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1 **T HELPER CELL CYTOKINE PROFILES FOLLOWING ENDURANCE EXERCISE**

2

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21

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23

1 **ABSTRACT**

2 Endurance exercise can cause immunosuppression and increase the risk of upper respiratory
3 illness. The present study examined changes in the secretion of T helper (Th) cell cytokines
4 following endurance exercise. Ten highly-trained road cyclists (mean \pm SEM: age 24.2 ± 1.7
5 yr; height 1.82 ± 0.02 m; body mass 73.8 ± 2.0 kg; peak oxygen uptake 65.9 ± 2.3 ml.kg⁻¹.min⁻¹)
6 performed 2 h of cycling exercise at 90 % of the second ventilatory threshold.
7 Peripheral blood mononuclear cells were isolated, and stimulated with phytohemagglutinin.
8 Plasma cortisol concentrations and the concentration of Th1/Th2/Th17 cell cytokines were
9 examined. Data were analysed using both traditional statistics and magnitude-based
10 inferences. Results revealed a significant decrease in plasma cortisol at 4 h to 24 h post-
11 exercise, compared to pre-exercise values. Qualitative analysis revealed post-exercise
12 changes in concentrations of plasma cortisol, IL-2, TNF, IL-4, IL-6, IL-10, and IL-17A
13 compared to pre-exercise values. A Th1/Th2 shift was evident immediately post-exercise.
14 Furthermore, for multiple cytokines including; IL-2 and TNF (Th1), IL-6 and IL-10 (Th2),
15 and IL-17 (Th17) no meaningful change in concentration occurred until more than 4 hours
16 post-exercise, highlighting the duration of exercise-induced changes in immune function.
17 These results demonstrate the importance of considering ‘clinically’ significant versus
18 statistically significant changes in immune cell function following exercise.

1 INTRODUCTION

2 Prolonged endurance exercise is associated with increased incidence of upper respiratory
3 illnesses (URIs) in elite athletes (Heath and others 1991; Peters and others 1996; Mackinnon
4 and others 1996; Nieman and others 1990). This increased rate of URIs may be observed
5 following acute bouts of prolonged heavy- to severe-intensity exercise (e.g., marathon
6 running, triathlon, road cycling) and periods of increased training load, potentially inducing a
7 chronic stress response (Walsh and others 2011a). The aetiology of exercise-induced URIs
8 appears to be multifactorial, as the associated stress response involves many different cell
9 types, pathways and proteins of the immune system (Walsh and others 2011b).

10

11 Stress-related changes in immune system function are typically mediated through activation
12 of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal axis.
13 Activation of the SNS induces catecholamine release, with a delayed release of
14 glucocorticoids. These stress hormones alter immune function after acute exercise and during
15 periods of heavy training (Pedersen and others 1998), in response to acute and chronic
16 psychological stress (Cohen and others 1991), and following surgery (Rosenberger and others
17 2009). The effects of exercise-induced stress hormones on T helper cell function during and
18 after prolonged heavy-intensity exercise has not been fully elucidated.

19

20 Glucocorticoids and catecholamines influence T helper cell (Th) cytokine release, principally
21 by stimulating glucocorticoid and β_2 adrenergic receptors, respectively (Calcagni and
22 Elenkov 2006). In turn, these stress hormones influence the activity of cellular (Th1),
23 humoral (Th2), and mucosal (Th17) branches of the immune response (Calcagni and Elenkov
24 2006; Elenkov 2004). Specifically, cortisol, adrenaline and noradrenaline may suppress
25 cytokine release from antigen presenting cells and Th1 cells, while up-regulating cytokine
26 production from Th2 cells, thereby causing a shift toward Th2 cytokine production
27 (Steensberg and others 2001; Lancaster and others 2004). The balance between Th1 and Th2
28 immunological responses is very important in maintaining optimum immune health. The Th1
29 pathway primarily acts against intracellular pathogens, particularly viruses and bacteria,
30 while the Th2 pathway is believed to protect against extracellular pathogens such as parasites
31 (Kidd, 2003). Previous research has extensively examined changes in the balance of Th1 and
32 Th2 cytokines in plasma in the first 1–2 h after exercise (Suzuki and others 2002). By
33 contrast, there is less research published concerning changes in T helper cytokine secretion

1 (including Th17 cytokines) during recovery from prolonged heavy exercise. While these
2 changes are transient, it may have implications related to potential immune suppression
3 following prolonged heavy intensity exercise (Nieman, 2000).

4
5 Exercise-induced glucocorticoid release occurs during prolonged exercise (Pedersen and
6 others 1998). An increase in glucocorticoid concentration after exercise may influence T
7 helper cytokine secretion differently to exercise of moderate duration and intensity, due to the
8 large effect of changes in cortisol on immune function (Walsh and others 2011b; Pedersen
9 and others 1998). Th17 cells play a key role in mucosal host defence of the upper respiratory
10 and gastrointestinal tracts against a myriad of airborne antigens (Korn and others 2009; Liang
11 and others 2006; Yao and others 1995). Functioning as part of the “first line of defence” in
12 the mucosa, Th17 cytokines have been linked to increased polymeric immunoglobulin
13 receptor (IgR) expression, saliva SIgA and human β -defensin 2 release (Johansen and others
14 1999; Jaffar and others 2009; Kao and others 2004; Brandtzaeg and others 1999). These
15 results demonstrate that Th17 cytokine pathways are influential in the innate immune
16 response in the mucosa. However, there is little evidence available about changes in Th17
17 cytokines after exercise.

