Effect of prolonged, submaximal exercise and carbohydrate ingestion on monocyte intracellular cytokine production in humans

R. L. Starkie, D. J. Angus, J. Rolland*, M. Hargreaves† and M. A. Febbraio

*Exercise Physiology and Metabolism Laboratory, Department of Physiology, The University of Melbourne, Parkville 3052, Victoria, *Department of Pathology and Immunology, Monash Medical School, Prahran 3181, Victoria and †School of Health Sciences, Deakin University, Burwood 3125, Victoria, Australia*

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1. The present study was undertaken to examine the effect of exercise and carbohydrate (CHO) ingestion on intracellular monocyte cytokine production. Subjects performed 2 h of cycling at 70% peak pulmonary O_2 uptake ($\dot{V}_{O_2,peak}$) while ingesting either an 8% CHO beverage or a sweet placebo. Whole blood was incubated with (stimulated) or without (spontaneous) lipopolysaccharide (LPS) and surface stained for monocyte surface antigens. The cells were permeabilised, stained for intracellular cytokines and analysed using flow cytometry.

2. Exercise had no effect on the number of monocytes spontaneously producing cytokines, but the number of stimulated IL-1α-, TNF-α- and IL-6-positive monocytes were elevated ($P < 0{\cdot}01$) immediately post-exercise and 2 h post-exercise. These stimulated cells produced less ($P < 0{\cdot}05$) TNF-α immediately post-exercise, and less ($P < 0{\cdot}05$) TNF-α and IL-1α 2 h post-exercise. There was a small, but significant increase ($P < 0{\cdot}05$) in the plasma IL-6 concentration immediately post-exercise.

3. Exercise resulted in an elevation ($P < 0{\cdot}01$) in the plasma adrenaline concentration in the placebo trial, and ingestion of CHO attenuated this increase. CHO ingestion had no effect on monocyte cytokine production, plasma IL-6 or circulating leukocyte numbers.

4. These data suggest that circulating monocytes are not the origin of increased levels of plasma IL-6 during exercise: prolonged cycling exercise increased the number of monocytes producing cytokines upon stimulation, but these cells produced less cytokines post-exercise. In addition, attenuation of plasma adrenaline levels had no effect on plasma IL-6 or monocyte cytokine production.

Cytokines are an important group of proteins produced by cells of the immune system upon activation. The specific and non-specific immune responses are governed by cytokines, whose coordinated presence is needed for the proper function of virtually all cells partaking in the immune response (Northoff *et al.* 1994). Recent evidence is consistent in showing increases in plasma proinflammatory cytokine concentrations during intense prolonged exercise. Plasma interleukin (IL)-1α (Ostrowski *et al.* 1999), tumour necrosis factor-α (TNF-α) (Weinstock *et al.* 1997; Ostrowski *et al.* 1999) and IL-6 (Sprenger *et al.* 1992; Nehlsen-Cannarella *et al.* 1997; Weinstock *et al.* 1997; Ostrowski *et al.* 1998*a*,*b*, 1999; Suzuki *et al.* 1999) increase in response to exercise. However, the literature is unclear with respect to the effect of exercise on *in vitro* stimulated TNF-α, IL-1 and IL-6 production since some (Northoff *et al.* 1994; Weinstock *et al.* 1997), but not all (Cannon *et al.* 1991; Haahr *et al.* 1991) studies report a decrease in production. Monocytes are a principle source of IL-1α, TNF-α and IL-6 and the effect of prolonged exercise on monocyte cytokine production is unclear. It has been hypothesised that circulating monocytes are stimulated to produce large quantities of proinflammatory cytokines (Bagby *et al.* 1996; Smith, 2000), therefore the effect of exercise on monocyte cytokine production warrants further investigation.

Changes in plasma cytokine levels are subtle and may not necessarily reflect alterations in production by leukocytes during exercise. Concentrations can be rapidly modified by alterations in receptor binding, breakdown of cytokines within reacting cells and excretion in urine (Shephard *et al.* 1994). Cytokines in urine are also extremely unstable, disappearing within a few hours (Northoff *et al.* 1994). Since numerous cells in a variety of tissues can produce cytokines (Turnbull & Rivier, 1999), alterations in plasma

concentrations may be indicative not of changes in production by circulating leukocytes but of altered output by other cells types. In addition, production of most cytokines is not limited to one white blood cell type (Jung *et al.* 1993). Consequently *in vitro* preparations of leukocyte cultures do not indicate which type of cell has altered its production, and do not reflect how many cells are producing the measured cytokine. Detection of intracellular cytokines not only directly identifies which type of leukocyte is producing the measured cytokine, but it also allows any changes in the number of cells producing cytokines, and the amount that they are producing, to be ascertained. The method employed in this study has the advantage of rapidly determining the cytokine production of a large number of individual cells (Prussin & Metcalfe, 1995). At present, no studies have examined the effect of exercise on cytokine production at a single cell level.

