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_{2peak} 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-1receptor 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.

26 **Key Words:** Cycling; Inflammation; Tobacco smokers; Cytokines.

27 The adverse effects of tobacco smoke are associated with the delivery of many
28 carcinogenic and cytotoxic stimuli (Domagala-Kulawik, 2008; Lee, Taneja, &Vassallo,
29 2012). Whilst the detrimental effects of long-term cigarette smoking are commonly reported
30 in middle- and older-aged groups, the highest prevalence rates are often observed in young
31 adult groups (Australian Bureau of Statistics, 2006; White, Siahpush, & Bobevski, 2003).
32 Further, despite research focus on the pulmonary consequences of cigarette smoking, the
33 injurious effects of cigarette smoking on endothelial function, cardiovascular physiology and
34 the immune system are also of high importance. The development of systemic injury (Blann,
35 Kirkpatrick, Devine, Naser, & McCollum, 1998) and subsequent systemic inflammation are
36 important precursors for the development of chronic diseases such as cardiovascular disease
37 (CVD) and diabetes. Thus, given the renowned physiological consequences of smoking, and
38 the potential for early intervention, further investigation into the cigarette smoke-induced
39 changes to the inflammatory profile in young smokers is warranted.
40
41 It is well established that habitual cigarette smoking results in the development of a
42 low grade systemic inflammatory state, consistent with a dose and duration dependent
43 fashion of consumption (Frohlich et al., 2003; Tracy et al., 1997). The complexity of the
44 composition of cigarette smoke presents a challenge in understanding both the pro-
45 inflammatory and immunosuppressive actions of mainstream cigarette smoke (Gonçalves et
46 al., 2011; Lee, Taneja, &Vassallo, 2012). Cigarette smoking modifies immune and
47 inflammatory processes (Stampfli & Anderson, 2009), particularly inhibiting natural killer
48 cell activity and creates an imbalance between pro- and anti-inflammatory cytokines – likely
49 to impede the ability for a normal immune response (Moszczynski et al., 2001; Zeidel et al.,
50 2002). Contrastingly, exercise training is reported as an effective therapeutic tool that
51 produces favourable health outcomes, including improved endothelial and respiratory

52 function in a smoking population (Rooks, McCully, & Dishman, 2011), and is further
53 reported to impose positive effects on immune function (Gleeson, 2007). An acute bout of
54 exercise is accompanied by an influx of anti-inflammatory cytokines (interleukin [IL]-6 and
55 IL-1 receptor antagonist [ra]), the magnitude of which is dependent upon modality, intensity
56 and duration (Gleeson, 2007). These exercise induced elevations in anti-inflammatory
57 cytokines, ie. IL-1ra, may provide the mechanism for long term protection against chronic
58 diseases (Fischer 2006; Gleeson, 2007; Gleeson et al., 2011).
59
60 However, despite current knowledge of the potent pro-inflammatory profile reported
61 to result from chronic cigarette smoking, and the anti-inflammatory effects of exercise
62 (Petersen & Pedersen, 2005; Thatcher, 2005) there is limited literature on the anti-
63 inflammatory response to acute exercise in a smoking group. Given the absence of such
64 research, it is important to draw upon insight from research on the effects of second hand
65 smoke exposure on exercise responses. Accordingly, secondhand smoke exposure prior to
66 exercise is suggested to compromise the immune system, resulting in the up-regulation of
67 inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin (IL)-4, IL-5, IL-6
68 and interferon-gamma (IFN-y) (Flouris et al., 2010; Flouris et al., 2012). Further, secondhand
69 smoke exposure following exercise results in changes in cardiovascular physiology and
70 compromises respiratory parameters; (Flouris et al., 2010; Flouris et al., 2012; McMurray,
71 Hicks & Thompson, 1985; Pimm and Silverman, 1978).
72
73 Given the potent pro-inflammatory profile reported to result from chronic cigarette
74 smoking, and the anti-inflammatory effects of acute exercise (Petersen & Pedersen, 2005;
75 Thatcher, 2005), no previous studies have determined the anti-inflammatory response to
76 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.,