18
19 The aim of the present study was to examine the effect of prolonged heavy exercise on
20 plasma cortisol concentration, and Th1, Th2, and Th17 cytokine release. We hypothesised
21 that the exercise-induced stress would increase plasma cortisol concentration after exercise,
22 resulting in the release of IL-17 and a shift from secretion of Th1 to Th2 cytokines.

23 24 **MATERIALS AND METHODS**

25 **Subjects**

26 Ten well-trained male cyclists volunteered to participate in the present study (mean \pm SEM:
27 age 24.2 ± 1.7 yr; height 1.82 ± 0.02 m; body mass 73.8 ± 2.0 kg; peak oxygen uptake $65.9 \pm$
28 2.3 ml.kg⁻¹.min⁻¹). Subjects were excluded if they exhibited any cardiovascular, metabolic,
29 neurological, immunological or autoimmune disorders. The cyclists provided written
30 informed consent for participation in the study, which was approved by the Bond University
31 Human Research Ethics Committee.

32 33 **Experimental Design**

1 The cyclists visited the laboratory on three occasions; for a screening/familiarisation session,
2 a maximal exercise test, and a constant load test with blood sampling before and after
3 exercise. Peak oxygen consumption ($\dot{V}O_{2peak}$) was determined during a graded exercise test
4 in which the cyclists were allowed to choose a preferred cadence within the range of 70–90
5 $rev \cdot min^{-1}$. The test was performed on an electromagnetically-braked cycle ergometer (Lode
6 Excalibur Sport, Groningen, Netherlands). The cyclists commenced cycling at 80 W for 5
7 min; thereafter intensity increased by 30 W/min until volitional fatigue and/or when cadence
8 could not be maintained at a minimum 70 $rev \cdot min^{-1}$. The criteria to determine maximal efforts
9 at the end of a test were: a plateau in $\dot{V}O_2$ (defined as an increase in $\dot{V}O_2$ values of less than
10 $1.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ between two consecutive 1-min periods in the final part of the test), a
11 maximal value of respiratory exchange ratio ≥ 1.1 , a maximal heart rate value above 95 % of
12 the age-predicted maximum ($220 - \text{age}$) (Doherty and others 2003). Each cyclist's second
13 ventilatory threshold (VT_2) was determined from the gas-exchange data collected during
14 incremental exercise (ParvoMedics' TrueOne® 2400, Utah, USA), using the ventilatory
15 equivalents methods (Wasserman and McIlroy 1964).

16
17 The cyclists refrained from exercise for 48 h prior to the maximal exercise test and
18 experimental trial, and avoided consumption of alcohol or caffeine in the previous 24 h, and
19 during the exercise and post-exercise sample period. Pre-exercise blood samples were
20 collected from the cyclists after an overnight fast. The cyclists then consumed a standardised
21 breakfast (2000 kJ) of cereal, milk, and juice and commenced the exercise trial at 0800 hrs.
22 The cyclists performed exercise for 2 h at 90 % VT_2 (Monark Ergomedic 828 E, Sweden).
23 Cardiac rate and rhythm were monitored continuously throughout exercise, and cardiac rate
24 was recorded each minute. Pulmonary gas exchange was sampled for 4 min at 20-min
25 intervals to ensure that the cyclists were exercising at the work rate associated with 90 % VT_2
26 and that no drift in oxygen uptake occurred. The cyclists were allowed to drink water *ad*
27 *libitum* during exercise. The cyclists were provided with a standardised meal (4500 kJ) of
28 sandwiches, and a milkshake at 2.5 h post-exercise.

29

30 **Blood collection**

31 Venous blood samples were collected pre-exercise, immediately post-exercise, 2 h, 4 h, 6 h, 8
32 h, and 24 h post-exercise. Blood was collected into tubes containing K_3EDTA (Becton
33 Dickinson, New Jersey, USA). One tube was immediately centrifuged at $1000 \times g$ for 15 min.

1 Plasma was stored in aliquots at -80°C. The remaining blood sample was used for the
2 cytokine stimulation assay.

3

4 **Plasma cortisol analysis**

5 Plasma cortisol was analysed by ultra high-performance liquid chromatography tandem mass
6 spectrometry (UHPLC MS/MS) described elsewhere (McWhinney and others 2010). Briefly,
7 plasma samples were treated with 1 M HCl at room temperature to displace cortisol and
8 cortisone from binding proteins. To each sample, the multi-analyte internal standard (d4-
9 dexamethasone, d2-11-deoxycortisol and d2-cortisone) and H₂O were added before loading
10 onto activated Oasis HLB 1 cc (30 mg) solid phase extraction cartridges (Waters, Milford,
11 USA). Samples were eluted with 100 % ethyl acetate, dried at 50°C and reconstituted in 45 %
12 methanol with 2 mmol/L ammonium acetate and 0.1 % formic acid. The flow rate of the
13 UHPLC analysis was 0.4 ml/min, sample injection volume 20 µl, and column temperature
14 50°C. The tandem mass spectrometer was operated in positive mode electron spray ionisation.
15 The total analytical run time on the UHPLC MS/MS was 3 min.

16

17 **Flow cytometry**

18 Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll-Paque PLUS (GE
19 Healthcare Life Sciences, Milan, Italy) and then counted (Countess[®] Automated Cell Counter,
20 Life Technologies, Carlsbad, CA). Cells were cultured at a concentration of 1×10^6 cells/ml
21 and stimulated with 1 µg/ml of the mitogen phytohemagglutinin (PHA) for 72 h. Following
22 incubation, the supernatant was removed and stored at -80°C for subsequent analysis. Th1,
23 Th2 and Th17 cytokine concentrations were quantified using a commercially available
24 cytometric bead array kit (BD Pharmingen, San Diego, CA) to measure the concentrations of
25 IL-2, IL-4, IL-6, IL-10, TNF, INF-γ and IL-17A from supernatant of stimulated samples. In
26 the absence of stimulation with PHA, the concentrations of these cytokines were not detected.

