

Plasma Endotoxin and Immune Responses During a 21-km Road Race Under a Warm and Humid Environment

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Abstract

Introduction: This study investigated the responses of plasma endotoxin and pro- and anti-inflammatory cytokines during a 21-km road race in warm and humid conditions. The influence of carbohydrate-electrolyte (CE)-water (WA) drink mix ingested on leukocyte subset responses and the association between plasma lipopolysaccharide (LPS) concentration and fluid balance, exercise intensity, and body core temperature (Tc) were also studied. **Materials and Methods:** Thirty runners provided blood samples before and after the half-marathon for leukocyte, LPS and cytokine analyses. Tc was measured by the ingestible telemetric temperature sensor and fluid intake and split-times were recorded at 3 km intervals. Exercise intensity was determined by matching running speed and heart rate during the race with the corresponding speed-oxygen uptake relationship and heart rate measured in the laboratory 2 to 6 weeks before the race. **Results:** Plasma LPS concentration increased from 1.9 ± 1.9 pg/mL before, to 2.5 ± 1.9 pg/mL after running ($P < 0.05$). Peak plasma LPS concentration was 7.5 pg/mL. Plasma IL-1 β and TNF-concentration did not change significantly, whereas significant increases in IL-10 (50%), IL-1ra (23.2%) and IL-6 (65.2%) were observed after the race. No significant correlation between plasma LPS concentration and exercise intensity, hydration and Tc was observed. **Conclusion:** Leukocyte subset responses were not related to the ratio of CE and water drink mix ingested. Running a half-marathon can induce mild endotoxaemia, which is not related to exercise intensity, fluid balance, and Tc responses. Mixing CE drink with water did not mitigate post-exercise leukocytosis and lymphopenia.

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Key words: Carbohydrate-electrolyte drink, Core temperature, Cytokines, Leukocytes, Lipopolysaccharide

Introduction

During intense exercise, immune disturbances can occur through an increase in plasma pro- and anti-inflammatory cytokines^{1,2} and lipopolysaccharide (LPS) concentrations,³⁻⁵ and post-exercise leukocytosis and lymphocytopenia.⁶⁻⁸ The increase in LPS in the central circulation, also known as endotoxaemia, has been attributed to the leakage of LPS from the gastrointestinal tract during intense exercise and heat stress.⁹⁻¹¹ When exaggerated, endotoxaemia can trigger systemic inflammation,⁹ which has been implicated in the pathology of heat stroke. Heat stroke patients have endotoxaemia and increased plasma concentrations of TNF- α and IL-1 α .¹² Besides hyperthermia and neurological deficits, the other symptoms observed in heat stroke patients,

such as systemic inflammation, haemorrhages and multi-organ failure¹²⁻¹⁴ are similar to those observed in patients with sepsis.^{11,15}

Plasma LPS concentrations of 5 to 284 pg/mL have been reported after marathons, triathlons and ultra-endurance races.^{3,4,5,16} LPS is attached to gram-negative bacteria, which resides mainly in the gut space and are prevented from entering the circulatory system by the epithelial tight junction of the gut.¹⁷ However, during intense exercise, poor fluid balance, hyperthermia, hypoxia and free radical stress at the gut¹⁸⁻²² can increase the permeability of the epithelial tight junction of the gut, resulting in the translocation of gut-LPS into the portal and central circulation. Pals et al²⁰ demonstrated that the permeability

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of the gut is increased with higher exercise intensity, and endotoxaemia (5 to 284 pg/mL) has been reported in endurance athletes running more than 42 km. However, studies investigating the relationship between exercise-induced endotoxaemia and exercise intensity, fluid balance and body core temperature (T_c) are lacking. Information of these relationships will help to shed more light on the pathophysiology of exercise-induced endotoxaemia.

Ingestion of carbohydrate-electrolyte (CE) drinks during intense exercise mitigates post-exercise leukocytosis (28%-59%), monocytosis (21%-34%), granulocytosis (30%-100%) and lymphocytopenia (5%-25%).^{6,8,23} Compared with drinking CE drinks, drinking water (WA) during intense exercise results in greater increases in leukocyte (83%-127%), monocyte (60%-62%), and granulocyte (120%-214%) counts and a greater decrease in lymphocyte (33%-41%) count after intense exercise.^{6,8,23} CE drink mitigates post-exercise immune responses by decreasing cortisol secretion by the hypothalamus, pituitary and adrenal axis.²⁴ However, subjects in these studies drank either WA or CE drinks, which do not reflect the fluid intake profile during endurance races, where most runners would ingest a mixture of both CE drinks and WA. The effects of CE-WA drink mix on leukocyte subset responses have not been previously investigated, although such a fluid intake profile is a better reflection of fluid intake habits of runners during endurance races.

