publishing Group* 9, 526–539. doi: 10.1038/nrcardio.2012.91

Nolen-Hoeksema, S. (2001). Gender differences in depression. *Curr. Dir. Psychol. Sci.* 10, 173–176. doi: 10.1111/1467-8721.00142

Oveis, C., Cohen, A. B., Gruber, J., Shiota, M. N., Haidt, J., and Keltner, D. (2009). Resting respiratory sinus arrhythmia is associated with tonic positive emotionality. *Emotion* 9, 265–270. doi: 10.1037/a0015383

Pavlov, V. A., and Tracey, K. J. (2012). The vagus nerve and the inflammatory reflex–linking immunity and metabolism. *Nat. Rev. Endocrinol.* 8, 743–754. doi: 10.1038/nrendo.2012.189

Porges, S. W. (1995). Cardiac vagal tone: a physiological index of stress. *Neurosci. Biobehav. Rev.* 19, 225–233. doi: 10.1016/0149-7634(94)00066-A

Powell, K. E., Paluch, A. E., and Blair, S. N. (2011). Physical activity for health: What kind. How much? How intense? On top of what? *Annu. Rev. Public Health* 32, 349–365. doi: 10.1146/annurev-publhealth-031210-101151

Quintana, D. S., Heathers, J. A. J., and Kemp, A. H. (2012). On the validity of using the Polar RS800 heart rate monitor for heart rate variability research. *Eur. J. Appl. Physiol.* 112, 4179–4180. doi: 10.1007/s00421-012-2453-2

Rajendra Acharya, U., Paul Joseph, K., Kannathal, N., Lim, C. M., and Suri, J. S. (2006). Heart rate variability: a review. *Med. Biol. Eng. Comput.* 44, 1031–1051. doi: 10.1007/s11517-006-0119-0

Rao, N. (2007). The clinical pharmacokinetics of escitalopram. *Clin. Pharmacokinet.* 46, 281–290. doi: 10.2165/00003088-200746040-00002

Rennie, K. L. (2003). Effects of moderate and vigorous physical activity on heart rate variability in a british study of civil servants. *Am. J. Epidemiol.* 158, 135–143. doi: 10.1093/aje/kwg120

Rudisch, B., and Nemeroff, C. B. (2003). Epidemiology of comorbid coronary artery disease and depression. *Biol. Psychiatry* 54, 227–240. doi: 10.1016/S0006-3223(03)00587-0

Russ, T. C., Stamatakis, E., Hamer, M., Starr, J. M., Kivimaki, M., and Batty, G. D. (2012). Association between psychological distress and mortality: individual participant pooled analysis of 10 prospective cohort studies. *BMJ* 345:e4933. doi: 10.1136/bmj.e4933

Senn, S. (1994). The AB/BA crossover: past, present and future. *Stat. Methods Med. Res.* 3, 303–324. doi: 10.1177/096228029400300402

Senn, S., D'Angelo, G., and Potvin, D. (2004). Carry-over in cross-over trials in bioequivalence: theoretical concerns and empirical evidence. *Pharm. Stat.* 3, 133–142. doi: 10.1002/pst.111

Slade, T., Johnston, A., Oakley Browne, M. A., Andrews, G., and Whiteford, H. (2009). 2007 National survey of mental health and wellbeing: methods and key findings. *Aus. N.Z. J. Psychiatry* 43, 594–605. doi: 10.1080/00048670902970882

Sogaard, B. (2005). The pharmacokinetics of escitalopram after oral and intravenous administration of single and multiple doses to healthy subjects. *J. Clin. Pharmacol.* 45, 1400–1406. doi: 10.1177/0091270005280860

Sondermeijer, H. P., van Marle, A. G. J., Kamen, P., and Krum, H. (2002). Acute effects of caffeine on heart rate variability. *Am. J. Cardiol.* 90, 906–907. doi: 10.1016/S0002-9149(02)02725-X

Spitzer, R. L., Kroenke, K., Williams, J. B. W., and Löwe, B. (2006). A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch. Intern. Med.* 166, 1092–1097. doi: 10.1001/archinte.166.10.1092

Steptoe, A., and Kivimäki, M. (2012). Stress and cardiovascular disease. *Nat. Rev. Cardiol.* 9, 360–370. doi: 10.1038/nrcardio.2012.45

Straneva-Meuse, P. (2004a). Bupropion and paroxetine differentially influence cardiovascular and neuroendocrine responses to stress in depressed patients. *J. Affect. Disord.* 79, 51–61. doi: 10.1016/S0165-0327(02)00352-X

Straneva-Meuse, P. (2004b). Bupropion and paroxetine differentially influence cardiovascular and neuroendocrine responses to stress in depressed patients. *J. Affect. Disord.* 79, 51–61. doi: 10.1016/S0165-0327(02)00352-X

Tabachnick, B. G., and Fidell, L. S. (2007). *Using multivariate statistics* 5th edn. Boston: Pearson Education, Inc.

Task Force of the European Society of Cardiology, and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability. standards of measurement, physiological interpretation, and clinical use. *Eur. Heart J.* 17, 354–381. doi: 10.1093/oxfordjournals.eurheartj.a014868

Thayer, J. F., and Brosschot, J. F. (2005). Psychosomatics and psychopathology: looking up and down from the brain. *Psychoneuroendocrinology* 30, 1050–1058. doi: 10.1016/j.psyneuen.2005.04.014

Thayer, J. F., Hansen, A. L., Saus-Rose, E., and Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

Thayer, J. F., and Sternberg, E. (2006). Beyond heart rate variability: vagal regulation of allostatic systems. *Ann. N.Y. Acad. Sci.* 1088, 361–372. doi: 10.1196/annals.1366.014

Thayer, J. F., Yamamoto, S. S., and Brosschot, J. F. (2010a). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int. J. Cardiol.* 141, 122–131. doi: 10.1016/j.ijcard.2009.09.543

Thayer, J. F., Yamamoto, S. S., and Brosschot, J. F. (2010b). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int. J. Cardiol.* 141, 122–131. doi: 10.1016/j.ijcard.2009.09.543

Tracey, K. (2002). The inflammatory reflex. *Nature* 420, 853–859. doi: 10.1038/nature01321

Vermetten, E. (2006). Alterations in stress reactivity after long-term treatment with paroxetine in women with posttraumatic stress disorder. *Ann. N.Y. Acad. Sci.* 1071, 184–202. doi: 10.1196/annals.1364.014

Weippert, M., Kumar, M., Kreuzfeld, S., Arndt, D., Rieger, A., and Stoll, R. (2010). Comparison of three mobile devices for measuring R–R intervals and heart rate variability: Polar S810i, Suunto t6 and an ambulatory ECG system. *Eur. J. Appl. Physiol.* 109, 779–786. doi: 10.1007/s00421-010-1415-9

Whang, W., Kubzansky, L. D., Kawachi, I., Rexrode, K. M., Kroenke, C. H., Glynn, R. J., et al. (2009). Depression and risk of sudden cardiac death and coronary heart disease in women. *J. Am. Coll. Cardiol.* 53, 950–958. doi: 10.1016/j.jacc.2008.10.060

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Traditional games resulted in post-exercise hypotension and a lower cardiovascular response to the cold pressor test in healthy children

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The present study aimed to verify if blood pressure (BP) reactivity could be reduced through a previous single session of active playing when compared to sedentary leisure. Sixteen pre-pubertal healthy children participated in this study. After familiarization with procedures and anthropometric evaluation, participants performed three sessions in randomized order: (1) 30 min of traditional Brazilian games (PLAY); (2) 30 min of video game playing (DDR); and (3) 30 min of watching TV (TV). Each session lasted 80 min, being 10 min of rest; 30 min of intervention activity; and 40 min of recovery. After recovery, the Cold Pressor Test (CPT) was used for the assessment of acute cardiovascular reactivity. BP was recorded at 30 s and 1 min during the CPT. Analysis of variance showed post-exercise hypotension (PEH) only after PLAY, and that systolic and diastolic BP were significantly increased in all conditions during CPT. However, the magnitude of the CPT-induced BP response was significantly less in PLAY compared to DDR and TV. The PEH observed during recovery and the reduced BP response to CPT following playing traditional games may be due its higher cardiovascular and metabolic demand as was indicated by the increased heart rate, oxygen consumption, and BP. It was concluded that BP reactivity to stress may be reduced through a previous single session of traditional games and that PEH was recorded only after this exercise form. This benefit indicates a potential role of playing strategies for cardiovascular health in childhood.

Keywords: cardiovascular system, blood pressure, heart rate, children, active playing

INTRODUCTION

Cardiovascular disease (CVD) is the primary cause of death in western countries (Ergin et al., 2004). More recently, researchers have provided evidence that CVD is becoming more frequent among pediatric populations (Bigi et al., 2011) and the increase of blood pressure (BP) during infancy and puberty is becoming more premature (Kark et al., 2009; Maximova et al., 2009), and has been identified as the first etiologic factor for the development of hypertension in adulthood (Chiolero et al., 2008; Tsioufis et al., 2011). Therefore, it is important to identify factors that could influence cardiovascular health during childhood, specifically those lifestyle-related that are potentially modifiable (Bell and Belsky, 2008; Daniels, 2011; Moraes et al., 2014).

Physical inactivity is considered to be one of the main causes of primary hypertension, independently of weight status (Martinez-Gomez et al., 2009; Daniels, 2011). Physical activity (PA) levels are becoming lower among children and adolescents worldwide (Rossow et al., 2010; Nettle and Sprogis, 2011), demonstrating that new generations are more sedentary than the previous ones (Chinapaw et al., 2011). This raises concerns about which strategies could help children and adolescents to increase their PA levels, as PA in childhood has been demonstrated to be an effective intervention to combat obesity and hypertension in adult life (Guy et al., 2011; Siegrist et al., 2013).

Among several mechanisms related to the effectiveness of PA on BP control, there is the so-called post-exercise hypotension (PEH) (MacDonald, 2002). The effect of a single exercise bout induced BP reduction in relation to a non-exercise control session has been systematically observed after different exercise modes can last several hours post-exercise (Ciolac et al., 2008; Mota et al., 2009; Rabelo et al., 2009; Morais et al., 2011), thus helping for control BP in both hypertensive (Mota et al., 2009) and healthy people (Richter et al., 2010). However, the occurrence of PEH after exercise in children was not investigated yet to our knowledge.

An important application of PEH is that, during its occurrence, individuals are supposed to present lowered BP reactivity to different stress situations (MacDonald et al., 2001; Brownley et al., 2003). Stress can be considered an important factor that typically promotes a rise in BP (Lehman et al., 2009), which is dependent on the nature of the stimulus and the individual characteristics of the subject (Probst et al., 1997). Previous

studies have demonstrated that an exercise bout of moderate intensity could attenuate the increase of BP and HR after a stressful event (Probst et al., 1997; Bond et al., 2002) in adults. More recently, the studies of Roemmich et al. (2009) and Lambiase et al. (2010) have showed a dampened cardiovascular reactivity to speech and stroop tasks after interval cycling and a self-paced walk respectively, in children. These important studies (Roemmich et al., 2009; Lambiase et al., 2010) highlighted the positive impact of different forms of exercise performed in laboratory conditions on acute stress reactivity. However, the short recovery interval used (20 min) in these previous studies did not allow a precise evaluation of the PEH phenomenon. Moreover, it would be interesting to evaluate also the impact of other activities more enjoyable to children, such as street games and active video game playing, that have been recently proposed to increase energy expenditure (Lanningham-Foster et al., 2006; Wang and Perry, 2006; Warburton et al., 2009; Rauber et al., 2013). This information would help to know the impact of other forms of exercise on both PEH and stress reactivity in children; and therefore to better understand the role of different exercise strategies for cardiovascular health during childhood.

On the other hand, the cold pressure test (CPT) is a simple and time-saving protocol proposed for the evaluation of cardiovascular reactivity to acute thermal stress (Hines and Brown, 1936; Wood et al., 1984) that requires passive coping in contrast to the active coping required in previous studies with children (Roemmich et al., 2009; Lambiase et al., 2010). Briefly, this test consists of the immersion of the left hand in cold water for the assessment of the BP responses (Hines and Brown, 1936). The validity of this test has been previously demonstrated as it is a good predictor of hypertension in adulthood from the recorded values during childhood (Wood et al., 1984; Menkes et al., 1989).

Thus, the purpose of present study was to analyze the occurrence of PEH after different games (i.e., video games and traditional Brazilian children's games), and to assess the potential protective effect of these games on cardiovascular reactivity to subsequent stress in children. The hypotheses were that both games would induce PEH and that cardiovascular reactivity would be lower after active games in comparison to the sedentary activity (TV).

METHODS

SAMPLE AND ETHIC PROCEDURES

Sixteen children aged 9–10 years (8 boys and 8 girls) participated in this study. After authorization from the school, the children's parents received an informed consent letter describing all procedures, risks and benefits of the study and signed the consent for children's participation.

The exclusion criteria were: any physical impairment that could prevent the participant from performing the programmed activities; having any previous cardiometabolic disease (e.g., diabetes, hypertension); and any previous experience with the video game "Dance Dance Revolution" (DDR). The study was approved by the local ethics committee for human research (protocol 147/2009).

GENERAL PROCEDURES

Four visits were necessary in order to perform the present study. The first one was for familiarization with the experimental procedures and for anthropometric evaluation. The other three visits were randomly performed between 8 and 11 a.m. Each visit lasted 80 min, divided in: rest (10 min); programmed activity (30 min); and recovery (40 min). Immediately after the 40th min of the post-activity recovery period, the CPT was applied over 1 min.

Familiarization and anthropometric evaluation

Evaluation of body weight (Electronic Scale Tech 05®, China), height (Portable Stadiometer, Sanny®, Brazil), and subscapular and triceps skinfolds (Adipometer, Lange Skinfold Caliper, USA) were performed for body composition estimation. Body fat percentage was estimated using the skinfolds method according to Slaughter et al. (1988). The CPT and BP measurements were also performed so the children were familiarized with these procedures. Subsequently, the children received orientations and practiced the DDR video game 10–15 times.

Traditional games (PLAY)

Volunteers participated of a physical education class together with other 10 children so the activity could be the most realistic possible. A physical education teacher guided an activity composed by three traditional games usually applied in physical education classes in Brazil, and organized as follows: 10 min of "run and catch;" 10 min of "dodge ball;" and 10 min of "capture the flag." These activities were performed in the same order in a gymnasium close to university's laboratory. The intensity of the games was monitored by HR (Polar Electro Oy FS1, Finland), as well as through the responses of oxygen uptake (VO_2), and respiratory exchange ratio (RER) (Cortex Metamax 3B, Germany).

"Dance Dance Revolution" video game (DDR)

Volunteers played an interactive video game (DDR) installed in a laptop computer. They selected the songs from a low-level difficulty list. Authors decided to use a basic level for standardization purposes, since their first contact with this video game was during the familiarization session.

Watching television (TV)

Volunteers watched two popular cartoons ("Sponge Bob" and "Ben 10") for 30 min (15 min per cartoon) while seated in a quiet room.

BP and HR measurements

Systolic (SBP), diastolic (DBP) BP and HR were collected during each session at the 5th and 10th min of rest before sessions; at the 10th, 20th, and 30th min during sessions; and at the 2nd, 10th, 20th, 30th, and 40th min after all sessions (see **Figure 1**). Mean arterial pressure (MAP) was calculated using the following equation: $MAP = DBP + [(SBP - DBP)/3]$. BP was measured, in the right arm, with the volunteer seated, through the auscultatory method using a pediatric sphygmomanometer with an appropriate cuff for children (Becton Dickinson, Brazil) and a children's stethoscope Duo Sonic (Missouri, Brazil). Heart rate was measured using a HR monitor (FS1, Polar Electro Oy, Finland).

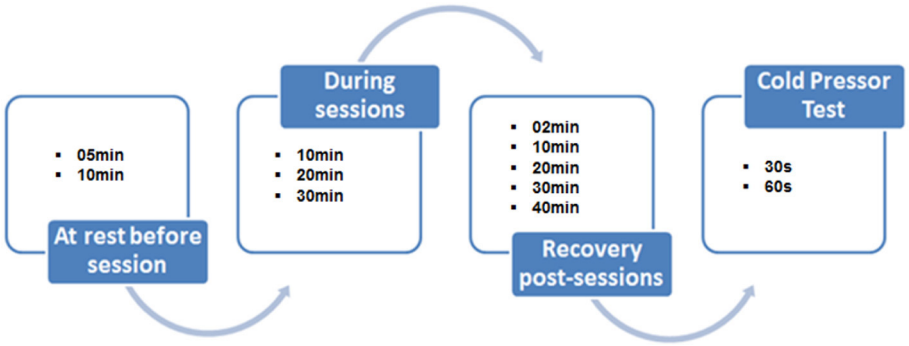

FIGURE 1 | Study design representing BP and HR measurements during sessions and when performing the Cold Pressor Test.

Cold Pressor Test (CPT)

This thermal stress test was performed after the completion of 40 min of recovery after each session. The volunteers immersed the left hand of the child in cold water (∼4 - 5°C) for 60 s. During this period, BP was measured at the 30th and 60th s, and subsequently compared with BP at rest. This test was performed in order to verify the BP response after the different sessions (PLAY, DDR, and TV). BP was measured using auscultatory method by the same evaluator, on the arm opposite to that immersed one, and HR was also measured using the same procedures and equipment cited above.

STATISTICAL ANALYSIS

Shapiro-Wilk's test was used to confirm the normality of data. One-Way ANOVA with Scheffé as a *post hoc* were applied to compare variables between sessions (PLAY, DDR, and TV). ANCOVA for repeated measures adjusted for exercise intensity (i.e., VO_2) was applied at different time points during recovery and CPT after TV, DDR, and PLAY sessions. The level of significance was set at $p \leq 0.05$. The data was analyzed using the Statistical Package for the Social Sciences (SPPS), version 15.0 for windows.

RESULTS

The characteristics of the children were: mean age 9.6 ± 0.5 years, 133.8 ± 9.9 cm of height, weight 32.4 ± 4.0 kg with a mean body mass index (BMI) of 18.4 ± 3.7 kg· m^{-2}, and body fat of 17.3 ± 3.8 %. All children were characterized as eutrophic.

Table 1 shows the cardiovascular and ventilatory responses at rest and during each experimental session. Heart rate, SBP, DBP, and MAP were significantly higher during PLAY and DDR when compared to TV. Also, PLAY elicited a significantly higher VO_2 and RER when compared to TV. Furthermore, all cardiovascular and ventilatory responses were significantly higher during PLAY in comparison to DDR session.

Figures 2–4 illustrate HR and BP responses during CPT. One of the volunteers was considered hyper-reactive to the test, having an increase in SBP of ≥25 mmHg during the test (Wood et al., 1984). Heart rate increased 4, 7.2, and 7.5 bpm at the 30th s, and 4.4, 8.2, and 8 bpm at the 60th s during the CPT after TV, DDR and PLAY, respectively. However, when adjusted for exercise intensity, only PLAY elicited a significant HR increase at both moments when compared to pre-exercise rest.

Figures 3, **4** show the responses of SBP and DBP, respectively, at the 30th and 60th s during CPT after each session. PLAY elicited a significantly smaller increase in SBP and DBP when compared to TV and in SBP when compared to DDR at both moments. When adjusted for exercise intensity, all sessions elicited a significant SBP increase at both moments when compared to pre-exercise rest with the extent of this change in SBP being greater in TV with respect to DDR and PLAY, and lower in PLAY with respect to TV and DDR. Meanwhile, only PLAY exhibited a lower DBP at both moments when compared to TV. Additionally, SBP at rest was significantly lower in PLAY when compared to TV.

Tables 2, **3** show the mean values of SBP and DBP over each session and subsequent recovery. With or without adjustment for exercise intensity, SBP and DBP in DDR and PLAY were significantly higher at the 10th, 20th, and 30th min of intervention when compared to TV. PLAY also showed significantly higher values when compared to DDR at all times for SBP and DBP, but only at the 10th and 20th for DBP when controlled for exercise intensity. During the recovery period, SBP and DBP were significantly reduced after adjustment for exercise intensity at the 30th min, and SBP at the 40th min when compared to rest only in PLAY. Interestingly, SBP at 2nd min of recovery in DDR was different from rest in the same session before adjustment for exercise intensity. Moreover, SBP at the 40th min of recovery and DBP at the 2nd of recovery in PLAY were significantly different from the values recorded after TV session before adjustment for exercise intensity. In contrast, DBP was not different from values recorded after TV at the 30th and 40th min of recovery before adjustment for exercise intensity.

DISCUSSION

The main findings of present study were that children experienced PEH after traditional games and that BP reactivity to stress induced by CPT was also lower after traditional games when compared to active video game playing and TV sessions. These responses may be related to exercise intensity once the more intense session (PLAY) also induced a more significant hypotensive effect during the post-exercise recovery period. Moreover, this lowered BP reactivity was observed 40 min after the end of the experimental session, which is longer than that observed in previous studies (Roemmich et al., 2009; Lambiase et al., 2010).

Table 1 | Cardiovascular and ventilatory responses at rest and during each session.