27

28 **Statistics**

29 Prior to statistical analysis, all data were tested to determine if they were normally distributed.
30 Data that were normally distributed (Figure 1) were analysed using one-way ANOVA with
31 repeated measures to determine any change over time, with a Bonferroni correction applied
32 post hoc when significance was evident. These data are presented as mean ± SEM. Data that
33 were not normally distributed (Table 3) were analysed using the non-parametric Friedman's

1 ANOVA and Wilcoxon signed-rank test. These data are presented as median \pm interquartile
2 range. The Bonferroni correction was used for all multiple comparisons, with significance set
3 at $p < 0.008$ ($p < 0.05$ divided by the number of time points). All statistical analyses were
4 performed using SPSS software version 19.0 (SPSS Inc., Chicago, USA). Data was further
5 analysed for 'clinical' significance using magnitude-based inferences (Hopkins and others
6 2009). All qualitative analyses based on effect size were conducted using a modified
7 statistical spreadsheet (available at www.sportsci.org/resource/stats/xPostOnlyCrossover.xls)
8 (Hopkins 2006). Measures were log-transformed before analysis to symmetrically distribute
9 the non-normally distributed data. Magnitudes of effects were determined by standardisation
10 of the log-transformed variable. Between-time point standardized differences and effect sizes
11 for all values were calculated using the pooled standard deviation (Cohen 1998). Threshold
12 values for Cohen Effect Size statistics were > 0.2 (small), > 0.5 (moderate), and > 0.8 (large).
13 For between-time point comparisons, probabilities were calculated to establish whether the
14 true (unknown) differences were lower, similar or higher than the smallest worthwhile
15 difference or change. The smallest worthwhile difference for each variable was derived by
16 standardisation: 0.20 multiplied by the pooled between-subject standard deviations at each
17 time point compared to pre-exercise, based on Cohen's Effect Size principle (Cohen 1998).
18 Quantitative chances of higher or lower differences were evaluated qualitatively as follows: $<$
19 1 %, almost certainly not; 1–5 %, very unlikely; 5–25 %, unlikely; 25–75 %, possible; 75–
20 95 %, likely; 95–99 %, very likely; > 99 %, almost certain. If the chance of higher or lower
21 differences was > 5 %, the true difference was assessed as unclear. Otherwise, the change
22 was interpreted as the observed chance (Hopkins and others 2009).

23

24 **RESULTS**

25 Physical characteristics and exercise values

26 Physical characteristics of the cyclists are presented in Table 1. Mean heart rate for the 2 h
27 exercise bout was 161 ± 3 beats.min⁻¹, and mean exercise intensity associated with 90 % VT₂
28 occurred at 75.1 ± 0.9 % of $\dot{V}O_{2peak}$.

29

30 <insert Table 1 here>

31

32 **Plasma cortisol**

1 Figure 1 displays plasma cortisol concentration following prolonged heavy-intensity exercise.
2 There was no statistically significant change immediately or 2 h post-exercise. Plasma
3 cortisol concentration significantly decreased 4 h post-exercise ($p < 0.001$), when compared
4 with the resting pre-exercise concentration, and remained significantly decreased at 6 h post-
5 exercise ($p < 0.01$), 8 h post-exercise ($p < 0.001$) and 24 h post-exercise ($p < 0.01$).

6

7 <insert Figure 1 here>

8

9 Magnitude-based inferences revealed that compared to pre-exercise, plasma cortisol
10 concentration was likely higher immediately post-exercise. The chances that the true
11 difference in cortisol was higher/trivial/lower were 94/5/1%. Cortisol was very likely lower at
12 2 h post-exercise (1/2/97 %). Furthermore, compared to pre-exercise, cortisol was almost
13 certainly lower at 4 h (0/0/100 %), 6 h (0/0/100 %), and 8 h (0/0/100 %). At 24 h post-
14 exercise, cortisol was likely lower (4/12/84 %) than pre-exercise values (Table 2).

15

16 <insert Table 2 here>

17

18 **T helper cell cytokines**

19 There were no statistically significant changes in PHA-stimulated production of the T helper
20 1 cytokines IL-2, TNF, and IFN- γ , T helper 2 cytokines IL-4 and IL-10, and T helper 17
21 cytokine IL-17A following exercise (Table 3). A significant time effect was observed for IL-
22 6; however, post hoc analysis revealed no significant change at individual time points after
23 exercise compared with the pre-exercise IL-6 concentration.

24

25 <insert Table 3 here>

26

27 Table 4 displays changes in Th1 cytokine concentrations. Compared with pre-exercise values,
28 the concentration of IL-2 was likely higher than pre-exercise at 4 h (85/15/0 %) and 8 h
29 (90/10/0 %) post-exercise. The concentration of TNF was also likely higher than pre-exercise
30 values at 4 h post-exercise (82/17/1 %). Changes in IFN- γ concentration were unclear at
31 every time point post-exercise.

32

1 <insert Table 4 here>

2

3 Compared to pre-exercise, the concentration of IL-4 was likely higher immediately post-
4 exercise (79/18/3 %), while the difference in IL-4 during the remaining recovery period were
5 unclear (Table 5). Compared to pre-exercise, the IL-6 concentration was possibly higher at 4
6 h (68/29/3 %), possibly lower at 6 h (4/42/54 %), and likely higher at 8 h post-exercise
7 (94/6/0 %). Compared to pre-exercise, IL-10 concentration was likely higher at 4 h post-
8 exercise (77/23/0 %).

