Abstract:

**Purpose:** This study examined post-exercise inflammatory and leukocyte responses in smokers and non-smokers, as well as the effects of cigarette smoking on the acute post-exercise inflammatory and leukocyte response in habitual smokers. **Method:** Eleven, recreationally-active, male smokers and eleven non-smokers, matched for age and aerobic fitness (23.2±3.04 & 24.0±2.41 years and 36.9±7.95 & 36.4±7.12 mL.kg\(^{-1}\).min\(^{-1}\) VO\(_{2}\text{peak}\) respectively) were familiarized and underwent baseline fitness testing. Participants then completed 40 min of cycling at 50% peak aerobic workload. Smokers performed two randomized exercise sessions, including an acute post-exercise smoking (two cigarettes in 15 min of 12 mg tar and 1 mg nicotine) and no-smoking condition, while non-smokers performed one exercise session without smoking. Venous blood was obtained pre- and post-exercise for analysis of interleukin (IL)-6, IL-1 receptor antagonist (ra), tumor necrosis factor-alpha (TNF-α) and c-reactive protein (CRP). **Results:** No differences existed between groups for resting CRP (d= 0.25-0.46; p=0.374-0.617). Despite no baseline difference (d= 0.03-0.07; p=0.149), exercise-induced increases were observed for IL-1ra in smokers (d=0.50; p=0.024-0.033), which was not observed in the never-smoker group. No between-group difference was observed for IL-6 across all points (d=0.09-0.5; p=0.102-0.728); however, all groups observed significant within-group change (d=0.27-1.09; p=0.001-0.042). Further, TNF-α for smokers-smoking was elevated above both smokers-no smoking and non-smokers at baseline (SNS) and across the protocol (d=1.20-1.80; d=0.20-1.0; p=0.001-0.035). Additionally, a marked post-exercise increase in leukocyte and neutrophil concentrations was evident in smokers-smoking compared to non-smokers and smokers-no smoking as indicated by a moderate to large effect size (d=0.72; d=0.78). **Conclusion:** Consequently, male smokers exhibit an altered post-exercise pro-inflammatory profile compared to age and fitness-matched non-smokers.
**Key Words:** Cycling; Inflammation; Tobacco smokers; Cytokines.
The adverse effects of tobacco smoke are associated with the delivery of many carcinogenic and cytotoxic stimuli (Domagala-Kulawik, 2008; Lee, Taneja, & Vassallo, 2012). Whilst the detrimental effects of long-term cigarette smoking are commonly reported in middle- and older-aged groups, the highest prevalence rates are often observed in young adult groups (Australian Bureau of Statistics, 2006; White, Siahpush, & Bobevski, 2003). Further, despite research focus on the pulmonary consequences of cigarette smoking, the injurious effects of cigarette smoking on endothelial function, cardiovascular physiology and the immune system are also of high importance. The development of systemic injury (Blann, Kirkpatrick, Devine, Naser, & McCollum, 1998) and subsequent systemic inflammation are important precursors for the development of chronic diseases such as cardiovascular disease (CVD) and diabetes. Thus, given the renowned physiological consequences of smoking, and the potential for early intervention, further investigation into the cigarette smoke-induced changes to the inflammatory profile in young smokers is warranted.

It is well established that habitual cigarette smoking results in the development of a low grade systemic inflammatory state, consistent with a dose and duration dependent fashion of consumption (Frohlich et al., 2003; Tracy et al., 1997). The complexity of the composition of cigarette smoke presents a challenge in understanding both the pro-inflammatory and immunosuppressive actions of mainstream cigarette smoke (Gonçalves et al., 2011; Lee, Taneja, & Vassallo, 2012). Cigarette smoking modifies immune and inflammatory processes (Stampfli & Anderson, 2009), particularly inhibiting natural killer cell activity and creates an imbalance between pro- and anti-inflammatory cytokines – likely to impede the ability for a normal immune response (Moszczynski et al., 2001; Zeidel et al., 2002). Contrastingly, exercise training is reported as an effective therapeutic tool that produces favourable health outcomes, including improved endothelial and respiratory
function in a smoking population (Rooks, McCully, & Dishman, 2011), and is further reported to impose positive effects on immune function (Gleeson, 2007). An acute bout of exercise is accompanied by an influx of anti-inflammatory cytokines (interleukin [IL]-6 and IL-1 receptor antagonist [ra]), the magnitude of which is dependent upon modality, intensity and duration (Gleeson, 2007). These exercise induced elevations in anti-inflammatory cytokines, ie. IL-1ra, may provide the mechanism for long term protection against chronic diseases (Fischer 2006; Gleeson, 2007; Gleeson et al., 2011).