Variables	TV		DDR		PLAY	
	Rest	During	Rest	During	Rest	During
HR (bpm)	82.9 ± 5.1	84.9 ± 4.7	85.4 ± 5.4	109.1 ± 9.3*	82.6 ± 5.2	144.9 ± 11.3*‡
SBP (mmHg)	99.6 ± 6.9	99.8 ± 6.2	99.9 ± 6.0	115.5 ± 5.2*	99.8 ± 5.8	138.2 ± 7.3*‡
DBP (mmHg)	63.6 ± 5.0	63.8 ± 5.9	63 ± 5.4	72.6 ± 5.0*	63.4 ± 4.7	79.1 ± 3.0*‡
MAP (mmHg)	75.6 ± 4.6	75.8 ± 5.0	75.3 ± 4.9	86.9 ± 4.1*	75.5 ± 4.2	98.8 ± 3.7*‡
VO_2 (ml·kg^{-1} · min^{-1})	7.1 ± 1.1	7.5 ± 1.0	7.2 ± 1.4	11.7 ± 1.3	7.1 ± 1.5	23.1 ± 4.0*‡
RER	0.77 ± 0.04	0.76 ± 0.03	0.78 ± 0.04	0.84 ± 0.04	0.76 ± 0.05	0.95 ± 0.08*‡

*Values expressed as mean ± standard deviation. TV, television; DDR, dance dance revolution; PLAY, traditional children's game; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; VO_2, oxygen uptake; RER, respiratory exchange ratio. *$p \leq 0.01$ in relation to TV; ‡$p \leq 0.01$ in relation to DDR.*

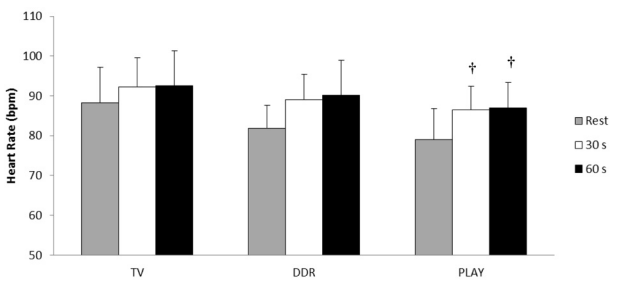

FIGURE 2 | Heart rate responses at the 30th and 60th s during Cold Pressor Test in each session. †$p \leq 0.05$ in relation to pre-exercise rest.

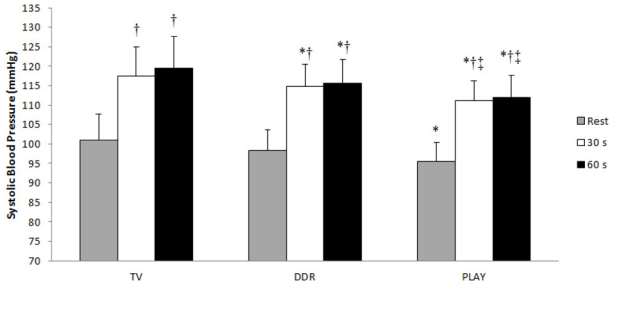

FIGURE 3 | Systolic blood pressure values during the Cold Pressor Test. TV, television; DDR, dance dance revolution; PLAY, traditional games; *$p \leq 0.05$ to TV in the same moment; †$p \leq 0.05$ to rest in the same session; ‡$p \leq 0.05$ to DDR in the same moment.

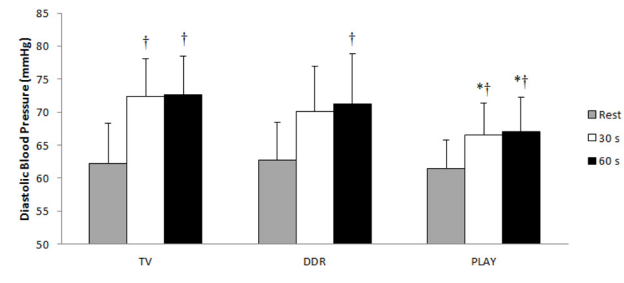

FIGURE 4 | Diastolic blood pressure values during the Cold Pressor Test. TV, television; DDR, dance dance revolution; PLAY, traditional games; *$p \leq 0.05$ vs. TV in the same moment; †$p \leq 0.05$ vs. rest in the same session.

Overall, these findings suggest that traditional games are better suited for cardiovascular health in children when compared to other activities of lower cardiovascular and metabolic stimuli like active video game playing or watching TV.

To the best of our knowledge, this is the first study reporting PEH in children. Thus, significant reductions in SBP and DBP were observed in children at the 30th and 40th min of recovery when compared to rest only after PLAY. Interestingly, DBP was significantly reduced when compared to TV session after adjustment for exercise intensity. In contrast, SBP at the 40th min of recovery was not different from SBP values recorded after TV session when controlled for exercise intensity. From a methodological point of view, these findings are interesting and suggest the necessity in further studies of comparing the effect of different activities matched for exercise intensity on cardiovascular responses. Additionally, as the recovery period lasted only 40 min in the current study, further studies should evaluate if the PEH exerts its positive influence over longer periods in children.

Previously, Roemmich et al. (2009) reported that previous interval exercise protocol in cycloergometer also dampened the subsequent cardiovascular response to a speech stressor test when compared to watching TV. Similarly, Lambiase et al. (2010) have reported that the cardiovascular reactivity during cognitive stress was dampened after a treadmill walking when compared to a simulated sedentary drive to school. These results are consistent with the results of the current study, and also with previous studies in adults (Probst et al., 1997; Bond et al., 2002), thus confirming the positive impact that acute exercise has on stress tolerance. However, this is the first study that reproduces traditional games which is a natural condition that imitates the habitual patterns of PA in children. Furthermore, the greater positive impact of traditional games (PLAY) could be due to its higher intensity as inferred from the higher exercising HR, BP, and VO_2 recorded when compared to active video game playing, which have exhibited similar values as in previous reports (Wang and Perry, 2006). Therefore, this suggests that street playing in a non-controlled

Table 2 | Systolic blood pressure mean values (±*SD*) of all sessions (TV, DDR, and PLAY) (*n* = 18).

	Moment		
	TV (mmHg)	DDR (mmHg)	PLAY (mmHg)
PRE-INTERVENTION REST			
	100.4 ± 7.1	101.6 ± 6.5	100.1 ± 5.8
SESSIONS			
10 min	101.1 ± 6.2	114.8 ± 4.9*†	137.9 ± 7.7*†‡
20 min	99.7 ± 6.5	116.3 ± 5.0*†	139.4 ± 6.6*†‡
30 min	100.4 ± 6.4	116.4 ± 5.1*†	138.2 ± 6.8*†‡
POST-INTERVENTION RECOVERY			
2 min	99.8 ± 6.6	105.6 ± 4.8*	109.1 ± 6.0*†
10 min	100.9 ± 6.7	101.4 ± 4.8§	101.5 ± 5.0§
20 min	100.7 ± 6.4	100.2 ± 5.1§	99.0 ± 5.2§#
30 min	101.8 ± 7.0	99.3 ± 5.6§	97.1 ± 5.7†§#
40 min	101.3 ± 6.8	98.8 ± 5.1§	95.8 ± 4.7†§#

*TV, television; DDR, dance dance revolution; PLAY, traditional games; *$p \leq 0.05$ vs. TV in the same moment; †$p \leq 0.05$ vs. rest in the same session; ‡$p \leq 0.05$ vs. DDR in the same moment; §$p \leq 0.05$ vs. 2 min recovery in the same session; #$p \leq 0.05$ vs. 10 min recovery in the same session.*

Table 3 | Diastolic blood pressure mean values (±*SD*) of all sessions (TV, DDR, and PLAY) (*n* = 18).

	Moment		
	TV (mmHg)	DDR (mmHg)	PLAY (mmHg)
PRE-INTERVENTION REST			
	64.5 ± 4.8	63.8 ± 5.3	64.1 ± 4.8
SESSIONS			
10 min	64.9 ± 5.8	71.9 ± 4.8*†	78.7 ± 3.7*†‡
20 min	64.7 ± 6.4	73.4 ± 4.7*†	80.2 ± 3.1*†‡
30 min	65.2 ± 6.4	73.9 ± 5.5*†	79.5 ± 2.3*†
RECOVERY			
2 min	64.5 ± 6.2	68.7 ± 5.9†	69.6 ± 4.2†
10 min	65.5 ± 5.7	66.0 ± 6.4§	65.2 ± 4.4§
20 min	65.8 ± 5.6	64.6 ± 6.0§	64.0 ± 4.8§
30 min	65.8 ± 5.7	64.2 ± 6.3§	62.7 ± 4.8*§
40 min	66.1 ± 6.0	63.9 ± 5.9§	62.1 ± 4.6*†§

*TV, television; DDR, dance dance revolution; PLAY, traditional games; *$p \leq 0.05$ vs. TV in the same moment; †$p \leq 0.05$ vs. rest in the same session; ‡$p \leq 0.05$ vs. DDR in the same moment; §$p \leq 0.05$ in relation to 2 min recovery in the same session.*

environment is an effective way for reducing the cardiovascular reactivity in children. PLAY seems to be more motivating, thus naturally increasing the levels of PA in an easy manner.

While we did not evaluate the underlying mechanisms that accounts for these differences, it may be suggested that the greater intensity achieved by children during street playing, as reflected in a greater HR increase (i.e., 72% for HR_{max} during PLAY vs. 54% HR_{max} during DDR; Machado and Denadai, 2011), and thus the occurrence of PEH after this more intense intervention, may have a role in a lowered post-exercise BP reactivity. Previous studies have reported that a greater sympathetic activation during exercise could mediate a lower post-exercise reactivity to stress (Lovallo, 2005). In this regard, Brownley et al. (2003) have demonstrated that a single bout of aerobic cycle exercise in adults reduces the post-exercise norepinephrine production in response to stress, favoring a lower cardiovascular reactivity that could also be related to a greater vasodilatory effect of exercise. Additionally, Negrão et al. (2003) have also suggested that exercise could induce a greater nitric oxide release after exercise that could elicit the vasodilatation response, and consequently favor both the PEH and the lower BP reactivity to post-exercise stress. As we did not evaluate possible mechanisms in the current study, it may be suggested that exercise intensity and subsequent metabolic demands could be the major factor accounting to these differences between conditions, as recently reported (Eicher et al., 2010; Simões et al., 2010). Additionally, the significant increase in HR during the CPT after PLAY that was only evident when cardiovascular responses were controlled for exercise intensity, may suggest the existence of other mechanisms (e.g., nitric oxide) different from autonomic control that may account for both the reduced BP reactivity and PEH as suggested in a previous study with women exhibiting PEH after resistance exercises (Tibana et al., 2013). Therefore, further studies are needed in order to determine the exact physiological mechanisms that account to these differences in cardiovascular reactivity in children after exercise, considering exercise mode and intensity.

The current study has some limitations. First, the number of boys and girls is limited and do not allow comparisons between genders. Second, the CPT does not reproduce the habitual source of stress in children's daily activities although is a valid, simple and time saving tool for cardiovascular stress reactivity evaluation. Therefore, further studies should be conducted for testing the potential differences between boys and girls regarding other stressors, such as those experienced at school, in a more realistic environment. Third, we only recorded cardiovascular responses within 40 min of recovery. Therefore, it is unknown how these responses could be maintained in the long-term (e.g., 24 h). Lastly, it is well known that genetic factors and ethnicity could be related to hypertension in adulthood (Douglas et al., 2003), but no evidence is available regarding such differences in children. In this scenario, as our sample was composed of blacks and whites, it may be interesting to look for such potentially genetic differences in further studies.

CONCLUSION

The most relevant finding of the present study was that the 30-min exercise session based on traditional games ("run and catch," "dodge ball," and "capture the flag") resulted both in PEH and lower BP reactivity to stress in children. These results could be due to greater exercise intensity and metabolic demands recorded during traditional games. Additional studies should be conduct to elucidate the influence of intensity, mode and duration of activities usually performed by children, the impact of these variables, and the possible mechanisms underlying the protective cardiovascular effect during subsequent stress-induced BP reactivity.

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REFERENCES

Bell, B. G., and Belsky, J. (2008). Parenting and children's cardiovascular functioning. *Child Care Health Dev.* 34, 194–203. doi: 10.1111/j.1365-2214.2007.00788.x

Bigi, S., Fischer, U., Wehrli, E., Mattle, H. P., Boltshauser, E., Bürki, S., et al. (2011). Acute ischemic stroke in children versus young adults. *Ann. Neurol.* 70, 245–254. doi: 10.1002/ana.22427

Bond, V., Stephens, Q., Adams, R. G., Vaccaro, P., Demeersman, R., Williams, D., et al. (2002). Aerobic exercise attenuates an exaggerated exercise blood pressure response in normotensive young adult African-American men. *Blood Press.* 11, 229–234. doi: 10.1080/08037050213765

Brownley, K. A., Hinderliter, A. L., West, S. G., Girdler, S. S., Sherwood, A., and Light, K. C. (2003). Sympathoadrenergic mechanisms in reduced hemodynamic stress responses after exercise. *Med. Sci. Sports Exerc.* 35, 978–986. doi: 10.1249/01.MSS.0000069335.12756.1B

Chinapaw, M. J., Proper, K. I., Brug, J., van Mechelen, W., and Singh, A. S. (2011). Relationship between young peoples' sedentary behaviour and biomedical health indicators: a systematic review of prospective studies. *Obes. Rev.* 12, e621–e632. doi: 10.1111/j.1467-789X.2011.00865.x

Chiolero, A., Burnier, M., Paradis, G., Paccaud, F., and Bovet, P. (2008). Blood pressure measurement and screening of hypertension in children. *Rev. Med. Suisse* 4, 1904–1906, 1908–1909.

Ciolac, E. G., Guimarães, G. V., D'Avila, V. M., Bortolotto, L. A., Doria, E. L., and Bocchi, E. A. (2008). Acute aerobic exercise reduces 24-h ambulatory blood pressure levels in long-term-treated hypertensive patients. *Clinics (Sao Paulo)* 63, 753–758. doi: 10.1590/S1807-59322008000600008

Daniels, S. R. (2011). International differences in secular trends in childhood blood pressure: a puzzle to be solved. *Circulation* 124, 378–380. doi: 10.1161/CIRCULATIONAHA.111.042747

Douglas, J. G., Bakris, G. L., Epstein, M., Ferdinand, K. C., Ferrario, C., Flack, J. M., et al. (2003). Management of high blood pressure in African Americans: consensus statement of the Hypertension in African Americans Working Group of the International Society on Hypertension in Blacks. *Arch. Intern. Med.* 163, 525–541. doi: 10.1001/archinte.163.5.525

Eicher, J. D., Maresh, C. M., Tsongalis, G. J., Thompson, P. D., and Pescatello, L. S. (2010). The additive blood pressure lowering effects of exercise intensity on post-exercise hypotension. *Am. Heart J.* 160, 513–520. doi: 10.1016/j.ahj.2010.06.005

Ergin, A., Muntner, P., Sherwin, R., and He, J. (2004). Secular trends in cardiovascular disease mortality, incidence, and case fatality rates in adults in the United States. *Am. J. Med.* 117, 219–227. doi: 10.1016/j.amjmed.2004.03.017

Guy, S., Ratzki-Leewing, A., and Gwadry-Sridhar, F. (2011). Moving beyond the stigma: systematic review of video games and their potential to combat obesity. *Int. J. Hypertens.* 2011, 179124. doi: 10.4061/2011/179124

Hines, E. A., and Brown, G. E. (1936). The cold pressor test for measuring the reactibility of the blood pressure: data concerning 571 normal and hypertensive subjects. *Am. Heart J.* 11, 1–9.

Kark, M., Tynelius, P., and Rasmussen, F. (2009). Associations between birthweight and weight change during infancy and later childhood, and systolic blood pressure at age 15 years: the COMPASS study. *Paediatr. Perinat. Epidemiol.* 23, 245–253. doi: 10.1111/j.1365-3016.2008.01007.x

Lambiase, M. J., Barry, H. M., and Roemmich, J. N. (2010). Effect of a simulated active commute to school on cardiovascular stress reactivity. *Med. Sci. Sports Exerc.* 42, 1609–1616. doi: 10.1249/MSS.0b013e3181d0c77b

Lanningham-Foster, L., Jensen, T. B., Foster, R. C., Redmond, A. B., Walker, B. A., Heinz, D., et al. (2006). Energy expenditure of sedentary screen time compared with active screen time for children. *Pediatrics* 118, e1831–e1835. doi: 10.1542/peds.2006-1087

Lehman, B. J., Taylor, S. E., Kiefe, C. I., and Seeman, T. E. (2009). Relationship of early life stress and psychological functioning to blood pressure in the CARDIA study. *Health Psychol.* 28, 338–346. doi: 10.1037/a0013785

Lovallo, W. R. (2005). Cardiovascular reactivity: mechanisms and pathways to cardiovascular disease. *Int. J. Psychophysiol.* 58, 119–132. doi: 10.1016/j.ijpsycho.2004.11.007

MacDonald, J. R. (2002). Potential causes, mechanisms, and implications of post exercise hypotension. *J. Hum. Hypertens.* 16, 225–236. doi: 10.1038/sj.jhh.1001377

MacDonald, J. R., Hogben, C. D., Tarnopolsky, M. A., and MacDougall, J. D. (2001). Post exercise hypotension is sustained during subsequent bouts of mild exercise and simulated activities of daily living. *J. Hum. Hypertens.* 15, 567–571. doi: 10.1038/sj.jhh.1001223

Machado, F. A., and Denadai, B. S. (2011). Validade das equações preditivas da frequência cardíaca máxima para crianças e adolescentes. *Arq. Bras. Cardiol.* 97, 136–140. doi: 10.1590/S0066-782X2011005000078

Martinez-Gomez, D., Tucker, J., Heelan, K. A., Welk, G. J., and Eisenmann, J. C. (2009). Associations between sedentary behavior and blood pressure in young children. *Arch. Pediatr. Adolesc. Med.* 163, 724–730. doi: 10.1001/archpediatrics.2009.90

Maximova, K., O'Loughlin, J., Paradis, G., Hanley, J. A., and Lynch, J. (2009). Declines in physical activity and higher systolic blood pressure in adolescence. *Am. J. Epidemiol.* 170, 1084–1094. doi: 10.1093/aje/kwp255

Menkes, M. S., Matthews, K. A., Krantz, D. S., Lundberg, U., Mead, L. A., Qaqish, B., et al. (1989). Cardiovascular reactivity to the cold pressor test as a predictor of hypertension. *Hypertension* 14, 524–530.

Moraes, J. F. V. N., Sales, M. M., Asano, R. Y., Carvalho, F. O., Moreira, S. R., and Campbell, C. S. G. (2014). Important association between body mass index and blood pressure reactivity in male adolescents. *J. Exerc. Physiol. Online* 17, 28–39.

Morais, P. K., CamBPell, C. S., Sales, M. M., Motta, D. F., Moreira, S. R., Cunha, V. N., et al. (2011). Acute resistance exercise is more effective than aerobic exercise for 24h blood pressure control in type 2 diabetics. *Diabetes Metab.* 37, 112–117. doi: 10.1016/j.diabet.2010.08.008

Mota, M. R., Pardono, E., Lima, L. C., Arsa, G., Bottaro, M., Campbell, C. S., et al. (2009). Effects of treadmill running and resistance exercises on lowering blood pressure during the daily work of hypertensive subjects. *J. Strength Cond. Res.* 23, 2331–2338. doi: 10.1519/JSC.0b013e3181bac418

Nettle, H., and Sprogis, E. (2011). Pediatric exercise: truth and/or consequences. *Sports Med. Arthrosc.* 19, 75–80. doi: 10.1097/JSA.0b013e318209cf2b

Negrão, C. E., Santos, A. C., and Alves, M. J. N. N. (2003). "Exercício físico e endotélio," in *Endotélio e Doenças Cardiovasculares*, eds P. L. Luz, F. R. M. Laurindo, and A. C. P. Chagas (São Paulo: Atheneu), 162–171.

Probst, M., Bulbulian, R., and Knapp, C. (1997). Hemodynamic responses to the stroop and cold pressor tests after submaximal cycling exercise in normotensive males. *Physiol. Behav.* 62, 1283–1290.

Rabelo, M., Pardono, E., Jesus, L., Arsa, G. C., Campbell, C. S. G., and Marques, M. F. B. (2009). Hypotensive effects of treadmill running and resistance exercise during the daily work of subjects with elevated blood pressure. *J. Strength Cond. Res.* 23, 2331–2338. doi: 10.1519/JSC.0b013e3181bac418

Rauber, S. B., Carvalho, F., Sousa, I. R. C., Mazzoccante, R. P., Franco, C. B. S., Farias, D. L., et al. (2013). Variáveis cardiovasculares durante e após a prática do VÍDEO GAME ativo 'Dance Dance Revolution' e televisão. *Motriz* 19, 358–367. doi: 10.1590/S1980-65742013000200013

Richter, C. M., Panigas, T. F., Bündchen, D. C., Dipp, T., Belli, K. C., and Viecili, P. R. N. (2010). Redução dos níveis pressóricos em indivíduos hiper-reativos após treinamento físico aeróbio. *Arq. Bras. Cardiol.* 95, 251–257. doi: 10.1590/S0066-782X2010005000085

Roemmich, J. N., Lambiase, M., Salvy, S. J., and Horvath, P. J. (2009). Protective effect of interval exercise on psychophysiological stress reactivity in children. *Psychophysiology* 46, 852–861. doi: 10.1111/j.1469-8986.2009.00808.x

Rossow, L., Yan, H., Fahs, C. A., Ranadive, S. M., Agiovlasitis, S., Wilund, K. R., et al. (2010). Postexercise hypotension in an endurance-trained population of men and women following high-intensity interval and steady-state cycling. *Am. J. Hypertens.* 23, 358–367. doi: 10.1038/ajh.2009.269

Siegrist, M., Lammel, C., Haller, B., Christle, J., and Halle, M. (2013). Effects of a physical education program on physical activity, fitness, and health in children: the JuvenTUM project. *Scand. J. Med. Sci. Sports* 23, 323–330. doi: 10.1111/j.1600-0838.2011.01387.x

Simões, G. C., Simões, H. G., Moreira, S. R., Kuschnick, M. R., and Campbell, C. S. G. (2010). Post-resistance exercise blood pressure reduction is influenced by exercise intensity in healthy individuals and in those with type-2

diabetes. *J. Strength Cond. Res.* 24, 1277–1284. doi: 10.1519/JSC.0b013e3181d67488

Slaughter, M. H., Lohman, T. G., Boileau, R. A., Horswill, C. A., Stillman, R. J., Van Loan, M. D., et al. (1988). Skinfold equations for estimation of body fatness in children and youth. *Hum. Biol.* 60, 709–723.