9

10 <insert Table 5 here>

11

12 IL-17A concentration compared to pre-exercise was possibly higher than pre-exercise at 8 h
13 post-exercise (73/25/2 %) (Table 6).

14

15 <insert Table 6 here>

16

17 **DISCUSSION**

18 This is the first study to analyse changes in Th1, Th2 and Th17 cytokine production during
19 recovery from exercise. It is established that exercise intensity and duration influence the
20 cortisol response to exercise. Previously, cycle exercise of comparable intensity (~75 %
21 $\dot{V}O_2$ peak) and duration (2 h) resulted in a significant increase in cortisol concentration
22 immediately post-exercise (Nieman and others 2007). Although traditional data analysis in
23 the present study did not reveal any statistically significant increase in plasma cortisol
24 immediately post-exercise, quantitative methods used suggested an increase in plasma
25 cortisol concentration was likely, and given results of previous research of similar exercise
26 duration and intensity, this result would be expected.

27

28 Plasma cortisol concentration decreased below pre-exercise levels from 4 h to 24 h post-
29 exercise. While the initial decrease (which occurred early in the afternoon) may be explained
30 by diurnal variation (Dhabhar and others 1994), plasma cortisol concentration remained
31 below resting values at 24 h post-exercise. This low plasma cortisol concentration may be
32 clinically important when considering the repetitive nature of endurance training day after
33 day. This result may indicate that the cyclists were well rested following the exercise bout,

1 and would have been able to respond positively to an additional bout of exercise on the
2 second morning.

3

4 Using traditional data analysis, there were no statistically significant changes in T helper cell
5 cytokine secretion at any time point during the post-exercise period in the present study
6 (Table 3). However, analysis using magnitude-based inferences suggested a multitude of
7 changes following exercise. Compared to pre-exercise, IL-2 (Th1) production was likely
8 higher at 4 h and again at 8 h post-exercise (Table 4), while TNF (Th1) production was likely
9 higher at 4 h post-exercise (Table 4). IL-4 (Th2) production was likely higher immediately
10 post-exercise (Table 5), while IL-6 (Th2) production was possibly higher at 4 h, possibly
11 lower at 6 h, and likely higher at 8 h post-exercise (Table 5). IL-10 (Th2) production was
12 likely higher at 4 h post-exercise (Table 5), while IL-17A (Th17) production was possibly
13 higher at 8 h post-exercise (Table 6).

14

15 Previously, intracellular cytokines IL-2 (Th1), IFN- γ (Th1), and IL-4 (Th2) produced by
16 CD4⁺ T cells were used to define T helper cell types and their functional changes. Using this
17 method, a Th1-to-Th2 shift was described following exercise and hormone infusion (Elenkov
18 2004; Elenkov 2008; Lancaster and others 2004; Steensberg and others 2001). This ‘shift’
19 occurs when IL-2- and/or IFN- γ -producing T helper cells (Th1) decrease in cell concentration,
20 while IL-4-producing T helper cells (Th2) remain unchanged. In the present study, no change
21 in IL-2 and IFN- γ occurred immediately post-exercise (Table 4), while IL-4 concentration
22 increased at the same time point (Table 5). Hence, a shift towards Th2 was observed
23 immediately post-exercise.

24

25 Although single intracellular cytokine analysis of T helper cells provides a concise
26 explanation of the Th response to exercise, it neglects to consider the magnitude of cytokines
27 involved in the T helper cell network. Cytokines are not solely secreted from T helper cells,
28 but include cytotoxic T cells, natural killer cells, dendritic cells, macrophages and even
29 skeletal muscle (Pedersen and Febbraio 2008; Zaldivar and others 2006). In the present study,
30 cytokine secretion both increased and decreased occurred immediately following exercise
31 (IL-4), and up to 8 hours post-exercise (IL-2, IL-6 and IL-17A). This presents an intricate
32 pattern of changes to consider. In particular, at 4 h post-exercise, the Th1 cytokines IL-2 and
33 TNF, and the Th2 cytokines IL-6 and IL-10 were all increased. This concomitant increase

1 and balancing of Th1 and Th2 cytokines has been observed previously by measuring
2 intracellular cytokine production from unstimulated peripheral blood mononuclear cells
3 following 30 min of moderate-intensity cycling (Zaldivar and others 2006). In contrast, the
4 present study examined the functional ability of peripheral blood mononuclear cells to
5 respond to a stimulus (PHA). Interestingly, the release of multiple cytokines was elevated at 4
6 h post-exercise. These responses demonstrate that exercise-induced stress can prime
7 peripheral blood mononuclear cells to respond to stimulation (Dhabhar 2009).

8

9 Recently, analysis by magnitude-based inference has received greater use in exercise science
10 and clinical settings. As previously stated, using traditional data analysis, there were no
11 statistically significant changes in T helper cell cytokine secretion at any time point during
12 the post-exercise period in the present study (Table 3). However, analysis using magnitude-
13 based inferences suggested various changes following exercise, which may prove clinically
14 significant. This approach may provide insights into what are clinically important, yet
15 statistically non-significant results in physiological and immunological processes. This study
16 assists in highlighting the importance of considering non-traditional methods for data analysis
17 when dealing with small sample sizes, with high between-subject variability.

