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 Improve Cardiometabolic Health in Rats**

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Complete List of Authors:	BATACAN, ROMEO; Central Queensland University, School of Medical and Applied Sciences Duncan, Mitch; The University of Newcastle, dSchool of Medicine & Public Health, Priority Research Centre for Physical Activity and Nutrition, Faculty of Health and Medicine Dalbo, Vincent; Central Queensland University Connolly, Kylie; Central Queensland University, School of Medical and Applied Sciences Fenning, Andrew; Central Queensland University, School of Medical and Applied Sciences
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Light Intensity and High Intensity Interval Training Improve Cardiometabolic Health in Rats

Romeo B. Batacan Jr.^{a,b*}, Mitch J. Duncan^d, Vincent J. Dalbo^{a,c}, Kylie J. Connolly^a, and Andrew S. Fenning^{a,b}

^aSchool of Medical and Applied Sciences, Central Queensland University, Rockhampton, QLD, Australia

^bCentre for Physical Activity Studies, Central Queensland University, Rockhampton, QLD, Australia

^cClinical Biochemistry Laboratory, Central Queensland University, Rockhampton, QLD, Australia

^dSchool of Medicine & Public Health, Priority Research Centre for Physical Activity and Nutrition, Faculty of Health and Medicine, The University of Newcastle, University Drive, Callaghan NSW, Australia

Draft

Correspondence should be addressed to:

Romeo Batacan Jr.

School of Medical and Applied Sciences □

CQUniversity Australia, Building 81, Rm 1.14, Bruce Highway,

North Rockhampton, QLD 4702 □

P +61 7 4923 2232 | **M** +61 421679472 | **E** r.j.batacan@cqu.edu.au

Abstract

Physical activity has the potential to reduce cardiometabolic risk factors but evaluation of different intensities of physical activity and the mechanisms behind their health effects still need to be fully established. This study examined the effects of sedentary behaviour, light-intensity training, and high-intensity interval training on biometric indices, glucose and lipid metabolism, inflammatory and oxidative stress markers, vascular and cardiac function in adult rats. Rats (12 week-old) were randomly assigned to one of four groups: control (CTL, no exercise), sedentary (SED, no exercise and housed in small cages to reduce activity), light-intensity trained (LIT, four 30 min exercise bouts/day at 8 m/min separated by 2-hr rest period, 5 days/wk) and high-intensity interval trained (HIIT, four 2.5 min work bouts/day at 50 m/min separated by 3-min rest period, 5days/wk). After 12 weeks of intervention, SED had greater visceral fat accumulation ($p < 0.01$) and slower cardiac conduction ($p = 0.04$) compared to the CTL group. LIT and HIIT demonstrated beneficial changes in body weight, visceral and epididymal fat weight, glucose regulation, LDL cholesterol, total cholesterol and mesenteric vessel contractile response compared to the CTL group ($p < 0.05$). LIT had significant improvements in insulin sensitivity and cardiac conduction compared to the CTL and SED groups whilst HIIT had significant improvements in SBP and endothelium-independent vasodilation to aorta and mesenteric artery compared to the CTL group ($p < 0.05$). LIT and HIIT induce health benefits by improving traditional cardiometabolic risk factors. LIT improves cardiac health while HIIT promotes improvements in vascular health.

Key words: Physical activity, sedentary behaviour, oxidative stress, inflammation, cardiovascular, metabolism

Introduction

Cardiometabolic syndrome, is an intricately related group of cardiovascular and metabolic disorders associated with increased risk of cardiovascular disease (CVD) morbidity and mortality (Castro et al. 2003). The beneficial effect of regular physical activity in reducing the risk of cardiometabolic disease and early all-cause mortality has been clearly established (Young et al. 2014). This research has predominantly focused on the influence of moderate-to-vigorous physical activity (MVPA) (3 - 9 metabolic equivalents (METs) or 40 - 85% maximal oxygen uptake ($\text{VO}_2 \text{ max}$) (Pescatello 1999).

The weight of evidence regarding the health benefit of physical activity led to the development of “Physical Activity Guidelines” which focused on accumulating a minimum of 30 min of MVPA per day broadly classified as moderate-intensity continuous training (MICT) (World Health Organization 2010). Recently, the benefit of lower intensity physical activity (20 - 40% $\text{VO}_2 \text{ max}$ or 1.6 - 3.0 METs) has also been acknowledged to improve health (Healy et al. 2007; Bailey & Locke 2015) and there is also a growing interest in the use of high intensity activity ($\geq 85\% \text{ VO}_2 \text{ max}$ or $\geq 9 \text{ METs}$) performed in brief intervals to achieve health benefits (Tjønnå et al. 2008; Ciolac et al. 2010).

A combination of societal, built environment and policy changes has contributed to an increase in sedentary behaviour (sitting or lying down activities with energy expenditure of $< 20\% \text{ VO}_2 \text{ max}$ or $< 1.6 \text{ METs}$) (Duncan et al. 2012; Owen et al. 2011; Norton et al. 2010). Numerous studies have shown that spending excessive time engaged in sedentary behaviour may carry a risk for cardiometabolic disease even when adjusting for time spent in MVPA (Henson et al. 2013; De Rezende et al. 2014). More recently, studies have also demonstrated

the health benefits of interrupting prolonged sedentary time with light intensity breaks (Bailey & Locke 2015).