Tibana, R. A., Boullosa, D. A., Leicht, A. S., and Prestes, J. (2013). Women with metabolic syndrome present different autonomic modulation and blood pressure response to an acute resistance exercise session compared with women without metabolic syndrome. *Clin. Physiol. Funct. Imaging* 33, 364–372. doi: 10.1111/cpf.12038

Tsioufis, C., Kyvelou, S., Tsiachris, D., Tolis, P., Hararis, G., Koufakis, N., et al. (2011). Relation between physical activity and blood pressure levels in young Greek adolescents: the Leontio Lyceum Study. *Eur. J. Public Health* 21, 63–68. doi: 10.1093/eurpub/ckq006

Wang, X., and Perry, A. C. (2006). Metabolic and physiologic responses to video game play in 7- to 10-year-old boys. *Arch. Pediatr. Adolesc. Med.* 160, 411–415. doi: 10.1001/archpedi.160.4.411

Warburton, D. E., Sarkany, D., Johnson, M., Rhodes, R. E., Whitford, W., Esch, B. T., et al. (2009). Metabolic requirements of interactive video game cycling. *Med. Sci. Sports Exerc.* 41, 920–926. doi: 10.1249/MSS.0b013e31819012bd

Wood, D. L., Sheps, S. G., Elveback, L. R., and Schirger, A. (1984). Cold pressor test as a predictor of hypertension. *Hypertension* 6, 301–306.

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Reduced vasodilator function following acute resistance exercise in obese women

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Obesity contributes to stress induced impairments in endothelium-dependent vasodilation (EDV), a precursor to atherosclerosis. Since obesity is associated with inflammation and oxidative stress, we sought to determine if a single bout of strenuous weight lifting (SWL) reduces EDV among sedentary obese adults. Participants included 9 obese (OB) (BMI 30.0–40.0 kg/m^2) and 8 lean (LN) (BMI 18.5–24.9 kg/m^2) sedentary young women. All participants underwent a single bout of SWL using a progressive leg-press protocol. Brachial artery flow-mediated dilation (FMD) (an index of EDV) was determined using ultrasonography before and after SWL. Sublingual nitroglycerin (NTG) was used to determine brachial artery endothelium-independent vasodilation following SWL. Brachial artery FMD was significantly reduced in OB and LN women (LN: 6.4 ± 1.6%, $p = 0.22$) after SWL. There was no difference in the magnitude of change pre- and post-SWL between groups (OB: −2.4 ± 0.6% and LN: −2.2 ± 1.6%, $p = 0.84$). Dilation to NTG was lower in OB (21.6 ± 1.3%) compared to LN women (27.6 ± 2.1%, $p = 0.02$) and associated with body weight ($r = -0.70$, $p = 0.01$). These data suggest that EDV is reduced in woman after acute resistance exercise. Dilations to NTG were lower in obese compared to lean woman and associated with body weight suggesting that changes in sensitivity of blood vessels to NO occurs during obesity. These findings may be important for understanding vascular risk following acute exercise in obesity.

Keywords: endothelium, vasodilation, acute exercise, obesity, women

INTRODUCTION

Rates of overweight and obesity have reached epidemic proportions (Flegal et al., 2012) with currently 2 in 3 adults either overweight or obese. In fact, atherosclerotic cardiovascular disease (CVD) is the third leading cause of death among women between the ages of 25 and 44 and obesity independently contributes to increased risk in this particular demographic (Mosca et al., 1997). Vascular dysfunction may play a pivotal role in the development of atherosclerosis in obesity (Sturm et al., 2009). Increased body fat is associated with increased reactive oxygen species production (ROS) (Anfossi et al., 2010) and impaired nitric oxide (NO)-mediated endothelium-dependent vasodilation (EDV) (Al et al., 2001). While impaired endothelium-independent dilation (EID), indicative of smooth muscle dysfunction, is also linked to the development of atherosclerosis, most research suggests that it is preserved (Arkin et al., 2008).

Resistance exercise training is recommended for preventing weight gain and reducing the risk of obesity associated cardiovascular risk (Mason et al., 2007) and may reduce the ill effects of chronic stress on cardiovascular function (i.e., psychological stressors) (Paine et al., 2013). A previous study found that resistance exercise training (2 days per week) for 18 weeks had no effect on endothelial function in healthy post-menopausal women (Casey et al., 2007), while others have found improvements in endothelial function after 16 weeks of resistance exercise when combined with other lifestyle interventions of aerobic exercise and calorie reduction (Cotie et al., 2014). Acute resistance exercise increases blood flow intermittently, yields increased shear stress and improves nitric oxide (NO)-mediated vasodilation (Tinken et al., 2010). However, acute resistance exercise is associated with elevated systolic and diastolic blood pressures (Sale et al., 1994) to levels known to impair vascular function (Jurva et al., 2006).

Previous studies on stress-induced cardiovascular dysfunction have focused on the neural and hormonal regulation of cardiovascular function following acute psychological challenges (Chrousos, 2000, 2009). Research on the direct effects of acute physical stressors on vasodilator function and the contribution of obesity to these responses has been limited. In previous studies from our laboratory brachial artery flow mediated dilation (FMD) was reduced following acute weight lifting in sedentary compared to exercise-trained men, despite similar elevations in blood pressure during exercise (Phillips et al., 2011). Resistance exercise is an important component of exercise prescription in overweight and obesity to promote lean body mass and insulin sensitivity (Laskowski, 2012). Therefore, there is a

need to determine the clinical impact of different modes of exercise (including resistance exercise) on vascular function in woman since (1) young women suffer a disproportionate burden of morbidity and mortality attributable to obesity and have higher morbidity and mortality rates after myocardial infarction compared to men (Maas et al., 2011) and (2) intense exercise which may be experienced particularly in the initial stages of an exercise program may be harmful to blood vessel function. For example, acute high intensity resistance exercise (>80% 1RM) can reduce brachial FMD (Phillips et al., 2011; Choi et al., 2014). In contrast, moderate intensity, aerobic exercise (50–65% VO_{2max}) has been shown to improve FMD in lean individuals (Rooks et al., 2011) or reduce FMD in overweight individuals (Harris et al., 2008). The purpose of this study was to determine whether a single bout of weight lifting reduces FMD in sedentary obese women compared to lean woman in order to determine if obesity (in the absence of other risk factors) translates to more severe reductions in FMD following a strenuous exercise session. In addition, we sought to determine if changes in FMD associated with SWL and are greater in obese compared to lean women. We hypothesized that acute physical stress (in the form of a single session of weight lifting) will reduce vascular function and that this change is augmented in obese compared to sedentary lean women.

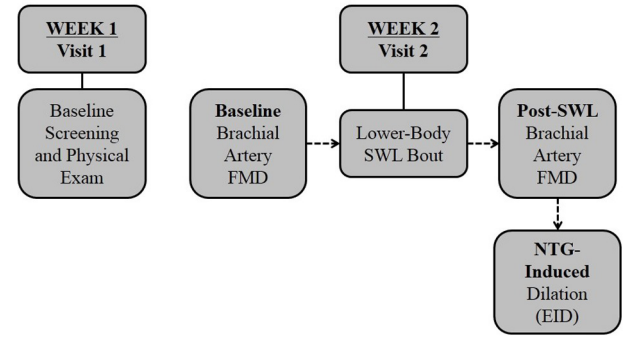

FIGURE 1 | Two-week research study timeline. Subjects were screened at baseline (Visit 1) by way of questionnaires and a physical examination. Approximately 1 week later (Visit 2) brachial artery flow-mediated dilation (FMD) was assessed in all subjects prior to and after performance of a single bout of bilateral lower-body strenuous weight lifting (SWL). Subjects were administered nitroglycerin approximately 10 min after FMD for measurement of brachial artery endothelium-independent dilation (EID).

MATERIALS AND METHODS

STUDY POPULATION

Seventeen women were studied and were recruited in the community. Initially, each participant was screened by telephone and if inclusion criteria were met, they were invited for an in-person screening at which time eligibility was confirmed upon completion of a medical and exercise history questionnaire and physical examination. We included woman who were 18–40 years of age, obese (OB) (BMI 30.0–40.0 kg/m^2) or lean (LN) (BMI 18.5–24.9 kg/m^2), and sedentary (less than 150 min of moderate physical activity/week).We excluded individuals with a history of CVD, diabetes mellitus or thyroid dysfunction, woman who were pregnant or recently pregnant and lactating, woman with a history of cancer, history of smoking (for at least 6 months prior to participation), amenorrhea or irregular menses, and use of vasoactive medications. Written informed consent was obtained prior to participation. The study protocol was approved by the Office for the Protection of Research Subjects and the Institutional Review Board of the University of Illinois at Chicago.

STUDY DESIGN, BIOCHEMICAL, BLOOD PRESSURE, AND BODY COMPOSITION MEASUREMENTS

All individuals were evaluated at the University of Illinois at Chicago, Clinical Research Center after a 12-h overnight fast between 0700 and 1100 in a temperature controlled study suite. **Figure 1** is a schematic summarizing the study design. Subjects were screened for eligibility at Visit 1. Approximately 1 week later (Visit 2) brachial artery flow-mediated dilation (FMD) was assessed in all subjects prior to and 30 min after performance of a single bout of bilateral lower-body strenuous weight lifting (SWL). Venous blood samples were drawn before and immediately following resistance exercise from an antecubital vein.

Plasma was separated by centrifugation for off-site laboratory analysis of total cholesterol, high-density lipoproteins (HDLs), low-density lipoproteins (LDLs), and glucose (Alverno Clinical Laboratories, LLC; Hammond, IN). Total cholesterol, HDLs, LDLs, and triglycerides were measured using spectrophotometric assays with intra-assay variances of 1.4, 3.0, 2.5, and 2.0%, respectively. Glucose concentration was measured using the glucose oxidase procedure (Beckman Autoanalyser II; Beckman Coulter Inc.; Fullerton, CA) with intra-assay variance of 1.5%. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after at least 10 min of rest using a standard manual mercury sphygmomanometer with an appropriate cuff size and during the last repetition of each set of exercise. Waist circumference was measured at the narrowest part of the waist (above the umbilicus and below the xiphoid process) (Ohrvall et al., 2000). Body composition was determined by dual energy x-ray absorptiometry (DEXA) using established methods (Mattila et al., 2007).

DETERMINATION OF VASODILATOR FUNCTION

Brachial artery FMD was used as a measure of endothelium dependent vasodilation (EDV) using techniques previously described (Corretti et al., 2002; Phillips et al., 2011). In premenopausal women, FMD may vary during the menstrual cycle (Hashimoto et al., 1995); therefore, subjects were recruited during the early follicular phase of menses. In the supine state, ultrasound imaging (MicroMaxx; Sonosite; Seattle, WA) of the brachial artery was performed in a longitudinal plane at a site 1–3 cm proximal to the antecubital fossa of the arm (Phillips et al., 2011). Baseline images were recorded and a blood pressure cuff was placed on the forearm and inflated to 50 mmHg above SBP for 5 min. Arterial diameter was determined during peak hyperemia after release of the blood pressure cuff from the

forearm. To assess dilation, 10 s of images were captured 1–3 min after cuff release. Flow velocity was recorded at baseline and just after cuff release where maximal velocity was observed. Brachial artery reactivity to FMD was assessed before and 30 min following a single bout of SWL. The original position of the ultrasound probe was marked and measured according to the distance from the antecubital crease and the post-exercise examination was performed in the same position. Ten minutes after recording the last brachial artery diameter measurement following exercise, EID was determined with 0.4 mg of sublingual nitroglycerin (NTG) for 5 min. Since there is a blood pressure lowering effect of NTG making it unsafe to administer immediately prior to maximal exercise, we analyzed NTG dilations in a separate group of age and BMI matched adults who did not perform exercise. All ultrasonographic images were recorded and transferred to a PC for offline analysis of FMD and NTG responses using edge-detection software (Medical Imaging; Iowa City, IA). The coefficient of variation for FMD% was 6.3 and 3.2% for NTG-induced dilation. To estimate brachial artery wall shear stress, peak shear rates (SR) were calculated during FMD using the following equation: peak SR = maximal flow velocity (mm/s) ÷ diameter (mm). FMD was normalized for the peak SR using the following equation: normalized FMD = FMD ÷ peak SR (Phillips et al., 2011).

EXERCISE PROTOCOL

The exercise protocol involved performance of a single bout of bilateral lower-body weight lifting using a variable-resistance leg press machine (Hoist HD-1610 Selectorized Leg Press; Hoist Fitness Systems; San Diego, CA). After becoming familiarized with the leg press machine, participants performed a warm up period of 1–2 sets of 10 repetitions at a perceived capacity of approximately 30–40% of 1-repetition maximum (RM; calculated with a prediction equation) (Kemmler et al., 2006). Then 3–4 sets of 10 repetitions at a perceived capacity of approximately 80–90% of 1-RM performed with a final isometric hold. A 2-min rest interval was allotted between each set. Exercise heart rate and the 10-point Borg rating of perceived exertion (RPE) scale were used as indices of intensity after each set. However, all volunteers completed a minimum of 4 sets of 10 repetitions or to failure on the final set. The RPE scale was not used as an intensity threshold since (1) the perceived capacity was 80–90% 1RM and (2) the goal was to elicit similar increases in BP between participants (Jurva et al., 2006).

STATISTICS

Results are expressed as mean ± standard deviation (SD), unless otherwise stated. Normality of the distribution was confirmed for all data using the Shapiro-Wilk test. Differences in physiological and physical characteristics (lipid panels, glucose measurements, vital signs, and anthropometric measurements) as well as dietary characteristics between OB and LN subjects were compared using student *t*-test. Pre-post differences in hemodynamic and vascular characteristics were compared using paired *t*-test within OB and LN groups. The mixed effects model was performed to examine the effects of acute resistance exercise between OB and LN subjects on hemodynamic and vascular variables including brachial artery diameter, maximum percentage change in diameter (FMD), and normalized FMD. For each outcome variable, group (OB vs. LN), time (pre vs. post) and group*time interaction were modeled as fixed effects and subject intercept was modeled as random effect. Using only baseline data, we also conducted linear regression analyses to investigate the association of obesity with brachial artery FMD, independent of potential confounding effects. Pearson's correlations were used to evaluate how brachial artery reactivity to FMD and NTG responses relate to physiological and physical characteristics. Throughout the paper the effect size was computed using Cohen (1988). Cohen's *d* is an appropriate effect size for the comparison between two means. It indicates the standardized difference between two means, and expresses this difference in SD units (Δ mean/SD). Due to its pilot nature, we did not perform a formal power analysis for the current study. With sample size of 8 and 9 in each group, the study will have approximately 80% power to detect a large effect size of 1.5 standard unit mean difference between OB and LN using a two-sided alpha of 0.05. The results of our data could provide a more precise power estimate for future studies. Throughout the paper, the level of statistical significance for all analyses was set at two-sided $P < 0.05$. Data were analyzed using SPSS software (Version 19.0; IBM Corp., Armonk, NY).

RESULTS

BASELINE AND VASCULAR CHARACTERISTICS

Baseline characteristics for the OB ($n = 9$) and LN ($n = 8$) women who underwent a single bout of SWL are presented in **Table 1**. As was expected, body weight was significantly different between sedentary OB and LN women. In addition, all body weight-related characteristics including BMI, body fat percentage, and waist circumference were significantly different between OB and LN women. Total cholesterol and LDL cholesterol were also higher in OB compared to LN women. **Table 2** shows hemodynamic and vascular characteristics before and after

Table 1 | Cardio-metabolic characteristics of study participants.

	Lean ($n = 8$)	Obese ($n = 9$)	*P*-value
Height, cm	164.7 ± 8.2	160.0 ± 7.8	0.238
Weight, kg	58.4 ± 6.5	88.5 ± 16.0*	<0.001
BMI, kg/m^2	21.5 ± 1.4	34.2 ± 3.3*	<0.001
Body fat (%)	27.3 ± 7.4	42.8 ± 4.5*	<0.001
Waist circumference, cm	71.7 ± 5.1	95.2 ± 7.8*	<0.001
Total cholesterol, mmol/l	4.0 ± 0.6	5.0 ± 0.6*	0.016
LDL, mmol/l	2.0 ± 0.6	2.8 ± 0.6*	0.005
HDL, mmol/l	1.6 ± 0.3	1.4 ± 0.3	0.163
Glucose, mmol/l	4.6 ± 0.6	4.9 ± 0.3	0.375
Maximum SBP, mm/Hg	187.8 ± 25.7	188.5 ± 18.0	0.740
Maximum DBP, mm/Hg	123.5 ± 12.2	114.0 ± 11.1	0.556
Maximum weight lifted, kg	103.6 ± 11.0	107.3 ± 10.2	0.639
Maximum heart rate (bpm)	130.9 ± 8.9	137.4 ± 7.1.	0.09

*All values expressed as mean (± SD). *P < 0.05, Statistically significant. BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure.*

Table 2 | Hemodynamic and vascular characteristics of participants.

	Lean ($n = 8$)				Obese ($n = 9$)			
	Before SWL	**After SWL**	**Cohen's *d***	***P*-value**	**Before SWL**	**After SWL**	**Cohen's *d***	***P*-value**
Resting heart rate, beats/min	65.0 ± 8.5	70.1 ± 9.6	0.56	0.078	69.7 ± 11	70.9 ± 11	0.11	0.790
Systolic BP, mm Hg	103.8 ± 7.4	105.3 ± 10	0.17	0.332	119.9 ± 10	119 ± 8	0.01	0.65
Diastolic BP, mm/Hg	60.6 ± 3.4	63.9 ± 6.2	0.66	0.063	76.6 ± 9	75 ± 13	0.14	0.24
Baseline diameter, mm	3.2 ± 0.3	3.1 ± 0.03	0.47	0.596	3.4 ± 0.3	3.4 ± 0.3	0.30	0.447
Brachial artery FMD (%)	8.5 ± 3.7	6.4 ± 4.5	0.51	0.193	10.7 ± 1.2	8.3 ± 1.8*	1.57	0.002
Baseline BFV, cm/s	85.3 ± 15.5	90.5 ± 40	0.17	0.615	64.4 ± 21.3†	72.7 ± 26	0.35	0.270
Peak BFV, cm/s	136.7 ± 40	140.7 ± 40	0.10	0.779	135.1 ± 37.8	143.4 ± 43	0.21	0.415
Baseline SR, s^{-1}	267.2 ± 47	287.8 ± 112	0.24	0.676	193.9 ± 63	218.8 ± 91	0.32	0.561
Peak SR, s^{-1}	385.6 ± 130	421.1 ± 81	0.33	0.468	375.0 ± 123	404.9 ± 189	0.19	0.365
Normalized FMD	0.018 ± 0.011	0.011 ± 0.01	0.67	0.102	0.032 ± 0.009	0.024 ± 0.009*	0.89	0.008

*All values expressed as mean (±SD). *P < 0.05, Statistically significant compared to Before SWL (P < 0.05). BP, blood pressure; FMD, flow-mediated dilation; SR, shear rate; BFV, blood flow velocity; FMD Δ, absolute change in FMD; NTG, nitroglycerin; ND, not determined.*

SWL. Although the participants were not hypertensive (SBP < 140 mmHg), between-group comparisons showed significantly increased SBP and DBP levels in the OB compared to LN women. In addition, FMD and normalized FMD was reduced after compared to before SWL in OB but not in LN women (**Table 2**). None of the interactions between group and time for hemodynamic and vascular variables listed in **Table 2** were found significant in the mixed effects ANOVA models.

EFFECTS OF ACUTE EXERCISE ON BRACHIAL FLOW AND NTG DILATIONS

The RPE during the last repetition was 9.4 ± 0.7 in obese and 9.5 ± 0.8 in lean women. Brachial artery FMD was significantly reduced after SWL in OB group but not in LN group (**Figure 2**). In the mixed models, however, the Group*SWL interaction was not significant, indicating that the slop of change in brachial artery FMD was not difference between LN and OB groups after SWL. There was a significant SWL effect ($b = -0.024, p = 0.038$) suggesting that the overall brachial artery FMD was significantly reduced after the SWL exercise (**Table 3**). NTG-dilation was lower in OB (21.6 ± 1.3%) compared to LN women (27.6 ± 2.1%, $p = 0.02$) (**Figure 3A**) and this response was negatively correlated with body weight ($r = -0.70$, $p = 0.01$) (**Figure 3B**), BMI ($r = 0.62$, $p = 0.02$), and WC ($r = 0.67$, $p = 0.03$) but not completely with %BF ($r = 0.41$, $p = 0.10$). Furthermore, correlations were independent of other physiological and physical characteristics. In separate studies ($n = 12$) obese women recruited to perform NTG dilations before exercise had similar NTG dilation (21.9 ± 1.9%) compared to after SWL.