18

19 The changes that occurred in T helper cell cytokine release suggest a multifactorial,
20 prolonged response to heavy endurance exercise. The fluctuations in cytokine production that
21 occurred during recovery from exercise could have important implications for resistance to
22 illness in athletes. While intracellular cytokine analysis following stimulation provides a
23 concise explanation of the Th1/Th2 shift, the current study suggests a much more complex
24 response to exercise.

25

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4

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7

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11

1 **REFERENCES**

2

3 Brandtzaeg P, Farstad IN, Johansen FE, Morton HC, Norderhaug IN, Yamanaka T. 1999. The
4 B-cell system of human mucosae and exocrine glands. *Immunol Rev* 171:45–87.

5 Calcagni E, Elenkov I. 2006. Stress system activity, innate and T helper cytokines, and
6 susceptibility to immune-related diseases. *Ann NY Acad Sci* 1069:62–76.

7 Cohen J. 1988. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Hillsdale, NJ:
8 Lawrence Erlbaum Associates.

9 Cohen S, Tyrrell DA, Smith AP. 1991. Psychological stress and susceptibility to the common
10 cold. *N Engl J Med* 325(9):606–12.

11 Dhabhar FS, Miller A, Stein M, McEwen BS, Spencer R. 1994. Diurnal and acute stress-
12 induced changes in distribution of peripheral blood leukocyte subpopulations. *Brain*
13 *Behav Immun* 8:66–79.

14 Dhabhar FS. 2009. A hassle a day may keep the pathogens away: The fight-or-flight stress
15 response and the augmentation of immune function. *Integr Comp Biol* 49(3):215–36.

16 Doherty M, Nobbs L, Noakes TD. 2003. Low frequency of the "plateau phenomenon" during
17 maximal exercise in elite British athletes. *Eur J Appl Physiol* 89(6):619–23.

18 Elenkov I. 2004. Glucocorticoids and the Th1/Th2 balance *Ann NY Acad Sci*. 1024:138–46.

19 Elenkov I. 2008. Effects of catecholamines on the immune response. In: del Rey A, Chrousos
20 G, Besedovsky H, editors. *NeuroImmune Biology: The Hypothalamus-Pituitary-Adrenal*
21 *Axis*. Elsevier B.V. p 189–206.

22 Heath GW, Ford ES, Craven TE, Macera CA, Jackson KL, Pate RR. 1991. Exercise and the
23 incidence of upper respiratory tract infections. *Med Sci Sports Exerc* 23:152–7.

- 1 Hopkins WG. 2006. Spreadsheets for analysis of controlled trials with adjustment for a
2 subject characteristic. *Sportsmedicine*. 10:46–50.
- 3 Hopkins WG, Marshall SW, Batterham AM, Hanin J. 2009. Progressive statistics for studies
4 in sports medicine and exercise science. *Medicine and science in sports and exercise*.
5 41(1):3–13.
- 6 Jaffar Z, Ferrini ME, Herritt LA, Roberts K. 2009. Cutting edge: lung mucosal Th17-
7 mediated responses induce polymeric Ig receptor expression by the airway epithelium
8 and elevate secretory IgA levels. *J Immunol* 182(8):4507–11.
- 9 Johansen FE, Pekna M, Norderhaug IN, Haneberg B, Hietala MA, Krajci P, Betsholtz C,
10 Brandtzaeg P. 1999. Absence of epithelial immunoglobulin A transport, with increased
11 mucosal leakiness, in polymeric immunoglobulin receptor/secretory component-deficient
12 mice. *J Exp Med* 190(7):915–22.
- 13 Kao CY, Chen Y, Thai P, Wachi S, Huang F, Kim C, Harper RW, Wu R. 2004. IL-17
14 markedly up-regulates beta-defensin-2 expression in human airway epithelium via JAK
15 and NF-kappaB signaling pathways. *J Immunol* 173(5):3482–91.
- 16 Kidd P. 2003. Th1/Th2 balance: the hypothesis, its limitations, and implications for health
17 and disease. *Altern Med Rev*. 8(3):223-46.
- 18 Korn T, Bettelli E, Oukka M, Kuchroo VK. 2009. IL-17 and Th17 Cells. *Annu Rev Immunol*
19 27:485–517.
- 20 Lancaster GI, Halson SL, Khan Q, Drysdale P, Wallace F, Jeukendrup AE, Drayson MT,
21 Gleeson M. 2004. Effects of acute exhaustive exercise and chronic training on type 1 and
22 type 2 T lymphocytes. *Exerc Immunol Rev* 10:91–106.

- 1 Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser
2 LA. 2006. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively
3 enhance expression of antimicrobial peptides. *J Exp Med* 203(10):2271–9.
- 4 Mackinnon LT, Hooper S. 1996. Plasma glutamine and upper respiratory tract infection
5 during intensified training in swimmers. *Med Sci Sports Exerc* 28:285–90.
- 6 McWhinney BC, Briscoe SE, Ungerer JP, Pretorius CJ. 2010. Measurement of cortisol,
7 cortisone, prednisolone, dexamethasone and 11 deoxycortisol with ultra high
8 performance liquid chromatography tandem mass spectrometry: Application from plasma,
9 ultrafiltrate, urine and saliva in a routine laboratory. *J Chromatogr B Analyt Technol*
10 *Biomed Life Sci* 878(28):2863–9.
- 11 Nieman DC, Johanssen LM, Lee JW, Arabatzis K. 1990. Infectious episodes in runners
12 before and after the Los Angeles Marathon. *J Sports Med Phys Fitness* 30(3):316–28.
- 13 Nieman DC. 2000. Exercise effects on systemic immunity. *Immunol Cell Biol.* 78, 496-501.
- 14 Nieman D, Henson D, Gojanovich G, Davis JM, Dumke C, Utter A, Murphy A, Pearce S,
15 McAnulty S. 2007. Immune Changes: 2 h of Continuous vs. Intermittent Cycling. *Int J*
16 *Sports Med* 28:625–30.
- 17 Pedersen BK, Rohde T, Ostrowski K. 1998. Recovery of the immune system after exercise.
18 *Acta Physiol Scand* 162:325–32.
- 19 Pedersen BK, Febbraio MA. 2008. Muscle as an endocrine organ: focus on muscle-derived
20 interleukin-6. *Physiol Rev* 88(4):1379–406.
- 21 Peters EM, Goetzsche JM, Joseph LE, Noakes TD. 1996. Vitamin C as effective as
22 combinations of anti-oxidant nutrients in reducing symptoms of upper respiratory tract
23 infections in ultramarathon runners. *South African Journal of Sports Medicine* 11:23–7.