102 recent influenza or surgery, periodontal disease, etc.) associated with systemic inflammatory
103 responses. Any participant that was confirmed as having these conditions, or taking anti-
104 inflammatory or any other potentially confounding medications were excluded from this
105 study. Smokers were classified as current active smokers, smoking no more than 1 pack per
106 day. Participants were matched based on their comparative age and aerobic fitness in
107 accordance with their smoking status, with anthropometric and descriptive baseline values
108 reported in Table 1. The self-reported smoking history for the smoker group was 6.9 ± 1.3 yr
109 of smoking and 12.9 ± 2.1 cigarettes per day. Smoking participants engaged in comparable
110 levels of recreational physical activity as the aforementioned non-smokers based on
111 qualitative feedback regarding exercise engagement. Prior to the commencement of the study
112 all participants were required to provide written and verbal consent following an outline of all
113 procedures and measures. This study conformed to the Declaration of Helsinki and was
114 approved by the Research in Human Ethics Committee at the University.

115

116 **Baseline Testing**

117 Participants completed a Physical Activity Readiness Questionnaire and a healthy
118 history questionnaire, and if satisfying the above study inclusion criteria, were recruited into
119 the study. Participants abstained from strenuous physical activity for 48 h prior, further
120 abstained from all physical activity for the 24 h prior to baseline testing and exercise
121 protocol, respectively, with all consumed food and beverages documented in a diary provided
122 by the research team. Moreover, for the 10 h prior to baseline testing and the exercise
123 protocol, participants avoided alcohol consumption and abstained from cigarette smoking and
124 caffeine. Further, smokers avoided all passive or active consumption of cigarette smoke
125 during the post-exercise data collection period (up to 3 h post) and for 10 h prior to the 24 h

126 post time point. Prior to arriving at 0700 h, participants consumed (at 0500 h) 50 g of a
127 nutritional supplement (Sustagen, Sport Chocolate, Mead Johnson Nutritionals, Nestle) in
128 300 ml of milk to standardise carbohydrate intake. Although it is recognised carbohydrate
129 may affect inflammatory response, the low amount and controlled intake ensured
130 standardised dietary intake across protocols. Upon arrival, anthropometric measures were
131 obtained, including stature (Stadiometer: Custom CSU, Bathurst, Australia), body mass (HW
132 150 K, A & D, Bradford, MA, USA), and waist and hip circumferences (steel tape, EC P3
133 metric graduation, Australia). In addition, a supine dual-energy x-ray absorptiometry (DXA)
134 was conducted for the determination of body composition (XR800, Norland, Cooper Surgical
135 Company, Trumbull, CT, USA). Scanning resolution and speed were set at 6.5 x 13.0 mm
136 and 130 $mm \cdot s^{-1}$, respectively. Whole body scans were analyzed (Illuminatus DXA, ver. 4.2.0,
137 USA) for total body lean mass and total body fat mass and are reported in absolute and
138 relative terms. Following spirometry, participants completed a GXT on an electronically-
139 braked cycle ergometer (LODE Excalibur Sport, LODE BV, Groningen, The Netherlands) to
140 determine VO_{2peak}. The incremental test began at 100 W and increased by 25 W every min
141 until volitional exhaustion and/or attainment of maximal heart rate (HR_{max}). Pulmonary gas
142 exchange was measured by determining O_2 and CO_2 concentrations and ventilation to
143 calculate VO_2 using a metabolic gas analysis system (Parvo-Medics, True2400, East Sandy,
144 UT, USA). The system was calibrated according to the manufacturer's instructions. This
145 involved the pneumotachometer calibration using a 3 L syringe. The gas analyzers were
146 calibrated using a two-point fully automated process involving room air and gas calibration
147 for fractional gas concentration with a gravimetric gas mixture of known concentrations
148 (CO_2, 4.1 (0.1)%; O_2, 15.7 (0.2)%).