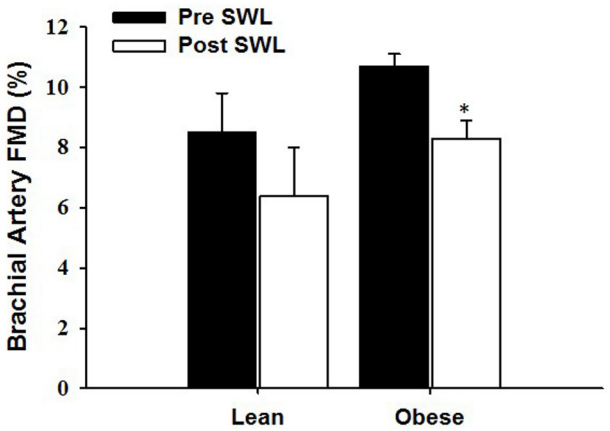

FIGURE 2 | The effect of a single bout of strenuous weight lifting (SWL) on brachial artery flow-mediated dilation (FMD) in lean (LN) and obese (OB) sedentary women. *Significant difference observed after SWL vs. before SWL ($p < 0.05$).

Table 3 | Mixed effects model on brachial artery FMD (%).

Effect	**Coefficient estimate (*b*)**	**Standard error (*SE*)**	***P*-value**
Intercept	0.083	0.010	<0.0001
Group (LN vs. OB)	−0.018	0.014	0.2185
SWL (post vs. pre)	−0.024	0.011	0.0383
Group*SWL interaction	0.002	0.015	0.9125

**Mixed effects model was performed using maximum likelihood method with unstructured covariance.*

DISCUSSION

The major findings of this study are that: (1) a single bout of strenuous physical stress (induced by weight lifting) reduces EDV in obese women who are sedentary, and (2) nitroglycerin-induced endothelium-independent dilation are reduced in sedentary obese compared to sedentary lean women measured after a single bout of SWL.

VASCULAR REACTIVITY AND ACUTE EXERTION IN OBESITY

Under normal physiologic conditions, NO is the chief vasodilator released by the endothelium and functions in modulating smooth muscle tone and inflammation (Vanhoutte et al., 2009).

In addition, NO has anti-proliferative effects on the vascular wall and plays a critical role in vasodilation under conditions of increased blood flow (Landmesser et al., 2004). During increased flow, ROS are also produced by endothelial nitric oxide synthase (eNOS) but are usually scavenged by vascular antioxidant enzymes and, subsequently, NO diffuses into adjacent smooth muscle cells and mediates vasodilation (Wolin, 2000).

Repetitive increases in blood flow during chronic exercise training have been shown to improve NO-mediated vasodilation as assessed by FMD (Walsh et al., 2003; Olson et al., 2006) and may protect against vascular dysfunction through regulation of eNOS and reduction of oxidative stress (Hambrecht et al., 2003). However, acute exercise induces oxidative stress in individuals who are unaccustomed to exercise training by enhancing vascular ROS production, which contributes to a decrease in NO bioavailability and impairs FMD. Similar to previous studies (Phillips et al., 2011), brachial artery FMD after a single bout of weight lifting was reduced in sedentary adults (**Table 2**). Since a sedentary lifestyle is closely linked to obesity and both are associated with vascular dysfunction and increased risk of CVD, we hypothesized the degree of FMD impairment to be augmented in OB compared to LN adults. Although FMD was reduced in sedentary OB adults after SWL, the absolute difference (pre- vs. post-SWL) was similar between groups. Other studies have found impaired FMD responses after acute aerobic exercise in sedentary overweight and obese men (Harris et al., 2008). Other studies found reduced FMD following hand grip exercise in individuals at risk of CVD (McGowan et al., 2006). To our knowledge, this is the first study to determine the effects of acute resistance exercise on FMD responses in obese women.

Previous studies showed that vascular smooth muscle responses to NTG are impaired in the presence of obesity (Ayer et al., 2011) and other CVD risk factors (Raitakari et al., 2004; Adams et al., 2005); however, other studies examining NTG-dilations in obesity independent of other risk factors suggest that it is preserved. Of considerable interest were the findings in this study that brachial artery EID responses to NTG were lower in OB compared to LN women (**Figure 3**). This result may represent a mechanistically different effect of exercise on FMD whereby vascular sensitivity to NO may contribute to reduce FMD in obesity compared to lean adults. Moreover, NTG-induced dilation after acute resistance exercise was associated with body weight, BMI, and WC with a tendency to correlate with %BF but not the other metabolic risk factors assessed (i.e., total cholesterol and LDL cholesterol). Although SBP and DBP were higher in OB women, there was no relationship between baseline values and NTG-induced vasodilation. Taken together, these data suggest an independent link between obesity and reduced vascular smooth muscle function after a single bout of SWL.

Both inflammation and oxidative stress could influence EID by reducing bioconversion of NTG to or scavenging of NO in smooth muscle (Schulz et al., 2002). In fact, obesity is associated with a pro-inflammatory milieu characterized by increased production of pro-atherogenic adipokines that enhance production of pro-inflammatory cytokines and increase ROS generation promoting oxidative stress (Tilg and Moschen, 2006). Acute resistance and aerobic exercise has been shown to increase plasma markers of oxidative stress in obesity (Vincent et al., 2004). Future studies will focus on the specific vascular effects of acute resistance exercise and other physical stressors on smooth muscle sensitivity to NO during obesity.

STUDY LIMITATIONS

There are some limitations of this study. First, the generalizability of the study is a limited to flow mediated dilation responses in women up to an hour following acute exertion. However, given that the burden of CVD is increasing in women, the results of this study contribute to a better understanding of the mechanisms by which exercise influences vascular health in women. Sex specific influences of physical stress on endothelium-dependent and endothelium-independent vasodilator responses may be important considerations in the future. Second, we were unable to evaluate the effects of NTG in sedentary OB and LN adults before a single bout of SWL due to the residual vasodilator effects of NTG on blood pressure during exercise. However, we

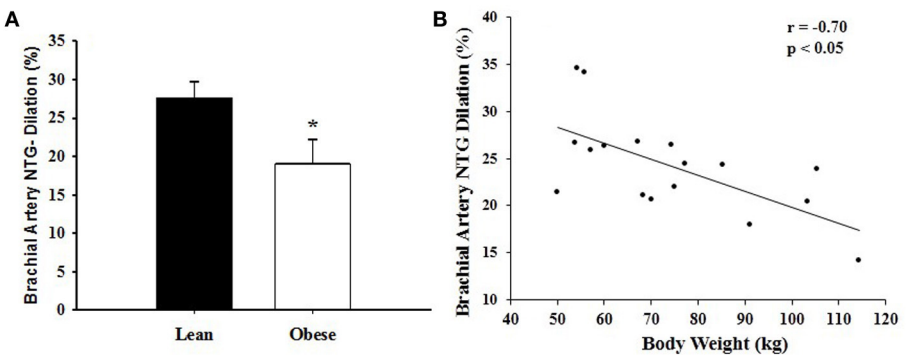

FIGURE 3 | (A) Nitroglycerin (NTG)-induced dilation of the brachial artery in lean (LN) and obese (OB) sedentary women after a single bout of strenuous weightlifting (SWL). *Significant difference observed in OB vs. LN ($p < 0.05$). **(B)** Correlation between nitroglycerin (NTG)-induced dilation of the brachial artery and body weight in sedentary women after a single bout of strenuous weight lifting (SWL). There was a negative correlation between brachial artery NTG-induced dilation and body weight ($r = -0.70$, $p < 0.01$).

have found that NTG dilations in a similar cohort of obese subjects were similar before and after exercise (Mean: 21% ± 1.4) suggesting that lower NTG dilation in obese women is not mediated by weight lifting. Third, CVD risk factors may have influenced NTG-dilations after SWL in OB adults since baseline blood pressure, total cholesterol, and LDL levels were higher compared to LN adults. However, in our analysis there were no relationships between these CVD risk factors and EID after acute resistance exercise. Finally, our results may have been confounded by nutritional variation and that the % body fat in the lean group was 27% a level that may be associated with metabolic dysfunction. There was no relationship between % body fat and FMD.

CLINICAL INSIGHTS AND CONCLUSIONS

In conclusion, our results suggest that acute physical stress induced by resistance exercise reduces FMD in obese women who are sedentary. Atherosclerotic CVD is a leading cause of preventable death among women between the ages of 25 and 44 and a large majority of women with CVD are asymptomatic (Maas et al., 2011) which makes early detection difficult. Since FMD correlates well with coronary artery function (Hashimoto et al., 2000) the results of our studies may extend to predict CVD risk among young women since acute exertion often triggers myocardial infarction in individuals who are sedentary (Mittleman et al., 1993).

In addition, endothelium-independent dilation to nitroglycerin was reduced in sedentary obese compared to lean women suggesting changes in vasodilator sensitivity of blood vessels to NO in obese adults. Reduced arterial function (FMD) and reduced sensitivity to NO donors (NTG) after acute physical exertion may be an important composite marker for future CV risk in young obese women without baseline arterial dysfunction or overt disease. Furthermore, these findings may be important in understanding the link between obesity and vascular dysfunction and may have important implications for how vascular and hemodynamic responses to other acute physiological perturbations such as acute hypertension, systemic hypoxemia, and hyperglycemia are altered during obesity.

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REFERENCES

Adams, V., Linke, A., Krankel, N., Erbs, S., Gielen, S., Mobius-winkler, S., et al. (2005). Impact of regular physical activity on the NAD(P)H oxidase and angiotensin receptor system in patients with coronary artery disease. *Circulation* 111, 555–562. doi: 10.1161/01.CIR.0000154560.88933.7E

Al, S. J., Higano, S. T., Holmes, D. R. Jr., Lennon, R., and Lerman, A. (2001). Obesity is independently associated with coronary endothelial dysfunction in patients with normal or mildly diseased coronary arteries. *J. Am. Coll. Cardiol.* 37, 1523–1528. doi: 10.1016/S0735-1097(01)01212-8

Anfossi, G., Russo, I., Doronzo, G., Pomero, A., and Trovati, M. (2010). Adipocytokines in atherothrombosis: focus on platelets and vascular smooth muscle cells. *Mediators Inflamm.* 2010:174341. doi: 10.1155/2010/174341

Arkin, J. M., Alsdorf, R., Bigornia, S., Palmisano, J., Beal, R., Istfan, N., et al. (2008). Relation of cumulative weight burden to vascular endothelial dysfunction in obesity. *Am. J. Cardiol.* 101, 98–101. doi: 10.1016/j.amjcard.2007.07.055

Ayer, J. G., Harmer, J. A., David, C., S Steinbeck, K., Seale, J. P., and Celermajer, D. S. (2011). Severe obesity is associated with impaired arterial smooth muscle function in young adults. *Obesity (Silver. Spring)* 19, 54–60. doi: 10.1038/oby.2010.114

Casey, D. P., Pierce, G. L., Howe, K. S., Mering, M. C., and Braith, R. W. (2007). Effect of resistance training on arterial wave reflection and brachial artery reactivity in normotensive postmenopausal women. *Eur. J. Appl. Physiol.* 100, 403–408. doi: 10.1007/s00421-007-0447-2

Choi, Y., Akazawa, N., Miyaki, A., Ra, S. G., Shiraki, H., Ajisaka, R., et al. (2014). Acute effect of high-intensity eccentric exercise on vascular endothelial function in young men. *J. Strength. Cond. Res.* doi: 10.1519/JSC.0000000000000536. [Epub ahead of print].

Chrousos, G. P. (2000). The role of stress and the hypothalamic-pituitary-adrenal axis in the pathogenesis of the metabolic syndrome: neuro-endocrine and target tissue-related causes. *Int. J. Obes. Relat. Metab. Disord.* 24(Suppl. 2), S50–S55. doi: 10.1038/sj.ijo.0801278

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nat. Rev. Endocrinol.* 5, 374–381. doi: 10.1038/nrendo.2009.106

Cohen, J. (1988). "Analysis of variance," in *Statistical Power Analysis for the Behavioral Sciences, 2nd Edn.* (Hillsdale, NJ: Lawrence Erlbaum Associates), 273–406.

Corretti, M. C., Anderson, T. J., Benjamin, E. J., Celermajer, D., Charbonneau, F., Creager, M., et al. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J. Am. Coll. Cardiol.* 39, 257–265. doi: 10.1016/S0735-1097(01)01746-6

Cotie, L. M., Josse, A. R., Phillips, S. M., and Macdonald, M. J. (2014). Endothelial function increases after a 16-week diet and exercise intervention in overweight and obese young women. *Biomed. Res. Int.* 2014:327395. doi: 10.1155/2014/327395

Flegal, K. M., Carroll, M. D., Kit, B. K., and Ogden, C. L. (2012). Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999–2010. *JAMA* 307, 491–497. doi: 10.1001/jama.2012.39

Hambrecht, R., Adams, V., Erbs, S., Linke, A., Krankel, N., Shu, Y., et al. (2003). Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation* 107, 3152–3158. doi: 10.1161/01.CIR.0000074229.93804.5C

Harris, R. A., Padilla, J., Hanlon, K. P., Rink, L. D., and Wallace, J. P. (2008). The flow-mediated dilation response to acute exercise in overweight active and inactive men. *Obesity.(Silver. Spring)* 16, 578–584. doi: 10.1038/oby.2007.87

Hashimoto, M., Akishita, M., Eto, M., Ishikawa, M., Kozaki, K., Toba, K., et al. (1995). Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. *Circulation* 92, 3431–3435. doi: 10.1161/01.CIR.92.12.3431

Hashimoto, M., Kozaki, K., Eto, M., Akishita, M., Ako, J., Iijima, K., et al. (2000). Association of coronary risk factors and endothelium-dependent flow-mediated dilatation of the brachial artery. *Hypertens. Res.* 23, 233–238. doi: 10.1291/hypres.23.233

Jurva, J. W., Phillips, S. A., Syed, A. Q., Syed, A. Y., Pitt, S., Weaver, A., et al. (2006). The effect of exertional hypertension evoked by weight lifting on vascular endothelial function. *J. Am. Coll. Cardiol.* 48, 588–589. doi: 10.1016/j.jacc.2006.05.004

Kemmler, W. K., Lauber, D., Wassermann, A., and Mayhew, J. L. (2006). Predicting maximal strength in trained postmenopausal woman. *J. Strength Cond. Res.* 20, 838–842. doi: 10.1519/R-18905.1

Landmesser, U., Hornig, B., and Drexler, H. (2004). Endothelial function: a critical determinant in atherosclerosis? *Circulation* 109, II27–II33. doi: 10.1161/01.CIR.0000129501.88485.1f

Laskowski, E. R. (2012). The role of exercise in the treatment of obesity. *PM R* 4, 840–844; quiz 844. doi: 10.1016/j.pmrj.2012.09.576

Maas, A. H., van der Schouw, Y. T., Regitz-Zagrosek, V., Swahn, E., Appelman, Y. E., Pasterkamp, G., et al. (2011). Red alert for women's heart: the urgent need for more research and knowledge on cardiovascular disease in women: proceedings of the workshop held in Brussels on gender differences in cardiovascular disease, 29 September 2010. *Eur. Heart J.* 32, 1362–1368. doi: 10.1093/eurheartj/ehr048

Mason, C., Brien, S. E., Craig, C. L., Gauvin, L., and Katzmarzyk, P. T. (2007). Musculoskeletal fitness and weight gain in Canada. *Med. Sci. Sports Exerc.* 39, 38–43. doi: 10.1249/01.mss.0000240325.46523.cf

Mattila, V. M., Tallroth, K., Marttinen, M., and Pihlajamaki, H. (2007). Physical fitness and performance. Body composition by DEXA and its association with physical fitness in 140 conscripts. *Med. Sci. Sports Exerc.* 39, 2242–2247. doi: 10.1249/mss.0b013e318155a813

McGowan, C. L., Levy, A. S., Millar, P. J., Guzman, J. C., Morillo, C. A., McCartney, N., et al. (2006). Acute vascular responses to isometric handgrip exercise and effects of training in persons medicated for hypertension. *Am. J. Physiol. Heart Circ. Physiol.* 291, H1797–H1802. doi: 10.1152/ajpheart.01113.2005

Mittleman, M. A., Maclure, M., Tofler, G. H., Sherwood, J. B., Goldberg, R. J., and Muller, J. E. (1993). Triggering of acute myocardial infarction by heavy physical exertion. Protection against triggering by regular exertion. Determinants of Myocardial Infarction Onset Study Investigators. *N. Engl. J. Med.* 329, 1677–1683. doi: 10.1056/NEJM199312023292301

Mosca, L., Manson, J. E., Sutherland, S. E., Langer, R. D., Manolio, T., and Barrett-Connor, E. (1997). Cardiovascular disease in women: a statement for healthcare professionals from the American Heart Association. Writing Group. *Circulation* 96, 2468–2482. doi: 10.1161/01.CIR.96.7.2468

Ohrvall, M., Berglund, L., and Vessby, B. (2000). Sagittal abdominal diameter compared with other anthropometric measurements in relation to cardiovascular risk. *Int. J. Obes. Relat. Metab. Disord.* 24, 497–501. doi: 10.1038/sj.ijo.0801186

Olson, T. P., Dengel, D. R., Leon, A. S., and Schmitz, K. H. (2006). Moderate resistance training and vascular health in overweight women. *Med. Sci. Sports Exerc.* 38, 1558–1564. doi: 10.1249/01.mss.0000227540.58916.0e

Paine, N. J., Ring, C., Aldred, S., Bosch, J. A., Wadley, A. J., and Veldhuijzen van Zanten, J. J. (2013). Eccentric-exercise induced inflammation attenuates the vascular responses to mental stress. *Brain Behav. Immun.* 30, 133–142. doi: 10.1016/j.bbi.2013.01.082

Phillips, S. A., Das, E., Wang, J., Pritchard, K., and Gutterman, D. D. (2011). Resistance and aerobic exercise protects against acute endothelial impairment induced by a single exposure to hypertension during exertion. *J. Appl. Physiol.* 110, 1013–1020. doi: 10.1152/japplphysiol.00438.2010

Raitakari, M., Ilvonen, T., Ahotupa, M., Lehtimaki, T., Harmoinen, A., Suominen, P., et al. (2004). Weight reduction with very-low-caloric diet and endothelial function in overweight adults: role of plasma glucose. *Arterioscler. Thromb. Vasc. Biol.* 24, 124–128. doi: 10.1161/01.ATV.0000109749.11042.7c

Rooks, C. R., McCully, K. K., and Dishman, R. K. (2011). Acute exercise improves endothelial function despite increasing vascular resistance during stress in smokers and nonsmokers. *Psychophysiology* 48, 1299–1308. doi: 10.1111/j.1469-8986.2011.01194.x

Sale, D. G., Moroz, D. E., McKelvie, R. S., Macdougall, J. D., and McCartney, N. (1994). Effect of training on the blood pressure response to weight lifting. *Can. J. Appl. Physiol.* 19, 60–74. doi: 10.1139/h94-004

Schulz, E., Tsilimingas, N., Rinze, R., Reiter, B., Wendt, M., Oelze, M., et al. (2002). Functional and biochemical analysis of endothelial (dys)function and NO/cGMP signaling in human blood vessels with and without nitroglycerin pretreatment. *Circulation* 105, 1170–1175. doi: 10.1161/hc1002.105186

Sturm, W., Sandhofer, A., Engl, J., Laimer, M., Molnar, C., Kaser, S., et al. (2009). Influence of visceral obesity and liver fat on vascular structure and function in obese subjects. *Obesity (Silver. Spring)* 17, 1783–1788. doi: 10.1038/oby.2009.81

Tilg, H., and Moschen, A. R. (2006). Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat. Rev. Immunol.* 6, 772–783. doi: 10.1038/nri1937

Tinken, T. M., Thijssen, D. H., Hopkins, N., Dawson, E. A., Cable, N. T., and Green, D. J. (2010). Shear stress mediates endothelial adaptations to exercise training in humans. *Hypertension* 55, 312–318. doi: 10.1161/HYPERTENSIONAHA.109.146282

Vanhoutte, P. M., Shimokawa, H., Tang, E. H., and Feletou, M. (2009). Endothelial dysfunction and vascular disease. *Acta Physiol. (Oxf).* 196, 193–222. doi: 10.1111/j.1748-1716.2009.01964.x

Vincent, H. K., Morgan, J. W., and Vincent, K. R. (2004). Obesity exacerbates oxidative stress levels after acute exercise. *Med. Sci. Sports Exerc.* 36, 772–779. doi: 10.1249/01.MSS.0000126576.53038.E9

Walsh, J. H., Bilsborough, W., Maiorana, A., Best, M., O'Driscoll, G. J., Taylor, R. R., et al. (2003). Exercise training improves conduit vessel function in patients with coronary artery disease. *J. Appl. Physiol.* 95, 20–25. doi: 10.1152/japplphysiol.00012.2003

Wolin, M. S. (2000). Interactions of oxidants with vascular signaling systems. *Arterioscler. Thromb. Vasc. Biol.* 20, 1430–1442. doi: 10.1161/01.ATV.20.6.1430

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Central gene expression changes associated with enhanced neuroendocrine and autonomic response habituation to repeated noise stress after voluntary wheel running in rats

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Accumulating evidence indicates that regular physical exercise benefits health in part by counteracting some of the negative physiological impacts of stress. While some studies identified reductions in some measures of acute stress responses with prior exercise, limited data were available concerning effects on cardiovascular function, and reported effects on hypothalamic-pituitary-adrenocortical (HPA) axis responses were largely inconsistent. Given that exposure to repeated or prolonged stress is strongly implicated in the precipitation and exacerbation of illness, we proposed the novel hypothesis that physical exercise might facilitate *adaptation* to repeated stress, and subsequently demonstrated significant enhancement of both HPA axis (glucocorticoid) and cardiovascular (tachycardia) response habituation to repeated noise stress in rats with long-term access to running wheels compared to sedentary controls. Stress habituation has been attributed to modifications of brain circuits, but the specific sites of adaptation and the molecular changes driving its expression remain unclear. Here, *in situ* hybridization histochemistry was used to examine regulation of select stress-associated signaling systems in brain regions representing likely candidates to underlie exercise-enhanced stress habituation. Analyzed brains were collected from active (6 weeks of wheel running) and sedentary rats following control, acute, or repeated noise exposures that induced a significantly faster rate of glucocorticoid response habituation in active animals but preserved acute noise responsiveness. Nearly identical experimental manipulations also induce a faster rate of cardiovascular response habituation in exercised, repeatedly stressed rats. The observed regulation of the corticotropin-releasing factor and brain-derived neurotrophic factor systems across several brain regions suggests widespread effects of voluntary exercise on central functions and related adaptations to stress across multiple response modalities.