The level of circulating hormones such as adrenaline, noradrenaline, growth hormone and cortisol all increase as a result of strenuous exercise (Hargreaves *et al.* 1996), and it is possible that these are the cause of the exercise-induced immunomodulation (Nehlsen-Cannarella *et al.* 1997). It is well documented that adrenaline (Tvede *et al.* 1994), growth hormone (Kappel *et al.* 1993) and cortisol (Onsrud & Thorsby, 1981) affect populations of circulating leukocytes. It has been suggested that adrenaline (Guirao *et al.* 1997) and cortisol (DeRijk *et al.* 1997) may mediate changes in cytokine production. *In vitro* studies have observed that incubation of whole blood with adrenaline (Bergmann *et al.* 1999) decreases TNF-α, IL-1 and IL-6 production. In addition, carbohydrate (CHO) feeding during cycling exercise results in an attenuated rise in circulating adrenaline (McConnell *et al.* 1994) and plasma IL-6 (Nieman *et al.* 1998), a finding the authors attributed to lower levels of stress hormones. However, it is not known whether CHO ingestion, and hence blunted plasma adrenaline, during exercise modifies the effect of exercise on monocyte cytokine production.

Therefore the aim of the present study was to investigate the effect of submaximal, prolonged cycling and CHO ingestion, which decreases the circulating adrenaline concentration, on spontaneous and stimulated monocyte cytokine production. In addition, the present study aimed to elucidate whether changes in spontaneous monocyte cytokine production correlated with plasma cytokine levels. We hypothesised that exercise would decrease monocyte cytokine production due to increased adrenaline and cortisol concentrations, and ingestion of CHO would attenuate these changes.

METHODS

Subjects

Six endurance-trained men (25 ± 5 years; 77 ± 7 kg; peak pulmonary oxygen uptake ($\dot{V}_{O_2,peak}$) $= 4{\cdot}78 \pm 0{\cdot}43$ l min^{-1}; mean $\pm$ S.D.) volunteered for this study. Each subject was informed of the experimental protocol, made aware of the possible risks and signed a letter of informed consent prior to participation. Subjects had been free of infection for 6 weeks prior to the study, were exempt from symptoms of respiratory illnesses and were not on any medication. Experiments were approved by the Human Research Ethics Committee of The University of Melbourne.

Experimental procedures

Cycling was chosen as an exercise model because it results in elevated plasma adrenaline levels (Starkie *et al.* 1999) and CHO ingestion has been demonstrated to blunt this increase (McConnell *et al.* 1994). In addition, CHO ingestion during cycling exercise has been shown to attenuate elevations in plasma IL-6 (Nieman *et al.* 1998)

At least 1 week prior to the first trial, $\dot{V}_{O_2,peak}$ was determined during a continuous, incremental cycling test on an electrically braked cycle ergometer (Lode, Groningen, The Netherlands). Subjects began cycling at 150 W, with the resistance being increased by 50 W every 3 min for 9 min and then increased by 25 W every minute until volitional exhaustion. A power output estimated to require 70% $\dot{V}_{O_2,peak}$ was determined from a linear regression equation which plotted the steady state submaximal $\dot{V}_{O_2}$ values against corresponding workloads.

Subjects arrived in the laboratory after an overnight fast to participate in experimental trials. Trials were conducted at least 1 week apart and commenced in the early morning (6.30–7 a.m.) to avoid circadian variations in circulating hormones. The subjects were instructed to abstain from alcohol, caffeine, tobacco and strenuous exercise and were given food packages to consume for 24 h prior to the trial.

An indwelling Teflon catheter (Terumo, 20G, Tokyo, Japan) was inserted into an antecubital vein for blood sampling. This was kept patent by flushing with saline after each sample collection. After resting quietly for 2 h, a pre-exercise venous blood sample was collected (pre). Subjects then commenced a 2 h cycling trial at the predetermined workload in comfortable ambient conditions (20–22 °C, $<$ 50% humidity). Venous blood samples were collected at 1 h during exercise (60 min), immediately post-exercise (post) and 2 h post-exercise (2 h post). Any water or food consumed during the 2 h period following exercise was recorded and consumed at the same time in the following trial. Pre-, post- and 2 h post-exercise blood samples were analysed for alterations in leukocyte counts and spontaneous and lipopolysaccharide (LPS)-stimulated monocyte cytokine production. Pre-, 60 min and post-exercise samples were analysed for plasma glucose, lactate, cortisol, catecholamines and plasma IL-6.

Within the first 5 min of exercise subjects consumed a bolus of $5{\cdot}7$ ml kg^{-1} (441 ± 15 ml, mean $\pm$ S.E.M.) of an 8% CHO or placebo beverage. Throughout the remainder of the trial $3{\cdot}3$ ml kg^{-1} (254 ± 9 ml, mean $\pm$ S.E.M.) was consumed every 15 min resulting in a total volume of $2{\cdot}2 \pm 0{\cdot}1$ l. Trials were randomised and the beverages were identical in appearance, flavour and electrolyte concentration.

Leukocyte counts

Blood samples (3 ml) were placed in EDTA tubes and kept at room temperature until analysis for differential counts as routinely performed by the haematology laboratory at the Alfred Hospital (Melbourne, Victoria, Australia). This included determination of total white blood cell (WBC) numbers, including neutrophil, monocyte and lymphocyte numbers, to detect changes in circulating white blood cell populations.