149

150 **Exercise Protocol**

151 The respective groups (smokers or non-smokers) underwent different testing formats.
152 The smokers group completed two aerobic exercise protocols in a randomized cross-over
153 design that were at a standardised time of day (0700 h) and were separated by a one week
154 recovery, either with or without post-exercise cigarette smoking. Conversely, the non-smoker
155 (NS) group only completed a singular exercise session. The exercise protocol completed by
156 both smokers and non-smokers consisted of 40 min of stationary cycle ergometry (Monark
157 828E, Monark Exercise AB, Varburg, Sweden) at 50% of VO_{2peak}. The workload was
158 calculated as 50% of the pedalling resistance (W) achieved during the GXT and was
159 converted into kilopond units and set as a fixed intensity for the exercise protocol. The
160 selection of this exercise protocol was based upon previous research (Mendham, Donges,
161 Liberts & Duffield, 2011) which demonstrated an inflammatory response to an acute bout of
162 exercise of the same intensity and duration as the current study. Telemetry-based heart rate
163 (HR) (Vantage NV, Polar, Finland) and rating of perceived exertion (RPE) (Borg CR10
164 scale) were recorded every 5 min during the exercise protocol and a session RPE was
165 obtained 30 min post-exercise.
166
167 **Smoking Protocol**
168 Cigarette consumption was randomized in a cross-over and counter-balanced design
169 within the smoking group only. Following the exercise protocol, on one occasion participants
170 were required to consume two cigarettes of the same brand (Winfield Blue, 12 mg tar, 1.0 mg
171 nicotine). Following post-exercise venous blood collection (~5 min), participants
172 immediately smoked the two cigarettes within 15 min and were avoided any secondhand
173 smoke exposure. During this period participants were encouraged to inhale deeply and
174 consistently, with adequacy of smoking ensured by visual observation by the research team in
175 order to standardise consumption. That said, it must be noted that the smoking protocol

176 would not be considered “normal” smoking behavior. The smoking protocol was chosen
177 based upon previous research published by Van der Vaart et al., (2005) who reported in their
178 methods two cigarettes of the same brand within 30 min and were encouraged to inhale
179 deeply. Given the lack of active smoking research, this was the guideline for selection of an
180 acute smoking protocol and was adjusted based upon the selected group (young habitual
181 cigarette smokers). Following the consumption of the cigarettes, and in both the no-smoking
182 and non-smokers conditions, participants passively rested until 3 h post-exercise blood
183 collection.

184

185 **Venous Blood Procedures**

186 Venous blood samples were collected pre-exercise, and immediately post, 30 min, 3 h
187 and 24 h after the exercise protocol. Accordingly, a 21GA catheter was inserted into a medial
188 antecubital vein and a 40 ml sample was drawn and aliquoted into SST for analysis of blood
189 lipid profile and CRP, and EDTA tubes for analysis of inflammatory cytokines, glycosylated
190 haemoglobin (HbA1c) and total and sub-population leukocyte count. EDTA tubes used for
191 cytokine analysis were centrifuged immediately post-aliquot at 3500 rpm for 15 min at 4°C,
192 whilst SST tubes were left to clot at room temperature for 20 min prior to centrifugation.
193 Supernatants were immediately stored at -80 ^{o}C or -20 ^{o}C for EDTA and SST, respectively.
194 Total and sub-population leukocyte count and HbA1c were kept refrigerated determined
195 within 4 h of venous blood collection. Blood samples were analyzed for IL-6, IL-1ra, TNF-α,
196 total cholesterol, triglycerides, high density lipoprotein (HDL), HbA1c, total and sub-
197 population leukocyte count and CRP. All biochemistry variables were analyzed in duplicate
198 according to manufacturer’s instructions. Total cholesterol was analyzed using an enzymatic
199 method and polychromatic endpoint technique measurement. HDL cholesterol was measured
200 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.