To clearly establish the effects of physical activity, it is important to understand the mechanisms that link physical activity with cardiometabolic risk. There are several mechanisms suggested behind MVPA-induced health outcomes (Mora et al. 2007). First, MVPA has the potential to reduce the risk of CVD events by improving traditional CVD risk factors (Mora et al. 2007). Second, it can reduce CVD risk by improving cardiac function characterized by increased cardiac contractility (Kemi et al. 2005). Third, MVPA can improve vascular endothelial function by increasing NO bioavailability thus promoting microcirculation (Green et al. 2004). Finally, more recent findings demonstrate that MVPA decreases novel biomarkers such as plasma interleukin-6 (Nicklas et al. 2008) and F2-isoprostanes (Campbell et al. 2010) providing evidence that the cardioprotective effects of MVPA might be attributed to reduced vascular inflammation and oxidative damage. In regards to sedentary behavior, LIT, and HIIT, adaptations associated with these different intensities of physical activity are yet to be established. Thus, to elucidate the mechanisms behind the health effects of these different intensities of physical activity, this study examined the effects of sedentary behaviour, LIT and HIIT on cardiometabolic markers, vascular and cardiac function in male adult rats.

Methods

Animals

All experimental procedures were approved by CQUniversity Animal Ethics Research Committee (A13/08-303). Male Wistar rats (N = 48) bred in the animal house of the institute

were maintained in an environmentally controlled room (temperature $22 \pm 1^{\circ}\text{C}$, relative humidity $50 \pm 2\%$) with a 12 hour light-dark cycle. Water and standard rat chow (Riverina Stockfeeds; South Brisbane, QLD, Australia) were provided *ad libitum*.

Experimental design

Rats (12 weeks old; 442.9 ± 48.1 g) were randomly divided into four groups each comprising of 12 animals: control (CTL), sedentary (SED), LIT, and HIIT. Rats in the CTL group were housed three to four rats per standard cage with 2400cm^2 floor area ($400\text{ cm}^2/400\text{g}$ rat) and were not exercised but were allowed to maintain normal cage activity. The SED group was not exercised and was housed three rats per small cage with 1080 cm^2 floor area ($240\text{ cm}^2/400\text{g}$ rat) to initiate a significant reduction in physical activity (Sharp et al. 2003). The LIT group was housed three to four rats per standard cage with 2400cm^2 floor area ($400\text{ cm}^2/400\text{g}$ rat) and ran on a motor-driven treadmill 125 min daily, at 8 m/min speed, 0% incline, divided into 4 bouts (30-30-30-35 min) separated by two hour rest period ($\sim 40\%$ VO_2 max), 5 days a week (Lee et al. 2001). The HIIT group was housed three to four rats per standard cage with 2400cm^2 floor area ($400\text{ cm}^2/400\text{g}$ rat) and was progressively trained to run from 10-15 min daily, at 10 m/min speed, 0% incline, to 10 min daily, at 50m/min speed, 10% incline, divided into four 2.5 min work bouts separated by a 3 min rest period ($>90\%$ VO_2 max), 5 days a week (Matsunaga et al. 2007).

Food and water intake were measured biweekly throughout the intervention period. Body weights, oral glucose tolerance, systolic blood pressure (SBP) and heart rate (HR) were recorded every 4 weeks during the intervention period. After 12 weeks of intervention, all rats (24 weeks old) were euthanized after a 24-hour rest period via intraperitoneal injection of Lethabarb (sodium pentobarbitone, 1.5mg/kg). Visceral and epididymal fat pads, heart,

kidney, liver, and spleen were removed and weighed (weight normalized to tibial length). Blood taken from the abdominal vena cava of each rat was collected, allowed to clot then centrifuged for 10 min at 4000 rpm. Serum was aliquoted to microtubes and frozen at -80°C until further analysis. Changes in cardiovascular function were determined by electrophysiological studies and isolated thoracic aortic and mesenteric ring organ baths.

Biochemical assays

Total nitric oxide (NO) concentration was determined using Sorte and Basak (2010) modified copper–cadmium reduction method. Serum triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) concentrations were measured using a commercially available assay kits (Abcam, Cambridge, United Kingdom) according to manufacturer-provided standards and protocols. Total cholesterol was calculated using a modified Friedlander formula (Friedlander et al. 1982). Concentrations of insulin were determined using a Mercodia Rat Insulin ELISA kit (Uppsala, Sweden). Quantification of interleukin-6 concentration was determined using R&D Systems Rat IL-6 DuoSet ELISA (Catalogue Number DY506). Total F2-isoprostane concentrations were measured using gas chromatography-tandem mass spectrometry as described by Briskey et al. (2014).