However, despite current knowledge of the potent pro-inflammatory profile reported to result from chronic cigarette smoking, and the anti-inflammatory effects of exercise (Petersen & Pedersen, 2005; Thatcher, 2005) there is limited literature on the anti-inflammatory response to acute exercise in a smoking group. Given the absence of such research, it is important to draw upon insight from research on the effects of second hand smoke exposure on exercise responses. Accordingly, secondhand smoke exposure prior to exercise is suggested to compromise the immune system, resulting in the up-regulation of inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin (IL)-4, IL-5, IL-6 and interferon-gamma (IFN-y) (Flouris et al., 2010; Flouris et al., 2012). Further, secondhand smoke exposure following exercise results in changes in cardiovascular physiology and compromises respiratory parameters; (Flouris et al., 2010; Flouris et al., 2012; McMurray, Hicks & Thompson, 1985; Pimm and Silverman, 1978).

Given the potent pro-inflammatory profile reported to result from chronic cigarette smoking, and the anti-inflammatory effects of acute exercise (Petersen & Pedersen, 2005; Thatcher, 2005), no previous studies have determined the anti-inflammatory response to acute exercise in a smoking group, which may provide indication of the effects of the
immune-inflammatory changes that accompany smoking and their effects on the exercise response. Additionally, no studies have examined the inflammatory profile (IL-6, IL-1ra, TNF-α, CRP) induced from an acute bout of exercise alongside acute cigarette smoke inhalation. It remains unknown as to whether the pro-inflammatory response to smoking (i.e. TNF-α) blunts the anti-inflammatory exercise-induced responses (IL-1ra) observed in young active smoking groups, and in turn may further highlight the smoking-induced changes to chronic systemic inflammation at a young age. Accordingly, this study aims to compare the acute post-exercise inflammatory and leukocyte responses in young adult smokers and non-smokers. A further aim is to examine the effects of cigarette smoking on the post-exercise inflammatory responses following an acute bout of exercise in young habituated smokers. It is hypothesized that smokers would exhibit elevated pro-inflammatory cytokine concentrations than non-smokers (Frohlich et al., 2003) and to the immunosuppressive effects of cigarette smokes (Arnson, Shoenfeld, & Amital, 2010) that smokers will exhibit a suppressed anti-inflammatory response to exercise.

Methods

Participants

The study group consisted of 22 recreationally active male participants who were either smokers (n=11) or non-smokers (n=11) (characteristics in Table 1). The non-smoker participants were classified as having never smoked, and were otherwise healthy individuals who reported modest regular physical activity (2-3 days of 30 min per week). Specifically, all participants reported undertaking no strenuous activity, and only light-to-moderate intensity exercise per week. Participants reported to be free from any known metabolic, cardiovascular or pulmonary disease, immunological irregularities or other conditions (i.e.,
recent influenza or surgery, periodontal disease, etc.) associated with systemic inflammatory responses. Any participant that was confirmed as having these conditions, or taking anti-inflammatory or any other potentially confounding medications were excluded from this study. Smokers were classified as current active smokers, smoking no more than 1 pack per day. Participants were matched based on their comparative age and aerobic fitness in accordance with their smoking status, with anthropometric and descriptive baseline values reported in Table 1. The self-reported smoking history for the smoker group was $6.9 \pm 1.3$ yr of smoking and $12.9 \pm 2.1$ cigarettes per day. Smoking participants engaged in comparable levels of recreational physical activity as the aforementioned non-smokers based on qualitative feedback regarding exercise engagement. Prior to the commencement of the study all participants were required to provide written and verbal consent following an outline of all procedures and measures. This study conformed to the Declaration of Helsinki and was approved by the Research in Human Ethics Committee at the University.

