Article Title: Small-sided games training reduce CRP, IL-6 and leptin in sedentary, middle-aged men.

Running Title: Small-sided games training and inflammation

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ABSTRACT

Purpose: Long-term physical activity is reported to improve chronic systemic inflammation, which provides protection against the ensuing development of chronic disease. Accordingly, the present study assessed changes in pro- and anti-inflammatory cytokines, aerobic capacity and body composition following 8-weeks of either small-sided games (SSG) or cycling (CYC) training compared to a sedentary control (CON) condition.

Methods: Thirty-three middle-aged, sedentary men were randomized into CYC (n=11), SSG (n=11), or CON (n=11) conditions. The CYC and SSG conditions trained 3 days/week for 8-weeks, whilst CON maintained habitual activity and dietary patterns. Pre- and post-intervention testing included a dual-energy x-ray absorptiometry scan, sub-maximal aerobic capacity (VO_2) and fasting venous blood. Venous blood measures for pro-inflammatory markers included C-reactive protein (CRP), interleukin (IL)-6, IL-1β, tumor necrosis factor-α, and leptin; anti-inflammatory markers included IL-10, IL-1 receptor agonist, and adiponectin.

Results: Both CYC and SSG increased submaximal power output and VO_2 ($P<0.05$), decreased total body fat-mass (TB-FM; $P<0.05$), and CRP (SSG, -0.45 ±0.42 $mg \cdot L^{-1}$; P=0.008; CYC, -0.44 ±0.59 $mg \cdot L^{-1}$; P=0.02). Only SSG increased total body fat-free mass (TB-FFM; +1.1 ±1.2 kg; P=0.001) and decreased concentration of plasma IL-6 (-0.69 ±0.62 $pg \cdot mL^{-1}$; P=0.002) and leptin (-2212 ±2531 $ng \cdot mL^{-1}$; P=0.014).

Conclusion: Cycling and SSG training were both effective at improving CRP, VO_2 and TB-FM. Furthermore, SSG training has also shown to be an effective training approach in reducing IL-6 and leptin and increasing muscle mass within sedentary, middle-aged men.

ABBREVIATIONS

BMI – Body mass index

CON – Control

CRP - C-reactive protein

CVD - Cardiovascular Disease

CYC – Cycling

DEXA - Dual-energy x-ray absorptiometry

GLUT4 - Glucose Transporter 4

GPS - Global Positioning Satellite

GXT - Graded Exercise Test

HR - Heart Rate

HR_{max} - Maximum heart rate

IL - Interleukin

Kp - Kilopond

VO_2 - Oxygen Consumption

Ra - Receptor Agonist

RPE – Rating of Perceived Exertion

RPM – Revolutions per minute

TB-FM - Total Body Fat Mass

TB-FFM – Total-body fat-free mass

TNF-α - Tumor Necrosis Factor Alpha

T2DM - Type 2 Diabetes Mellitus

SSG – Small-sided games

VO_2 – Oxygen consumption

WHR – Waist to hip ratio

INTRODUCTION

Physical inactivity and obesity are risk-factors associated with a heightened systemic inflammatory state and the overall development of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (You et al. 2013; Pradhan et al. 2001). A sub-clinical inflammatory state is evident from elevated plasma concentrations of pro-inflammatory markers such as leptin, interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)-α and C-reactive protein (CRP) (Pradhan et al. 2001; Popa et al. 2007). In response, the anti-inflammatory marker IL-1ra is elevated in an attempt to suppress IL-1β and retain homeostasis of the innate immune system (Chernoff et al. 1995; Petersen & Pedersen 2005). Conversely, anti-inflammatory markers such as adiponectin and IL-10 are known to be associated with improved endothelial function, insulin sensitivity and the inhibition of pro-inflammatory mediators (Bouassida et al. 2010; Arita et al. 1999). However, these anti-inflammatory markers are suppressed in obese and insulin resistance populations, and in coordination with increased pro-inflammatory markers, results in a sub-clinical inflammatory state and potential development of metabolic and cardiovascular abnormalities (Bouassida et al. 2010; Arita et al. 1999).