Cardiac function

The left ventricle papillary muscle was pinned to the bottom of an experimental chamber filled with Tyrodes solution. The papillary muscle was slowly stretched to a maximum pre-load (5-10 mN). Electrical stimulation was induced using a Grass SD-9 stimulator at a frequency of 1 Hz, pulse width of 0.5 msec and stimulus strength of 20% above threshold, delivered through bipolar earth-isolated platinum electrodes. After a 5 min equilibration period, the papillary muscle was impaled by glass microelectrodes (World Precision

Instruments filamented borosilicate glass, outer diameter 1.5 mm, tip resistance of 5-15 m Ω) filled with 3M potassium chloride connected to a differential voltage follower via silver/silver chloride platinum electrode. Stimulated action potentials were recorded with a Cyto 721 electrometer (World Precision Instruments) connected to a PowerLab data recording system (ADInstruments, Australia).

Vascular function

Thoracic aortic rings were suspended in individual 25 ml organ baths filled with Tyrodes solution. Mesenteric arteries dissected from the intestine vasculature were threaded with 40 μ m stainless steel wire and were suspended in 10 ml myograph chambers containing Tyrodes buffer. All vessels were allowed to equilibrate for 30 min at a resting tension of $\sim$ 10 mN. Following equilibration, mesenteric arteries were normalized using an automated normalization function, were contracted with 10mM potassium chloride and relaxed with 1e-5 M acetylcholine. Cumulative concentration-contraction curves were measured for norepinephrine and cumulative concentration-relaxation curves were measured for acetylcholine and sodium nitroprusside (SNP) following submaximal (70%) contraction to norepinephrine.

Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD). Food and water intake, body weight, SBP, and HR were analysed using separate (group x time) Mixed Factorial Repeated Measures Analysis of Variance (RM ANOVAs). If there was a significant interaction, separate RM ANOVAs were used to examine within group differences over time and separate one-way ANOVAs were used to examine between group differences at each time point. Vascular functional parameters were analysed using separate (group x concentration) factorial

ANOVAs. If there was a significant interaction, separate one-way ANOVAs were used to examine between group differences at each concentration, and separate one-way ANOVAs were used to examine differences in concentration within each group. Cardiometabolic, cardiac action potential parameters and vascular reactivity were analysed using separate one-way ANOVAs. Bonferroni post-hoc tests were used in all analysis to adjust for multiple comparisons. An alpha level of 0.05 was set for significant differences. Analyses were performed using Graphpad Prism v6 (GraphPad Software La Jolla, CA, USA).

Results

Food and water intake

Mean 24-hour food and water intake among groups at weeks 0, 4, 8, and 12 of the intervention are presented in Table 1. There were no significant between group differences in food intake at week 0 of intervention. A significant group x time interaction was observed for food intake ($p < 0.01$). Follow-up tests within-groups revealed that for the LIT group, there was a significant increase in food intake at week 12 compared to week 8 ($p < 0.01$) and significant decrease in food intake at week 4 compared to week 0 ($p = 0.02$) and at week 8 compared to week 0 ($p < 0.01$). For the HIIT group, there was a significant decrease in food intake at week 8 compared to week 0 ($p < 0.01$) and at week 12 compared to week 0 ($p < 0.01$). Follow-up tests between-groups revealed the LIT group food intake was significantly lower compared to the CTL ($p < 0.01$) and SED ($p < 0.01$) groups at week 4 and significantly lower compared to the CTL ($p < 0.01$) and SED ($p < 0.01$) groups at week 8 of the intervention. However, at week 12, no difference in food intake was observed in the LIT group compared to the CTL and SED groups. The HIIT group food intake was significantly lower compared to the CTL ($p = 0.01$) and SED ($p < 0.01$) groups at week 4, significantly

lower compared to the CTL ($p < 0.01$) and SED ($p = 0.01$) groups at week 8, and significantly lower compared to the CTL ($p = 0.03$) and SED ($p = 0.04$) groups at week 12 of intervention. No significant differences in food intake were observed between CTL and SED groups at weeks 4, 8 and 12. No significant main effects or interactions were observed for water intake throughout the intervention period.

Body and organ weights

The effects of the intervention on body weights at weeks 0, 4, 8 and 12 and organ weights at week 12 are presented in Table 2. Initial body weights ranged from 425.7 ± 41.1 g to 459.0 ± 50.0 g with no significant differences between groups at week 0. A significant group $\times$ time interaction was observed for body weight ($p < 0.01$). Follow-up tests within-groups revealed that all groups had significantly higher body weights at weeks 4, 8, and 12 compared to week 0 ($p < 0.01$ for all groups), CTL group had significantly higher body weights at weeks 8 and 12 compared to week 4 ($p < 0.01$), and the SED group had significantly higher body weight at week 12 compared to weeks 4 ($p < 0.01$) and 8 ($p = 0.01$). Follow-up tests between-groups revealed the LIT group body weights were significantly lower compared to the SED ($p < 0.01$) group at week 4, significantly lower compared to the CTL ($p < 0.01$) and SED ($p < 0.01$) groups at week 8, and significantly lower compared to the CTL ($p < 0.01$) and SED ($p < 0.01$) groups at week 12 of the intervention. The HIIT group body weights were significantly lower compared to the SED ($p = 0.01$) group at week 4, significantly lower compared to the CTL ($p < 0.01$) and SED ($p < 0.01$) groups at week 8, and significantly lower compared to the CTL ($p < 0.01$) and SED ($p < 0.01$) groups at week 12 of the intervention. No significant difference in body weights between CTL and SED groups were observed at weeks 4, 8 and 12 of the intervention. Mean normalized organ and fat weights were also compared among groups. The SED group visceral fat weight was significantly higher compared to the CTL ($p <$

0.01) group. The LIT group visceral fat, epididymal fat, and liver weights were significantly lower compared to the CTL ($p < 0.01$ for all outcome variables) and SED ($p < 0.01$ for all outcome variables) groups. The HIIT group demonstrated significantly lower visceral fat and epididymal fat weights compared to the CTL ($p < 0.01$) and SED ($p < 0.01$) groups. No significant between group differences in left and right ventricles, kidney and spleen weights were observed at week 12 of the intervention.