### Baseline Testing

Participants completed a Physical Activity Readiness Questionnaire and a healthy history questionnaire, and if satisfying the above study inclusion criteria, were recruited into the study. Participants abstained from strenuous physical activity for 48 h prior, further abstained from all physical activity for the 24 h prior to baseline testing and exercise protocol, respectively, with all consumed food and beverages documented in a diary provided by the research team. Moreover, for the 10 h prior to baseline testing and the exercise protocol, participants avoided alcohol consumption and abstained from cigarette smoking and caffeine. Further, smokers avoided all passive or active consumption of cigarette smoke during the post-exercise data collection period (up to 3 h post) and for 10 h prior to the 24 h
Prior to arriving at 0700 h, participants consumed (at 0500 h) 50 g of a nutritional supplement (Sustagen, Sport Chocolate, Mead Johnson Nutritionals, Nestle) in 300 ml of milk to standardise carbohydrate intake. Although it is recognised carbohydrate may affect inflammatory response, the low amount and controlled intake ensured standardised dietary intake across protocols. Upon arrival, anthropometric measures were obtained, including stature (Stadiometer: Custom CSU, Bathurst, Australia), body mass (HW 150 K, A & D, Bradford, MA, USA), and waist and hip circumferences (steel tape, EC P3 metric graduation, Australia). In addition, a supine dual-energy x-ray absorptiometry (DXA) was conducted for the determination of body composition (XR800, Norland, Cooper Surgical Company, Trumbull, CT, USA). Scanning resolution and speed were set at 6.5 x 13.0 mm and 130 mm s\(^{-1}\), respectively. Whole body scans were analyzed (Illuminatus DXA, ver. 4.2.0, USA) for total body lean mass and total body fat mass and are reported in absolute and relative terms. Following spirometry, participants completed a GXT on an electronically-braked cycle ergometer (LODE Excalibur Sport, LODE BV, Groningen, The Netherlands) to determine VO\(_{2}\text{peak}\). The incremental test began at 100 W and increased by 25 W every min until volitional exhaustion and/or attainment of maximal heart rate (HR\(_{\text{max}}\)). 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 3 L 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)%).

**Exercise Protocol**
The respective groups (smokers or non-smokers) underwent different testing formats. The smokers group completed two aerobic exercise protocols in a randomized cross-over design that were at a standardized time of day (0700 h) and were separated by a one week recovery, either with or without post-exercise cigarette smoking. Conversely, the non-smoker (NS) group only completed a singular exercise session. The exercise protocol completed by both smokers and non-smokers consisted of 40 min of stationary cycle ergometry (Monark 828E, Monark Exercise AB, Varburg, Sweden) at 50% of VO\textsubscript{2peak}. The workload was calculated as 50% of the pedalling resistance (W) achieved during the GXT and was converted into kilopond units and set as a fixed intensity for the exercise protocol. The selection of this exercise protocol was based upon previous research (Mendham, Donges, Liberts & Duffield, 2011) which demonstrated an inflammatory response to an acute bout of exercise of the same intensity and duration as the current study. Telemetry-based heart rate (HR) (Vantage NV, Polar, Finland) and rating of perceived exertion (RPE) (Borg CR10 scale) were recorded every 5 min during the exercise protocol and a session RPE was obtained 30 min post-exercise.

**Smoking Protocol**

Cigarette consumption was randomized in a cross-over and counter-balanced design within the smoking group only. Following the exercise protocol, on one occasion participants were required to consume two cigarettes of the same brand (Winfield Blue, 12 mg tar, 1.0 mg nicotine). Following post-exercise venous blood collection (~5 min), participants immediately smoked the two cigarettes within 15 min and were avoided any secondhand smoke exposure. During this period participants were encouraged to inhale deeply and consistently, with adequacy of smoking ensured by visual observation by the research team in order to standardise consumption. That said, it must be noted that the smoking protocol
would not be considered “normal” smoking behavior. The smoking protocol was chosen based upon previous research published by Van der Vaart et al., (2005) who reported in their methods two cigarettes of the same brand within 30 min and were encouraged to inhale deeply. Given the lack of active smoking research, this was the guideline for selection of an acute smoking protocol and was adjusted based upon the selected group (young habitual cigarette smokers). Following the consumption of the cigarettes, and in both the no-smoking and non-smokers conditions, participants passively rested until 3 h post-exercise blood collection.