Long-term physical activity can improve the chronic inflammatory state and protect against the development of chronic disease (Petersen & Pedersen 2005). Participants who engage in high levels of physical activity reportedly have lower basal concentrations of CRP (29%), IL-6 (32%) and TNF-α (20%) when compared to sedentary participants (Panagiotakos et al. 2005). Furthermore, elevated concentrations of IL-6 (>1.80 $pg \cdot mL^{-1}$) and TNF-α (>3.20 $pg \cdot mL^{-1}$) have been shown to be associated with low muscle mass and increased fat-mass (Visser et al. 2002). Given that the chronic inflammatory state is reportedly mediated by the ratio of adiposity to lean muscle mass, the extent to which the exercise mode alters these variables of body composition may have a bearing on altering the inflammatory state (Carson et al. 2012; Fried et al. 1998; Visser et al. 2002). Ongoing engagement in either aerobic (walking, running, cycling) and/or resistance training is proposed to result in reductions in pro-inflammatory (i.e. CRP, IL-6, TNFα, leptin) and an increased anti-inflammatory (i.e. IL-10, adiponectin) markers, although these current findings remain equivocal (Donges et al. 2010; You et al. 2013; Bouassida et al. 2010). Possible reasons for these discrepancies in the inflammatory responses are the divergent adaptations in body composition (i.e. fat mass and lean muscle mass) between the exercise modes (Carson et al. 2012; Fried et al. 1998; Visser et al. 2002).

Small-sided games (SSG) are a popular health promotion initiative within sedentary populations (Krustrup et al. 2009; Krustrup et al. 2010b). For those impartial towards individual aerobic exercise sessions such as stationary cycling, SSG is a group training approach that delivers repeated bouts of high-intensity efforts over prolonged durations (Randers et al. 2010). Training studies which have assessed soccer-specific SSG in sedentary populations have shown positive adaptations in aerobic capacity and body composition (reduced fat-mass and increased lean muscle mass) (Krustrup et al. 2010a), which may have a potential influence on the chronic inflammatory state (Bouassida et al. 2010; Gleeson et al. 2011). However, to our knowledge no studies have assessed the pro- and anti-inflammatory response to rugby-specific SSG training and/or in comparison to traditional training modes such as stationary cycling within a sedentary cohort. The aim of the present study was to assess the training-induced changes in pro- and anti-inflammatory cytokines, aerobic capacity and body composition in response to 8-weeks of SSG, stationary cycling (CYC) or control (CON) conditions. It was hypothesized that both SSG and CYC training will improve the anti- and pro-inflammatory state, body composition and aerobic function.

MATERIALS AND METHODS

Participants

Thirty-three men (Table 1; age 48.6 ±6.6 y; stature 176.7 ±5.9 cm; mass 89.8 ±12.3 kg) were randomly assigned to a stationary CYC (n=11), SSG (n=11) or control (CON, n=11) condition. Participants were recruited through verbal communication and newspaper advertisements within a regional Australian community. Based on verbal communication and the self-reporting of activity patterns, participant recruitment ensured a non-smoking population representative of a sedentary lifestyle (no regular pattern of planned or incidental activity >60 min per week). Participant exclusion criteria extended to those on or having received any medication, flu injections or vitamin supplementations and those clinically diagnosed with any pre-existing CVD, metabolic or inflammatory related disorders. Participants with orthopaedic limitations were advised against involvement due to the musculoskeletal demands of the respective exercise protocols. Prior to pre-intervention testing procedures clearance was obtained from the University Ethics in Human Research Committee and participants attended an information and familiarization session where verbal and written consent for all testing and training procedures was obtained.