Based on the evidence presented, we hypothesise that a higher exercise intensity, T_c , and state of dehydration will promote endotoxaemia, which in turn triggers responses of the pro- and anti-inflammatory cytokines. We also hypothesise that a higher CE drink to WA intake ratio during endurance exercise will mitigate post-exercise leukocytosis and lymphocytopenia. This study investigated changes in plasma LPS concentration and immune responses (leukocyte subsets and cytokines) during a half-marathon race in warm and humid conditions. The correlation between plasma LPS concentration and T_c , fluid balance, and exercise intensity, and between CE-WA drink mix ingestion and leukocyte subset responses were also investigated.

Materials and Methods

Subjects

This study was conducted on 32 male endurance runners (with an age range of 21 to 32 years) comprising 30 military commando servicemen and 2 civilians who are trained endurance athletes. The volunteers gave their informed consent to participate in the study after being briefed on the nature, benefits, and risks of the study. The volunteers were also informed of their rights to withdraw from the study without any obligation. The procedures of this study were approved by the ethics committee at the Defence Medical

and Environmental Research Institute and are in compliance with the Belmont Report. Table 1 summarises the physical characteristics and race performance of the subjects.

Pre-race Laboratory Trials

Peak oxygen uptake ($\dot{V}O_{2\text{ peak}}$) was measured 2 to 6 weeks before the race day in the laboratory. The $\dot{V}O_{2\text{ peak}}$ test was conducted on a treadmill with indirect calorimetry based on expired air measurement with a metabolic cart (MetaLyzer® 3B-R2, CORTEX Biophysik, Germany). The test was conducted in a climatic chamber that was programmed at 27°C ambient temperature and 80% relative humidity, which reflect the environmental condition of the half-marathon race.²⁵ Continuous $\dot{V}O_{2\text{ peak}}$ test used comprised 4 running stages, with each stage lasting 4 min at submaximal effort. This was followed by an additional four maximal running stages, each lasting 3 min, after a 10 min break. The runners ran to volitional exhaustion during the maximal running stages. Figure 1 provides a schematic of the protocol.

For the 4 submaximal stages, the starting speed for each volunteer was derived from their most recent 2.4 km running time. The finishing time of <8 min, <9 min and >9 min in the 2.4 km run corresponded with a starting running speed of 10 km/h, 11 km/h and 12 km/h respectively, and the speed was increased by 1 km/h at each 4 min stage. Expired air, heart rate (HR) and the rating of perceived exertion (RPE)²⁶ were recorded at the last minute of each stage. The average $\dot{V}O_2$ in the last minute of each stage was then computed. A linear regression of speed versus $\dot{V}O_2$ responses was then plotted for each runner. This $\dot{V}O_2$ - speed relationship was used later to estimate the runner's exercise intensity during the race.

Upon completion of the 4 submaximal stages, the runners rested for 10 min before resuming the run to volitional exhaustion over another 4 x 3 min stages. Based on the HR and RPE scores during the submaximal stages, an estimated

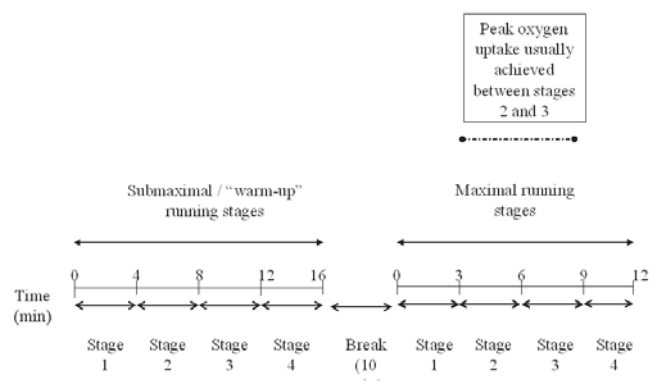


Fig. 1. Time-line schematic of the continuous protocol used for the $\dot{V}O_{2\text{ peak}}$ test conducted on a treadmill.