**Venous Blood Procedures**

Venous blood samples were collected pre-exercise, and immediately post, 30 min, 3 h and 24 h after the exercise protocol. Accordingly, a 21GA catheter was inserted into a medial antecubital vein and a 40 ml sample was drawn and aliquoted into SST for analysis of blood lipid profile and CRP, and EDTA tubes for analysis of inflammatory cytokines, glycosylated haemoglobin (HbA1c) and total and sub-population leukocyte count. EDTA tubes used for cytokine analysis were centrifuged immediately post-aliquot at 3500 rpm for 15 min at 4°C, whilst SST tubes were left to clot at room temperature for 20 min prior to centrifugation. Supernatants were immediately stored at -80°C or -20°C for EDTA and SST, respectively. Total and sub-population leukocyte count and HbA1c were kept refrigerated determined within 4 h of venous blood collection. Blood samples were analyzed for IL-6, IL-1ra, TNF-α, total cholesterol, triglycerides, high density lipoprotein (HDL), HbA1c, total and sub-population leukocyte count and CRP. All biochemistry variables were analyzed in duplicate according to manufacturer’s instructions. Total cholesterol was analyzed using an enzymatic method and polychromatic endpoint technique measurement. HDL cholesterol was measured using accelerator selective detergent methodology. Triglycerides were assessed using an
enzymatic method and biochromatic endpoint technique measurement (Dimension Xpand Plus, Siemens Healthcare Diagnostics, Sydney, Australia). HbA1c was measured using automated high-performance liquid chromatography (HPLC) methodology (Bio-Rad Variant, Sydney, Australia). CRP concentrations were determined using a solid-phase chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corp., Los Angeles, USA). Concentrations of IL-6, IL-1ra and TNF-α were determined through a sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) according to manufacturer’s instructions. Intra and inter-assay coefficients of variation for all analytes were between 1.6 – 6.9%.

Statistical Analysis
Normal distribution was determined by Shapiro-Wilk’s test and non-normally distributed data (IL-6) was logarithmically transformed prior to analysis. All data are reported as mean ± standard deviation (SD). Repeated measures analysis of variance (ANOVA) (condition x time) was used to determine within- and between-group differences. Where a main effect was noted, one-way ANOVA tests were applied to determine the source of statistical significance. Further, a covariate analysis (ANCOVA) was conducted with baseline inflammatory markers and body fat as the covariates. Significance was accepted at p<0.05. All statistical procedures were performed using Predictive Analytic Software (PASW) (Statistical Package for the Social Sciences for Windows version 18.0, Chicago, IL, USA). Standardized effect sizes (ES; Cohen d) analyses were used in interpreting the magnitude of differences between groups and conditions. An ES was classified as trivial (<0.20), small (0.21–0.50), moderate (0.51–0.89), or large (>0.90). An a-priori power analysis was completed using G*Power (G*Power for Windows, version 3) based upon data obtained from previous similar studies (Mendham et al.
Results

Baseline variables for body composition, blood lipid profile and anthropometric variables are reported in Table 1. The smoker group demonstrated lower waist and hip circumferences and percentage fat mass than the never-smoker group \((d=0.87-1.32; p<0.05)\). There were no differences between groups for age or \(\text{VO}_{2}\text{peak}\) \((d=0.06; p>0.05)\), although the never-smoker group were heavier and demonstrated increased absolute fat mass \((d=0.87; p<0.05; \text{Table 1})\). Exercise-induced HR responses (% of \(\text{HR}_{\text{max}}\)) were not significantly different between groups \((79 \pm 3\%, 78\pm3\%, 80 \pm 2\% \text{ for smokers- no smoking, smokers-smoking and non-smokers respectively; } p>0.05)\). Furthermore, there were no significant differences in session RPE between groups \((4.7 \pm 0.5, 4.6\pm0.5, 5.1 \pm 0.4 \text{ for smokers- no smoking, smokers-smoking and non-smokers, respectively } d=0.10-0.36; p>0.05)\).