Experimental design

Participants attended two pre- and two post-intervention testing sessions. The first testing session comprised of a pre-screening health questionnaire, anthropometry, a dual-energy x-ray absorptiometry (DEXA) scan, and a fasting (10-12 h) blood sample, while the second testing session involved a graded exercise test (GXT). The training interventions consisted of 8 weeks of SSG (modified rugby) or CYC and participants were required to attend 90% of all training sessions for inclusion in the post-training testing. Given the different exercise modes used during respective training programs, it is recognized the inherent difficulties of matching external training load or metabolic cost. However, despite this limitation, in an attempt to match training load between conditions the respective training programs were designed to elicit similar internal training loads. Internal training load was quantified via heart rate (Vantage NV, Polar, Kempele, Finland), which was recorded at 5-min intervals and reported as mean percent of age-predicted (220 – age) maximal heart rate (% HR_{max}) and session-Rating of Perceived Exertion (session-RPE; Borg 6-20 scale), which was collected 10 min after the conclusion of each training session (Foster et al. 2001; Uchida et al. 2014).

Nutrition and physical activity standardization

Prior to pre and post training interventions participants refrained from any physical activity for 72 h, and the consumption of alcohol and caffeine for 24 h. Participants documented dietary and physical activity patterns 24 h prior to pre intervention testing. This diary was photocopied and issued to the participants to ensure diet was replicated for the 24-h period prior to post-intervention testing. Participants were informed of the importance in maintaining their normal dietary and nutritional patterns throughout the 8-week training period. Participants were required to maintain food and beverage type and timing of consumption, including cooking preparation and portion size. Physical activity was standardized to ensure all participants did not engage in any additional planned or incidental physical activity, nor reduce any incidental activity during the 8-week intervention.

Stationary cycling condition

The training program involved participants performing continuous cycling (Monark 828E, Monark Exercise AB, Varburg, Sweden) for three sessions per week (details of external training load and progression are presented in Table 2). To quantify external training load, kilopond (kp), revolutions per minute (RPM) and total distance (km) were recorded at 5-min intervals during each session, with training load progression manipulated through alternate increases in session duration and resistance (kp), respectively.

Small-sided games condition

The SSG condition involved three sessions per week of modified rugby league (most popular football code in this geographical region). Each modified rugby session was played under Touch Football rules (Kennett et al. 2012). The rules allowed each team six 'plays' whilst in possession of the ball; each play required players to pass the ball backwards to an 'on side' team member with the aim to score at opposing ends of the field. Defending players were required to touch their opponent with one hand. Following a successful touch, game play would restart with a 'play the ball', at this time requiring the line of defending players to be 5 m from the position of each 'play the ball' (Kennett et al. 2012). Each training session comprised of 4 x 10 min quarters, interspersed by 2-min passive recovery periods (see Table 2 for details of external training load and progression). To quantify external training load, a Global Positioning Satellite (GPS) device (SPI-Pro, 1 Hz, GPSports, Canberra, ACT, Australia) was worn in a customized harness between the scapulae to quantify total distance (m), mean speed ($m \cdot min^{-1}$) and peak speed ($km \cdot h^{-1}$) of each training session. During the 8 weeks, training load progressively increased via manipulation of session duration and field size, including consistent game rules, verbal feedback and player numbers at 5 v 5 or 6 v 6 (depending on participant availability).

Control condition

The CON condition completed all pre and post-intervention testing sessions and was required to continue their sedentary life and habitual dietary and nutritional patterns for the 8-week intervention period. Participants were provided with a dietary and a physical activity diary that documented any dietary or physical activity changes. Participants received instruction expressing the importance of maintaining these patterns. The chief investigator reviewed the diaries prior to post-intervention testing to ensure individual conformity to the control condition.