Table 1. Physical Characteristics and Race Performance of the Subjects

Position	Age (y)	Height (m)	Weight (kg)	Body fat (%)	$\dot{V}O_2$ peak (mL/min/kg)	Running speed (km/h)
1	26	1.64	53.6	9.7	62.3	13.8
2	28	1.67	64.9	10.1	65.8	13.4
3	22	1.70	72.9	12.9	61.0	13.0
4	23	1.74	62.9	14.9	60.2	13.0
5	22	1.79	73.0	14.9	60.1	12.8
6	26	1.69	57.0	15.5	60.6	12.7
7	23	1.71	63.5	12.8	63.9	12.5
8	23	1.74	76.2	15.8	62.9	12.4
9	22	1.65	54.9	9.1	57.7	12.2
10	28	1.73	61.7	11.0	58.6	12.1
11	26	1.77	70.4	13.5	55.2	12.0
12	30	1.74	61.9	9.1	62.9	12.0
13	23	1.79	70.4	15.5	51.1	11.9
14	22	1.78	68.0	15.8	55.7	11.9
15	26	1.69	71.7	18.2	57.1	11.8
16	23	1.76	69.1	11.8	64.5	11.8
17	28	1.73	67.7	17.6	61.2	11.7
18	26	1.72	55.2	13.1	53.2	11.6
19	30	1.68	69.0	17.1	63.3	11.6
20	21	1.76	70.0	19.6	58.6	11.4
21	26	1.77	67.7	11.7	57.1	11.3
22	22	1.70	61.3	11.2	48.7	11.2
23	32	1.64	61.0	19.3	54.4	11.1
24	31	1.79	76.1	17.4	62.0	11.1
25	23	1.67	67.4	17.9	59.9	11.0
26	23	1.73	65.3	13.9	59.7	11.0
27	21	1.70	61.3	11.2	58.3	10.9
28	25	1.74	71.9	11.6	52.6	10.8
29	26	1.63	57.6	15.8	55.7	10.7
30	30	1.72	68.9	17.2	59.7	9.2
Mean \pm SD	25 \pm 3.2	1.72 \pm 0.1	65.8 \pm 6.3	14.2 \pm 3.1	58.8 \pm 4.1	11.8 \pm 0.9
Min	21	1.63	53.6	9.1	48.7	9.2
Max	32	1.79	76.2	19.6	65.8	13.8

SD: standard deviation

Age, height, weight, percentage body fat, $\dot{V}O_2$ peak measured during the laboratory trial and race performance of the 30 subjects who completed the study.

constant running speed that would induce exhaustion between 6 and 9 min was used. With the speed held constant, the gradient of the treadmill was increased to 3.5% in stage 1, 6% in stage 2, 8.5% in stage 3 and 11% in stage 4.

$\dot{V}O_2$ peak is defined as the highest $\dot{V}O_2$ value attained during the run to exhaustion, and it must satisfy at least 2 of the following criteria: (i) a respiratory exchange ratio >1.15 ,²⁷ (ii) 100% of age predicted HR²⁸ and (iii) RPE of 19 to 20.²⁶

Height, body weight, skinfold measurements at the biceps, triceps, subcapsular and suprailiac (triplicates) were also performed during the laboratory trials. Percentage body fat was derived from the skinfold measurement using the tables provided by Durnin and Wormersley.²⁹

Race Details

The subjects reported to the race site at 0330 h on the day of the race. They were first asked to complete a health declaration form. In a designated room for the research team, subjects were first asked to produce a urine sample

before nude body mass was measured to the nearest 0.001 kg using a digital scale (ID 1Plus, Mettler-Toledo (S) Pte Ltd, Singapore) and blood samples [5 mL to plain tubes and 5 mL to pyrogen-free ethylene diamine tetraacetic acid (EDTA) tubes] were obtained from the forearm vein in the standing position. For easy identification along the race route, the subjects wore a standard bright orange running singlet with number tags, white shorts, socks and running shoes. WA was ingested *ad libitum* before the race. The race was flagged off at 0545 h. A portable climate-monitoring device (Questemp-15 Area Heat Stress Monitor, Quest Technologies, WI) was used to measure the dry bulb, wet bulb, relative humidity and wind velocity at the start/finish area.