Intracellular cytokines

Blood samples (2 ml) were placed in sodium heparin tubes and kept at room temperature until the end of the trial for measurement of

Table 1. Circulating leukocyte numbers

	Control			CHO		
	Pre	Post	2 h post	Pre	Post	2 h post
WBCs (1×10^9 l^{-1})	4·5 ± 0·3	9·3 ± 1·4*	9·1 ± 1·2*	4·3 ± 0·3	7·2 ± 0·4*	7·8 ± 0·9*
Neutrophils (1×10^9 l^{-1})	2·3 ± 0·3	5·7 ± 1·3*	7·0 ± 1·2*	2·2 ± 0·2	4·0 ± 0·5*	5·7 ± 0·9*
Lymphocytes (1×10^9 l^{-1})	1·7 ± 0·3	2·8 ± 0·5*	1·4 ± 0·1	1·7 ± 0·3	2·4 ± 0·4*	1·5 ± 0·1
Monocytes (1×10^9 l^{-1})	0·3 ± 0·0	0·6 ± 0·1*	0·5 ± 0·1*	0·3 ± 0·0	0·5 ± 0·1*	0·5 ± 0·1*

Values are mean ± S.E.M. ($n = 6$) circulating total WBCs, neutrophils, lymphocytes and monocytes in peripheral blood prior to (Pre), immediately following (Post), and 120 min into recovery (2 h post) from 2 h of bicycle exercise with (CHO) or without (Control) the ingestion of an 8% carbohydrate solution throughout exercise. * $P < 0·01$, significantly different from pre-exercise values.

intracellular cytokine production. The tubes were gently inverted and rolled periodically. Whole blood was incubated for 4 h with (stimulated) or without (spontaneous) 1 μg lipopolysaccharide (LPS; *Escherichia coli* derived, Sigma Aldrich, Australia) at 37 °C in a humidified incubator. Brefeldin-A (10 μg ml^{-1}) was added at the beginning of culture to all samples to inhibit intracellular transport of proteins, thus retaining cytokines produced inside the cell. Aliquots (100 μl) of stimulated and unstimulated blood were incubated for 30 min with CD33 (R-phycoerythrin Cy-Chrome; PECy5) conjugated monoclonal antibody (Immunotech, Marseille, France) for staining of monocytes. Red blood cells were lysed (0·15 M ammonium chloride, 10 mM potassium bicarbonate, 1 mM EDTA) for 10 min and the samples were spun in a centrifuge (350 *g*) for 5 min. The supernatant was decanted and the pellet was resuspended in 500 μl of 4% paraformaldehyde for 20 min. Samples were again spun (350 *g*) for 5 min and the supernatant decanted. The fixed cells were permeabilised with 500 μl permeabilising solution (Becton Dickinson, San Jose, CA, USA) for 20 min, washed (1% fetal calf serum, phosphate-buffered saline, 0·02 M sodium azide), spun (350 *g*) for 5 min and the supernatant was decanted. The cells were then incubated with conjugated monoclonal antibodies against IL-6 (fluorescein isothiocyanate (FITC); Pharmingen, San Diego, CA, USA), IL-1α (R-phycoerythrin; PE), TNF-α (FITC) or control ($_{\gamma 2a}$FITC/$_{\gamma 1}$PE) (Becton Dickinson) for 30 min. After the samples had been washed and spun (350 *g*) for 5 min, the pellet was resuspended in 500 μl of wash buffer. All incubations took place at room temperature in the dark. The percentage of cytokine-positive monocytes was determined by flow cytometry (FACScan; Becton Dickinson). Monocytes were separately gated on viable cells on a side scatter *vs.* CD33 (FL3) cytogram. Data for 2×10^3 events within this gate were acquired. Analysis of collected samples was performed using Cell Quest (Becton Dickinson) with gates for positive set on isotype controls. Results are expressed as the percentage and number of cytokine-producing cells in CD33-positive (CD33$^+$) populations. The absolute count was determined by multiplying the percentage of cytokine-positive monocytes by the concentration of monocytes in peripheral blood. For quantification of the amount of cytokine within positive cells, the mean fluorescence intensity of positive events was obtained.

Plasma IL-6

Blood samples (3 ml) were collected into heparin tubes and spun for 4 min at 6000 *g*. The supernatant was removed and stored at −80 °C until analysis. The concentration of IL-6 was measured using a commercially available ELISA kit (R&D systems, Minneapolis, MN, USA; coefficient of variation (CV) = 2·6%) which detected both soluble and receptor-bound IL-6. All measurements were performed in duplicate.

Hormones

Upon sampling, blood for analysis of cortisol was placed in lithium heparin tubes and blood for catecholamines in tubes containing 20 μl ml^{-1} of EGTA and reduced glutathione (GSH). Blood was then spun for 4 min at 6000 *g*. The supernatant was removed and stored at −80 °C until analysis. The cortisol concentration was determined by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA; CV = 4·3%). Samples were analysed for plasma catecholamines by a modification of the single isotope [^{3}H] radioenzymatic assay (Amersham, USA; CV = 10%).