Measures

Anthropometry and DEXA

All testing procedures were conducted at a standardized time for each participant between 0600 and 0900 h. Anthropometric measures included stature (Stadiometer, CSU, Bathurst, Australia), body mass on calibrated scales (HW 150 K; A&D, Bradford, MA, USA) and waist and hip girths (EC P3 steel tape Sydney, Australia) for calculation of BMI and WHR (Marfell-Jones et al. 2012). A supine whole body DEXA scan (XR800, Norland, Cooper Surgical Company, USA) was conducted with scanning resolution set at 6.5 x 13.0 mm, and

scanning speed was set at 130 $mm \cdot s^{-1}$. Whole body scans were analyzed (Illuminatus DEXA, ver. 4.2.0, USA) for total body fat-mass (TB-FM) and total body fat-free-mass (TB-FFM).

Graded exercise test

Aerobic fitness measures were obtained via a GXT to determine sub-maximal oxygen consumption (VO_2) and power output at 80% HR_{max}. A sub-maximal GXT was used in preference to maximal testing to minimize risks associated with such methodology in sedentary middle-aged men (Wallman & Campbell 2007). The GXT was performed on an electronically braked cycle ergometer (Excalibur Sport, LODE BV, Groningen, The Netherlands), commencing at 25 W and increasing by 25 W every min. Pulmonary gas exchange was measured by determining O_2 and CO_2 concentrations and ventilation to calculate VO_2 using a metabolic gas analysis system (Parvo Medics, True2400, East Sandy, UT, USA). The system was calibrated according to the manufacturer's instructions. This involved the pneumotachometer calibration using a 3L syringe. The gas analyzers were calibrated using a two-point fully automated process involving room air and gas calibration for fractional gas concentration with a gravimetric gas mixture of known concentrations (CO_2, 4.1 (0.1)%; O_2, 15.7 (0.2)%). Heart rate was continuously monitored and recorded each min throughout the protocol, and participants exercised until attainment of 80% HR_{max}.

Venous blood collection and analysis

A fasting venous blood sample was collected for analysis of TNF-α, IL-6, IL-1β, IL-1ra, IL-10, leptin, adiponectin (Immunoassay ELISA: Quantikine, R & D Systems, Minneapolis, MN, USA) and CRP (Particle enhanced turbidimetric immunoassay: Dimension Xpand Plus, Siemens Healthcare Diagnostics, Sydney, Australia). Intra and inter-assay coefficients of variation were between 2.2-4.9%. Following the clotting of the sample (SST) or immediately following collection (EDTA) samples were centrifuged at 3500 rpm for 15 min at 4°C. Aliquots were frozen immediately at -80°C until further analysis.

Statistical analyses

All data are reported as mean ± SD. Non-normally distributed variables CRP, IL-10, IL-6, TNF-α, IL-1β, leptin and adiponectin were log transformed prior to all analysis. A general linear model two-way repeated measures ANOVA was used to compare conditions over time. Post hoc analysis to determine the location of differences when a significant main effect or interactions were detected was performed using a paired *t-test* with Tukey's

adjustment within conditions over time and between conditions at each time point. Significance was accepted at $P \leq 0.05$. All statistical analyses were performed using PASW™ for MS-Windows version 20.0 (Statistical Package for the Social Sciences, Chicago, IL, USA).

RESULTS

Training load and compliance

Participant numbers for completion of the study were CON (n=11), CYC (n=11) and SSG (n=10). One participant could not complete the SSG training due to a knee injury sustained during training (week 3). Mean weekly training load variables and progression for SSG and CYC conditions are shown in Table 2. Internal training load quantified through mean $\%HR_{max}$ (SSG, 85.2 ±0.3%; CYC, 84.6 ±0.4%; P=0.11) and session-RPE (SSG, 12.4 ±0.1 AU; CYC, 12.5 ±0.1 AU; P=0.64) were not significantly different between conditions. Compliance to both training interventions was also not significantly different between conditions (91 ±2% and 95 ±2% for SSG and CYC, respectively; P=0.13).