- 1 Rosenberger PH, Ickovics JR, Epel E, Nadler E, Jokl P, Fulkerson JP, Tillie JM, Dhabhar FS.
2 2009. Surgical stress-induced immune cell redistribution profiles predict short-term and
3 long-term postsurgical recovery. A prospective study. *J Bone Joint Surg Am*
4 91(12):2783–94.
- 5 Steensberg A, Toft AD, Bruunsgaard H, Sandmand M, Halkjaer-Kristensen J, Pedersen BK.
6 2001. Strenuous exercise decreases the percentage of type 1 T cells in the circulation. *J*
7 *Appl Physiol* 91:1708–12.
- 8 Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, Sugawara K. 2002. Systemic
9 inflammatory response to exhaustive exercise. Cytokine kinetics. *Exerc Immunol Rev*
10 8:6–48.
- 11 Walsh NP, Gleeson M, Pyne DB, Nieman DC, Dhabhar FS, Shephard RJ, Oliver SJ, Bermon
12 S, Kajeniene A. 2011a. Position Statement Part two: Maintaining immune health. *Exerc*
13 *Immunol Rev* 17:64–103.
- 14 Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop NC, Fleshner M, Green
15 C, Pedersen BK, Hoffman-Goetz L, Rogers CJ, Northoff H, Abbasi A, Simon P. 2011b.
16 Position Statement Part one: Immune function and exercise. *Exerc Immunol Rev* 17:6–63.
- 17 Wasserman K, McIlroy MB. 1964. Detecting the threshold of anaerobic metabolism in
18 cardiac patients during exercise. *Am J Cardiol* 14:844–52.
- 19 Yao Z, Fanslow WC, Seldin MF, Rousseau AM, Painter SL, Comeau MR, Cohen JI, Spriggs
20 MK. 1995. Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel
21 cytokine receptor. *Immunity* 3(6):811–21.

- 1 Zaldivar F, Wang-Rodriguez J, Nemet D, Schwindt C, Galassetti P, Mills PJ, Wilson LD,
- 2 Cooper DM. 2006. Constitutive pro- and anti-inflammatory cytokine and growth factor
- 3 response to exercise in leukocytes. *J Appl Physiol* 100:1124–33.

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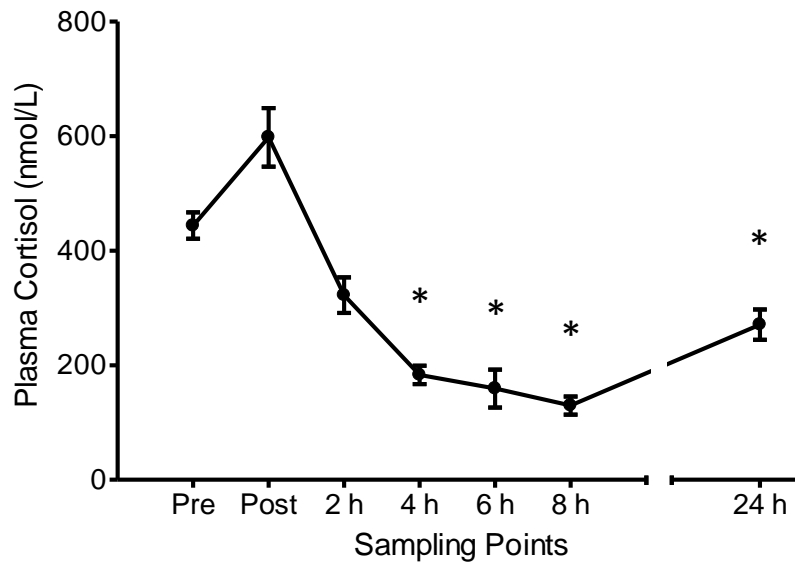


Figure 1. Plasma cortisol concentration before and after prolonged heavy intensity cycling. Data represent mean \pm SEM. *, significantly change compared to 'Pre' ($p < 0.05$)

1 Table 1. Physical characteristics and aerobic capacity of the cyclists

| 2 | Subject Characteristics (n=10) | Mean \pm SEM |
|---|---|------------------|
| 3 | Age (years) | 24.2 \pm 1.7 |
| 4 | Height (m) | 1.82 \pm 0.02 |
| 5 | Body mass (kg) | 73.8 \pm 2.0 |
| 6 | BMI (kg.m ⁻²) | 22.2 \pm 0.4 |
| 7 | Absolute $\dot{V}O_{2peak}$ (L.min ⁻¹) | 4.83 \pm 0.1 |
| 8 | Relative $\dot{V}O_{2peak}$ (ml.kg ⁻¹ .min ⁻¹) | 65.9 \pm 2.3 |
| 8 | Training (km/week) | 292.5 \pm 28.4 |

9 Data are mean \pm standard error of the mean. BMI: body mass index. $\dot{V}O_{2peak}$: peak oxygen
10 uptake. Training: volume in kilometres performed during training per week.