During the race, the subjects drank only from the fluid stations that were set up by the research team at approximately 3 km intervals throughout the race route. At each drink station, specific water bottles for both CE drink and WA were designated for each subject and they were free to choose between drinking CE drink (100 Plus, F&N, Singapore) in yellow water bottles and/or WA, in blue water bottles. The bottles were handed to the corresponding subjects by the fluid station volunteers as they approach the stations. The amount of fluid ingested at each fluid station was derived from the difference in the weight of the water bottles, before and after consumption, and was later converted to volume. Split times were recorded by 4 to 6 members of the research team at each fluid station and by 2 researchers at the end-point of the race. Urine output during the race was also collected and weighed. At the end of the race, the subjects were led back to the preparatory room immediately where blood was drawn within 5 to 10 min with the same method as the pre-race blood sample. The subjects then towelled dry before nude body mass were recorded. No fluid consumption was allowed till both blood and body mass were taken.

Measurements

Leukocyte subsets count: Sixty blood samples (30 pre- and 30 post-race blood) were kept on ice from the site of collection and during transportation to the laboratory, which took about 3 h. Once at the laboratory, all blood samples were spun at 3500 rpm for 10 min at 4°C. Blood stored in plain tubes was then mixed thoroughly for a full blood count in duplicates using an haematology analyser (Coulter A^c.T diff 2 Analyzer, Coulter Corp, A Beckman Coulter Co, Miami, Florida). Plasma was extracted from the pyrogen-free tubes containing the anti-coagulant EDTA and stored at -80°C in pyrogen-free micro-tubes for the LPS and cytokine bioassays.

LPS and cytokine assays: The LPS bioassay was performed in duplicates using the Limulus Amebocyte

Lysate Chromogenic Endpoint assay (Hycult biotechnology b.v, Uden, Netherlands) according to the instructions of the manufacturer, and was read kinetically with a spectrophotometer (Microplate Scanning Spectrophotometer, PowerWave_x ITS, Bio-tek Instruments Inc, Winooski, Vermont). Kinetic readings were recorded every 1 min for 90 min or till the highest concentration of standards have reached a plateau.

Cytokines were assayed using a multiplex bead assay (Bio-Rad Laboratories, Inc, Hercules, California) according to the instructions of the manufacturer. The LiquiChip Reader and LiquiChip Microplate Handler, and Fluid Module (Luminex xMAP Technology, Qiagen Instruments, Venlo, Netherlands) were used for microplate reading.

Hydration parameters: The fluid intake volume at all stations was converted from weight (g) to volume (mL) by multiplying the weight of the fluid (g) to the specific gravity of the fluid. The specific gravity for the CE drink is 1.024 and for water is 1. The ratio of CE to WA consumed during the race was computed as a percentage. Fluid balance was estimated by subtracting total exercise urine volume, excreted during the race, from the difference in body weight before and after the race.

Body core temperature: T_c was measured using the CORTEMP™ (COR-100 Wireless Ingestible Temperature Sensor, HQ Inc, Palmetto, FL) or Jonah™ (Mini Mitter Co, Inc, Bend, Oregon) telemetric temperature sensors. Temperature signals are transmitted by low powered radio frequency to an external temperature-recording device (CORTEMPi CT2000, HQ Inc or VitalSense, Mini Mitter Co, Inc). On the day prior to the race, the sensors were calibrated at 4 points, 36°C, 38°C, 40°C and 42°C, using a heated water bath and a certified calibrated mercury thermometer (GH Zeal Ltd., London, UK). The volunteers ingested the sensors approximately 6 h 15 min to 7 h 15 min before the race. The ambulatory T_c recording device was placed in a sealed water-proof bag, and fitted into a padded pouch around the waists of the volunteers. The device weighed approximately 303 g for the CORTEMPi CT2000 and 323 g for the VitalSense® Integrated Physiological Monitoring System. During the race, T_c data were recorded at 15 s intervals and downloaded for analysis after the race. Peak T_c achieved for each runner was derived.