The inflammatory responses of IL-6, CRP, IL-1ra and TNF-\(\alpha\) are presented in Figure 1. There were no baseline differences between groups for IL-6, IL-1ra or CRP \((p>0.05; d=0.02-0.65)\). In response to the exercise protocol, an increase in IL-6 was evident in all conditions \((d=0.64-1.30; p<0.05)\). For smokers- no smoking, IL-6 concentration peaked at 30 min post-exercise \((d=0.27; p<0.05)\), although in the smokers-smoking condition a significant decline in IL-6 from 30 min to 24 h post exercise \((d=0.64; p<0.05)\) was noted. Smokers-smoking and smokers- no smoking observed an exercise-induced increase immediately post-exercise in IL-1ra values \((d=0.50; d=0.50; p<0.05)\), not observed in non-smokers. Additionally, smokers-smoking had elevated concentrations from pre, 30 min and 3 h post-
exercise, as indicated by moderate-large effect sizes ($d=0.37$; $d=0.80$; $p<0.05$). For non-smokers, IL-1ra concentrations peaked at 3h followed by a decline to pre-values at 24 h post-exercise ($d=1.04$). Smokers-smoking had elevated baseline concentrations of TNF-α compared to that of non-smokers and smokers- no smoking ($d=0.60$; $d=1.28$; $p<0.05$). Post-exercise elevations in TNF-α were observed in smokers-smoking and non-smokers as denoted by moderate effect sizes ($d=0.47$; $d=0.57$), with smokers-smoking experiencing a decline at 30 min ($d=0.10$; $p<0.05$). Concentrations of TNF-α for non-smokers peaked at 3h ($d=1.89$; $d=1.33$; $p<0.05$), which were not observed in smokers- no smoking or for smokers-smoking. Further, TNF-α responses in smokers-smoking were elevated above both smokers-no smoking and non-smokers across all time points ($d=1.20$-$1.80$; $d=0.20$-$1.0$; $p<0.05$). CRP concentrations were not different between groups at baseline or post-exercise ($d=0.05$-$0.72$; $p>0.05$), despite a significant increase in CRP from pre- to 24h post-exercise in the smokers-no smoking condition ($d=0.68$; $p<0.05$). Finally, although IL-8 was analyzed, all measures resulted in values below the minimum detectable range of the ELISA kit and hence were excluded from statistical analysis.

Baseline total leukocyte count did not differ between conditions ($d=0.50$; $0.30$; $0.05$; $p>0.05$). Post-exercise elevations were observed in all conditions for total leukocyte count, neutrophils, basophils, lymphocytes and monocytes ($p<0.05$; Figure 2). Total leukocyte count and neutrophil concentration peaked at 3 h post-exercise for all conditions, without significant differences and with small effect sizes between smokers- no smoking and non-smokers ($d=0.18$; $p>0.05$). However, moderate to large effect sizes suggested the smokers-smoking condition resulted in a greater total leukocyte and neutrophil count than smokers- no smoking and non-smokers at 3 h ($d=0.72$; $d=0.78$). A decline in neutrophil concentration at 30 min was observed in smokers- no smoking ($d=0.57$; $p<0.05$), but not in non-smokers. The
smokers- no smoking group exhibited a post-exercise increase in eosinophils, \((d=0.30; p<0.05)\), with no significant difference and trivial effect sizes in the never-smoker group \((d=0.01; p>0.05)\). Values for eosinophils, basophils were lower than pre-values at 3h for smokers-smoking, smokers- no smoking and non-smokers \((d=0.90; d=1.07; d=0.56; p<0.05)\). Despite post-exercise elevations in monocytes in both conditions there were no significant differences between groups \((d=0.24; 0.06; 0.19; p>0.05)\). All immunological markers returned to baseline in both conditions by 24h post-exercise \((p>0.05)\).