Systolic blood pressure and heart rate

The effects of intervention on SBP and HR at weeks 0, 4, 8 and 12 are presented in Figure 1. There were no significant differences between groups on SBP at week 0 of intervention. No group x time interaction was observed for SBP. No main effect was observed for time, but there was a significant main effect for group ($p < 0.01$). Follow-up tests between-groups revealed the HIIT group SBP was significantly lower compared to the CTL ($p < 0.01$), SED ($p < 0.01$) and LIT ($p < 0.01$) groups at week 8 and significantly lower compared to the CTL ($p < 0.01$), SED ($p < 0.01$) and LIT ($p < 0.01$) groups at week 12 of intervention. No significant main effects or interactions were observed for HR throughout the intervention period.

Cardiometabolic parameters

The effects of 12 weeks of intervention on cardiometabolic parameters are presented in Table 3. No significant differences between the CTL and SED groups in all cardiometabolic parameters were observed after 12 weeks of the intervention. The LIT group had significantly lower LDL cholesterol compared to the CTL group ($p < 0.01$) and the HIIT group had significantly lower LDL cholesterol compared to the CTL ($p < 0.01$) and SED ($p < 0.01$) groups. No significant differences in serum HDL and triglycerides were observed between

groups after 12 weeks of the intervention. The LIT group had significantly lower total cholesterol compared to the CTL group ($p = 0.03$) and the HIIT group had significantly lower total cholesterol compared to the CTL ($p < 0.01$) and SED ($p = 0.01$) groups. In regard to glucose metabolism, the LIT group insulin levels were significantly lower compared to the CTL ($p = 0.03$) and SED ($p < 0.01$) groups and area under the curve in OGTT was lower in the LIT group compared to the CTL group ($p = 0.01$) after 12 weeks of the intervention. In contrast, the HIIT group insulin levels were not statistically different compared to all other groups but the area under the curve in the OGTT in the HIIT group was lower compared to the CTL group ($p = 0.02$). All groups after 12 weeks of the intervention demonstrated no significant differences in the level of serum NO, interleukin-6 and total F2-isoprostanes.

Cardiac function

Data in Table 4 presents the effect of 12 weeks of intervention on cardiac function. No significant differences in all cardiac functional parameters were observed between groups after 12 weeks of the intervention except for action potential amplitude (APA). The SED group APA was significantly lower compared to the CTL group ($p = 0.04$) while the LIT group APA was significantly higher compared to all other groups ($p < 0.01$ vs CTL, SED and HIIT).

Vascular function

Figure 2 presents the effect of physical activity prescriptions on vascular reactivity in isolated tissues after 12 weeks of the intervention. No significant differences in vascular reactivity between the CTL and SED groups were observed after 12 weeks of the intervention. Both the LIT and HIIT groups demonstrated decreased mesenteric vessel contractile responses to norepinephrine compared to CTL group ($p < 0.05$). Results regarding vasorelaxation of

norepinephrine pre-contracted vessel segments demonstrated HIIT enhanced sensitivity to aortic relaxation response to SNP compared to the CTL ($p < 0.05$) and LIT ($p < 0.05$) groups and enhanced sensitivity to mesenteric artery relaxation response to SNP compared to the CTL group ($p < 0.05$). No significant differences in aortic and mesenteric relaxation to acetylcholine were observed between groups after 12 weeks of the intervention.

Discussion

This study examined the effect of sedentary behaviour, LIT, and HIIT on cardiometabolic risk markers, cardiac function, and vascular function in young adult male standard chow fed rats. Results demonstrated LIT and HIIT promote an improvement in cardiovascular function in similar ways in terms of favourable changes in body weight, adipose tissue, blood pressure, glucose regulation and cholesterol metabolism and in different ways in terms of their influence in the cardiac cells and blood vessels.

Food intake was initially similar among groups. At 4, 8, and 12 weeks of intervention, the SED group had the same food intake compared to the CTL group suggesting that sedentary behaviour did not change food intake in rats despite low energy expenditure. The HIIT group had significantly lower food intake compared to the CTL and SED groups at 4, 8 and 12 weeks. This observation is consistent with studies that have reported that rats generally decrease food intake in response to high intensity exercise ($> 80\% \text{VO}_{2\text{max}}$) (Katch et al. 1979; Flores et al. 2006). It has been suggested appetite suppression resulting from HIIT is due to a rise in epinephrine and norepinephrine levels during chronic physical training, with both neurotransmitters contributing to post-training inhibition of food intake (Guilland et al. 1988). Another mechanism thought to be responsible for the reduced energy consumption

effect of physical training is the increase release of corticotropin releasing factor (CRF) by the hypothalamus, a potent anorectic peptide (Rivest & Richard 1990).