225 2010). The output parameters demonstrate a sample size of 16 to provide actual power of
226 0.67, and as such we recognize the potential limitation of reduced power of this study.
227
228
229 **Results**
230 Baseline variables for body composition, blood lipid profile and anthropometric
231 variables are reported in Table 1. The smoker group demonstrated lower waist and hip
232 circumferences and percentage fat mass than the never-smoker group (*d*=0.87- 1.32; p<0.05).
233 There were no differences between groups for age or VO_{2peak} (*d*=0.06; p>0.05), although the
234 never-smoker group were heavier and demonstrated increased absolute fat mass (*d*=0.87;
235 p<0.05; Table 1). Exercise-induced HR responses (% of HR_{max}) were not significantly
236 different between groups (79 ± 3%, 78±3%, 80 ±2% for smokers- no smoking, smokers-
237 smoking and non-smokers respectively; p>0.05). Furthermore, there were no significant
238 differences in session RPE between groups (4.7 ±0.5, 4.6±0.5, 5.1 ±0.4 for smokers- no
239 smoking, smokers-smoking and non-smokers, respectively (*d*=0.10-0.36; p>0.05).
240
241 The inflammatory responses of IL-6, CRP, IL-1ra and TNF-α are presented in Figure
242 1. There were no baseline differences between groups for IL-6, IL-1ra or CRP (p>0.05;
243 *d*=0.02-0.65). In response to the exercise protocol, an increase in IL-6 was evident in all
244 conditions (*d*=0.64-1.30; p<0.05). For smokers- no smoking, IL-6 concentration peaked at 30
245 min post-exercise (*d*=0.27; p<0.05), although in the smokers-smoking condition a significant
246 decline in IL-6 from 30 min to 24 h post exercise (*d*=0.64; p<0.05) was noted. Smokers-
247 smoking and smokers- no smoking observed an exercise-induced increase immediately post-
248 exercise in IL-1ra values (*d*=0.50; *d*=0.50; p<0.05), not observed in non-smokers.
249 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).

300 The present study suggests that young adult smokers have a baseline inflammatory
301 profile comparable to that of their age – and fitness-matched young adult never-smoking
302 counterparts. Consequently, the relatively short smoking history may not be sufficient to
303 exacerbate resting inflammatory profiles, as observed in long term smokers (Kuschner,
304 Alessandro, Wong & Blanc, 1996; Tracy et al., 1997). Kuschner et al. (1996) reported that
305 middle-aged smoker's exhibit higher TNF-α concentrations than non-smokers, further Tracy
306 et al. (1997) reported elevated concentrations of CRP as a result of a lifetime of smoking,
307 which may suggest that chronic inflammatory states are associated with smoking duration
308 and dependence. In the present study there were no observed baseline differences in
309 concentrations of CRP, IL-6 or IL-1ra; however, concentrations of TNF-α in the smokers-
310 smoking condition were elevated compared to other conditions, which may present as a
311 limitation to this study. Further, it should be noted that to match fitness and age between
312 respective groups, the non-smokers were heavier and had greater waist and hip
313 circumferences. An implication here is that the non-smokers had higher adiposity, and in
314 turn higher adiposity has been reported to relate to exacerbated pro-inflammatory responses
315 (Maury & Brichard, 2010). However, despite a noted main effect for body mass during
316 covariate analysis for IL-6, IL-1ra and TNF-α, the non-smokers did not demonstrate higher
317 basal inflammatory values, suggesting differences in adiposity between groups had minimal
318 influence on the inflammatory markers at rest and following the protocol.

319 All conditions observed a small to moderate increase in post-exercise IL-6, which is
320 consistent with previous literature suggesting IL-6 is sensitive to exercise intensity and
321 duration (Gleeson et al., 2011; Petersen & Pedersen, 2005). A marked increase in IL-6
322 following exercise has been consistently reported to confer anti-inflammatory properties,
323 which in turn is reported to induce a cascade of anti-inflammatory cytokines including IL-1ra
324 and IL-10 (Petersen & Pedersen, 2005). Whilst both groups (smokers and non-smokers)

325 presented increased IL-6 responses post-exercise as represented by moderate effect sizes, the
326 expected continued post-exercise elevation in IL-1ra was observed in smokers- no smoking
327 and smokers-smoking, with no elevations observed in non-smokers. Further, smokers-
328 smoking and non-smokers observed a peak in concentrations of IL-1ra at 3h, which was not
329 observed in smokers- no smoking - suggesting an acute dose of cigarette smoke is sufficient
330 to induce inflammatory changes in the smoker group. However, in the absence of an
331 additional inflammatory stimulus such as cigarette smoke, smokers may exhibit a suppressed
332 immune response to exercise, as indicated by the IL-1ra response of the smokers- no smoking
333 group.
334 Similarly, although concentrations of TNF-α in the smokers-smoking group were
335 elevated, as denoted by a large effect, across all time points when compared to smokers- no
336 smoking, non-smokers observed an increase in TNF-α at 3 h post-exercise, which was not
337 observed in smokers- no smoking. In agreement with previous research in healthy non-
338 smoking groups (Ostrowski, Rohde, Asp, Schjerling, & Pedersen, 1999) an elevation in TNF-
339 α following moderate to high intensity cycling was observed in non-smokers. Further, the
340 elevated concentrations of TNF-α across all time points for smokers-smoking when compared
341 to smokers- no smoking suggest that the acute stimulus of cigarette smoke induces an influx
342 of TNF-α, which may be a potent contributor to the elevated concentrations of TNF-α
343 associated with long term cigarette smoking (Kuschner et al., 1996). Although these findings
344 are indicative of an elevated inflammatory state, the results observed in the current study may
345 be amplified to that of real life situation due to the large dose of cigarette smoke delivered
346 (two cigarettes within 15 minutes). Further, it must be noted that the dose delivered in the
347 current study may not be consistent with regular smoking behavior. Regardless, such findings
348 demonstrate that the insult of cigarette smoke may adversely affect the inflammatory profile
349 of young habitual cigarette smokers.