**Discussion**

Given the contrasting inflammatory responses to cigarette smoking and exercise, the aim of this study was to compare the acute post-exercise inflammatory and leukocyte responses in young adult smokers and non-smokers. An additional aim was to examine the effect of cigarette smoking on post-exercise inflammatory responses in young smokers. Accordingly, the main findings from this study revealed that despite the young age (~23yr old), cigarette smokers already exhibit an abnormal immune-inflammatory response to exercise compared to age- and fitness-matched never-smoked controls. Even in the absence of acute exposure to cigarette smoke, young smokers’ exhibit altered exercise-induced leukocyte responses, as observed by elevated eosinophil and suppressed neutrophil responses compared to non-smokers. Further findings from this study suggest that acute cigarette smoking elevates the post-exercise total leukocyte and neutrophil responses in young adult smokers. Such responses are previously highlighted as risk factors associated with future systemic and pulmonary disease development and the present data highlights the role of acute effects of exercise in potentially mediating such risks (Frohlich et al., 2003; Petersen & Pedersen, 2005).
The present study suggests that young adult smokers have a baseline inflammatory profile comparable to that of their age- and fitness-matched young adult never-smoking counterparts. Consequently, the relatively short smoking history may not be sufficient to exacerbate resting inflammatory profiles, as observed in long term smokers (Kuschner, Alessandro, Wong & Blanc, 1996; Tracy et al., 1997). Kuschner et al. (1996) reported that middle-aged smoker’s exhibit higher TNF-α concentrations than non-smokers, further Tracy et al. (1997) reported elevated concentrations of CRP as a result of a lifetime of smoking, which may suggest that chronic inflammatory states are associated with smoking duration and dependence. In the present study there were no observed baseline differences in concentrations of CRP, IL-6 or IL-1ra; however, concentrations of TNF-α in the smokers-smoking condition were elevated compared to other conditions, which may present as a limitation to this study. Further, it should be noted that to match fitness and age between respective groups, the non-smokers were heavier and had greater waist and hip circumferences. An implication here is that the non-smokers had higher adiposity, and in turn higher adiposity has been reported to relate to exacerbated pro-inflammatory responses (Maury & Brichard, 2010). However, despite a noted main effect for body mass during covariate analysis for IL-6, IL-1ra and TNF-α, the non-smokers did not demonstrate higher basal inflammatory values, suggesting differences in adiposity between groups had minimal influence on the inflammatory markers at rest and following the protocol.

All conditions observed a small to moderate increase in post-exercise IL-6, which is consistent with previous literature suggesting IL-6 is sensitive to exercise intensity and duration (Gleeson et al., 2011; Petersen & Pedersen, 2005). A marked increase in IL-6 following exercise has been consistently reported to confer anti-inflammatory properties, which in turn is reported to induce a cascade of anti-inflammatory cytokines including IL-1ra and IL-10 (Petersen & Pedersen, 2005). Whilst both groups (smokers and non-smokers)
presented increased IL-6 responses post-exercise as represented by moderate effect sizes, the expected continued post-exercise elevation in IL-1ra was observed in smokers- no smoking and smokers-smoking, with no elevations observed in non-smokers. Further, smokers-smoking and non-smokers observed a peak in concentrations of IL-1ra at 3h, which was not observed in smokers- no smoking - suggesting an acute dose of cigarette smoke is sufficient to induce inflammatory changes in the smoker group. However, in the absence of an additional inflammatory stimulus such as cigarette smoke, smokers may exhibit a suppressed immune response to exercise, as indicated by the IL-1ra response of the smokers- no smoking group.