Graded exercise test

Results for the GXT are shown in Table 1. Both CYC and SSG significantly increased end stage power output and test duration in the sub-maximal cycling test (P<0.05), which was significantly different to CON condition (condition x time interaction P=0.001). A significant condition x time interaction (P=0.0001) was evident in relative VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$) at 80% HR_{max}. Both CYC (+4.0 ±3.5 $mL \cdot kg^{-1} \cdot min^{-1}$; P=0.002) and SSG (+4.2 ±3.6 $mL \cdot kg^{-1} \cdot min^{-1}$; P<0.001) conditions increased significantly, compared to no change in CON (-1.0 ±1.7 $mL \cdot kg^{-1} \cdot min^{-1}$; P=0.09). Correspondingly, sub-maximal power output increased significantly within CYC (+47.7 ±28.4 W; P=0.009) and SSG (+37.5 ±35.8 W; P=0.001), compared to no change in CON (+2.3 ±13.5 W; P=0.56).

Anthropometry and DEXA

There were no significant changes within or between conditions for measurements of body mass, BMI and WHR (P>0.05; Table 1). TB-FM showed a significant condition x time interaction (P=0.0001). Relative (%) and absolute (kg) TB-FM decreased within both SSG (-3.7 ±4.5% and -0.79 ±0.87 kg; P<0.001) and CYC (-2.9 ±3.5% and -0.75 ±0.84 kg; P<0.001), compared to a significant increase in the CON (3.0 ±3.9% and +0.79 ±1.32 kg; P=0.012) condition. TB-FFM showed a significant condition x time interaction (P=0.017). TB-FFM significantly increased within the SSG (+1.1 ±1.2 kg; P=0.001), compared to the CON (-0.79 ±1.9 kg; P=0.19)

condition, whilst CYC showed no significant change (+0.76 ±1.39 kg; P=0.10) compared to SSG (P=0.855) and CON (P=0.062) conditions.

Fasting blood chemistry

Fasting blood chemistry for inflammatory markers is provided in Table 3. CRP showed a significant condition x time interaction (P=0.001), which showed a significant decrease in SSG (-0.45 ±0.42 $mg \cdot L^{-1}$; P=0.008) and CYC (-0.44 ±0.59 $mg \cdot L^{-1}$; P=0.02), without change in CON (-0.05 ±0.48 $mg \cdot L^{-1}$; P=0.94). IL-6 showed a significant condition x time interaction (P=0.001), which indicated a decrease following SSG (-0.69 ±0.62 $pg \cdot mL^{-1}$; P=0.002), without change within CYC (-0.28 ±0.49 $pg \cdot mL^{-1}$; P=0.06) and CON (+0.13 ±0.51 $pg \cdot mL^{-1}$; P=0.27) conditions. Leptin indicated a significant condition x time interaction (P=0.02) that showed a significant decrease within SSG (-2212 ±2531 $ng \cdot mL^{-1}$; P=0.014), without change in the CYC (642 ±3637 $ng \cdot mL^{-1}$; P=0.96) or CON (356 ±5263 $ng \cdot mL^{-1}$; P=0.46) conditions. No significant changes were evident within or between conditions for TNFα, IL-1β, IL-10, IL-1ra and adiponectin (P>0.05).

DISCUSSION

Sedentary, middle-aged men recruited for the present study were classified as overweight and had an aerobic capacity, CRP and IL-6 concentrations which placed them at 'high risk' of developing metabolic and cardiovascular diseases (Alberti et al. 2006; Pradhan et al. 2001). This study demonstrates both SSG and CYC training in sedentary men are capable of inducing positive inflammatory and body composition adaptations, albeit to differing extents. Specifically, 8-weeks of SSG and CYC training increases submaximal power output and VO_2, decreases in TB-FM and systemic concentrations of CRP. Notably, additional benefits of SSG training include increases in TB-FFM and decreases in basal IL-6 and leptin concentrations.