Similar to the HIIT results, the LIT group had significantly lower food intake compared to the CTL and SED groups at weeks 4 and 8 of intervention (and significantly increased food intake at week 12 compared to week 8). However, in contrast to the HIIT group, no significant difference in food intake was observed compared to the CTL and SED groups at week 12. This finding is consistent with studies that demonstrated reduced food intake in male rats as an adaptation to chronic low intensity physical training (Katch et al. 1979; Gleeson et al. 1982). However, it appears that at 12 weeks of intervention, the LIT group had already adapted to the training intervention showing a similar food intake to the CTL and SED groups.

The SED group did not show any difference in body weight compared to the CTL group. Despite no change in body weight the SED group was found to have significantly higher visceral fat deposition compared to the CTL group. This finding is consistent with existing literature demonstrating an increase in visceral fat accumulation in response to less energy expenditure in sedentary rats (Dantas et al. 2010). Since excess visceral fat has been associated with enhanced cardiometabolic disease risk profile (Sironi et al. 2012), increased visceral fat observed as an effect of sedentary behaviour may explain the association of this behaviour to increase cardiometabolic disease risk.

In this study, the LIT and HIIT groups had significantly lower body weight in comparison to CTL and SED group at weeks 8 and 12 of the intervention. These changes in body weights induced by LIT and HIIT were further reflected in the organ weight data. The LIT and HIIT

groups were found to have reduced visceral and epididymal fat weights compared to the CTL and SED groups. This finding is in agreement with previous rat studies showing 9 weeks of LIT (Dantas et al. 2010) and 6 weeks of HIIT (Rhamos-Filho et al. 2015) reduce visceral fat. Similarly, in a randomized clinical trial significant decrease in abdominal visceral fat was observed in overweight women following 12 weeks of HIIT (Zhang et al. 2015). There may be several mechanisms underlying this training-induced body weight/fat loss effect observed in human (Boutcher 2011; Terada et al. 2004) and animal studies (Katch et al. 1979; Yano et al. 1998; Panveloski-Costa et al. 2012). These include increased whole body and skeletal muscle capacity for fatty acid oxidation and decreased post-training appetite (Boutcher 2011; Terada et al. 2004; Katch et al. 1979). For the HIIT group, it is suggested that generated catecholamines or the need to remove lactate and H^+ and to resynthesize glycogen could be the mechanism influencing post exercise fat oxidation (Boutcher 2011; Panveloski-Costa et al. 2012; Yano et al. 1998). In addition, lower liver weight was observed in the LIT group compared to the CTL and SED groups. This is probably due to loss of fat stored in the liver (Sene-Fiorese et al. 2008), an effect observed in LIT and not following HIIT suggesting possibly a higher liver fat oxidation at low exercise intensity compared to HIIT in rats.

In the present study, no significant difference in SBP between SED and CTL groups were observed throughout the intervention period. These rats were relatively young with normal vascular function so it is reasonable to expect that the 12 week restricted activity protocol may not have showed adverse cardiometabolic health outcomes. In exercise-trained rats, no difference in SBP was observed in the LIT group compared to the CTL and SED groups while the HIIT group significantly reduced SBP compared to the CTL, SED and LIT groups after 8 and 12 weeks of intervention. Several studies have examined the effect of low and high intensity exercise on SBP with findings demonstrating low-intensity (Petriz et al. 2015; Sun et

al. 2008) and high-intensity (Petriz et al. 2015; Huang et al. 2012) exercise improved SBP in hypertensive rats. A potential reason for the lack of effect observed on SBP in the LIT group is that SBP of normotensive rats respond less to physical training compared to hypertensive rats (Melo et al. 2003). Whilst evidence showing high intensity stimulus causes greater norepinephrine reductions (less sympathetic activation) post exercise resulting to lower SBP even in normotensive individuals is obtained in humans (Ciolac et al. 2010). The precise mechanism is not completely understood but central renin-angiotensin system (Cameron et al. 2012) and sympathetic activation (Ciolac 2012) are considered likely contributors of this HIIT-induced blood pressure reduction. Additionally, the increased stressful pulsatile blood flow would trigger enhanced endothelium-dependent vasodilatory mechanisms which would further enhance a decrease in blood pressure (Paniagua et al. 2001).

No significant effects on HR were observed in this study. This is intriguing as other studies (Gava et al. 1995; Barnard et al. 1976) have shown exercise training in animals over similar time periods elicits reductions in HR. Of the two training groups, the HIIT group showed the greatest decline in HR although this was not statistically significant. This may be due the variability observed in HR of animals in this study (Figure 1), the training stimulus not being of a sufficient dose over to elicit changes or other unmeasured central neural mechanisms that mediated HR changes.