Similarly, although concentrations of TNF-α in the smokers-smoking group were elevated, as denoted by a large effect, across all time points when compared to smokers- no smoking, non-smokers observed an increase in TNF-α at 3 h post-exercise, which was not observed in smokers- no smoking. In agreement with previous research in healthy non-smoking groups (Ostrowski, Rohde, Asp, Schjerling, & Pedersen, 1999) an elevation in TNF-α following moderate to high intensity cycling was observed in non-smokers. Further, the elevated concentrations of TNF-α across all time points for smokers-smoking when compared to smokers- no smoking suggest that the acute stimulus of cigarette smoke induces an influx of TNF-α, which may be a potent contributor to the elevated concentrations of TNF-α associated with long term cigarette smoking (Kuschner et al., 1996). Although these findings are indicative of an elevated inflammatory state, the results observed in the current study may be amplified to that of real life situation due to the large dose of cigarette smoke delivered (two cigarettes within 15 minutes). Further, it must be noted that the dose delivered in the current study may not be consistent with regular smoking behavior. Regardless, such findings demonstrate that the insult of cigarette smoke may adversely affect the inflammatory profile of young habitual cigarette smokers.
The concurrent peaks in concentrations of TNF-α and IL-1ra highlight the complexity of the anti- and pro-inflammatory interaction between acute smoking and acute exercise. The elevation in TNF-α following acute cigarette smoking signifies a pro-inflammatory stimulus, following the presence of a pro-inflammatory factor, is the simultaneous release of a cytokine inhibitor IL-1ra (Pedersen, 2000) to counteract the pro-inflammatory state, thus the similar profiles of TNF-α and IL-1ra in the present study. However, it must be noted that smokers- no smoking, in the absence of a pro-inflammatory stimulus such as acute cigarette smoking, exhibit a suppressed inflammatory response to exercise. Despite no baseline immune-inflammatory differences, such a finding may be an early indication of the future altered responses to immune and inflammatory function observed in long term smokers (Zeidel et al., 2002). As these data represent novel findings of the interaction between smoking and exercise, the only comparable context relates to the effects of secondhand smoke exposure. Flouris et al. (2012) reported the effects of secondhand smoke exposure followed by physical activity in 16 non-smokers and found elevations in inflammatory cytokines IL-4, TNF-α and IFN-γ. Further, early research by Pimm and Silvermann (1978) suggested that physical activity following secondhand smoke exposure elevated cardiovascular demands when compared to no smoke exposure. Consistent with Flouris et al. (2012) the present study observed an elevation in TNF-α as a result of cigarette smoke exposure, suggesting that cigarette smoke impedes normal immune-inflammatory processes following exercise.

The effects of cigarette smoke on inflammatory mediators is particularly complex, the diversity of compounds contained with cigarette smoke present both immunostimulatory and suppressive actions (Sopori, 2002). Whilst the literature is lacking in vivo human models, in
vitro and murine models suggest that exposure to cigarette smoke elevates pro-inflammatory markers such as TNF-α (Churg et al., 2003), although others have reported no effect of acute cigarette smoking (Van der Vaart et al., 2005). Nicotine, the component of cigarette smoke responsible for addiction (Jain & Mukherjee, 2003), is suggested to exert anti-inflammatory actions via α7-nicotinic acetylcholine receptors (Park et al., 2007), which may explain the elevation in IL-1ra at 3h post-exercise, not observed in the smokers-no smoking condition. Contrastingly, the less pronounced inflammatory responses in smokers-no smoking may be a result of the modification of the HPA axis in smokers (Rohleder & Kirschbaum, 2006). Chronic exposure to cigarette smoke results in significant alterations to the responsiveness of the HPA-axis, which is an important regulator of inflammation (Rohleder & Kirschbaum, 2006). Further, a shift in the T-helper (Th)-1 and T-helper(Th)-2 cytokine balance hypothesis may explain the less pronounced inflammatory response, which suggests the regulation of the immune system as maintained by Th-1 and Th-2 activity, is altered by chronic smoking (Kidd, 2003; Mehta, Nazzal & Sadikot, 2008). Although these mechanisms were not measured here, the present findings suggest that habitual cigarette smoking may alter the exercise-induced inflammatory response in young male smokers. Whilst immunological markers are reported to be acutely increased in response to exercise in an intensity-dependent manner (Pedersen & Hoffman-Goetz, 2000), previous studies report habituated cigarette smokers to exhibit elevated baseline concentrations of leukocytes as a result of chronic cigarette smoking (Frohlich et al., 2003). Garey, Neuhauser, Robbins, Danziger, & Rubinstein (2004) suggest the increased immunological chemotactic activity in smokers is representative of a state of elevated inflammation, characterised by intense neutrophilic infiltration into the airway mucosa. In the present study, exercise increased immunological markers in all conditions; however, despite a matched exercise duration and
intensity, a moderate systemic response to cigarette smoking was observed in both leukocyte and neutrophil responses 3h post-exercise compared to smokers- no smoking. Further the present study also reports smokers- no smoking to exhibit elevated concentrations of leukocytes post-exercise than never- smokers, which suggest that smokers may present a heightened leukocyte response to an exercise stimulus.