Keywords: audiogenic stress, exercise, habituation, glucocorticoid, cardiovascular, *in situ* hybridization

INTRODUCTION

Stress is a significant risk factor for numerous physical (Brindley and Rolland, 1989; Khansari et al., 1990; Forsen, 1991; Pasternac and Talajic, 1991; Vanitallie, 2002; Kalantaridou et al., 2004) and psychological (Dunner et al., 1979; Brown et al., 1987; Hammen et al., 1992; Arborelius et al., 1999; Vanitallie, 2002; Swaab et al., 2005) disorders. Remarkably, many of the same disorders precipitated or exacerbated by stress can be prevented or improved by regular physical exercise (Manson et al., 1992; Paffenbarger et al., 1992; Chodzko-Zajko and Moore, 1994; Bérard et al., 1997; Wannamethee et al., 1998; Dunn et al., 2001; Goodwin, 2003), suggesting that routine physical activity may benefit health in part through a stress-mitigating effect (Roth and Holmes, 1985; Brown and Lawton, 1986; Moraska and Fleshner, 2001; Fleshner, 2005). Indeed, exercise can reduce some of the physiological consequences of acute stress under certain experimental conditions (Brown and Siegel, 1988; Dishman et al., 1995, 1997; Moraska and Fleshner, 2001; Greenwood et al., 2003a,b, 2007a,b; Adlard and Cotman, 2004; Campeau et al., 2010; Masini et al., 2011). However, results are inconsistent across stress stimuli when hypothalamic-pituitary-adrenocortical (HPA) axis-mediated glucocorticoid responses are assessed (Dishman et al., 1995, 1997; Fleshner, 2000; Campisi and Fleshner, 2003; Droste et al., 2003, 2006, 2007; Fediuc et al., 2006; Sasse et al., 2008; Nyhuis et al., 2010), and only limited data are available with regard to cardiovascular reactivity (Morimoto et al., 2000; Masini et al., 2011). Collectively, these findings argue against generalized reductions in acute stress sensitivity by prior exercise, and rather point to complex regulation involving selective modulation of responses to some, but not all, acute stress stimuli. How such a high degree of regulatory specificity is conferred by regular exercise remains to be understood.

While many stress-responsive systems regulate vital physiological functions under both normal and acute stress conditions, it is their sustained, excessive or dysregulated activation by repeated stress that is most strongly associated with pathogenesis (Chrousos and Gold, 1992; Tsigos and Chrousos, 1994; Charmandari et al., 2005). Repeated exposure to the same stressor

is often accompanied by a progressive decrease in response amplitude, or habituation, which is considered an adaptation that lessens the physiological toll of repeatedly activating stress-responsive systems (Armario et al., 1984, 1986; Kant et al., 1985; Melia et al., 1994; Campeau et al., 2002). Dysfunctional response habituation could contribute to increased susceptibility to stress-related pathology. Prior work in our laboratory indicated that habituation of glucocorticoid (Sasse et al., 2008; Nyhuis et al., 2010) and tachycardia (Masini et al., 2011) responses to repeated audiogenic stress is facilitated after chronic voluntary wheel running in rats, lending support to the hypothesis that the health benefits of regular exercise include enhanced adaptive mechanisms that reduce the cumulative impact of repeated or prolonged stress. Habituation of stress responses involves active plastic processes mediated by changes within the central nervous system (Armario et al., 1988; Melia et al., 1994; Akana and Dallman, 1997; Ezzeddine and Glanzman, 2003; Esdin et al., 2010). Enhanced glucocorticoid and cardiovascular response habituation to repeated stress in exercised animals is thus likely to result from regulation at a central level by physical activity. To explore this possibility, *in situ* hybridization histochemistry was performed on brains collected from animals that displayed exercise-induced facilitation of glucocorticoid response habituation to repeated audiogenic stress (Sasse et al., 2008; Experiment 2). Similar experimental manipulations also produce enhanced cardiovascular response habituation in exercised, repeatedly stressed rats (Masini et al., 2011). The primary goal was to define neuroanatomical regions and associated neurochemical substrates potentially underlying facilitation of response habituation to repeated audiogenic stress by prior exercise. Although correlational, any reliable changes in central gene expression could subsequently be tested for their specific relevance to the regulation of stress adaptation by physical activity.

First, messenger RNA (mRNA) expression of the immediate-early gene FBJ osteosarcoma oncogene (*Fos*, also known as c-*fos*) was characterized in select regions based on their prior association with audiogenic stress activation. These included the central control station of HPA axis activation, the paraventricular nucleus of the hypothalamus (PVN), in addition to various cortical (cingulate [CG], infralimbic [IL], orbitofrontal/claustrum [OFC/CL], and prelimbic [PL] regions of the medial prefrontal cortex [mPFC]) and forebrain (septohypothalamic nucleus [SHy] and closely associated ventrolateral septum [LSv], anterior bed nucleus of the stria terminalis [BNST]) areas (Campeau and Watson, 1997; Campeau et al., 2002; Burow et al., 2005). Regions differentially activated by repeated audiogenic stress in exercised vs. sedentary brains could reflect important sites of exercise-induced regulation associated with stress response habituation. Within many of these regions, additional molecules reported to mediate glucocorticoid or cardiovascular responses to stress and/or to be regulated by stress or regular exercise were also examined for putative transcriptional regulation. These included transcripts of corticotropin-releasing hormone (*Crh*) and arginine vasopression (*Avp*) at the level of the PVN (Timofeeva et al., 2003; Kawashima et al., 2004; Park et al., 2005), *Crh* in stress-related extra-hypothalamic regions (e.g., BNST, central [CeA] and basolateral [BLA] amygdaloid nuclei—Antoni, 1993; Gu et al., 2003; Dong and Swanson, 2006; Hauger et al., 2006; Radley et al., 2009; Ulrich-Lai and Herman, 2009), *Crh* receptor subtypes *Crhr1* and *Crhr2* (LSv, PVN, BLA and medial amgydaloid nucleus [MeA], and ventromedial hypothalamic nucleus [VMH]—Luo et al., 1994; Chalmers et al., 1995; Makino et al., 1995; Imaki et al., 1996; Van Pett et al., 2000), brain-derived neurotrophic factor (*Bdnf*; hippocampus, PVN—Smith et al., 1995a,b; Neeper et al., 1996; Oliff et al., 1998; Nibuya et al., 1999; Adlard and Cotman, 2004; Farmer et al., 2004; Gomez-Pinella et al., 2008; Nyhuis et al., 2010), and tyrosine receptor kinase B subtype (*Trkb*), the main receptor for *Bdnf*, in the hippocampus (Nibuya et al., 1999). Our group previously examined neurotrophin regulation by prior exercise and repeated stress (Nyhuis et al., 2010), but this analysis did not include comparisons in non-stressed and acutely stressed animals, which were available in the current study.

MATERIALS AND METHODS

SUBJECTS AND EXPERIMENTAL DESIGN

Readers are referred to Sasse et al. (2008) (Experiment 2) for explicit details regarding manipulation of animal subjects and experimental design. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Colorado and conformed to the National Research Council's *Guide for the Care and Use of Laboratory Animals* (8th Edn., 2011). All efforts were made to minimize animal suffering and the number of animals used. Briefly, following an acclimation period to our colony facility, the 48 young adult (∼2 months of age upon arrival), male Sprague–Dawley rats (Harlan, Indianapolis, IN) were divided into six groups matched for body weight ($n = 8$/group) and individually housed. Three groups were assigned to the Exercise (Ex) condition and given 24 h unlimited access to a stainless steel running wheel (Nalge Nunc International) attached to the wire lid of their home cages for the 6 weeks prior to and the 11 days of the experimental testing phase. During the same time period, the remaining 3 groups were individually housed in similar cages in the same colony room, but under Sedentary (Sed) conditions, without running wheels in their home cages.

Figure 1 provides a graphic summary of the experimental design. On each of the 3 days prior to the onset of testing, all Ex and Sed animals were pre-exposed to the testing conditions, which involved transporting rats to a separate testing room, placing them within their home cages into assigned acoustic chambers, and exposing them only to the background noise (∼60 decibel-A scale [dB]) generated by the ventilation fans for 30 min. Importantly, Ex rats would be denied running wheel access during all 30 min stress (or no stress) exposures, so they were habituated to this additional experimental manipulation during these pre-exposures by placing a long metal rod through both ends of each wheel that completely blocked rotation. Then, on each of 11 consecutive days, rats were transported to the testing room, placed within their home cages into their assigned acoustic chambers, and exposed to one of the following *Stress Treatment* conditions for 30 min: 98 dB noise stress (Ex- or Sed-Repeated Noise, $n = 8$/group), 60 dB background noise (Ex- or Sed-No Noise, $n = 8$/group), or 60 dB background noise exposures on the first 10 days followed by a single 98 dB noise stress presentation on Day

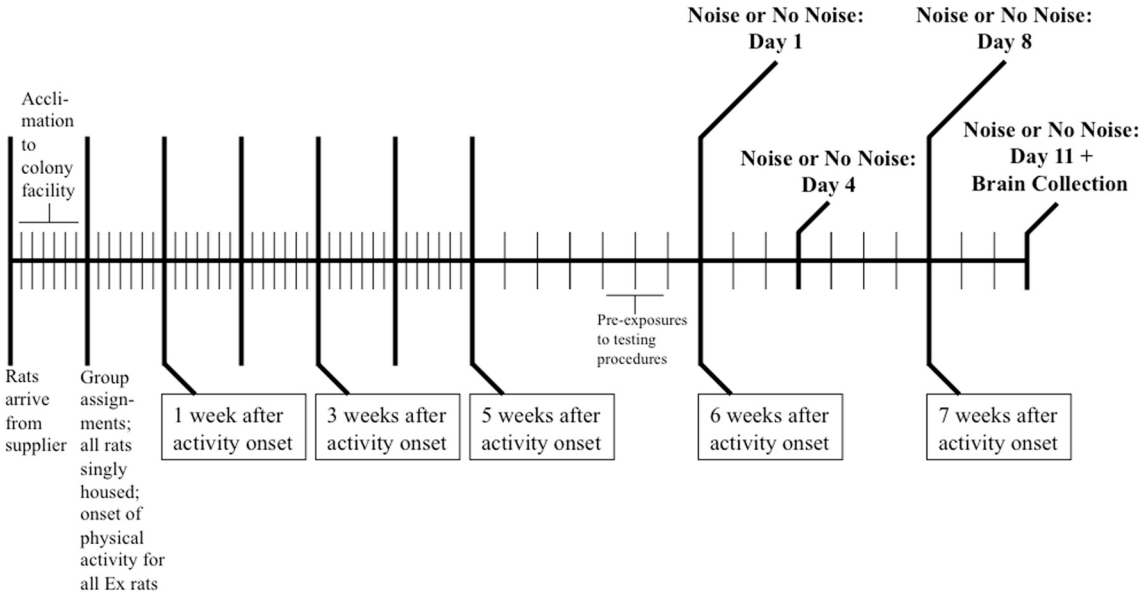

FIGURE 1 | Schematic depicting the experimental design used in the Sasse et al. (2008) **study to generate the brain tissue samples utilized in the present study**. Following a 7-day acclimation period to the colony facility, rats were assigned to one of six groups matched for body weight ($n = 8$/group) and individually housed. Half of the rats were given unrestricted voluntary access to running wheels in their home cages (Ex) while the remaining animals lived in similar cages without running wheels under sedentary conditions (Sed) for 6 weeks. Rats were then exposed to 11 consecutive daily 30 min 98 dB noise stress (Ex- or Sed-Repeated Noise; $n = 8$/group) or 60 dB background noise (Ex- or Sed-No Noise; $n = 8$/group) presentations, or were presented with 10 consecutive daily 30 min exposures to 60 dB background noise followed by a single 30 min 98 dB noise stress exposure on Day 11 (Ex- or Sed-Acute Noise; $n = 8$/group). On each of the 3 days prior to the onset of testing, all rats were pre-exposed to the acoustic chambers in order to familiarize them with the testing conditions. Immediately following the stress (or no stress) exposure on Day 11, animals were decapitated and brains were collected for analysis by *in situ* hybridization histochemistry. Each vertical line in the schematic represents 1 day.

11 (Ex- or Sed-Acute Noise, $n = 8$/group). Running wheels of all Ex rats were locked during each noise (or no noise) exposure, after which wheels were immediately unlocked and all animals were returned to the colony. Immediately following the final 30 min 98 dB or background noise exposure on Day 11, all rats were killed by decapitation (without anesthesia), and brains were removed immediately and frozen in isopentane chilled to −30 to −40°C prior to storage at −80°C until further processing.

In situ HYBRIDIZATION HISTOCHEMISTRY

Ten micron coronal sections of collected brains were cut on a cryostat (Leica model 1850, Wetzlar, Germany), thaw-mounted onto poly-L-lysine-coated slides, and stored at −80°C until processed. Slides were first fixed in a phosphate-buffered 4% paraformaldehyde solution for 1 h, and then rinsed 3 times in 2X sodium saline citrate (SSC), acetylated for 10 min in 0.1 M triethanolamine containing 0.25% acetic anhydride, rinsed in distilled water, and dehydrated in graded ethyl alcohol concentrations. ^{35}S-labeled cRNA riboprobes were generated for *Fos*, *Crh*, *Crhr1*, *Crhr2*, *Avp*, *Bdnf*, and *Trkb* from cDNA subclones in transcription vectors using standard *in vitro* transcription methodology. The rat *Fos* (courtesy of Dr. T. Curran, St. Jude Children's Research Hospital, Memphis, TN) and *Crh* (courtesy of Dr. R. T. Thompson, University of Michigan) cDNA clones were subcloned in pGem 3Z and cut with HindIII to yield 680 nucleotide (nt) and 770 nt cDNA templates, respectively. The rat *Crhr1* cDNA clone (Dr. J. P. Herman, University of Cincinnati) was subcloned in pCR-Blunt II-Topo and cut with BamHI to yield a 345 nt cDNA template. The rat *Crhr2* (courtesy of Dr. R. T. Thompson, University of Michigan) and *Trkb* (courtesy of Dr. D. McKinnon, SUNY, Stony Brook) cDNA clones were subcloned in pBluescript SK and cut with HindIII to yield 899 nt and 306 nt cDNA templates, respectively. The rat *Avp* cDNA clone (courtesy of Dr. T. G. Sherman, Georgetown University Medical Center) was subcloned into pGem 3 and cut with EcoRI to yield a 235 nt cDNA template. The rat *Bdnf* cDNA clone (courtesy of Dr. J. P. Herman, University of Cincinnati) was subcloned in pBluescript and cut with PvuII to yield a 759 nt cDNA template.

Copy riboprobes were labeled in a reaction mixture consisting of 1 μg of the appropriate linearized plasmid, 1X T3, T7, or SP6 transcription buffer (Promega), 125 μCi ^{35}S-UTP, 150 μM NTP's (ATP, CTP, and GTP), 12.5 mM dithiothreitol, 20 U RNase inhibitor, and 6 U of T3 (for *Crhr2*, *Bdnf*, and *Trkb*), T7 (for *Fos*, *Crh*, and *Crhr1*), or SP6 (for *Avp*) RNA polymerase. The reaction was allowed to proceed for 2 h at 37°C, after which probe was separated from free nucleotides over a Sephadex G50–50 column. Riboprobes were diluted in hybridization buffer consisting of 50% formamide, 10% dextran sulfate, 2X SSC, 50 mM sodium phosphate buffer (pH 7.4), 1X Denhardt's solution, and 0.1 mg/ml yeast tRNA, to yield ∼1–2 × 10^6 dpm/70 μl buffer. Diluted probe (70 μl) was applied to tissue sections on each slide, which were then coverslipped, placed in sealed plastic boxes lined with filter paper moistened with 60% formamide in distilled water, and incubated overnight (12–18 h) at 55°C. Coverslips were then

removed, and slides were rinsed 3 times in 2X SSC before being incubated in a solution containing RNase A (2.0 μg/ml) for 1 h at 37°C. Slides were next washed successively in 2X, 1X, 0.5X, and 0.1X SSC for 5 min each, and then incubated in 0.1X SSC for 1 h at 65°C. They were subsequently returned to room temperature in 2 rinses of 0.1X SSC for 5–10 min each prior to being dehydrated in graded ethyl alcohols and air-dried. Slides were then exposed to Kodak MR X-ray film for optimized exposure times.

Control experiments were performed on tissue sections pretreated with RNase A (2.0 μg/ml at 37°C for 1 h) prior to hybridization; this treatment prevented labeling. Additionally, some control sections were hybridized with the sense cRNA strands, which in all cases did not lead to significant hybridization to tissue sections (data not shown). For each riboprobe, two to three slides (4–6 sections/slide) representing a given brain region from each rat included in the study were processed simultaneously to allow for direct comparisons in the same regions. In more cases than not, multiple *in situ* hybridizations were performed for the same target mRNA at different levels of the brain with all animals represented, which reduced the effects of technical variation within regions, but limited the ability to make direct comparisons between different regions.

IMAGE ANALYSIS

Semi-quantitative densitometric analyses were performed on digitized images from X-ray films in the linear range of the gray values obtained with our acquisition system (Northern Light lightbox model B95 [Imaging Res. Inc., St. Catharines, Ontario], a SONY TV camera model XC-ST70 fitted with a Navitar 7000 zoom lens [Rochester, NY], connected to an LG3-01 frame grabber [Scion Corp., Frederick, MD] inside a Dell Dimension 500, captured with Scion Image beta rel. 4.02). Signal pixels in a region of interest were defined as being 3.5 standard deviations above the mean gray value of the representative background, which was set by a cell- and/or signal- poor area close to the region of interest. Mean integrated gray values were computed from the product of the number of pixels comprising the positive signal and their average gray level within the region of interest.

The panels in the left column of **Figures 2A,E,I,M** provide schematic representations of the brain that were adapted from *The Rat Brain in Stereotaxic Coordinates* (CD-ROM; Paxinos and Watson, 2005) atlas, with permission from Elsevier. The right sides of these coronal sections are labeled for the regions of interest in which semi-quantitative analyses were performed, while the left sides of the same sections illustrate the templates that were consistently used to define these regions. For example, panel **A** represents a section at the level of the prefrontal cortex (PFC; 3.24 mm anterior to bregma, as designated in the lower right corner), in which *Fos* mRNA expression was analyzed in: the prelimbic (PL) PFC, designated by a 50 × 60 pixel rectangle template; IL PFC, designated by a 40 × 50 pixel ellipse; the region encompassing the orbitofrontal cortex and claustrum (OFC/CL), designated by a 110 × 60 pixel ellipse; and the cingulate (CG) PFC, for which a trapezoidal template was designed using the Paxinos and Watson atlas for guidance. For each riboprobe, 4–12 bilateral measurements were made within each relevant region of interest (as defined by the template boundaries illustrated in **Figure 2**) on appropriate sections from each animal. These values were averaged to obtain the mean integrated gray value per region for each rat, which provided the relative mRNA expression level. This analytic method gives relative semi-quantitative results that are comparable to performing a quantitative grain analysis on photographic emulsion-dipped sections (Day et al., 2005). Occasionally, sections or slides for a specific animal were missing, damaged, or otherwise inappropriate for analysis following these multiple levels of processing and were therefore excluded. These instances will be explicitly stated in the appropriate Results section.

STATISTICAL ANALYSES

All statistical analyses were performed using the *Statistical Analysis Software (SAS)* program. Two-way (*Activity Status* × *Stress Treatment*) ANOVAs were used to analyze the various target mRNA expression levels in the brain regions of interest. These were followed by Scheffé's *post-hoc* multiple means comparisons to determine the source of reliable effects when present. Statistical significance for all analyses was set at $p \leq 0.05$.