Statistical analysis

A two-way (time × treatment) analysis of variance (ANOVA) with repeated measures was used to compare blood metabolites, hormones, WBC counts and cytokine production. Newman-Keuls *post hoc* tests were used to locate differences when the ANOVA revealed a significant interaction. A Statistica software package (Statsoft Inc., Tulsa, OK, USA) was used to compute these statistics. The level of significance to reject the null hypothesis was set at $P < 0·05$.

RESULTS

Exercise resulted in an increase ($P < 0·01$) in total circulating leukocyte numbers and these remained elevated ($P < 0·01$) 2 h post-exercise (Table 1). This leukocytosis post-exercise was due to an increase ($P < 0·01$) in circulating neutrophils, monocytes and lymphocytes. The maintained leukocytosis 2 h post-exercise was due to a sustained elevation ($P < 0·01$) in neutrophils and monocytes, whereas lymphocyte numbers had returned to pre-exercise levels within 2 h (Table 1).

There was a decrease ($P < 0·05$) in the percentage of monocytes spontaneously producing IL-6 and TNF-α post-exercise, but the percentage of monocytes spontaneously producing IL-1α was not affected by exercise (Table 2). When calculated as numbers of monocytes in circulation, exercise had no effect on spontaneous cytokine production (Figs 1, 2 and 3), indicating that cells entering circulation during exercise were not spontaneously producing

Table 2. Percentage of cytokine-positive cells in unstimulated blood

	Control			CHO		
	Pre	Post	2 h post	Pre	Post	2 h post
$CD33^+/IL\text{-}6^+$ (%)	8·0 ± 3·1	4·5 ± 1·9*	5·1 ± 1·8	7·5 ± 3·5	4·5 ± 2·8*	6·5 ± 4·1
$CD33^+/TNF\text{-}\alpha$ (%)	9·3 ± 3·8	4·7 ± 1·9*	5·5 ± 2·2	8·5 ± 3·5	4·8 ± 2·0*	6·6 ± 2·7
$CD33^+/IL\text{-}1\alpha$ (%)	12·8 ± 4·1	7·5 ± 1·8	8·9 ± 2·5	12·1 ± 4·5	7·7 ± 3·3	13·0 ± 6·4

Values are mean ± S.E.M. ($n = 6$) percentages of unstimulated IL-6-, TNF-α- and IL-1α-positive CD33-positive cells prior to (Pre), immediately following (Post), and 120 min into recovery (2 h post) from 2 h of bicycle exercise with (CHO) or without (Control) the ingestion of an 8% carbohydrate solution throughout exercise. * $P < 0{\cdot}05$, significantly different from pre-exercise values.

cytokines. In addition, exercise had no effect on the amount of cytokine, as indicated by fluorescence intensity, in cytokine-positive cells (Table 4). Upon stimulation with LPS, the percentage of IL-6-positive monocytes was elevated ($P < 0{\cdot}01$) post- and 2 h post-exercise compared with pre-exercise, whereas there was no change in the percentage of monocytes producing TNF-α or IL-1α upon stimulation (Table 3). The number of stimulated cells positive for IL-6, TNF-α and IL-1α production was elevated ($P < 0{\cdot}01$) post- and 2 h post-exercise (Figs 1, 2 and 3), but cells produced less ($P < 0{\cdot}05$) TNF-α post-exercise and less ($P < 0{\cdot}05$) TNF-α and IL-1α 2 h post-exercise (Table 5). A

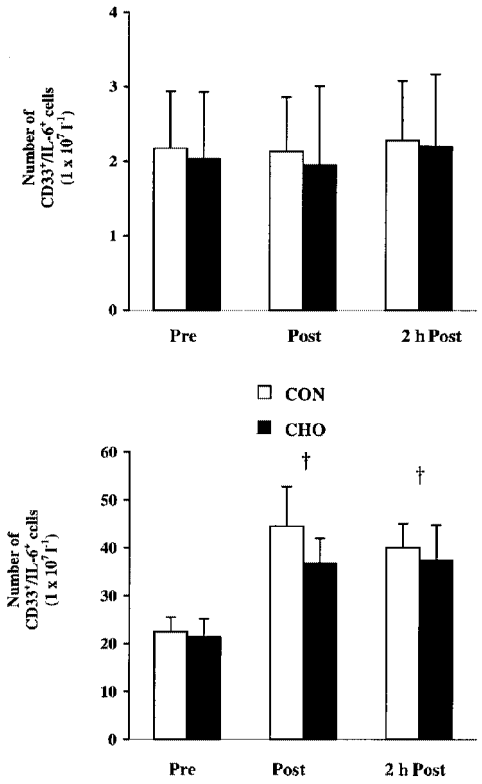

Figure 1. Number of IL-6-positive cells
Spontaneous (top) and LPS-stimulated (bottom) $CD33^+/IL\text{-}6^+$ cells prior to (Pre), immediately following (Post), and 120 min into recovery (2 h post) from 2 h of bicycle exercise with (CHO) or without (CON) the ingestion of an 8% carbohydrate solution throughout exercise. Values are means ± S.E.M. ($n = 6$). † $P < 0{\cdot}01$, significantly different from pre-exercise values.