In the current study, LIT and HIIT decreased total and LDL cholesterol after 12 weeks of intervention possibly as a consequence of lower body and fat weights. These observations are consistent with evidence that decreasing body/fat weight and enhanced fat oxidation may be the mechanism behind this lipid lowering effect of low intensity exercise and HIIT (Terada et al. 2004). In addition to demonstrating improvement in lipid profile in both

training programs, the present study revealed LIT and HIIT decreased area under the curve in 2-hour post glucose load at week 12 of the intervention. These improvements in glucose metabolism following LIT and HIIT has been linked to enhanced skeletal muscle glucose uptake and improved insulin sensitivity (Marliss & Vranic 2002; Sun et al. 2008). Whilst the LIT group demonstrated lower insulin level compared to the CTL and SED groups, the HIIT group demonstrated no difference in insulin level compared to all other groups. Similar results were observed in a study in rats subjected to acute bouts of low intensity and HIIT delivered as swimming where LIT was found to have greater increased in insulin responsiveness to glucose uptake compared to the HIIT prescription (Koshinaka et al. 2009). It has been suggested that the enhanced Akt (key enzyme in the insulin signalling pathway that stimulates glucose uptake in skeletal muscle cells) Thr³⁰⁸ phosphorylation may be the reason for the improved insulin sensitivity after LIT but not after HIIT (Koshinaka et al. 2009).

Although there is emerging evidence that oxidative stress and inflammation are linked to CVD and metabolic disorders (Heitzer et al. 2001; van den Oever et al. 2010), this study showed no significant differences in the serum concentration of interleukin-6, NO, or total F2-isoprostanes between groups after 12 weeks of the intervention. Other studies (Kiliç et al. 2014; Kim et al. 2014; Tucker et al. 2015) examining changes in these outcomes in response to exercise provide mixed evidence on the impact of exercise training in animal models. Therefore, further research is required to understand the impact of exercise on oxidative stress and inflammation.

To date, the effect on cardiac electrophysiology of LIT and HIIT has not been described. In this study, no significant differences in action potential duration, resting membrane potential, and force of contraction were observed between groups at week 12 of intervention. LIT

increased APA compared to the CTL, SED, and HIIT groups at 12 weeks. As APA is an important determinant of the propagation velocity along the myocardial fibres, the increase in APA induced by LIT indicates improved cardiac conduction leading to potentially improved cardiac electrophysiological function (Yan et al. 2007). The mechanism behind this finding is unknown but higher myofilament Ca^{2+} sensitivity and enhanced Ca^{2+} handling have been suggested (Chicco et al. 2008). Conversely, the SED group decreased APA compared to the CTL group leaving the maximum voltage reached outside the stimulus region below the threshold for the L-type Ca^{2+} current activation leading to slower conduction or even conduction block (Clayton et al. 2011). This can have a number of consequences such as altered sequence of heart activation, impaired ventricular pressure development, and increased myocardial oxygen demand all of which are known to increase CVD risk (Vranka et al. 2007).

Arterial compliance, an index of vascular function is an important cardiovascular risk marker (Jani & Rajkumar 2006). Impaired arterial compliance enhances vasoconstriction and reduces vasodilation (Brandes et al. 1997) and can promote hypertension. In response to physical training, LIT and HIIT rats decreased mesenteric vessel contractile response to norepinephrine compared to the CTL group. The reason behind this improved vasoreactivity in the mesenteric vessel is still unclear but LIT- and HIIT-induced decreases in LDL and total cholesterol which are known mediators of vascular dysfunction (Cox & Cohen 1996) may have contributed to this outcome. The training effect may have also lessened the responsiveness to sympathetic mediators.

Findings from this study found that HIIT rats showed enhanced aortic and mesenteric endothelium-independent relaxation to SNP compared to the CTL group. These findings suggest enhanced sensitivity to NO in arterial smooth muscle in the HIIT group (Arvola et al.

1999). However, since no significant differences in endothelium-dependent dilation to acetylcholine were observed between groups, this implies HIIT did not enhance NO release from the endothelium which is consistent with the maintained NO concentrations (Table 3) observed between groups. Earlier reports on HIIT (85-90% $\text{VO}_{2\text{max}}$, 1hr/day, 5 days/week for 8 weeks) (Haram et al. 2009) have demonstrated that improved vasodilation following physical training is endothelium-mediated with no effect on endothelium-independent dilation. The reason behind these different vascular adaptations observed in HIIT may be explained by differences in the exercise-training protocol or in exercise-training intensity. Additionally, the current study utilised healthy, young adult rats with normal endothelial function so it is difficult to detect any increases in endothelial function or NO release beyond the CTL values.

The current study demonstrated that after 12 weeks of intervention, the SED group had greater visceral fat accumulation and slower cardiac conduction compared to the CTL group. The LIT and HIIT groups showed beneficial changes in body weight, fat accumulation, glucose regulation, blood lipids (HDL and total cholesterol) and mesenteric vessel contractile response to norepinephrine compared to the CTL group. Significant improvements in insulin sensitivity and cardiac conduction were also observed in the LIT group compared to the CTL group and significant improvements in SBP and endothelium-independent vasodilation to aorta and mesenteric artery were observed in the HIIT group compared to the CTL group. It appears that LIT and HIIT induce health benefits by improving traditional risk factors for cardiometabolic disease and by stimulating cardiovascular adaptations achieved via different mechanisms. LIT is more directed towards the cardiac system and HIIT towards the vascular system. Results from our study suggest the most effective form of physical activity for the promotion of cardiovascular health is likely a combination of LIT and HIIT as these training programs will likely have a synergist effect on the promotion of cardiovascular health. Future

research should begin to examine how to best develop a training program of LIT and HIIT to promote cardiovascular health as these findings have significant clinical relevance.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Table 1 Mean 24-hour food and water intake of control, sedentary, light-intensity trained, and high-intensity interval trained groups at 0, 4, 8 and 12 weeks of intervention.