Additionally, although measured in sputum in a rested state, Van Der Vaart et al. (2005) reported neutrophil concentrations increased in response to acute cigarette smoking. Further, Blann et al. (1998) reported that acute cigarette smoke exposure activates leukocyte activity, causing endothelial damage and contributing to the development of systemic inflammation. Such exacerbated immunological responses in the pulmonary system are suggested to result from the noxious stimuli of cigarette smoke, although in the present study these responses were noted in the circulatory system, in addition to the exercise-induced.

Though exercise is known to result in an immediate elevation in immunological markers, the present study further corroborates an acute smoking induced increase in systemic concentrations of leukocytes and neutrophils. Accordingly, even in a post-exercise state, exposure to cigarette smoke results in elevated leukocyte responses compared to exercise alone in young habituated smokers, suggesting that a relatively short smoking history is sufficient to modify host defense responses to exercise.

Despite these findings certain limitations must be acknowledged. Firstly, it should be noted that the smoking protocol (2 cigarettes within 15min) in some cases may not be considered “normal” smoking behavior. Regardless, such procedures allowed the comparison of a standardised and sufficient cigarette dose. Moreover, while the authors attempted to standardize tobacco smoke exposure, following the 3h measures, smoking was not controlled
until a 10h abstinence prior to the 24h sample, and we also recognize this period of uncontrolled time as a limitation. However, it must be stated that denying active smokers from engaging in cigarette consumption for 24h is highly unlikely to garner adherence to such requests. Additionally, given the small sample size, it is acknowledged such underpowered results may present as a limitation for deterministic conclusions. Finally, the non-smokers demonstrated greater absolute and relative fat mass, whereby the implication being a higher adiposity may relate to exacerbated pro-inflammatory states (Maury & Brichard, 2010). However, with marginal clinical differences and within normal ranges, we would suggest such factors are unlikely to explicitly affect inflammatory responses to smoking or exercise.

Conclusions

In conclusion, this study investigated the effect of acute exercise and acute cigarette smoking on the inflammatory responses in young adult male smoker and non-smokers. Although there were no baseline differences between groups, results indicated that smokers-no smoking and smokers-smoking exhibit abnormal immune-inflammatory responses to exercise. Whether such responses can be attributed to a modification of the HPA-axis or the anti-inflammatory effects of some of the compounds found in cigarette smoke requires further investigation. The present study also suggests that even a relatively short period of habitual smoking is sufficient to induce alterations to the inflammatory profile in young cigarette smokers. Given the reported benefits of exercise to public health and well-being, further investigation into the pro- and anti-inflammatory relationship between chronic cigarette smoking and exercise may determine whether the anti-inflammatory effects of exercise may potentially reduce or inhibit pulmonary and systemic disease processes.
What does this article add?

The findings from the current study provide insight into the acute exercise-induced inflammatory and leukocyte responses between young smokers and non-smokers. Additionally this study also demonstrates the effect of acute cigarette smoking on the exercise-induced response in smokers. The current study also provides further understanding of the combined acute pro- and anti-inflammatory responses to smoking and exercise. The acute descriptive responses to smoking in young groups reported here may add further explanation of the disease progression observed in smoking groups.
Conflict of Interest

The authors declare no conflict of interest.
References


Lee, J., Taneja, V., & Vassallo, R. (2012). Cigarette Smoking and Inflammation:
Cellular and Molecular Mechanisms. *Journal of Dental Research, 91*, 142–149.


Petersen, A. M. W., & Pedersen, B. K. (2005). The anti-inflammatory effect of


White, V., Hill, D., Siahpush, M., & Bobevski, I. (2001). How has the prevalence of