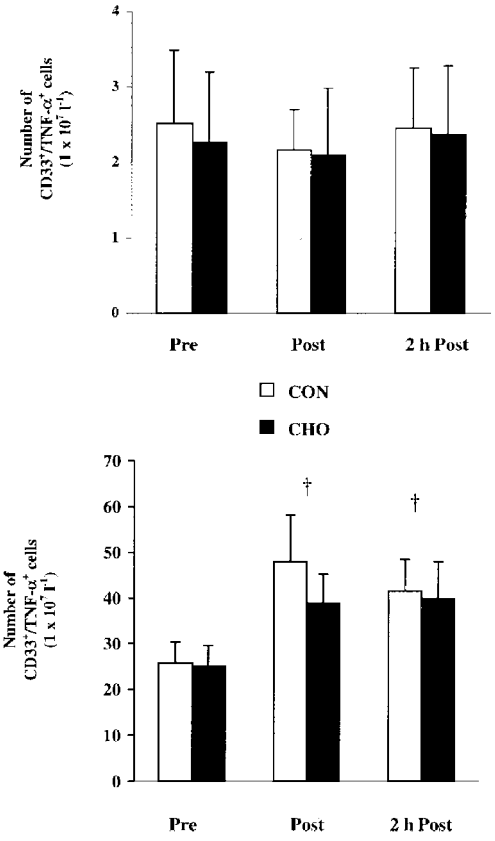

Figure 2. Number of TNF-α-positive cells
Spontaneous (top) and LPS-stimulated (bottom) $CD33^+/TNF\text{-}\alpha^+$ cells prior to (Pre), immediately following (Post), and 120 min into recovery (2 h post) from 2 h of bicycle exercise with (CHO) or without (CON) the ingestion of an 8% carbohydrate solution throughout exercise. Values are means ± S.E.M. ($n = 6$). † $P < 0{\cdot}01$, significantly different from pre-exercise values.

Table 3. Percentage of cytokine-positive cells in stimulated blood

	Control			CHO		
	Pre	Post	2 h post	Pre	Post	2 h post
$CD33^+/IL\text{-}6^+$ (%)	72·6 ± 3·3	77·8 ± 2·0*	80·1 ± 1·8*	70·3 ± 4·1	77·0 ± 3·4*	72·4 ± 7·4*
$CD33^+/TNF\text{-}\alpha$ (%)	81·5 ± 4·1	82·0 ± 1·4	82·4 ± 2·1	82·1 ± 3·9	80·5 ± 3·5	78·6 ± 6·0
$CD33^+/IL\text{-}1\alpha$ (%)	71·4 ± 2·7	70·0 ± 2·9	65·8 ± 3·2	67·3 ± 3·3	68·5 ± 4·0	63·7 ± 5·3

Values are mean ± S.E.M. ($n = 6$) percentages of stimulated IL-6-, TNF-α- and IL-1α-positive CD33-positive cells prior to (Pre), immediately following (Post), and 120 min into recovery (2 h post) from 2 h of bicycle exercise with (CHO) or without (Control) the ingestion of an 8% carbohydrate solution throughout exercise. * $P < 0{\cdot}01$, significantly different from pre-exercise values.

Table 4. Mean fluorescence intensity of cytokine-positive cells in unstimulated blood

	Control			CHO		
	Pre	Post	2 h post	Pre	Post	2 h post
$CD33^+/IL\text{-}6^+$ (%)	15·8 ± 2·4	13·3 ± 1·8	14·1 ± 1·5	16·9 ± 3·2	15·5 ± 1·7	17·7 ± 3·1*
$CD33^+/TNF\text{-}\alpha$ (%)	16·5 ± 1·2	15·1 ± 1·8	15·4 ± 1·2	16·9 ± 1·9	23·3 ± 5·8	15·8 ± 1·7
$CD33^+/IL\text{-}1\alpha$ (%)	30·1 ± 4·1	50·7 ± 13·5	41·0 ± 7·8	38·6 ± 9·7	38·5 ± 5·5	30·4 ± 3·2

Values are mean ± S.E.M. ($n = 6$) fluorescence intensities of unstimulated IL-6-, TNF-α- and IL-1α-positive CD33-positive cells prior to (Pre), immediately following (Post), and 120 min into recovery (2 h post) from 2 h of bicycle exercise with (CHO) or without (Control) the ingestion of an 8% carbohydrate solution throughout exercise. * $P < 0{\cdot}01$, significantly different from pre-exercise values.