Measures	CTL	SED	LIT	HIIT
Food intake (g/24h)				
0 wk	35.0 ± 3.3	37.1 ± 2.3	35.2 ± 4.0	37.2 ± 3.1
4 wk	40.0 ± 4.6	41.6 ± 3.3	29.6 ± 2.0 ^{*†a}	32.0 ± 2.8 ^{*†a}
8 wk	37.7 ± 2.7	37.1 ± 2.5	26.0 ± 0.7 ^{*†a}	29.0 ± 3.0 ^{*†a}
12 wk	36.1 ± 3.6	35.8 ± 2.6	34.6 ± 2.8 ^c	28.9 ± 3.1 ^{*†a}
Water intake (ml/24h)				
0 wk	96.7 ± 6.3	113.9 ± 30.0	95.8 ± 3.2	91.8 ± 9.7
4 wk	84.9 ± 13.3	105.4 ± 5.2	80.8 ± 11.4	80.0 ± 11.3
8 wk	84.7 ± 8.1	90.5 ± 11.6	85.7 ± 25.9	76.2 ± 9.6
12 wk	99.2 ± 23.8	99.9 ± 28.9	83.7 ± 7.0	76.8 ± 9.4

Data expressed as Mean ± SD. wk, week; CTL (n = 11), control; SED (n = 12), sedentary; LIT (n = 11), light-intensity trained; HIIT (n = 12), high-intensity interval trained. ^{*}p < 0.05 vs CTL, [†]p < 0.05 vs SED, ^ap < 0.05 vs 0 wk (within group), ^cp < 0.05 vs 8 wk (within group).

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Table 2 Body weights at 0, 4, 8 and 12 weeks and organs weights of control, sedentary, light-intensity trained, and high-intensity interval trained groups at 12 weeks of intervention.

Measures	CTL	SED	LIT	HIIT
Body weight (g)				
0 wk	440.5 ± 47.5	459.0 ± 50.0	425.7 ± 41.1	454.5 ± 41.8
4 wk	532.0 ± 60.7 ^a	565.4 ± 43.2 ^a	494.3 ± 42.2 ^{†a}	503.1 ± 36.1 ^{†a}
8 wk	588.1 ± 51.0 ^{ab}	597.5 ± 50.8 ^a	498.4 ± 46.4 ^{*†a}	520.5 ± 40.5 ^{*†a}
12 wk	624.6 ± 67.9 ^{ab}	641.0 ± 79.3 ^{abc}	509.2 ± 54.9 ^{*†a}	530.2 ± 32.7 ^{*†a}
Organ weights				
Visceral fat (mg/mm)	413.2 ± 143.8	493.0 ± 97.1 [*]	229.8 ± 91.5 ^{*†}	297.6 ± 82.2 ^{*†}
Epididymal fat (mg/mm)	329.0 ± 110.8	328.8 ± 97.6	173.7 ± 68.1 ^{*†}	203.5 ± 34.1 ^{*†}
LV (mg/mm)	29.3 ± 3.0	30.5 ± 2.3	26.1 ± 3.1	27.8 ± 2.1
RV (mg/mm)	7.6 ± 1.1	8.4 ± 1.4	6.7 ± 1.1	7.4 ± 1.6
Kidney (mg/mm)	99.7 ± 8.9	102.4 ± 6.4	86.0 ± 6.9	93.4 ± 8.9
Spleen (mg/mm)	34.3 ± 5.0	32.2 ± 5.6	27.2 ± 4.5	28.0 ± 3.4
Liver (mg/mm)	401.5 ± 46.3	401.8 ± 45.9	310.3 ± 32.2 ^{*†}	359.6 ± 37.0

Data expressed as Mean ± SD. LV, left ventricle; RV, right ventricle; wk, week; CTL (n = 11), control; SED (n = 12), sedentary; LIT (n = 11), light-intensity trained; HIIT (n = 12), high-intensity interval trained. ^{*}p < 0.05 vs CTL, [†]p < 0.05 vs SED, ^ap < 0.05 vs 0 wk (within group), ^bp < 0.05 vs 4 wk (within group), ^cp < 0.05 vs 8 wk (within group).

Table 3 Cardiometabolic parameter outcomes of control, sedentary, light-intensity trained, and high-intensity interval trained groups after 12 weeks of intervention.