11

1 Table 2. Qualitative analysis of changes in cortisol concentration following prolonged heavy
 2 intensity exercise

| | Pre – Post | Pre – 2 h | Pre – 4 h | Pre – 6 h | Pre – 8 h | Pre – 24 h |
|---|---------------|----------------------|---------------------------|---------------------------|---------------------------|----------------|
| % Difference | 39.4 | -24.6 | -58.3 | -63.0 | -70.0 | -38.8 |
| (90% CL) | (13.2; 65.6) | (-39.8; -9.5) | (-64.7; -52.0) | (-76.7; -49.3) | (-76.8, -63.3) | (-48.0; -29.6) |
| Effect Size | 1.3 (large) | -1.4 (large) | -4.2 (large) | -3.2 (large) | -5.1 (large) | -2.2 (large) |
| % Chance for value to be higher/trivial/lower than Pre | 94/5/1 | 1/2/97 | 0/0/100 | 0/0/100 | 0/0/100 | 4/12/84 |
| Qualitative inference | Likely higher | Very likely lower | Almost certainly lower | Almost certainly lower | Almost certainly lower | Likely lower |

3 Data presented as percentage difference compared to the pre-exercise concentration. CL:
 4 Confidence level.

5

1 Table 3. T helper cytokine release before and after a bout of prolonged heavy intensity-
 2 exercise exercise

| | Pre-exercise | Post-exercise | 2 h | 4 h | 6 h | 8 h | 24 h | P value |
|---------------|------------------------|-------------------------|-----------------------|-------------------------|------------------------|------------------------|------------------------|---------|
| IL-2 | 18.9 (3.9, 59.8) | 11.5 (2.8, 24.0) | 11.2 (2.5, 51.7) | 65.8 (8.0, 110.0) | 50.7 (10.6, 107.0) | 47.4 (13.6, 96.3) | 7.1 (1.7, 162.0) | 0.141 |
| TNF | 2.0 (1.5, 2.4) | 1.8 (1.4, 2.0) | 1.8 (1.5, 2.1) | 2.3 (2.0, 3.3) | 2.9 (1.7, 3.9) | 2.0 (1.6, 2.6) | 1.9 (1.4, 2.8) | 0.488 |
| IFN- γ | 1.1 (1.1, 1.3) | 1.1 (1.0, 1.2) | 1.2 (1.1, 1.4) | 1.3 (1.1, 2.4) | 1.6 (1.2, 2.3) | 1.8 (1.1, 4.8) | 1.1 (1.0, 1.4) | 0.103 |
| IL-4 | 2.0 (1.9, 2.1) | 2.1 (2.0, 2.2) | 2.1 (1.9, 2.3) | 2.0 (1.9, 2.2) | 2.0 (1.9, 2.1) | 2.0 (2.0, 2.2) | 1.9 (1.9, 2.0) | 0.227 |
| IL-6 | 347.7 (14.3, 835.8) | 142.7 (32.4, 1061.8) | 98.3 (30.5, 433.4) | 249.9 (112.6, 706.2) | 458.4 (125.1, 1006) | 718.2 (95.7, 990.3) | 131.7 (49.9, 606.0) | 0.039 |
| IL-10 | 4.4 (1.8, 7.5) | 2.7 (1.8, 7.2) | 4.6 (1.8, 7.4) | 5.2 (2.2, 16.1) | 11.5 (2.7, 17.0) | 6.6 (2.2, 19.3) | 3.7 (1.5, 30.0) | 0.126 |
| IL-17A | 2.8 (2.8, 3.0) | 2.9 (2.5, 3.3) | 2.7 (2.7, 3.2) | 2.8 (2.8, 3.2) | 2.8 (2.6, 3.1) | 3.0 (2.7, 3.1) | 2.8 (2.6, 3.0) | 0.500 |

3 Th1 cytokines: IL-2, TNF, IFN- γ . Th2 cytokines: IL-4, IL-6, IL-10. Th17 cytokine: IL-17A.

4 Data represent median (1st and 3rd quartile).

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1 Table 4. Qualitative analysis of changes in Th1 cytokine concentrations following prolonged
 2 heavy-intensity exercise