Table 5. Mean fluorescence intensity of cytokine-positive cells in stimulated blood

	Control			CHO		
	Pre	Post	2 h post	Pre	Post	2 h post
$CD33^+/IL\text{-}6^+$ (%)	28·7 ± 3·3	27·5 ± 3·1	28·4 ± 3·6	30·5 ± 4·1	30·2 ± 3·8	28·3 ± 4·2
$CD33^+/TNF\text{-}\alpha$ (%)	57·7 ± 7·8	35·8 ± 2·2*	39·3 ± 2·7*	69·0 ± 12·2	44·4 ± 8·6*	52·8 ± 5·2*
$CD33^+/IL\text{-}1\alpha$ (%)	47·8 ± 6·1	40·3 ± 3·1	40·1 ± 5·1*	49·6 ± 5·5	46·0 ± 5·6	37·6 ± 3·5*

Values are mean ± S.E.M. ($n = 6$) fluorescence intensities of stimulated IL-6-, TNF-α- and IL-1α-positive CD33-positive cells prior to (Pre), immediately following (Post), and 120 min into recovery (2 h post) from 2 h of bicycle exercise with (CHO) or without (Control) the ingestion of an 8% carbohydrate solution throughout exercise. * $P < 0{\cdot}05$, significantly different from pre-exercise values.

two-fold increase ($P < 0{\cdot}05$) in plasma IL-6 was observed post-exercise (Fig. 4). CHO ingestion had no effect on any of these alterations in circulating leukocyte numbers or cytokine production in response to exercise.

Plasma glucose levels were not different at rest when comparing trials. During the control trial, plasma glucose decreased ($P < 0{\cdot}05$), but ingestion of CHO maintained plasma glucose levels (Table 6). Plasma lactate concentrations were higher ($P < 0{\cdot}05$) pre-exercise in the control trial than in the CHO trial. There was an increase ($P < 0{\cdot}05$) in lactate levels after 60 min in the control trial and at 60 min and post-exercise ($P < 0{\cdot}01$) in the CHO trial (Table 6).

During the control trial, plasma adrenaline concentration was elevated ($P < 0{\cdot}05$) at 60 min and post-exercise compared with pre-exercise values. Ingestion of CHO, however, resulted in lower ($P < 0{\cdot}01$) adrenaline levels (Fig. 5). The noradrenaline concentration increased ($P < 0{\cdot}05$) with exercise but was not effected by CHO ingestion (Table 6). Plasma cortisol concentrations were not altered in response to exercise or beverage ingestion (Table 6).

Table 6. Plasma hormone and metabolite concentrations

	Control			CHO		
	Pre	Post	2 h post	Pre	Post	2 h post
Cortisol (nmol l^{-1})	476 ± 42	516 ± 59	525 ± 42	534 ± 56	585 ± 49	514 ± 8
Noradrenaline (nmol l^{-1})	2·6 ± 0·6	8·7 ± 1·2*	9·6 ± 1·4*	1·5 ± 0·6	8·6 ± 1·3*	8·1 ± 1·5*
Glucose (mmol l^{-1})	5·1 ± 0·3	5·1 ± 0·2	4·4 ± 0·3	5·3 ± 0·2	5·3 ± 0·2	5·4 ± 0·1†
Lactate (mmol l^{-1})	1·4 ± 0·2	2·2 ± 0·3*	1·9 ± 0·2*	1·0 ± 0·1†	2·5 ± 0·2*	1·9 ± 0·2*

Values are means ± S.E.M. ($n = 6$) plasma cortisol, noradrenaline, glucose and lactate concentrations prior to (Pre), after 60 min of exercise (60 min), and immediately following (Post) 2 h of bicycle exercise with (CHO) or without (Control) the ingestion of an 8% carbohydrate solution throughout exercise. * $P < 0·01$, significantly different from pre-exercise values; † $P < 0·05$, significantly different from control.

DISCUSSION

Results from the present study demonstrate that circulating monocytes are not the origin of the elevated levels of plasma IL-6 observed post-exercise. This is illustrated by the lack of change in the number of monocytes spontaneously producing IL-6 and the amount of cytokine produced by cytokine-positive cells as a result of exercise. In addition, during cycling exercise in trained men, CHO ingestion had no effect on intracellular cytokine production or plasma IL-6, despite a suppression of circulating adrenaline.

The concentration of plasma IL-6 observed post-exercise was two-fold higher than was observed pre-exercise, but the concentration was low (< 2 pg ml^{-1}) compared with levels observed in previous studies (> 70 pg ml^{-1}; Nehlsen-Cannarella *et al.* 1997; Ostrowski *et al.* 1998*b*, 1999). One reason for this discrepancy is likely to be differences in the mode of exercise used between the studies. The previous experiments employed running as an exercise mode which requires weight bearing, eccentric contractions that are known to cause muscle damage. In the present study well-trained men performed 2 h of cycling exercise, which is predominantly concentric in nature. Hence it is likely that minimal muscle damage would have occurred. Bruunsgaard *et al.* (1997) compared concentric and eccentric exercise and observed no increase in plasma IL-6 levels following