350

351 The concurrent peaks in concentrations of TNF- α and IL-1ra highlight the
352 complexity of the anti- and pro-inflammatory interaction between acute smoking and acute
353 exercise. The elevation in TNF-α following acute cigarette smoking signifies a pro-
354 inflammatory stimulus, following the presence of a pro-inflammatory factor, is the
355 simultaneous release of a cytokine inhibitor IL-1ra (Pedersen, 2000) to counteract the pro-
356 inflammatory state, thus the similar profiles of TNF-α and IL-1ra in the present study.
357 However, it must be noted that smokers- no smoking, in the absence of a pro-inflammatory
358 stimulus such as acute cigarette smoking, exhibit a suppressed inflammatory response to
359 exercise. Despite no baseline immune-inflammatory differences, such a finding may be an
360 early indication of the future altered responses to immune and inflammatory
361 function observed in long term smokers (Zeidel et al., 2002). As these data represent novel
362 findings of the interaction between smoking and exercise, the only comparable context relates
363 to the effects of secondhand smoke exposure. Flouris et al. (2012) reported the effects of
364 secondhand smoke exposure followed by physical activity in 16 non-smokers and found
365 elevations in inflammatory cytokines IL-4, TNF-α and IFN-y. Further, early research by
366 Pimm and Silvermann (1978) suggested that physical activity following secondhand smoke
367 exposure elevated cardiovascular demands when compared to no smoke exposure. Consistent
368 with Flouris et al. (2012) the present study observed an elevation in TNF-α as a result of
369 cigarette smoke exposure, suggesting that cigarette smoke impedes normal immune-
370 inflammatory processes following exercise.

371

372 The effects of cigarette smoke on inflammatory mediators is particularly complex, the
373 diversity of compounds contained with cigarette smoke present both immunostimulatory and
374 suppressive actions (Sopori, 2002). Whilst the literature is lacking *in vivo* human models, *in*

375 *vitro* and murine models suggest that exposure to cigarette smoke elevates pro-inflammatory
376 markers such as TNF-α (Churg et al., 2003), although others have reported no effect of acute
377 cigarette smoking (Van der Vaart et al., 2005). Nicotine, the component of cigarette smoke
378 responsible for addiction (Jain & Mukherjee, 2003), is suggested to exert anti-inflammatory
379 actions via α7-nicotinic acetylcholine receptors (Park et al., 2007), which may explain the
380 elevation in IL-1ra at 3h post-exercise, not observed in the smokers- no smoking condition.
381 Contrastingly, the less pronounced inflammatory responses in smokers- no smoking may be a
382 result of the modification of the HPA axis in smokers (Rohleder & Kirschbaum, 2006).
383 Chronic exposure to cigarette smoke results in significant alterations to the responsiveness of
384 the HPA-axis, which is an important regulator of inflammation (Rohleder & Kirschbaum,
385 2006). Further, a shift in the T-helper (Th)-1 and T-helper(Th)- 2 cytokine balance hypothesis
386 may explain the less pronounced inflammatory response, which suggests the regulation of the
387 immune system as maintained by Th-1 and Th-2 activity, is altered by chronic smoking
388 (Kidd, 2003; Mehta, Nazzal & Sadikot, 2008). Although these mechanisms were not
389 measured here, the present findings suggest that habitual cigarette smoking may alter the
390 exercise-induced inflammatory response in young male smokers.
391
392 Whilst immunological markers are reported to be acutely increased in response to exercise in
393 an intensity-dependent manner (Pedersen & Hoffman-Goetz, 2000), previous studies report
394 habituated cigarette smokers to exhibit elevated baseline concentrations of leukocytes as a
395 result of chronic cigarette smoking (Frohlich et al., 2003). Garey, Neuhauser, Robbins,
396 Danziger, & Rubinstein (2004) suggest the increased immunological chemotactic activity in
397 smokers is representative of a state of elevated inflammation, characterised by intense
398 neurtophilic infiltration into the airway mucosa. In the present study, exercise increased
399 immunological markers in all conditions; however, despite a matched exercise duration and