Moderate-intensity aerobic exercise (i.e. >40 min at ~65% VO_{2peak}) completed over a training program ($\geq$4 weeks) improves aerobic capacity in an intensity and duration dependent manner (Gollnick et al. 1974). Typically, an increase in aerobic capacity is a consequence of increased skeletal muscle substrate (i.e. blood glucose, plasma free fatty acids and intramuscular lipids) utilisation to sustain energy requirements, coupled with the increased oxidative capacity or availability of oxygen (Gollnick et al. 1974). Several separate studies have shown increases in maximal aerobic capacity following SSG (soccer) and CYC training ($\leq$ 12 weeks) of 13% and 10%, respectively (Krustrup et al. 2009; Burgomaster et al. 2008). The present results show a similar

response in aerobic function, however, no previous studies have directly compared the change in aerobic function between these two training methods in sedentary men. In the current study, a significant increase in submaximal VO_2 was evident for both SSG (18.9%) and CYC (19.1%) conditions which was associated with a higher power output and duration to 80% HR_{max} (Table 1). Given the training load and intensity (% HR_{max} and session-RPE) were matched between modes it is not surprising that comparable improvements in aerobic capacity were evident, irrespective of the training mode (SSG or CYC). From a clinical perspective, men with sufficiently high cardiorespiratory fitness ($\geq$35.7 $mL \cdot kg^{-1} \cdot min^{-1}$) report to be two-thirds less likely to develop metabolic syndrome (Laaksonen et al. 2002). As such, improvements in aerobic capacity induced by both SSG and CYC within the present study have the potential for preventing the development of metabolic abnormalities and associated comorbidities (Laaksonen et al. 2002).

A decrease in cardiovascular fitness and the accumulation of body fat increases the chronic inflammatory state and may result in the development of metabolic abnormalities (Gleeson et al. 2011; Ouchi et al. 2011). In the present study both CYC and SSG training resulted in significant decreases in TB-FM in overweight middle-aged men. Previous observations report 12 weeks of SSG (soccer) training results in a significant decrement in TB-FM (3.0%) (Krustrup et al. 2010b). This response in SSG is comparable with cycling training of similar duration, which showed a 3.4% decrease in TB-FM (Donges et al. 2010). The present study showed comparative reductions in TB-FM (SSG at -2.6 ±0.9% and CYC at -2.9 ±1.1%), while SSG was the only training condition that increased TB-FFM (1.1 ±0.3 kg) compared to no change in CYC (0.7 ±0.4 kg) and CON (-0.8 ±0.6kg). These results are consistent with previous reports that show 12 weeks of SSG (soccer) training increases TB-FFM (1.7 ±0.4 kg) (Krustrup et al. 2009), while CYC training in sedentary individuals have shown minimal to no changes in TB-FFM (i.e. -0.6 kg and +0.7 kg) (Donges et al. 2010; Balducci et al. 2010; Samjoo et al. 2013). Eccentric loading is known to stimulate a higher rate of muscle protein synthesis and associated muscular hypertrophy, especially compared to concentric contractions (Coffey & Hawley 2007). Accordingly, it could be assumed the presence of eccentric contractions during running based demands of SSG, that are otherwise absent in CYC, could explain the increased TB-FFM in SSG.

Pro-inflammatory cytokines are secreted from adipose tissue, with an increase in TB-FM associated with an increase in pro-inflammatory cytokines i.e. CRP and IL-6 (Balducci et al. 2010; Fried et al. 1998; Petersen & Pedersen 2005). Consequently, a sedentary lifestyle and excess accumulation of adipose tissue causes elevated