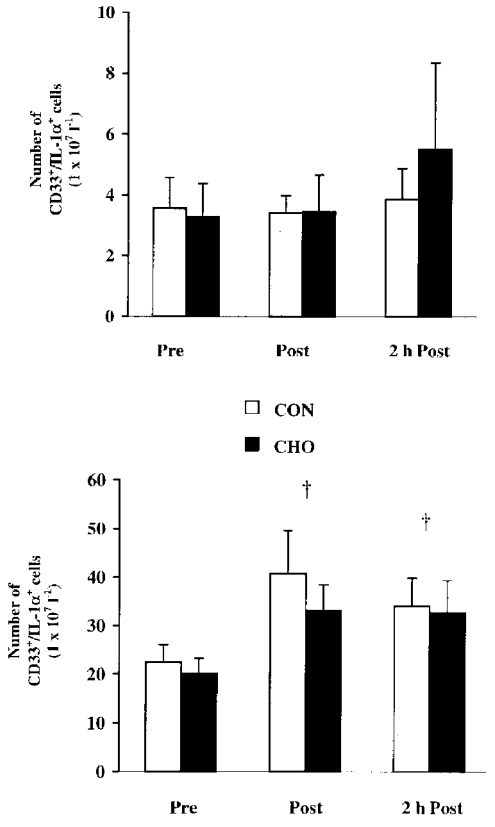

Figure 3. Number of IL-6α-positive cells

Spontaneous (top) and LPS-stimulated (bottom) $CD33^+/IL\text{-}1\alpha^+$ cells prior to (Pre), immediately following (Post), and 120 min into recovery (2 h post) from 2 h of bicycle exercise with (CHO) or without (CON) the ingestion of an 8% carbohydrate solution throughout exercise. Values are means ± S.E.M. ($n = 6$). † $P < 0·01$, significantly different from pre-exercise values.

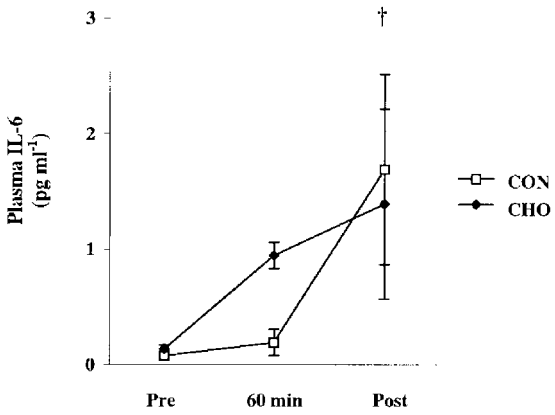

Figure 4. Plasma IL-6 concentration

Plasma IL-6 concentrations prior to (Pre), after 60 min (60 min), and immediately following (Post) 2 h of bicycle exercise with (CHO) or without (CON) the ingestion of an 8% carbohydrate solution throughout exercise. Values are means ± S.E.M. ($n = 6$). † $P < 0·01$, significantly different from pre-exercise values.

concentric cycling, yet an increase in plasma IL-6 and creatine kinase (a marker of muscle damage) concentrations following eccentric exercise. It should be noted that Nieman *et al.* (1998) employed cycling as an exercise mode and observed plasma IL-6 at concentrations of 25 pg ml^{-1}. Given that exercise intensities and durations were comparable between their study and ours, a possible reason for the higher plasma IL-6 levels observed in our study may be the different training status of the subjects employed. Subjects in the Nieman *et al.* (1998) study (maximal O_2 uptake ($\dot{V}_{O_2,max}$) = 52·8 ± 3·0 ml kg^{-1} min^{-1}; mean ± S.E.M.) were not as highly trained as those employed in the present study ($\dot{V}_{O_2,peak}$ = 4·78 ± 0·43 l min^{-1}). Monocytes are involved in the muscle tissue inflammatory response to injury (Woods & Davis, 1994) and damaged muscle may stimulate infiltration and activation of monocytes resulting in an elevation of plasma IL-6. Ostrowski *et al.* (1998*b*) reported increased IL-6 mRNA in muscle after 2 h of running, providing evidence that cytokines may be produced within the muscle in response to eccentric types of exercise. Thus IL-6 produced in muscle may be responsible for the elevations in plasma cytokines observed in previous studies. It is not known whether exercise which causes substantial muscle damage has a similar effect on monocyte cytokine production as observed in the present study.