RESULTS

REGIONAL Fos mRNA INDUCTION

Expression levels and basic statistical comparisons of *Fos* mRNA measured in several brain regions of exercised and sedentary rats immediately following the last of 11 consecutive daily noise (Repeated Noise) or background noise (No Noise) exposures, or an acute noise stress presentation on Day 11 (Acute Noise), are presented in **Table 1**. Of the eight regions analyzed, six displayed a similar pattern of *Fos* mRNA induction, in which only rats from the acutely exposed groups demonstrated reliable induction that was greater than that of the repeatedly exposed and no noise control groups, which did not differ from each other (see panels **B–D** of **Figure 2**). These regions included the PVN [main effect of *Stress Treatment*: $F_{(2,\ 38)} = 35.73$, $p < 0.0001$], LSv [*Stress Treatment*: $F_{(2,\ 42)} = 55.77$, $p < 0.0001$], SHy [*Stress Treatment*: $F_{(2,\ 42)} = 82.27$, $p < 0.0001$], ventrolateral aspect of the anterior BNST [vBNST; *Stress Treatment*: $F_{(2,\ 42)} = 23.64$, $p < 0.0001$], and the PL [*Stress Treatment*: $F_{(2,\ 38)} = 6.93$, $p = 0.0027$] and IL [*Stress Treatment*: $F_{(2,\ 38)} = 17.15$, $p < 0.0001$] regions of the mPFC, as evidenced by Two-Way ANOVAs and confirmed and further characterized by Scheffé's *post-hoc* analyses (all p's ≤ 0.05). Additional Two-Way ANOVAs showed that group differences in *Fos* mRNA expression in the OFC/CL region did not reach statistical significance, and while there was a significant *Activity Status* × *Stress Treatment* interaction effect on *Fos* mRNA expression in the CG [$F_{(2,\ 38)} = 3.28$, $p = 0.0487$], Scheffé's *post-hoc* analyses did not reveal any reliable differences among pair-wise comparisons. Of the four mPFC regions examined for *Fos* mRNA induction, the following groups had at least one animal excluded from the analysis: Sed-Repeated Noise ($n = 7$), Ex-No Noise ($n = 7$), and Ex-Repeated Noise ($n = 6$). The following groups had at least one animal excluded from the analysis in the PVN: Sed-Repeated Noise ($n = 7$) and Ex-Repeated Noise ($n = 7$).

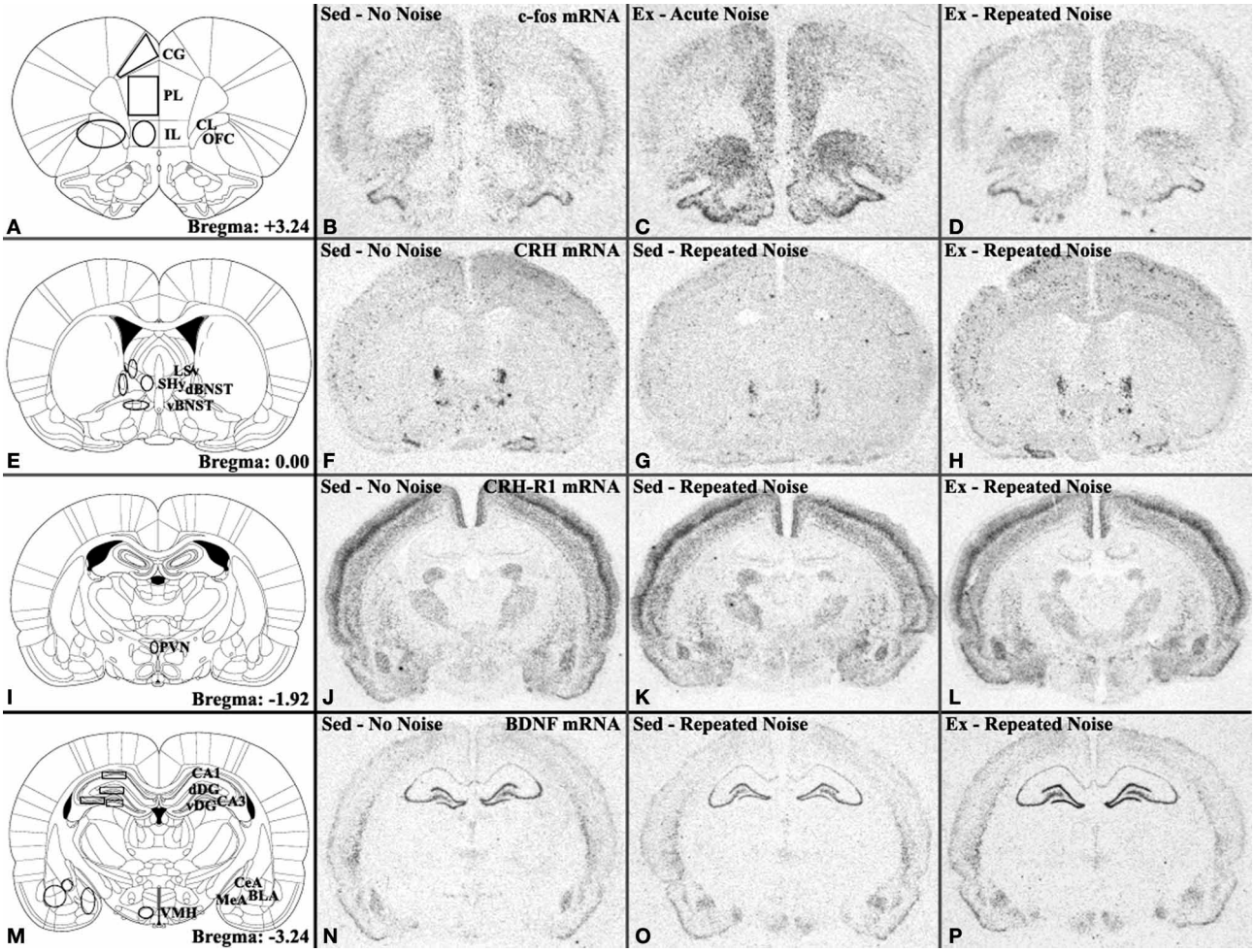

FIGURE 2 | Diagram specifying brain regions analyzed by *in situ* hybridization and corresponding representative autoradiograms depicting important groups differences. Panels in the left column provide images adapted from the Paxinos and Watson (2005) atlas (with permission to reprint from Elsevier) of coronal brain sections at the level of the **(A)** medial prefrontal cortex (mPFC), **(E)** ventrolateral septum (LSv) and anterior bed nucleus of the stria terminalis (BNST), **(I)** paraventricular nucleus of the hypothalamus (PVN), and **(M)** hippocampus and amygdala. All regions of interest analyzed in this study are labeled on the right sides of these sections while the left sides depict the templates used to consistently define their boundaries. Remaining panels in each row show representative autoradiogram images of specified mRNAs in brain sections corresponding to these atlas diagrams. Panels **(B–D)** *Fos* mRNA expression illustrating robust induction of *Fos* mRNA by acute noise stress and nearly complete habituation of *Fos* responses to levels similar to No Noise controls following repeated noise exposures in some (prelimbic [PL] and infralimbic [IL]), but not all (orbitofrontal cortex/claustrum [OFC/CL] and cingulate [CG]) mPFC regions. Panels **(F–H)** *Crh* mRNA in the dorsal (d) and ventral (v) aspects of the BNST demonstrating repeated noise-induced reduction of *Crh* mRNA expression in sedentary but not exercised rats, which exhibited levels similar to No Noise controls. Panels **(J–L)** *Crhr1* mRNA expression in the PVN illustrating the trend for mRNA induction that was specific to exercised, repeatedly stressed rats. Panels **(N–P)** *Bdnf* mRNA expression showing the trend for voluntary exercise to prevent the repeated stress-induced reduction in hippocampal *Bdnf* mRNA expression observed in sedentary rats. Other abbreviations: BLA, basolateral nucleus of amygdala; CeA, central nucleus of amygdala; dDG, dorsal dentate gyrus; MeA, medial nucleus of amygdala; SHy, septohypothalamic nucleus; vDG, ventral dentate gyrus; VMH, ventromedial nucleus of hypothalamus.

HYPOTHALAMIC Crh AND Avp mRNA EXPRESSION

Crh mRNA expression levels in the PVN are presented in **Table 2**. A Two-Way ANOVA revealed significant *Activity Status* [$F_{(1,\ 42)} = 6.07$, $p = 0.0180$], *Stress Treatment* [$F_{(2,\ 42)} = 7.28$, $p = 0.0019$], and *Activity Status* × *Stress Treatment* [$F_{(2,\ 42)} = 5.17$, $p = 0.0098$] effects on *Crh* mRNA expression in this key locus of stress response integration. Specifically, when controlling for *Stress Treatment*, Ex rats exhibited reliably lower PVN-*Crh* mRNA expression than Sed rats, and while holding *Activity Status* constant, animals in the Repeated Noise groups had significantly greater mRNA expression than those exposed to Acute Noise, although neither of these two groups differed reliably from the No Noise controls (Scheffé, p's ≤ 0.05). The significant interaction effect reflects that, while acute and repeated noise stress exposure had little effect on *Crh* mRNA expression in the PVN of Ex rats, the Sed rats acutely exposed to

Table 1 | Stress and voluntary exercise effects on central *Fos* mRNA expression.

	Sed			Ex		
	No	**Acute**	**Repeated**	**No**	**Acute**	**Repeated**
Fos mRNA						
Cortex						
CG	141 (50)	243 (58)	194 (41)	288 (93)	163 (23)	94 (17)
IL*†	19 (5.4)^{a}	101 (18)^{b}	37 (9.5)^{a}	38 (13)^{a}	78 (9.6)^{b}	34 (7.2)^{a}
OFC/CL	369 (86)	568 (105)	577 (159)	554 (146)	412 (145)	339 (51)
PL*†	105 (38)^{a}	333 (67)^{b}	184 (60)^{a}	182 (52)^{a}	281 (36)^{b}	132 (24)^{a}
Forebrain						
vBNST*†	4.3 (1.2)^{a}	51 (11)^{b}	5.6 (1.0)^{a}	6.0 (2.3)^{a}	43 (11)^{b}	12 (5.3)^{a}
SHy*†	2.3 (1.2)^{a}	178 (18)^{b}	16 (5.8)^{a}	3.8 (1.5)^{a}	144 (27)^{b}	18 (6.3)^{a}
LSv*†	6.7 (2.6)^{a}	248 (35)^{b}	40 (22)^{a}	20 (6.4)^{a}	225 (33)^{b}	57 (16)^{a}
Hypothalamus						
PVN*†	6.4 (1.3)^{a}	61 (7.8)^{b}	10 (2.1)^{a}	5.7 (0.9)^{a}	48 (13)^{b}	8.4 (2.3)^{a}

Mean relative Fos mRNA expression in indicated brain regions of exercised (Ex) and sedentary (Sed) rats exposed to no, acute, or repeated noise stress. Group means are mean integrated gray values/100 (±1 s.e.m.), presented as arbitrary units. CG, cingulate cortex; IL, infralimbic cortex; OFC/CL, orbitofrontal cortex/claustrum; PL, prelimbic cortex; vBNST, ventrolateral bed nucleus of stria terminalis; SHy, septohypothalamic nucleus; LSv,ventrolateral septum; PVN, paraventricular nucleus of hypothalamus.

**Omnibus ANOVA, $p \leq 0.05$.*

†Two-Way ANOVA: significant main effect of Stress Treatment ($p \leq 0.05$); ^{a,b}across each row, Stress Treatment group means with same (or no) letter symbol were not statistically different (Scheffé, $p \leq 0.05$).

Table 2 | Effects of stress and wheel running on central CRH signaling.

	Sed			Ex		
	No	**Acute**	**Repeated**	**No**	**Acute**	**Repeated**
Crh mRNA						
Forebrain						
dBNST*¶	77 (9.1)	74 (11)	31 (2.7)	55 (9.7)	47 (6.0)	68 (10)
vBNST*¶	56 (7.5)	43 (5.2)	28 (6.9)	34 (3.8)	32 (3.7)	52 (6.4)
Hypothal.						
PVN*‡†¶	289 (11)^{a,b}	225 (9.1)^{b}	293 (12)^{a}	234 (13)^{a,b}	242 (10)^{b}	261 (13)^{a}
Amygdala						
CeA*¶	136 (16)	137 (24)	73 (10)	93 (16)	67 (15)	119 (18)
Crhr1 mRNA						
Hypothal.						
PVN	31 (4.5)	33 (8.1)	33 (4.5)	28 (3.5)	24 (4.0)	47 (8.3)
Amygdala						
MeA	191 (19)	159 (17)	173 (20)	191 (22)	152 (30)	156 (12)
BLA	148 (19)	99 (8.5)	147 (14)	132 (19)	110 (27)	116 (12)
Crhr2 mRNA						
Forebrain						
LSv	673 (83)	636 (106)	490 (111)	445 (65)	585 (122)	775 (129)
Hypothal.						
VMH*‡	329 (19)	322 (29)	309 (20)	250 (9.9)	284 (16)	258 (14)
Amygdala						
MeA	369 (39)	344 (42)	332 (32)	358 (24)	378 (36)	335 (48)

Mean relative expression of Crh, Crhr1, and Crhr2 mRNAs in indicated brain regions of Ex and Sed rats following no, acute, or repeated audiogenic stress. Group means are mean integrated gray values/100 (±1 s.e.m.), presented as arbitrary units. dBNST, dorsal BNST; Hypothal., hypothalamus; CeA, central nucleus of amygdala; MeA and BLA, medial and basolateral nuclei of amygdala; VMH, ventromedial nucleus of hypothalamus.

**Omnibus ANOVA, $p \leq 0.05$.*

*Two-Way ANOVA: ‡significant main effect of Activity Status, †significant main effect of Stress Treatment ($p \leq 0.05$); ^{a,b}across each row, Stress Treatment group means with same (or no) letter symbol(s) were not statistically different (Scheffé, $p \leq 0.05$). ¶significant Activity Status x Stress Treatment interaction ($p \leq 0.05$); group differences are not indicated by letter symbols here but rather presented in the Results section and illustrated in **Figure 3**.*

noise showed a reliable reduction in mRNA expression as compared to the other two Sed groups, which did not differ (Scheffé, $p \leq 0.05$). A graphic representation of *Crh* mRNA expression in the PVN is depicted in **Figure 3A** to help illustrate this interaction. *Avp* mRNA expression was abundant in both the paraventricular and supraoptic nuclei of the hypothalamus in all groups. However, the extent of this expression was not reliably affected by *Activity Status* or *Stress Treatment* in either structure, as evidenced by non-significant Two-Way ANOVAs (data not shown).

EXTRA-HYPOTHALAMIC Crh mRNA EXPRESSION

Table 2 also depicts relative *Crh* mRNA expression levels in specified extra-hypothalamic regions. In the dorsal aspect of the anterior BNST (dBNST), a significant *Activity Status* × *Stress Treatment* interaction effect was found on *Crh* mRNA expression [$F_{(2,\ 42)} = 8.42$, $p = 0.0008$]. In general, while expression was decreased by the repeated noise stress in Sed rats, it was relatively unaffected, and even somewhat increased, in Ex animals exposed to the same repeated stress paradigm (see panels **F–H** of **Figure 2**). The only statistically significant group difference revealed by Sheffé's *post-hoc* analyses, however, was that *Crh* mRNA expression was reliably lower in the Sed-Repeated Noise group than all other groups ($p \leq 0.05$), which did not differ amongst each other. *Crh* mRNA levels in the dBNST are depicted in **Figure 3B** to aid in the visualization of this interaction. A similar expression pattern was observed in the vBNST and the CeA, and while significant *Activity Status* × *Stress Treatment* interaction effects were uncovered for both regions by Two-Way ANOVAs [vBNST: $F_{(2,\ 42)} = 9.27$, $p = 0.0005$; CeA: $F_{(2,\ 41)} = 6.62$, $p = 0.0032$], none of the *post-hoc* pair-wise comparisons revealed reliable group differences (Scheffé, all p's > 0.05). One animal in the Ex-Acute Noise group was excluded from the analysis of *Crh* mRNA expression in the CeA ($n = 7$).

Crh RECEPTOR mRNAs

Two-Way ANOVAs revealed no significant differences in *Crhr1* mRNA levels in the MeA or BLA. Similarly, no reliable effects of *Activity Status* or *Stress Treatment* were found with respect to *Crhr1* mRNA expression in the PVN when analyzing all six experimental groups (see **Table 2**). Interestingly, when comparing only the Ex groups using a One-Way ANOVA for *Stress Treatment*,

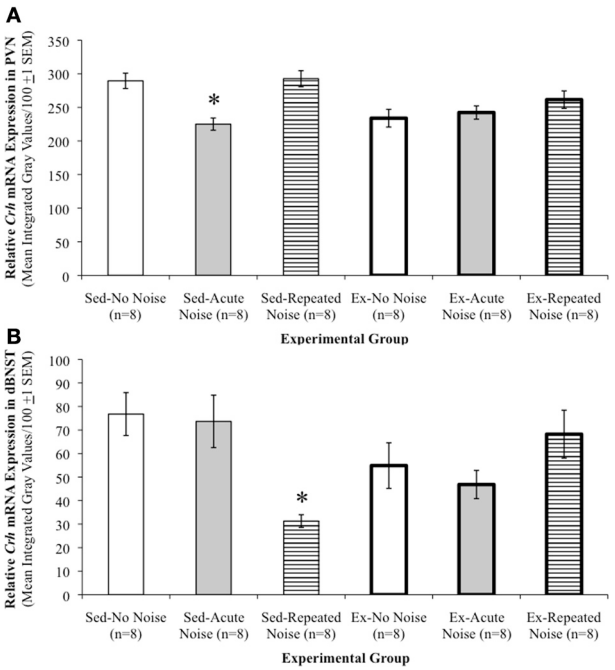

FIGURE 3 | Stress-induced *Crh* regulation in the PVN and dBNST is different in exercised compared to sedentary rats. (A) Relative *Crh* mRNA expression levels in the paraventricular nucleus of the hypothalamus (PVN). Significant main effects of *Activity Status* and *Stress Treatment* were observed, with reliably lower PVN-*Crh* mRNA expression levels in Ex as compared to Sed rats, and significantly greater expression levels in Repeated as compared to Acute Noise groups, although neither of these two groups differed reliably from the No Noise controls. The interaction effect was also significant, such that, while acute and repeated noise stress exposure had little effect on *Crh* mRNA expression in the PVN of Ex rats, the Sed rats acutely exposed to noise showed a reliable reduction in mRNA expression as compared to the other two Sed groups, which did not differ. Group means are mean integrated gray values/100 (±1 s.e.m.), presented as arbitrary units. $^{*}p \leq 0.05$ vs. Sed-No Noise and Sed-Repeated Noise. **(B)** Relative *Crh* mRNA expression levels in the dorsal aspect of the anterior bed nucleus of the stria terminalis (dBNST). A significant *Activity Status* × *Stress Treatment* interaction effect was found such that, while expression levels were decreased by the repeated noise stress in Sed rats, they were relatively unaffected, and even slightly increased, in Ex animals exposed to the same repeated stress paradigm. A similar pattern in CRH mRNA expression was also observed in the anteroventral portion of the BNST (vBNST), as well as the central nucleus of the amygdala (CeA; see **Table 2**). Group means are mean integrated gray values/100 (±1 S.E.M.), presented as arbitrary units. $^{*}p \leq 0.05$ vs. all other experimental groups, which were not reliably different amongst each other.

Table 3 | Stress and wheel running regulation of central *Bdnf* and *Trkb* mRNA expression.

	Sed			Ex		
	No	**Acute**	**Repeated**	**No**	**Acute**	**Repeated**
***Bdnf* mRNA**						
Hippocampus						
CA1	8.8 (1.7)	6.9 (1.1)	4.9 (0.9)	8.1 (1.5)	9.5 (2.3)	9.7 (2.1)
CA3*‡	100 (8.0)	91 (4.9)	82 (8.4)	114 (8.4)	124 (12)	124 (9.2)
dDG*‡	138 (7.4)	125 (7.4)	115 (16)	169 (14)	187 (14)	189 (12)
vDG*‡	113 (10)	95 (5.9)	93 (14)	148 (17)	170 (19)	163 (15)
Hypothalamus						
PVN	111 (15)	113 (13)	117 (11)	100 (16)	118 (16)	126 (16)
***Trkb* mRNA**						
Hippocampus						
CA1*‡	106 (7.2)	96 (4.7)	100 (7.3)	121 (8.2)	118 (4.4)	107 (3.0)
CA3‡	82 (7.9)	72 (7.5)	80 (6.6)	97 (10)	95 (9.0)	87 (5.8)
dDG	133 (9.2)	121 (8.0)	129 (6.7)	139 (9.5)	144 (11)	136 (3.7)
vDG	145 (11)	132 (9.9)	143 (8.2)	148 (8.6)	155 (9.5)	136 (4.6)

Mean relative Bdnf and Trkb mRNA expression levels in indicated brain regions of Ex and Sed rats following no, acute, or repeated stress. Group means are mean integrated gray values/100 (±1 s.e.m.), presented as arbitrary units. dDG and vDG, dorsal and ventral blades of dentate gyrus, respectively.

*Omnibus ANOVA, $p \leq 0.05$.