| | Pre – Post | Pre – 2 h | Pre – 4 h | Pre – 6 h | Pre – 8 h | Pre – 24 h |
|--|-----------------|----------------|----------------|----------------|---------------|-----------------|
| IL-2 | | | | | | |
| % Difference | 69.3 | -4.0 | 162.2 | 142.4 | 115.3 | 132.6 |
| (90% CL) | (-75.7; 214.4) | (-33.0; 25.1) | (42.5; 281.9) | (64.0; 220.7) | (34.0; 196.5) | (-41.5; 306.6) |
| Effect Size (rating) | -0.1 (trivial) | 0.04 (trivial) | 0.5 (moderate) | 0.5 (moderate) | 0.3 (small) | 0.5 (moderate) |
| % Chance for value to be higher/trivial/lower than Pre | 7/49/44 | 1/77/22 | 85/15/0 | 26/59/15 | 90/10/1 | 4/94/2 |
| Qualitative inference | Unclear | Trivial | Likely higher | Unclear | Likely higher | Trivial |
| TNF | | | | | | |
| % Difference | -3.2 | 80.7 | 45.4 | 145.2 | 11.4 | 20.5 |
| (90% CL) | (-24.7; 18.2) | (-55.9; 217.4) | (-1.1; 92.0) | (-43.8; 334.3) | (-9.4; 32.2) | (-17.4; 58.4) |
| Effect Size (rating) | -0.4 (small) | 0.5 (moderate) | 0.6 (moderate) | 0.7 (moderate) | 0.1 (trivial) | 0.2 (small) |
| % Chance for value to be higher/trivial/lower than Pre | 6/47/47 | 56/31/14 | 82/17/1 | 69/21/11 | 44/46/10 | 60/26/14 |
| Qualitative inference | Unclear | Unclear | Likely higher | Unclear | Unclear | Unclear |
| IFN-γ | | | | | | |
| % Difference | 5.2 | 7.3 | 11.8 | 25.5 | 77.0 | 21.8 |
| (90% CL) | (-32.7; 43.2) | (-28.2; 42.8) | (-11.9; 35.5) | (-4.6; 55.6) | (-0.0; 154.1) | (-35.5; 79.2) |
| Effect Size (rating) | -0.5 (moderate) | -0.4 (small) | -0.2 (small) | -0.1 (trivial) | 0.2 (small) | -0.03 (trivial) |
| % Chance for value to be higher/trivial/lower than Pre | 11/28/61 | 11/47/42 | 18/77/6 | 45/50/5 | 50/43/7 | 33/54/14 |
| Qualitative inference | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear |

3 Data presented as percentage difference compared to the pre-exercise concentration. CL:
 4 Confidence level.

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1 Table 5. Qualitative analysis of changes in Th2 cytokine concentrations following prolonged
 2 heavy-intensity exercise
 3

| | Pre – Post | Pre – 2 h | Pre – 4 h | Pre – 6 h | Pre – 8 h | Pre – 24 h |
|---|----------------|-----------------|------------------|----------------|---------------|-----------------|
| IL-4 | | | | | | |
| % Difference | 5.4 | 5.1 | 4.2 | -0.1 | 2.6 | -3.4 |
| (90% CL) | (-0.4; 11.2) | (-3.2; 13.4) | (-3.6; 11.9) | (-4.3; 4.0) | (-3.9; 9.2) | (-8.9; 2.0) |
| Effect Size (rating) | 0.5 (moderate) | 0.4 (small) | 0.3 (small) | -0.1 (trivial) | 0.2 (small) | -0.6 (moderate) |
| % Chance for value to be higher/trivial/lower than Pre | 79/18/3 | 66/23/10 | 62/28/11 | 33/26/41 | 21/43/35 | 14/23/62 |
| Qualitative inference | Likely higher | Unclear | Unclear | Unclear | Unclear | Unclear |
| IL-6 | | | | | | |
| % Difference | 250.8 | 159.2 | 640.3 | 397.5 | 461.1 | 359.7 |
| (90% CL) | (79.8; 581.3) | (-123.0; 441.4) | (-195.6; 1476.1) | (109.1; 686.0) | (87.8; 834.5) | (-75.3; 794.7) |
| Effect Size (rating) | 0.1 (trivial) | -0.6 (moderate) | -0.2 (small) | 0.2 (small) | 0.4 (small) | 0.04 (trivial) |
| % Chance for value to be higher/trivial/lower than Pre | 39/54/7 | 11/60/29 | 68/29/3 | 4/42/54 | 94/6/0 | 35/64/1 |
| Qualitative inference | Unclear | Unclear | Possibly higher | Possibly lower | Likely higher | Trivial |
| IL-10 | | | | | | |
| % Difference | 10.9 | 46.7 | 63.7 | 116.5 | 139.7 | 153.0 |
| (90% CL) | (-27.8; 49.7) | (-28.5; 121.8) | (19.0; 108.4) | (3.9; 229.1) | (49.5; 229.8) | (10.2; 295.8) |
| Effect Size (rating) | -0.1 (trivial) | 0.4 (small) | 0.5 (moderate) | 0.6 (moderate) | 0.8 (large) | 0.9 (large) |
| % Chance for value to be higher/trivial/lower than Pre | 7/69/25 | 26/70/4 | 77/23/0 | 46/43/11 | 46/53/0 | 25/72/3 |
| Qualitative inference | Unclear | Trivial | Likely higher | Unclear | Trivial | Trivial |

4 Data presented as percentage difference compared to the pre-exercise concentration. CL:
 5 Confidence level.

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1 Table 6. Qualitative analysis of changes in Th17 cytokine IL-17A concentration following
 2 prolonged heavy-intensity exercise
 3

| | Pre – Post | Pre – 2 h | Pre – 4 h | Pre – 6 h | Pre – 8 h | Pre – 24 h |
|---|---------------|----------------|--------------|--------------|--------------------|--------------|
| IL-17A | | | | | | |
| % Difference | 1.9 | -1.2 | 5.2 | 4.8 | 8.4 | -2.0 |
| (90% CL) | (-3.8; 7.7) | (-10.3; 7.9) | (-6.4; 16.9) | (-6.5; 16.0) | (-5.3; 22.0) | (-11.4; 7.4) |
| Effect Size (rating) | 0.1 (trivial) | -0.1 (trivial) | 0.3 (small) | 0.2 (small) | 0.4 (small) | -0.4 (small) |
| % Chance for value to be higher/trivial/lower than Pre | 29/62/9 | 15/42/44 | 51/35/15 | 17/30/53 | 73/25/2 | 31/32/37 |
| Qualitative inference | Unclear | Unclear | Unclear | Unclear | Possibly higher | Unclear |

4 Data presented as percentage difference compared to the pre-exercise concentration. CL:
 5 Confidence level.

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