Measures	CTL	SED	LIT	HIIT
LDL cholesterol (mg/dL)	102.1 ± 55.1	87.1 ± 12.7	56.7 ± 11.1 [*]	44.6 ± 20.5 ^{*†}
HDL cholesterol (mg/dL)	45.6 ± 20.7	51.6 ± 16.0	52.3 ± 17.1	54.8 ± 10.0
Triglycerides (mg/dL)	94.3 ± 16.0	98.1 ± 10.1	92.0 ± 16.9	103.4 ± 22.4
Total cholesterol(mg/dL)	166.6 ± 75.6	158.3 ± 15.9	133.8 ± 35.1 [*]	120.1 ± 10.4 ^{*†}
OGTT AUC 0 wk (mmol/L)	1008.0 ± 94.3	1068.0 ± 18.7	930.0 ± 51.3	1008.0 ± 61.5
OGTT AUC 4 wk (mmol/L)	963.0 ± 90.0	981.0 ± 48.0	879.0 ± 92.1	882.0 ± 33.6
OGTT AUC 8 wk (mmol/L)	921.0 ± 21.0	882.0 ± 45.0	882.0 ± 45.0	969.0 ± 88.5
OGTT AUC 12wk (mmol/L)	945.0 ± 28.8	888.0 ± 5.9	801.0 ± 18.0 [*]	813.0 ± 25.4 [*]
Insulin (ug/L)	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.1 ^{*†}	0.5 ± 0.2
NO (uM)	20.7 ± 7.8	26.9 ± 16.1	23.7 ± 10.5	21.3 ± 12.5
Interleukin-6 (pg/mL)	47.2 ± 6.0	49.1 ± 3.5	49.6 ± 5.6	48.1 ± 4.6
F2-isoprostane (pg/mL)	689.5 ± 563.5	546.8 ± 570.9	717.8 ± 502.2	398.8 ± 301.1

Data expressed as Mean ± SD. AUC, area under the curve; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; wk, week; CTL (n = 11), control; SED (n = 12), sedentary; LIT (n = 11), light-intensity trained; HIIT (n = 12), high-intensity interval trained. ^{*}p < 0.05 vs CTL, [†]p < 0.05 vs SED.

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Table 4 Cardiac electrophysiology outcomes of control, sedentary, light-intensity trained, and high-intensity interval trained groups after 12 weeks of intervention.

Cardiac action potential parameters	CTL	SED	LIT	HIIT
APD20	16.7 ± 3.1	14.4 ± 2.2	16.6 ± 3.2	15.4 ± 1.7
APD50	26.1 ± 4.5	23.8 ± 4.0	26.0 ± 6.1	23.9 ± 4.8
APD90	87.7 ± 21.9	99.1 ± 23.5	78.6 ± 23.3	73.3 ± 33.4
RMP	-66.6 ± 12.4	-69.3 ± 3.6	-71.0 ± 3.2	-66.5 ± 5.1
APA	55.1 ± 6.6	46.3 ± 6.8 [*]	69.9 ± 8.3 ^{*†}	50.5 ± 6.1 [‡]
Fc	3.3 ± 2.1	2.2 ± 1.5	3.3 ± 1.7	2.1 ± 1.4

Data expressed as Mean ± SD. APD20, action potential duration at 20%; APD50, action potential duration at 50%; APD90, action potential duration at 90%; APA, action potential amplitude; Fc, force of contraction; RMP, resting membrane potential; CTL (n = 11), control; SED (n = 12), sedentary; LIT (n = 11), light-intensity trained; HIIT (n = 12), high-intensity interval trained. ^{*}p < 0.05 vs CTL, [†]p < 0.05 vs SED, [‡]p < 0.05 vs LIT.

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Figure 1 Systolic blood pressure and heart rate of control, sedentary, light-intensity trained, and high-intensity interval trained groups at 0, 4, 8 and 12 weeks of intervention. Data expressed as Mean $\pm$ SD. CTL (n = 11), control; SED (n = 12), sedentary; LIT (n = 11), light-intensity trained; HIIT (n = 12), high-intensity interval trained. *p < 0.05 vs CTL, †p < 0.05 vs SED, ‡p < 0.05 vs LIT.

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Figure 2 Vascular functional changes after 12 weeks of intervention. A. Norepinephrine mediated contraction of the thoracic aorta B. Endothelium-dependent relaxation to acetylcholine of norepinephrine pre-contracted thoracic aorta C. Endothelium-independent relaxation to sodium nitroprusside of norepinephrine pre-contracted thoracic aorta D. Norepinephrine mediated contraction of the mesenteric artery E. Endothelium-dependent relaxation to acetylcholine of norepinephrine pre-contracted mesenteric artery F. Endothelium-independent relaxation to sodium nitroprusside of norepinephrine pre-contracted mesenteric artery. Data expressed as Mean $\pm$ SD. CTL (n = 11), control; SED (n = 12), sedentary; LIT (n = 11), light-intensity trained; HIIT (n = 12), high-intensity interval trained. *p < 0.05 vs CTL, [†]p < 0.05 vs SED, [‡]p < 0.05 vs LIT.

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Figure 1 Systolic blood pressure and heart rate of control, sedentary, light-intensity trained, and high-intensity interval trained groups at 0, 4, 8 and 12 weeks of intervention. Data expressed as Mean $\pm$ SD. CTL (n = 11), control; SED (n = 12), sedentary; LIT (n = 11), light-intensity trained; HIIT (n = 12), high-intensity interval trained. *p < 0.05 vs CTL, †p < 0.05 vs SED, ‡p < 0.05 vs LIT.
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