levels of circulating inflammatory biomarkers (Petersen & Pedersen 2005; You et al. 2013; Fried et al. 1998). Typically, chronic systemic concentrations of IL-6 stimulate an acute-phase response through the hepatic secretion of CRP (Gleeson et al. 2011). The relative risk categories represented through mean CRP concentrations associated with developing T2DM are reported as quartile ranges – which include Q1, 0.5 (<1.0), Q2, 1.7 (1.0-2.6), Q3, 4.35 (2.7-6.1), Q4, 9.30 (>6.1) $mg \cdot L^{-1}$, with a relative risk for increasing quartiles of CRP at 1.0, 2.2, 8.7 and 15.7, respectively ($p<0.001$ for trend) (Pradhan et al. 2001).. These data suggest that the relative risk for future diabetes increased 64% per quartile increase in CRP, highlighting the role for inflammation in predicting the development of T2DM (Pradhan et al. 2001). In the present study, CRP values for SSG and CYC conditions started in quartile 4, whilst CON started in quartile 3. Although no change in quartile range was evident in CON condition, both SSG (quartile 2, n=7, quartile 3, n=3) and CYC (quartile 2, n=10; quartile 3, n=1) conditions reduced following training. Furthermore, the relative risk profiles for basal IL-6 association with developing T2DM were placed also in quartile ranges. These ranges include; Q1, 0.968 (<0.909), Q2, 1.133 (0.91-1.382), Q3, 1.646 (1.383-2.050), Q4, 2.709 (>2.050) $pg \cdot mL^{-1}$, with a relative risk for increasing quartiles of IL-6 at 1.0, 2.5, 4.1 and 7.5, respectively ($p<0.001$ for trend) (Pradhan et al. 2001). Again, the current data show that the relative risk for future diabetes increased 28% per quartile increase in basal IL-6 (Pradhan et al. 2001). In the present study all conditions started in quartile 3, however SSG was the only condition to decrease in quartile ranges (quartile 1, n=1; quartile 2, n=8; quartile 3, n=1), indicating that SSG was more effective in producing a positive effect on pro-inflammatory responses.

The chronic adaptations of CRP and IL-6 to continuous, aerobic exercise training (i.e. running, cycling) in obese participants remain equivocal (You et al. 2013; Kohut et al. 2006; Oberbach et al. 2006; Nicklas et al. 2004), while only one study has reported no change in CRP to SSG (soccer) training in a clinical cohort (although was inclusive of smokers, n=5; and those on medications n=2, statins; n=15, antihypertensive medications) (Andersen et al. 2010). Accordingly, the present study supports previous findings which report a reduction in CRP concentration when aerobic capacity is increased and fat mass is decreased (Aronson et al. 2004; Donges et al. 2010). Moreover, high resting plasma concentrations of IL-6 are associated with lower muscle mass, hence, the increase in TB-FFM within the SSG condition could account for the decrease in IL-6 (Visser et al. 2002). Taken together, when compared to CYC and CON, SSG was the dominate training condition for reducing pro-inflammatory cytokines (IL-6 and CRP) alongside increasing TB-FFM and thus representing a reduction in multiple risk-factors associated with the development of T2DM and CVD (Pradhan et al. 2001). The

preservation of lean muscle mass through exercise training can up-regulate glucose regulatory mechanisms in skeletal muscle (i.e. GLUT4 translocation) which can further influence the pro- and anti-inflammatory state to ameliorate metabolic dysfunction (Gleeson et al. 2011; Petersen & Pedersen 2005). As such, increases in lean muscle mass to SSG training may also have additional glucose regulatory effects at a systemic and skeletal muscle level; however, future research is required to test this hypothesis.

The first two cytokines produced in the pro-inflammatory cytokine cascade are TNF-α and IL-1β, and in addition to IL-6, stimulate the hepatic production of CRP (Petersen & Pedersen 2005). Despite a reduction in IL-6 and/or CRP in the present study, there were no changes in TNF-α or IL-1β. Previous research reporting the response of TNF-α and IL-1β with exercise training provide equivocal findings in either healthy or diabetic participants (Kadoglou et al. 2007; Balducci et al. 2010). The present results suggest that 8 weeks of exercise training resulting in reduced TB-FM were not adequate to mediate changes in TNF-α or IL-1β. Recently, Fernández-Riejos et al. (Fernández-Riejos et al. 2010) demonstrated a dose-dependent leptin-induced stimulation of pro-inflammatory cytokines TNF-α and IL-6 by monocytes; although, many training studies have reported that leptin is only decreased in accordance with reduced fat-mass (Pasman et al. 1998; Kraemer et al. 2002). In the present study, SSG was the only condition to reduce leptin concentrations, even though both SSG and CYC reduced TB-FM. Although speculative, these results suggest that the change in leptin may have occurred independently to the relatively small changes in fat-mass and consequently, a myriad of other mechanisms such as increased lean muscle mass (Visser et al. 2002) glucose regulation (Silha et al. 2003), and/or changes in other pro-inflammatory cytokines (Fernández-Riejos et al. 2010) may be involved. Accordingly, future research may be required to further investigate the contribution of reduced fat-mass and increased lean-muscle mass to changes in leptin concentration.