400 intensity, a moderate systemic response to cigarette smoking was observed in both leukocyte
401 and neutrophil responses 3h post-exercise compared to smokers- no smoking. Further the
402 present study also reports smokers- no smoking to exhibit elevated concentrations of
403 leukocytes post-exercise than never- smokers, which suggest that smokers may present a
404 heightened leukocyte response to an exercise stimulus.
405
406 Additionally, although measured in sputum in a rested state, Van Der Vaart et al.
407 (2005) reported neutrophil concentrations increased in response to acute cigarette smoking.
408 Further, Blann et al. (1998) reported that acute cigarette smoke exposure activates leukocyte
409 activity, causing endothelial damage and contributing to the development of systemic
410 inflammation. Such exacerbated immunological responses in the pulmonary system are
411 suggested to result from the noxious stimuli of cigarette smoke, although in the present study
412 these responses were noted in the circulatory system, in addition to the exercise-induced.
413 Though exercise is known to result in an immediate elevation in immunological markers, the
414 present study further corroborates an acute smoking induced increase in systemic
415 concentrations of leukocytes and neutrophils. Accordingly, even in a post-exercise state,
416 exposure to cigarette smoke results in elevated leukocyte responses compared to exercise
417 alone in young habituated smokers, suggesting that a relatively short smoking history is
418 sufficient to modify host defense responses to exercise.
419
420 Despite these findings certain limitations must be acknowledged. Firstly, it should be noted
421 that the smoking protocol (2 cigarettes within 15min) in some cases may not be considered
422 “normal” smoking behavior. Regardless, such procedures allowed the comparison of a
423 standardised and sufficient cigarette dose. Moreover, while the authors attempted to
424 standardize tobacco smoke exposure, following the 3h measures, smoking was not controlled

425 until a 10h abstinence prior to the 24h sample, and we also recognize this period of
426 uncontrolled time as a limitation. However, it must be stated that denying active smokers
427 from engaging in cigarette consumption for 24h is highly unlikely to garner adherence to
428 such requests. Additionally, given the small sample size, it is acknowledged such
429 underpowered results may present as a limitation for deterministic conclusions. Finally, the
430 non-smokers demonstrated greater absolute and relative fat mass, whereby the implication
431 being a higher adiposity may relate to exacerbated pro-inflammatory states (Maury &
432 Brichard, 2010). However, with marginal clinical differences and within normal ranges, we
433 would suggest such factors are unlikely to explicitly affect inflammatory responses to
434 smoking or exercise.
435

436 **Conclusions**

437 In conclusion, this study investigated the effect of acute exercise and acute cigarette
438 smoking on the inflammatory responses in young adult male smoker and non-smokers.
439 Although there were no baseline differences between groups, results indicated that smokers-
440 no smoking and smokers-smoking exhibit abnormal immune-inflammatory responses to
441 exercise. Whether such responses can be attributed to a modification of the HPA-axis or the
442 anti-inflammatory effects of some of the compounds found in cigarette smoke requires
443 further investigation. The present study also suggests that even a relatively short period of
444 habitual smoking is sufficient to induce alterations to the inflammatory profile in young
445 cigarette smokers. Given the reported benefits of exercise to public health and well-being,
446 further investigation into the pro- and anti-inflammatory relationship between chronic
447 cigarette smoking and exercise may determine whether the anti-inflammatory effects of
448 exercise may potentially reduce or inhibit pulmonary and systemic disease processes.
449

450 **What does this article add?**

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

458 **Conflict of Interest**

459 The authors declare no conflict of interest.

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