Two-Way ANOVA: ‡significant main effect of Activity Status ($p \leq 0.05$).

a significant main effect was uncovered [$F_{(2,\ 21)} = 4.67$, $p = 0.0210$], such that *Crhr1* mRNA expression was significantly greater in the PVN of Ex-Repeated Noise as compared to Ex-Acute Noise animals (Scheffé, $p \leq 0.05$), although neither of these groups differed reliably from the Ex-No Noise group (see panels **J–L** of **Figure 2**). A Two-Way ANOVA revealed a significant main effect of *Activity Status* [$F_{(1,\ 40)} = 13.88$, $p = 0.0006$] on *Crhr2* mRNA expression in the VMH, with reliably lower expression in brains of Ex compared to Sed animals. The following groups had at least one animal excluded from the analysis in the VMH: Sed-Acute Noise ($n = 7$) and Sed-Repeated Noise ($n = 7$). *Crhr2* mRNA levels were not reliably different between treatment groups in the LSv or MeA (**Table 2**).

Bdnf AND Trkb mRNA EXPRESSION

Expression levels and basic statistical comparisons of *Bdnf* and *Trkb* transcripts are presented in **Table 3**. In three of the four hippocampal sub-regions examined for *Bdnf* mRNA expression, Ex rats displayed significantly greater expression than Sed animals when controlling for *Stress Treatment*, as shown by Two-Way (*Activity Status* × *Stress Treatment)* ANOVAs. These included the CA3 region [main effect of *Activity Status*: $F_{(1,\ 42)} = 17.22$, $p = 0.0002$] and the dorsal [*Activity Status*: $F_{(1,\ 40)} = 33.04$, $p < 0.0001$] and ventral [*Activity Status*: $F_{(1,\ 42)} = 27.70$, $p < 0.0001$] blades of the dentate gyrus. Relatively low levels of *Bdnf* mRNA were expressed in the CA1 region that did not differ reliably between the experimental groups. Although not statistically significant, a general pattern in hippocampal *Bdnf* mRNA expression emerged such that, in Sed rats, acute and repeated noise had a tendency to decrease expression relative to non-stressed controls, while Ex animals appeared to be buffered from this stress-induced reduction in hippocampal *Bdnf* transcription (see panels **N–P** of **Figure 2**). **Figure 4** depicts *Bdnf* mRNA expression levels in the CA1 region, which serves to clearly illustrate this pattern of results. Expression of *Bdnf* mRNA was also examined in the PVN, in which a Two-Way ANOVA uncovered no reliable group differences. A significant main effect of *Activity Status* was revealed by Two-Way ANOVAs on *Trkb* mRNA expression in the CA1 [$F_{(1,\ 42)} = 8.63$, $p = 0.0053$] and CA3 [$F_{(1,\ 42)} = 5.37$, $p = 0.0254$] hippocampal sub-regions, such that mRNA levels

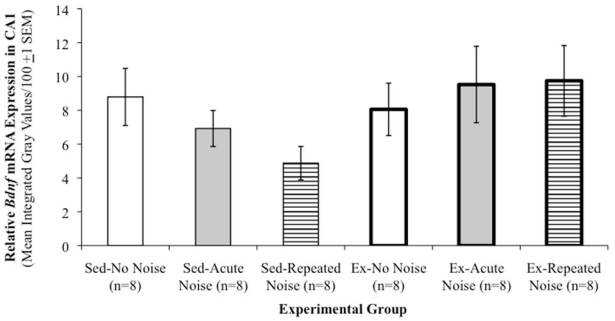

FIGURE 4 | Exercised rats appear less susceptible to stress-induced decreases in hippocampal *Bdnf*. Relative *Bdnf* mRNA expression levels in the CA1 sub-region of the hippocampus. Although group differences did not achieve statistical significance, a general pattern in *Bdnf* mRNA expression clearly emerged in this and the other three hippocampal sub-regions examined that is well-demonstrated by this particular figure, in that Ex rats did not appear to be susceptible to the same acute or repeated stress-induced decreases in hippocampal *Bdnf* mRNA expression that were exhibited in the Sed animals. Group means are mean integrated gray values/100 (±1 s.e.m.), presented as arbitrary units.

were significantly greater in Ex compared to Sed rats (Scheffé, p's ≤ 0.05). No reliable group differences in *Trkb* mRNA expression were observed in either the dorsal or ventral blades of the dentate gyrus.

DISCUSSION

The results of this study indicate that physical activity can significantly alter central nervous system expression patterns of several genes strongly associated with stress responsiveness. As summarized below, a number of studies have already identified various genes that are differentially regulated in the brains of physically active compared to sedentary rats under control (no stress) conditions. Importantly, some of this regulation can be further differentiated in exercised and sedentary brains in response to acute or repeated stress exposures. In view of our recent findings that prior physical activity enhances the rate of neuroendocrine and autonomic response habituation to repeated stress (Sasse et al., 2008; Nyhuis et al., 2010; Masini et al., 2011), we reasoned that these previously reported sites and molecular targets of regulation could also underlie this particular mode of stress adaptation. While the brains analyzed in the current study were obtained following measurements of HPA axis activation (Sasse et al., 2008), a nearly identical experimental protocol led to significantly facilitated habituation of heart rate and core body temperature responses in exercised rats (Masini et al., 2011). It is therefore highly likely that the central gene regulation observed in the current study could be associated with modulation of either or both the neuroendocrine and autonomic response adaptations observed in our prior studies.

REGIONAL *Fos* mRNA INDUCTION AND HABITUATION

As reported previously (Campeau et al., 1997, 2002; Campeau and Watson, 1997; Burow et al., 2005), acute loud noise exposure induced widespread *Fos* mRNA induction, but importantly, the amplitude of this expression did not differ between exercised and sedentary rats. This finding supports a number of prior studies reporting that acute HPA axis (Dishman et al., 1995, 1997, 1998; Fleshner, 2000; Fediuc et al., 2006; Sasse et al., 2008; Campeau et al., 2010; Nyhuis et al., 2010) and autonomic (Morimoto et al., 2000; Salam et al., 2009; Masini et al., 2011) responses to stress do not always differ between sedentary and more active animals. These indices of neuronal activation provide further evidence that general central responsiveness to acute stress can be preserved in exercising animals, which is important given the critical role of stress responses in adaptation and survival (Munck et al., 1984; Levine and Ursin, 1991; Chrousos and Gold, 1992; Akil and Morano, 1995). Notably, whereas our previous work demonstrated enhanced HPA axis (Sasse et al., 2008; Nyhuis et al., 2010) and autonomic (Masini et al., 2011) response habituation to repeated stress exposures in exercised animals, the quantified brain regions of Ex and Sed rats did not exhibit significant differences in *Fos* mRNA induction immediately following the final (11th) repeated noise exposure. This general result may reflect the fact that brains were collected at a point during repeated stress exposures at which the neuroendocrine and autonomic response differences between exercised and sedentary rats have mostly dissipated (Sasse et al., 2008; Nyhuis et al., 2010; Masini et al., 2011). Future studies should include earlier time-points during repeated stress exposures (3rd–8th exposures) when HPA axis and autonomic response differences are observed. In addition, although non-significant, an interesting pattern emerged in the brains of exercised animals, such that acute and especially repeated noise stress appeared to *decrease Fos* mRNA induction in prefrontal cortical regions relative to that of No Noise controls (see **Table 1**). Prior associations with motivated behaviors (Cardinal et al., 2002) and stress adaptation (Campeau et al., 2002; Weinberg et al., 2009) make these regions important targets for future studies of the impact of physical activity on stress adaptation.

Crh AND Avp EXPRESSION IN THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS

In the PVN, stress-induced release of CRH and AVP peptides are typically accompanied by increases in their gene transcription (Herman et al., 1992; Kovács and Sawchenko, 1996; Ma et al., 1997; Girotti et al., 2006) and subsequent elevations of steady state mRNA levels (Herman et al., 1992; Luo et al., 1994; Makino et al., 1995). It was therefore surprising to observe the lowest PVN-*Crh* mRNA levels in the Sed-Acute Noise group relative to that of the other Sed groups. However, PVN-*Crh* mRNA increases are typically observed 1–4 h after stress onset (Herman et al., 1992; Luo et al., 1994; Ma et al., 1997) and thus may have been missed given the shorter 30 min interval employed in the current study. This may also explain the lack of a *Stress Treatment* effect on the expression of *Avp* mRNA in the PVN, as elevation in *Avp* transcription is further delayed compared to that of *Crh* (Herman et al., 1992; Kovács and Sawchenko, 1996; Ma et al., 1997). An alternative explanation might be provided by unusually elevated *Crh* mRNA expression levels in the Sed-No Noise rats. Importantly, the lack of differences in plasma corticosterone concentrations between exercised and sedentary rats under basal conditions, after acute stress exposure, and following 11 days of

repeated stress exposures (Sasse et al., 2008; Nyhuis et al., 2010) suggests that observed differences in PVN-*Crh* mRNA exerted little influence on HPA axis tone or sensitivity. Repeated stress is also reported to elevate *Crh* mRNA expression in the PVN (Aguilera, 1994; Herman et al., 1995; Gómez et al., 1996), yet the comparatively milder repeated noise stress paradigm used here did not produce significant increases in PVN-*Crh* or *Avp* mRNA expression in sedentary or exercised rats. The similar expression levels of these principal hypothalamic regulators of stress responses between exercised and sedentary rats therefore argues against an important role for these hypophysiotropic signals in the differential rates of stress adaptation we have observed (Sasse et al., 2008; Nyhuis et al., 2010; Masini et al., 2011).

EXTRA-HYPOTHALAMIC *Crh* REGULATION

Crh mRNA expression was examined in three additional, extra-hypothalamic regions, including the central amygdala and the dorsal and ventral BNST. Previous studies have demonstrated that both acute and repeated stress activate the CRH systems in these regions (Chappell et al., 1986; Merali et al., 1998; Makino et al., 1999; Figueiredo et al., 2003) and have established important roles for their recruitment in mediating the expression and integration of behavioral and autonomic components of the stress response (Fisher et al., 1982; Koob et al., 1993). Each of these three regions exhibited a similar and interesting pattern of *Crh* regulation, in which expression was generally decreased by repeated noise stress in sedentary rats but was relatively unaffected, if not increased (although non-significantly), in voluntarily exercising animals exposed to the same repeated stress paradigm. The relatively short, 30 min interval between stress onset and euthanasia may again account for the lack of clear acute stress effects in these regions when compared to prior studies (Chappell et al., 1986; Merali et al., 1998; Makino et al., 1999; Figueiredo et al., 2003). Based on the results obtained in the Sed groups, one might hypothesize that a decrease in extra-hypothalamic CRH contributes to response habituation to repeated stress exposures. If this were the case, an even greater reduction in extra-hypothalamic *Crh* transcript would be expected in the Ex-Repeated Noise group, since response habituation to repeated noise was enhanced in these animals relative to their sedentary counterparts (Sasse et al., 2008; Nyhuis et al., 2010; Masini et al., 2011), but this was not observed. Whether and how differential regulation of these extra-hypothalamic influences contributes to the different rates of response habituation exhibited by exercised and sedentary rats remains an open question.

REGULATION OF *Crh* RECEPTORS

Expression levels of *Crhr1* mRNA in the medial and basolateral amygdala were similar between Ex and Sed groups, and were relatively insensitive to the different *Stress Treatment* conditions of this experiment, which is consistent with previous observations (Van Pett et al., 2000). In the PVN, *Crhr1* mRNA is typically expressed at very low levels basally, although several groups have reported inducible expression of PVN-*Crhr1* following exposure to a variety of different stressors (Luo et al., 1994; Makino et al., 1995; Rivest et al., 1995; Imaki et al., 1996; Van Pett et al., 2000). The only reliable difference in *Crhr1* expression observed in this study was exhibited in the PVN specifically of Ex rats, with significantly greater expression in the Repeated Noise compared to the Acute Noise group (although neither group differed significantly from No Noise controls). While others have demonstrated *Crhr1* mRNA induction in the PVN several hours following stressor onset (e.g., Van Pett et al., 2000), its expression was below the threshold of detection in the PVN of both Ex and Sed rats 30 min after acute noise onset. It thus remains possible that differential regulation of PVN-*Crhr1* might be revealed at later time-points following acute stress exposure.

Exploration of *Crhr2* mRNA expression, which is regionally more limited than that of *Crhr1* (Chalmers et al., 1995; Van Pett et al., 2000), did not reveal any differences in the lateral septum or medial amygdala. However, *Crhr2* mRNA expression was significantly reduced in the ventromedial hypothalamic nucleus of exercised compared to sedentary groups. Since *Crhr2* signaling in this region has previously been implicated in the regulation of food intake and energy balance (Steller, 1954; Spina et al., 1996), and significant alterations in these same measures are consistently reported in voluntarily exercising rats (Tokuyama et al., 1982; Rodnick et al., 1989; Afonso and Eikelboom, 2003), this finding may be important for future studies aimed at unraveling the complex metabolic adaptations associated with regular exercise.

EFFECTS WITHIN THE BDNF-TRKB SIGNALING SYSTEM

In three of the four hippocampal sub-regions in which *Bdnf* mRNA levels were assessed, expression was significantly greater in Ex compared to Sed rats. This finding is consistent with several other reports of increased hippocampal *Bdnf* expression in voluntarily exercising animals, both at the mRNA (Neeper et al., 1996; Oliff et al., 1998; Farmer et al., 2004; Gomez-Pinella et al., 2008; Nyhuis et al., 2010) and protein levels (Adlard and Cotman, 2004). Further, although stress has been demonstrated to reduce *Bdnf* expression in the hippocampus and PVN (Smith et al., 1995a,b; Nibuya et al., 1999; Adlard and Cotman, 2004), only non-significant reductions were observed here, particularly in the Sed-Repeated Noise rats. Importantly, the general trends we observed in the hippocampus were consistent with previously published results, in that voluntary exercise appeared to prevent the non-significant stress-induced decreases in hippocampal *Bdnf* mRNA expression observed in sedentary animals. Similarly, greater *Trkb* mRNA levels were expressed in the CA1 and CA3 pyramidal cell layers of Ex compared to Sed rats. Taken together, the exercise-induced increases in both ligand (*Bdnf*) and receptor (*Trkb*) transcripts in the present study are suggestive of an enhanced capacity for expression of the neuroprotective and synapse-strengthening functions of this signaling system. However, the overall lack of significant stress or interaction effects implies that the observed differences in hippocampal *Bdnf* and *Trkb* mRNA expression are not likely to provide the key neural substrate(s) directly mediating differential stress response habituation in exercised and sedentary rats.

CONCLUSIONS

This study provided a starting point for the elucidation of neurochemical mechanisms potentially underlying exercise-induced

facilitation of glucocorticoid and cardiovascular response habituation to repeated audiogenic stress exposures that were recently reported (Sasse et al., 2008; Nyhuis et al., 2010; Masini et al., 2011). The primary conclusion that can be drawn from these results is that the brains of sedentary and physically active animals respond in multiple different ways to acute and repeated stress exposures. It is likely that some of the central changes reported here mediate various exercise-induced physiological adaptations (i.e., metabolic regulation). Additional direct intervention studies will be required to determine whether or how these observed brain modifications contribute to the enhanced rate of stress response habituation observed in exercised animals. Because it is the situations involving prolonged or repeated stress that are most strongly associated with illness, the possibility that interventions as cost-effective as regular exercise could enhance the rate of adaptation of potentially deleterious physiological responses to such situations strongly indicates the need for further investigation.

AUTHOR CONTRIBUTIONS

Sarah K. Sasse performed the animal experiment with significant technical contributions from Tara J. Nyhuis, Cher V. Masini, Heidi E. W. Day and Serge Campeau. All *in situ* hybridization histochemistry was conducted by Sarah K. Sasse and Tara J. Nyhuis. Data analysis was performed by Sarah K. Sasse and interpreted by Sarah K. Sasse and Serge Campeau. Sarah K. Sasse and Serge Campeau wrote the manuscript.

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REFERENCES

Adlard, P. A., and Cotman, C. W. (2004). Voluntary exercise protects against stress-induced decreases in brain derived neurotrophic factor protein expression. *Neuroscience* 124, 985–992. doi: 10.1016/j.neuroscience.2003.12.039

Afonso, V. M., and Eikelboom, R. (2003). Relationship between wheel running, feeding, drinking, and body weight in male rats. *Physiol. Behav.* 80, 19–26. doi: 10.1016/S0031-9384(03)00216-6

Aguilera, G. (1994). Regulation of pituitary ACTH secretion during chronic stress. *Front. Neuroendocrinol.* 15, 321–350. doi: 10.1006/frne.1994.1013

Akana, S. F., and Dallman, M. F. (1997). Chronic cold in adrenalectomized, corticosterone (B)-treated rats: facilitated corticotropin responses to acute restraint emerge as B increases. *Endocrinology* 138, 3249–3258. doi: 10.1210/en.138.8.3249

Akil, H., and Morano, M. I. (1995). "Stress," in *Psychopharmacology: The Fourth Generation of Progress,* eds F. Bloom and D. Kupfer (New York, NY: Raven Press, Ltd.), 773–785.

Antoni, F. A. (1993). Vasopressinergic control of pituitary adrenocorticotropin secretion comes of age. *Front. Neuroendocrinol.* 14, 76–122. doi: 10.1006/frne.1993.1004

Arborelius, L., Owens, M. J., Plotsky, P. M., and Nemeroff, C. B. (1999). The role of corticotropin-releasing factor in depression and anxiety disorders. *J. Endocrinol.* 160, 1–12. doi: 10.1677/joe.0.1600001

Armario, A., Castellanos, J. M., and Balasch, J. (1984). Adaptation of anterior pituitary hormones to chronic noise stress in male rats. *Behav. Neural Biol.* 41, 71–76. doi: 10.1016/S0163-1047(84)90745-3

Armario, A., Hidalgo, J., and Giralt, M. (1988). Evidence that the pituitary-adrenal axis does not cross-adapt to stressors: comparison to other physiological variables. *Neuroendocrinology* 47, 263–267. doi: 10.1159/000124921

Armario, A., Lopez-Calderón, A., Jolin, T., and Balasch, J. (1986). Response of anterior pituitary hormones to chronic stress. The specificity of adaptation. *Neurosci. Biobehav. Rev.* 10, 245–250. doi: 10.1016/0149-7634(86)90011-4

Bérard, A., Bravo, G., and Gauthier, P. (1997). Meta-analysis of the effectiveness of physical activity for the prevention of bone loss in postmenopausal women. *Osteoporos. Int.* 7, 331–337. doi: 10.1007/BF01623773

Brindley, D., and Rolland, Y. (1989). Possible connections between stress, diabetes, obesity, hypertension and altered lipoprotein metabolism that may result in atherosclerosis. *Clin. Sci. (Lond.)* 77, 453–461.

Brown, G. W., Bifulco, A., and Harris, T. O. (1987). Life events, vulnerability and onset of depression. *Brit. J. Psychiat.* 150, 30–42. doi: 10.1192/bjp.150.1.30

Brown, J. D., and Lawton, M. (1986). Stress and well-being in adolescence: the moderating role of physical role of physical exercise. *J. Hum. Stress* 12, 125–131.

Brown, J. D., and Siegel, J. M. (1988). Exercise as a buffer of life stress: a prospective study of adolescent health. *Health Psychol.* 7, 341–353. doi: 10.1037/0278-6133.7.4.341

Burow, A., Day, H. E., and Campeau, S. (2005). A detailed characterization of loud noise stress: Intensity analysis of hypothalamo-pituitary-adrenocortical axis and brain activation. *Brain Res.* 1062, 63–73. doi: 10.1016/j.brainres.2005.09.031

Campeau, S., Akil, H., and Watson, S. J. (1997). Lesions of the medial geniculate nuclei specifically block corticosterone release and induction of c-fos mRNA in the forebrain associated with audiogenic stress in rats. *J. Neurosci.* 17, 5979–5992.

Campeau, S., Dolan, D., Akil, H., and Watson, S. J. (2002). c-fos mRNA induction in acute and chronic audiogenic stress: possible role of the orbitofrontal cortex in habituation. *Stress* 5, 121–130. doi: 10.1080/10253890290027895

Campeau, S., Nyhuis, T. J., Sasse, S. K., Kryskow, E. M., Herlihy, L., Babb, J. A., et al. (2010). Hypothalamic pituitary adrenal axis responses to low-intensity stressors are reduced after voluntary wheel running in rats. *J. Neuroendocrinol.* 22, 872–888. doi: 10.1111/j.1365-2826.2010.02007.x

Campeau, S., and Watson, S. J. (1997). Neuroendocrine and behavioral responses and brain pattern of c-fos induction associated with audiogenic stress. *J. Neuroendocrinol.* 9, 577–588.

Campisi, J., and Fleshner, M. (2003). Role of extracellular HSP72 in acute stress-induced potentiation of innate immunity in active rats. *J. Appl. Physiol.* 94, 43–52. doi: 10.1152/japplphysiol.00681.2002

Cardinal, R. N., Parkinson, J. A., Hall, J., and Everitt, B. J. (2002). Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci. Biobehav. Rev.* 26, 321–352. doi: 10.1016/S0149-7634(02)00007-6

Chalmers, D. T., Lovenberg, T. W., and De Souza, E. B. (1995). Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. *J. Neurosci.* 15, 6340–6350.

Chappell, P. B., Smith, M. A., Kilts, C. D., Bissette, G., Ritchie, J., Anderson, C., et al. (1986). Alterations in corticotropin-releasing factor-like immunoreactivity in discrete brain regions after acute and chronic stress. *J. Neurosci.* 6, 2908–2914.