Neuroendocrine hormones appear to be involved in the immune response and bi-directional communication exists between the immune and neuroendocrine systems (Turnbull & Rivier, 1999). Adrenaline is elevated in stressful situations and may be an important endogenous regulator of cytokine production. It has been observed that incubation of whole blood with adrenaline decreases LPS-induced TNF (Severn *et al.* 1992; Guirao *et al.* 1997; Bergmann *et al.* 1999), IL-1 and IL-6 production (Bergmann *et al.* 1999). In addition, infusion of adrenaline into healthy subjects resulted not only in decreased TNF-α production after *in vitro* LPS stimulation but also in a decrease in plasma TNF-α concentrations in response to LPS administration (van der Poll *et al.* 1996). These studies demonstrate that adrenaline has an inhibitory effect on the production of IL-1, TNF-α and IL-6. It is possible that elevations in plasma adrenaline during exercise could suppress circulating monocyte cytokine production, hence affecting immune function, and attenuation of adrenaline could prevent this suppression. In the present study, plasma adrenaline concentrations during exercise were elevated during both the CHO and placebo trials, but levels were significantly lower in the CHO trial than in the placebo trial (Fig. 5). Cells entering circulation during exercise were not spontaneously producing IL-1α, TNF-α and IL-6, as indicated by the lack of change in the number of cytokine-positive cells despite the increase in the number of monocytes in circulation. It is possible that elevated levels of adrenaline inhibited these cells from spontaneously producing cytokines. In addition, exercise may have resulted in an increase in circulating monocyte numbers, thereby increasing the number of cells capable of responding to LPS stimulation, but it is important to note that these cells produced less TNF-α post-exercise, and less TNF-α and IL-1α 2 h post-exercise. This decrease in the amount of cytokine produced as a result of exercise may be due to elevations in plasma adrenaline. This leaves us with the question of why CHO ingestion, which attenuated the increase in plasma adrenaline, did not result in the removal of the suppression of cytokine production. It is important to note that despite an attenuated adrenaline concentration in response to CHO ingestion, there was still a significant increase in adrenaline as a result of exercise during this trial. It is possible that these levels were sufficient to have an effect on circulating monocytes. An additional factor may be the difference in the time during which cells were subjected to differences in adrenaline between the two trials. Adrenaline concentrations in the placebo trial did not rise above concentrations observed in the CHO trial until after 60 min of exercise (Fig. 5). Therefore circulating monocytes were only subjected to different adrenaline concentrations for a maximum of 1 h. In the previously mentioned studies (Severn *et al.* 1992; Guirao *et al.* 1997; Bergmann *et al.* 1999) whole blood was exposed to adrenaline for periods ranging from 7 to 24 h. Van der Poll *et al.* (1996) infused adrenaline for 3 h before administering LPS and observing decreased plasma TNF-α. Therefore it is possible that in the present study circulating monocytes were not subjected to elevated levels of adrenaline for a sufficient period of time to observe a difference between the two trials. Also of interest is the exercise-induced increase in adrenaline observed during the current study, which was markedly lower than that observed by van der Poll *et al.* (1996) after adrenaline infusion. The effect of exercise, which results in a more significant elevation in plasma adrenaline for a longer period of time, on intracellular monocyte production remains to be elucidated. These aspects of hormonal–immunological interaction warrant further investigation. It has previously

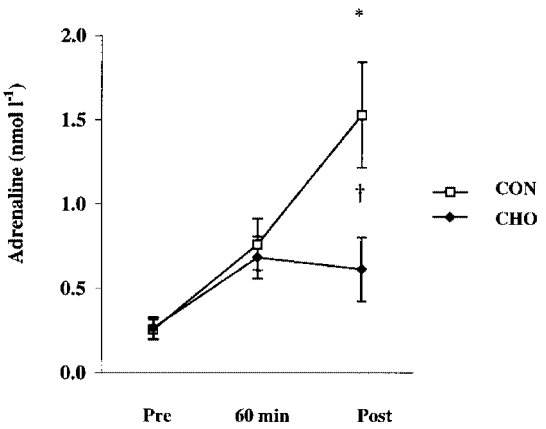

Figure 5. Plasma adrenaline concentration

Plasma adrenaline concentrations prior to (Pre), after 60 min (60 min), and immediately following (Post) 2 h of bicycle exercise with (CHO) or without (CON) the ingestion of an 8% carbohydrate solution throughout exercise. Values are means ± S.E.M. (n = 6). *P < 0·01, significantly different from pre-exercise values; †P < 0·01, significantly different from control.

been reported that CHO ingestion results in a decrease in plasma IL-6 during prolonged running exercise (Nehlsen-Cannarella *et al.* 1997; Nieman *et al.* 1998) and cycling (Nieman *et al.* 1998). In contrast, CHO administration during cycling exercise in the present study did not attenuate plasma IL-6 concentration (Fig. 4). A reason for this discrepancy may be that the predominant cell source of plasma IL-6 may be more sensitive to CHO during prolonged running than during cycling. Alternatively, it may be that, in contrast to previous studies, in the present study exercise or CHO ingestion had no effect on cortisol concentrations. Cortisol is produced during prolonged, strenuous exercise and has previously been demonstrated to decrease IL-1α, IL-6 (Amano *et al.* 1993; DeRijk *et al.* 1997) and TNF-α production (DeRijk *et al.* 1997). Nehlsen-Cannarella *et al.* (1997) and Nieman *et al.* (1998) observed that CHO ingestion decreased not only cortisol concentrations, but also plasma IL-6. If circulating monocytes are the source of the elevated plasma IL-6, it is likely that an increase in plasma cytokines and a decrease in cortisol with CHO ingestion would be observed. This provides further evidence that circulating monocytes are not the source of elevated plasma cytokine levels.

In conclusion, 2 h of submaximal cycling exercise resulted in an increase in the number of circulating monocytes producing proinflammatory cytokines in response to LPS stimulation, but these cells were producing less cytokine post-exercise, possibly due to elevated levels of plasma adrenaline. Circulating monocytes are not the source of the observed increase in plasma IL-6, and CHO ingestion, which blunted the rise in plasma adrenaline, had no effect on leukocytosis, plasma IL-6 or monocyte intracellular cytokine production.

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Corresponding author

M. A. Febbraio: Exercise Physiology and Metabolism Laboratory, Department of Physiology, The University of Melbourne, Parkville 3052, Victoria, Australia.

Email: m.febbraio@physiology.unimelb.edu.au