An imbalance of pro- and anti-inflammatory cytokines secreted from adipose tissue contribute to metabolic dysfunction (Ouchi et al. 2011; Arita et al. 1999). Adiponectin is another hormone secreted by adipocytes which stimulates an increase in the anti-inflammatory cytokines IL-10 and IL-1ra in monocytes and macrophages, while inhibiting systemic levels of IL-6 and TNF-α (Ouchi et al. 2011; Bouassida et al. 2010). The expression of adiponectin protects against metabolic and cardiovascular disorders and is decreased in plasma and adipose tissue in obese, compared to lean individuals (Ouchi et al. 2011; Carson et al. 2012). The current study showed no change in anti-inflammatory markers (adiponectin, IL-1ra and IL-10) in response to SSG or CYC training,

despite the observed reductions in IL-6 and/or CRP noted earlier. Furthermore, previous reports suggest a strong positive correlation between the change in IL-1ra and change in lean muscle mass (Meier et al. 2002), although this was not evident in the current study, which showed no change in IL-1ra despite an increase in TB-FFM in the SSG condition. Moreover, previous exercise training studies report inconsistent results regarding adiponectin and IL-10 (Kadoglou et al. 2007; Balducci et al. 2010; Bouassida et al. 2010). These studies document an increase in IL-10 which was related to a reduction in fat-mass, and no change in adiponectin, or no change in either variable in response to 6 and 12 months of aerobic exercise training (Balducci et al. 2010; Kadoglou et al. 2007), respectively. Although exercise alone has reported increases in adiponectin and IL-10, diet interventions in combination with exercise have been shown to increase these markers more robustly (Bouassida et al. 2010). As such, the current study shows 8-weeks of CYC or SSG does not stimulate an anti-inflammatory response and this may be due to the uncontrolled diet and calories intake within the current study (Bouassida et al. 2010)

Limitations

Despite the potential benefit of exercise for reduction of chronic systemic inflammatory markers, some limitations in the present study should be acknowledged. Firstly, the number of participants was small and the underpowered nature of the data is accepted as a limitation. Further, a sub-maximal VO_2 test was conducted and thus the ability to compare results to previous studies, which measure VO_{2max} is compromised – though unlikely to affect the findings of the current study. Additionally, the use of age-predicted (220bpm – age) HR_{max} may create variability in training load between participants and the determination of sub-maximal VO_2 during the GXT. However, it should be noted that the use of age-predicted HR_{max} and sub-maximal VO_2 are sensitive to experimental manipulation. Finally, the knee injury sustained as results from SSG training is a limitation of this training method.

Conclusions

This study reports 8 weeks of SSG or CYC training increased aerobic capacity, reduced CRP concentration, and fat-mass. In addition, SSG training was the only condition to increase TB-FFM, and decrease pro-inflammatory markers, leptin and IL-6. These changes in body composition and pro-inflammatory markers were not associated with anti-inflammatory markers IL-10, IL-1ra or adiponectin, which showed no change in response to exercise training. Collectively, while CYC training was effective at reducing several risk-factors, the differences

between conditions show SSG to be a more effective training approach in reducing the inflammatory risk profile (CRP and IL-6) and increasing muscle mass within sedentary, middle-aged men.

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