Charmandari, E., Tsigos, C., and Chrousos, G. P. (2005). Endocrinology of the stress response. *Annu. Rev. Physiol.* 67, 259–284. doi: 10.1146/annurev.physiol.67.040403.120816

Chodzko-Zajko, W. J., and Moore, K. A. (1994). Physical fitness and cognitive functioning in aging. *Exerc. Sport Sci. Rev.* 22, 195–220. doi: 10.1249/00003677-199401000-00009

Chrousos, G. P., and Gold, P. W. (1992). The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *J. Am. Med. Assoc.* 267, 1244–1252. doi: 10.1001/jama.1992.03480090092034

Day, H. E., Nebel, S., Sasse, S., and Campeau, S. (2005). Inhibition of the central extended amygdala by loud noise and restraint stress. *Eur. J. Neurosci.* 21, 441–454. doi: 10.1111/j.1460-9568.2005.03865.x

Dishman, R. K., Bunnell, B. N., Youngstedt, S. D., Yoo, H. S., Mougey, E. H., and Meyerhoff, J. L. (1998). Activity wheel running blunts increased plasma adrenocorticotrophin (ACTH) after footshock and cage-switch stress. *Physiol. Behav.* 63, 911–917. doi: 10.1016/S0031-9384(98)00017-1

Dishman, R. K., Renner, K. J., Youngstedt, S. D., Reigle, T. G., Bunnell, B. N., Burke, K. A., et al. (1997). Activity wheel running reduces escape latency and alters brain monoamine levels after footshock. *Brain Res. Bull.* 42, 399–406. doi: 10.1016/S0361-9230(96)00329-2

Dishman, R. K., Warren, J. M., Youngstedt, S. D., Yoo, H., Bunnell, B. N., Mougey, E. H., et al. (1995). Activity-wheel running attenuates suppression of natural killer cell activity after footshock. *J. Appl. Physiol.* 78, 1547–1554.

Dong, H. W., and Swanson, L. W. (2006). Projections from bed nuclei of the stria terminalis, dorsomedial nucleus: implications for cerebral hemisphere

integration of neuroendocrine, autonomic, and drinking responses. *J. Comp. Neurol.* 494, 75–107. doi: 10.1002/cne.20790

Droste, S. K., Chandramohan, Y., Hill, L. E., Linthorst, A. C., and Reul, J. M. (2007). Voluntary exercise impacts on the rat hypothalamic-pituitary-adrenocortical axis mainly at the adrenal level. *Neuroendocrinology* 86, 26–37. doi: 10.1159/000104770

Droste, S. K., Gesing, A., Ulbricht, S., Müller, M. B., Linthorst, A. C., and Reul, J. M. (2003). Effects of long-term voluntary exercise on the mouse hypothalamic-pituitary-adrenocortical axis. *Endocrinology* 144, 3012–3023. doi: 10.1210/en.2003-0097

Droste, S. K., Schweizer, M. C., Ulbricht, S., and Reul, J. M. (2006). Long-term voluntary exercise and the mouse hypothalamic-pituitary-adrenocortical axis: impact of concurrent treatment with the antidepressant drug tianeptine. *J. Neuroendocrinol.* 18, 915–925. doi: 10.1111/j.1365-2826.2006.01489.x

Dunn, A. L., Trivedi, M. H., and O'Neal, H. A. (2001). Physical activity dose–response effects on outcomes of depression and anxiety. *Med. Sci. Sports Exerc.* 33(6 Suppl.), S587–S597. doi: 10.1097/00005768-200106001-00027

Dunner, D., Patrick, V., and Fieve, R. R. (1979). Life events at the onset of bipolar affective illness. *Am. J. Psychiat.* 136, 508–511.

Esdin, J., Pearce, K., and Glanzman, D. L. (2010). Long-term habituation of the gill-withdrawal reflex in aplysia requires gene transcription, calcineurin and L-type voltage-gated calcium channels. *Front. Behav. Neurosci.* 4:181. doi: 10.3389/fnbeh.2010.00181

Ezzeddine, Y., and Glanzman, D. L. (2003). Prolonged habituation of the gill-withdrawal reflex in Aplysia depends on protein synthesis, protein phosphatase activity, and postsynaptic glutamate receptors. *J Neurosci.* 23, 9585–9594.

Farmer, J., Zhao, X., van Praag, H., Wodtke, K., Gage, F. H., and Christie, B. R. (2004). Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male sprague-dawley rats *in vivo*. *Neuroscience* 124, 71–79. doi: 10.1016/j.neuroscience.2003.09.029

Fediuc, S., Campbell, J. E., and Riddell, M. C. (2006). Effect of voluntary wheel running on circadian corticosterone release and on HPA axis responsiveness to restraint stress in Sprague-Dawley rats. *J. Appl. Physiol.* 100, 1867–1875. doi: 10.1152/japplphysiol.01416.2005

Figueiredo, H. F., Bodie, B. L., Tauchi, M., Dolgas, C. M., and Herman, J. P. (2003). Stress integration after acute and chronic predator stress: differential activation of central stress circuitry and sensitization of the hypothalamo-pituitary-adrenocortical axis. *Endocrinology* 144, 5249–5258. doi: 10.1210/en.2003-0713

Fisher, L. A., Rivier, J., Rivier, C., Spiess, J., Vale, W., and Brown, M. R. (1982). Corticotropin-releasing factor (CRF): central effects on mean arterial pressure and heart rate in rats. *Encocrinology* 110, 2222–2224. doi: 10.1210/endo-110-6-2222

Fleshner, M. (2000). Exercise and neuroendocrine regulation of antibody production: protective effect of physical activity on stress-induced suppression of the specific antibody response. *Int. J. Sports Med.* 21(Suppl. 1), S14–S19. doi: 10.1055/s-2000-1454

Fleshner, M. (2005). Physical activity and stress resistance: sympathetic nervous system adaptations prevent stress-induced immunosuppression. *Exerc. Sport. Sci. Rev.* 33, 120–126. doi: 10.1097/00003677-200507000-00004

Forsen, A. (1991). Psychosocial stress as a risk for breast cancer. *Psychother. Psychosom.* 55, 176–185. doi: 10.1159/000288427

Girotti, M., Pace, T. W., Gaylord, R. I., Rubin, B. A., Herman, J. P., and Spencer, R. L. (2006). Habituation to repeated restraint stress is associated with lack of stress-induced c-fos expression in primary sensory processing areas of the rat brain. *Neuroscience* 138, 1067–1081. doi: 10.1016/j.neuroscience.2005.12.002

Gómez, F., Lahmame, A., de Kloet, E. R., and Armario, A. (1996). Hypothalamic-pituitary-adrenal response to chronic stress in five inbred rat strains: differential responses are mainly located at the adrenocortical level. *Neuroendocrinology* 63, 327–337. doi: 10.1159/000126973

Gomez-Pinella, F., Vaynman, S., and Ying, Z. (2008). Brain-derived neurotrophic factor functions as a metabotrophin to mediate the effects of exercise on cognition. *Eur. J. Neurosci.* 28, 2278–2287. doi: 10.1111/j.1460-9568.2008.06524.x

Goodwin, R. D. (2003). Association between physical activity and mental disorders among adults in the United States. *Prev. Med.* 36, 698–703. doi: 10.1016/S0091-7435(03)00042-2

Greenwood, B. N., Foley, T. E., Day, H. E. W., Campisi, J., Hammack, S. H., Campeau, S., et al. (2003a). Freewheel running prevents learned helplessness/behavioral depression: role of dorsal raphe serotonergic neurons. *J. Neurosci.* 23, 2889–2898.

Greenwood, B. N., Kennedy, S., Smith, T. P., Campeau, S., Day, H. E. W., and Fleshner, M. (2003b). Voluntary freewheel running selectively modulates catecholamine content in peripheral tissue and c-fos expression in the central sympathetic circuit following exposure to uncontrollable stress in rats. *Neuroscience* 120, 269–281. doi: 10.1016/S0306-4522(03)00047-2

Greenwood, B. N., Strong, P. V., Dorey, A. A., and Fleshner, M. (2007a). Therapeutic effects of exercise: wheel running reverses stress-induced interference with shuttle box escape. *Behav. Neurosci.* 121, 992–1000. doi: 10.1037/0735-7044.121.5.992

Greenwood, B. N., Strong, P. V., Foley, T. E., Thompson, R. S., and Fleshner, M. (2007b). Learned helplessness is independent of levels of brain-derived neurotrophic factor in the hippocampus. *Neuroscience* 144, 1193–1208. doi: 10.1016/j.neuroscience.2006.11.007

Gu, G., Cornea, A., and Simerly, R. B. (2003). Sexual differentiation of projections from the principal nucleus of the bed nuclei of the stria terminalis. *J. Comp. Neurol.* 460, 542–562. doi: 10.1002/cne.10677

Hammen, C., Davila, J., Brown, G., Ellicott, A., and Gitlin, M. (1992). Psychiatric history and stress: predictors of severity of unipolar depression. *J. Abnorm. Psychol.* 101, 45–52. doi: 10.1037/0021-843X.101.1.45

Hauger, R. L., Risbrough, V., Brauns, O., and Dautzenberg, F. M. (2006). Corticotropin releasing factor (CRF) receptor signaling in the central nervous system: new molecular targets. *CNS Neurol. Disord. Drug Targets* 5, 453–479. doi: 10.2174/187152706777950684

Herman, J. P., Adams, D., and Prewitt, C. (1995). Regulatory changes in neuroendocrine stress-integrative circuitry produced by a variable stress paradigm. *Neuroendocrinology* 61, 180–190. doi: 10.1159/000126839

Herman, J. P., Schafer, M. K., Thompson, R. C., and Watson, S. J. (1992). Rapid regulation of corticotropin-releasing hormone gene transcription *in vivo*. *Mol. Endocrinol.* 6, 1061–1069. doi: 10.1210/me.6.7.1061

Imaki, T., Naruse, M., Harada, S., Chikada, N., Imaki, J., Onodera, H., et al. (1996). Corticotropin-releasing factor up-regulates its own receptor mRNA in the paraventricular nucleus of the hypothalamus. *Brain Res. Mol. Brain Res.* 38, 166–170. doi: 10.1016/0169-328X(96)00011-3

Kalantaridou, S. N., Makrigiannakis, A., Zoumakis, E., and Chrousos, G. P. (2004). Stress and the female reproductive system. *J. Reprod. Immunol.* 62, 61–68. doi: 10.1016/j.jri.2003.09.004

Kant, G. J., Eggleston, T., Landman-Roberts, L., Kenion, C. C., Driver, G. C., and Meyerhoff, J. L. (1985). Habituation to repeated stress is stressor specific. *Pharmacol. Biochem. Behav.* 22, 631–634. doi: 10.1016/0091-3057(85)90286-2

Kawashima, H., Saito, T., Yoshizato, H., Fujikawa, T., Sato, Y., McEwen, B. S., et al. (2004). Endurance treadmill training in rats alters CRH activity in the hypothalamic paraventricular nucleus at rest and during acute running according to its period. *Life Sci.* 76, 763–774. doi: 10.1016/j.lfs.2004.09.014

Khansari, D. N., Murgo, A. J., and Faith, R. E. (1990). Effects of stress on the immune system. *Immunol. Today* 11, 170–175. doi: 10.1016/0167-5699(90)90069-L

Koob, G. F., Heinrichs, S. C., Pich, E. M., Menzaghi, F., Baldwin, H., Miczek, K., et al. (1993). The role of corticotropin-releasing factor in behavioural responses to stress. *Ciba Foun. Symp.* 172, 277–295.

Kovács, K. J., and Sawchenko, P. E. (1996). Regulation of stress-induced transcriptional changes in the hypothalamic neurosecretory neurons. *J. Mol. Neurosci.* 7, 125–133. doi: 10.1007/BF02736792

Levine, S., and Ursin, H. (1991). "What is stress?", in *Stress: Neurobiology and Neuroendocrinology*, eds M. R. Brown, G. F. Koob, and C. Rivier (New York, NY: Marcel Dekker, Inc.), 3–21.

Luo, X., Kiss, A., Makara, G., Lolait, S. J., and Aguilera, G. (1994). Stress-specific regulation of corticotropin releasing hormone receptor expression in the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *J. Neuroendocrinol.* 6, 689–696. doi: 10.1111/j.1365-2826.1994.tb00636.x

Makino, S., Schulkin, J., Smith, M. A., Pacák, K., Palkovits, M., and Gold, P. W. (1995). Regulation of corticotropin-releasing hormone receptor messenger ribonucleic acid in the rat brain and pituitary by glucocorticoids and stress. *Endocrinology* 136, 4517–4525. doi: 10.1210/en.136.10.4517

Makino, S., Shibasaki, T., Yamauchi, N., Nishioka, T., Mimoto, T., Wakabayashi, I., et al. (1999). Psychological stress increased corticotropin-releasing hormone mRNA and content in the central nucleus of the amygdala but not in the

hypothalamic paraventricular nucleus in the rat. *Brain Res.* 850, 136–143. doi: 10.1016/S0006-8993(99)02114-9

Manson, J. E., Nathan, D. M., Krolewski, A. S., Stampfer, M. J., Willett, W. C., and Hennekens, C. H. (1992). A prospective study of exercise and incidence of diabetes among US male physicians. *J. Am. Med. Assoc.* 268, 63–67. doi: 10.1001/jama.1992.03490010065031

Masini, C. V., Nyhuis, T. J., Sasse, S. K., Day, H. E., and Campeau, S. (2011). Effects of voluntary wheel running on heart rate, body temperature, and locomotor activity in response to acute and repeated stressor exposures in rats. *Stress* 14, 324–334. doi: 10.3109/10253890.2010.548013

Ma, X. M., Levy, A., and Lightman, S. L. (1997). Rapid changes in heteronuclear RNA for corticotrophin-releasing hormone and arginine vasopressin in response to acute stress. *J. Endocrinol.* 152, 81–89. doi: 10.1677/joe.0.1520081

Melia, K. R., Ryabinin, A. E., Schroeder, R., Bloom, F. E., and Wilson, M. C. (1994). Induction and habituation of immediate early gene expression in rat brain by acute and repeated restraint stress. *J. Neurosci.* 14, 5929–5938.

Merali, Z., McIntosh, J., Kent, P., Michaud, D., and Anisman, H. (1998). Aversive and appetitive events evoke the release of corticotropin-releasing hormone and bombesin-like peptides at the central nucleus of the amygdala. *J. Neurosci.* 18, 4758–4766.

Moraska, A., and Fleshner, M. (2001). Voluntary physical activity prevents stress-induced behavioral depression and anti-KLH antibody suppression. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 281, R484–R489.

Morimoto, K., Tan, N., Nishiyasu, T., Sone, R., and Murakami, N. (2000). Spontaneous wheel running attenuates cardiovascular responses to stress in rats. *Pflugers Arch.* 440, 216–222.

Munck, A., Guyre, P. M., and Holbrook, N. J. (1984). Physiological functions of glucocorticoids and their relation to pharmacological actions. *Endocr. Rev.* 5, 25–44. doi: 10.1210/edrv-5-1-25

National Research Council. (2011). *Guide for the Care and Use of Laboratory Animals 8th, Edn*, Washington, DC: National Academies Press.

Neeper, S. A., Gómez-Pinella, F., Choi, J., and Cotman, C. W. (1996). Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res.* 726, 49–56. doi: 10.1016/0006-8993(96)00273-9

Nibuya, M., Takahashi, M., Russell, D. S., and Duman, R. S. (1999). Repeated stress increases catalytic TrkB mRNA in rat hippocampus. *Neurosci. Lett.* 267, 81–84. doi: 10.1016/S0304-3940(99)00335-3

Nyhuis, T. J., Masini, C. V., Sasse, S. K., Day, H. E., and Campeau, S. (2010). Physical activity, but not environmental complexity, facilitates HPA axis response habituation to repeated audiogenic stress despite neurotrophin mRNA regulation in both conditions. *Brain Res.* 1362, 68–77. doi: 10.1016/j.brainres.2010.09.038

Oliff, H. S., Berchtold, N. C., Isackson, P., and Cotman, C. W. (1998). Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus. *Brain Res. Mol. Brain Res.* 61, 147–153. doi: 10.1016/S0169-328X(98)00222-8

Paffenbarger, R. S. Jr., Lee, I. M., and Wing, A. L. (1992). The influence of physical activity on the incidence of site-specific cancers in college alumni. *Adv. Exp. Med. Biol.* 322, 7–15. doi: 10.1007/978-1-4684-7953-9_2

Park, E., Chan, O., Li, Q., Kiraly, M., Matthews, S. G., Vranic, M., et al. (2005). Changes in basal hypothalamo-pituitary-adrenal activity during exercise training are centrally mediated. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R1360–R1371. doi: 10.1152/ajpregu.00103.2005

Pasternac, A., and Talajic, M. (1991). The effects of stress, emotion, and behavior on the heart. *Methods Achiev. Exp. Pathol.* 15, 47–57.

Paxinos, G., and Watson, C. (2005). *The Rat Brain in Stereotaxic Coordinates, CD-ROM*. San Diego, CA: Academic Press.

Radley, J. J., Gosselink, K. L., and Sawchenko, P. E. (2009). A discrete GABAergic relay mediates medial prefrontal cortical inhibition of the neuroendocrine stress response. *J. Neurosci.* 29, 7330–7340. doi: 10.1523/JNEUROSCI.5924-08.2009

Rivest, S., Laflamme, N., and Nappi, R. E. (1995). Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. *J. Neurosci.* 15, 2680–2695.

Rodnick, K. J., Reaven, G. M., Haskell, W. L., Sims, C. R., and Mondon, C. E. (1989). Variations in running activity and enzymatic adaptations in voluntary running rats. *J. Appl. Physiol.* 66, 1250–1257.

Roth, D. L., and Holmes, D. S. (1985). Influence of physical fitness in determining the impact of stressful life events and physical and psychological health. *Psychosom. Med.* 47, 164–173.

Salam, J. N., Fox, J. H., Detroy, E. M., Guignon, M. H., Wohl, D. F., and Falls, W. A. (2009). Voluntary exercise in C57 mice is anxiolytic across several measures of anxiety. *Behav. Brain Res.* 197, 31–40. doi: 10.1016/j.bbr.2008.07.036

Sasse, S. K., Greenwood, B. N., Masini, C. V., Nyhuis, T. N., Fleshner, M., Day, H. E. W., et al. (2008). Chronic voluntary wheel running facilitates corticosterone response habituation to repeated audiogenic stress exposure in male rats. *Stress* 11, 425–437. doi: 10.1080/10253890801887453

Smith, M. A., Makino, S., Kim, S. Y., and Kvetnansky, R. (1995a). Stress increases brain-derived neurotrophic factor messenger ribonucleic acid in the hypothalamus and pituitary. *Endocrinology* 136, 3743–3750. doi: 10.1210/en.136.9.3743

Smith, M. A., Makino, S., Kvetnansky, R., and Post, R. M. (1995b). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J. Neurosci.* 15(3 Pt 1), 1768–1777.

Spina, M., Merlo-Pich, E., Chan, R. K. W., Basso, A. M., Rivier, J., Vale, W., et al. (1996). Appetite-suppressing effects of urocortin, a CRF-related neuropeptide. *Science* 273, 1561–1564. doi: 10.1126/science.273.5281.1561

Steller, E. (1954). The physiology of motivation. *Physiol. Rev.* 61, 5–22.

Swaab, D. F., Bao, A. M., and Lucassen, P. J. (2005). The stress system and the human brain in depression and neurodegeneration. *Ageing Res. Rev.* 4, 141–194. doi: 10.1016/j.arr.2005.03.003

Timofeeva, E., Huang, Q., and Richard, D. (2003). Effects of treadmill running on brain activation and the corticotropin-releasing hormone system. *Neuroendocrinology* 77, 388–405. doi: 10.1159/000071311

Tokuyama, K., Saito, M., and Okuda, H. (1982). Effects of wheel running on food intake and weight gain of male and female rats. *Physiol. Behav.* 28, 899–903. doi: 10.1016/0031-9384(82)90211-6

Tsigos, C., and Chrousos, G. P. (1994). Physiology of the hypothalamic-pituitary-adrenal axis in health and dysregulation in psychiatric and autoimmune disorders. *Endocrinol. Metab. Clin. North Am.* 23, 451–466.

Ulrich-Lai, Y. M., and Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nat. Rev. Neurosci.* 10, 397–409. doi: 10.1038/nrn2647

Vanitallie, T. B. (2002). Stress: a risk factor for serious illness. *Metabolis.* 51, 40–45. doi: 10.1053/meta.2002.33191

Van Pett, K., Viau, V., Bittencourt, J. C., Chan, R. K., Li, H. Y., Arias, C., et al. (2000). Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. *J. Comp. Neurol.* 428, 191–212. doi: 10.1002/1096-9861(20001211)428:2<191::AID-CNE1>3.0.CO;2-U

Wannamethee, S. G., Shaper, A. G., and Walker, M. (1998). Changes in physical activity, mortality, and incidence of coronary heart disease in older men. *Lancet* 351, 1603–1608. doi: 10.1016/S0140-6736(97)12355-8

Weinberg, M. S., Bhatt, A. P., Girotti, M., Masini, C. V., Day, H. E., Campeau, S., et al. (2009). Repeated ferret odor exposure induces different temporal patterns of same-stressor habituation and novel-stressor sensitization in both hypothalamic-pituitary-adrenal axis activity and forebrain c-fos expression in the rat. *Endocrinology* 150, 749–761. doi: 10.1210/en.2008-0958

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