Exercise intensity: Exercise intensity was based on race time, estimated percentage of $\dot{V}O_{2\text{ peak}}$, and percentage of maximum HR during the race. Percentage of $\dot{V}O_{2\text{ peak}}$ was estimated by matching the average running speed for each subject with his individual speed- $\dot{V}O_{2}$ regression plot established in the laboratory trial. The subjects also wore a HR monitor (Polar Vantage, Polar Electro Oy, Kempele,

Finland) during the race. The percentage HR_{max} during the race was estimated by matching HR during the race with maximum HR recorded during the $\dot{V}O_{2\ peak}$ test.

Statistics

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 15.0. Each parameter was tested for normality with the Shapiro-Wilk test and data that were not normally distributed were transformed to natural logarithm before further analyses. Paired *t*-test was used to test for significant difference between pre- and post-race data. Parameters that were not normally distributed and could not be transformed using the natural logarithm because of negative values were analysed non-parametrically with the Wilcoxon-Signed Rank test. Pearson's product-moment correlation coefficient (for parametric distributions) and Spearman's Rho correlation (for non-parametric distributions) were used to examine the bi-variate relationships between post-race plasma LPS concentration and Tc, fluid balance and exercise intensity. Besides correlation, the Tc, fluid balance and exercise intensity scores of subjects in the upper and lower 50th percentile of plasma LPS concentration were compared, using an independent *t*-test or the Wilcoxon rank sum test for data that were not normally distributed. The 0.05 level of significance was used for all statistical analysis.

Results

The summarised data for the climatic condition on the day of the race are presented in Figure 2. Data for 30 of the 32 subjects were eventually used for analysis. One subject was unwell on the race day, and another subject was removed from the study because of difficulties in obtaining his post-race blood sample. All 30 runners completed the race, subject 26 however walked for the last 1 km race due to muscle cramps. The exercise intensity data of subject 26 was thus excluded from data analysis. However, the other data of subject 26 (LPS, cytokines, leukocyte subsets and peak Tc) were included in the analyses as these data are within physiological ranges and the data did not have a

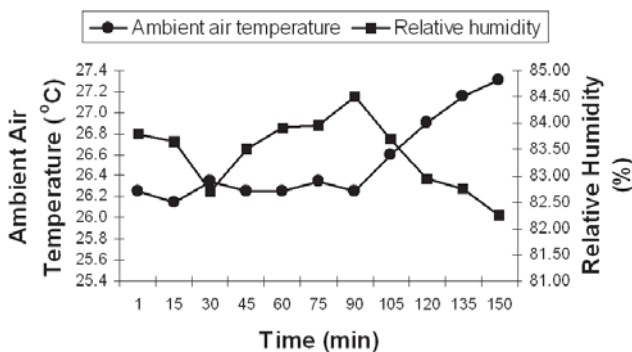


Fig. 2. Ambient temperature and relative humidity at the race site.

significant impact on the statistical analyses. Non-parametric correlation analyses were performed for tests involving percentage CE to WA drink mix parameters as they were not normally distributed and could not be transformed to natural logarithm due to zero values. The subjects completed the race at a mean of 107.12 ± 8.85 min. The average HR during the race is 172 ± 7.0 bpm and the average Tc is $39.6 \pm 0.6^\circ\text{C}$ (average peak Tc, 40.7°C) range at the end of the race.

Leukocyte Subset Concentration

Leukocyte count increased by 66.2%, from 6.8 ± 1.1 $10^9/\text{L}$ before the race to 11.3 ± 2.2 $10^9/\text{L}$ after the race ($P < 0.05$), and granulocyte count also increased significantly from 4.1 ± 1.0 $10^9/\text{L}$ before the race to 9.0 ± 3.2 $10^9/\text{L}$ after the race. In contrast, lymphocyte count decreased by 25%, from 2.4 ± 0.5 $10^9/\text{L}$ before the race to 1.8 ± 0.5 $10^9/\text{L}$ after the race ($P < 0.05$), whereas no significant changes were observed in monocyte count. Leukocyte subset responses are also not correlated significantly with the different ratio of CE-WA drink mix ingested (Table 2).

Plasma LPS Concentration

Running in the half-marathon induced a 31.6% increase in plasma LPS concentration, from 1.9 ± 1.9 pg/mL before the race to 2.5 ± 1.9 pg/mL ($P < 0.05$). The highest post-race plasma LPS concentration was 7.5 pg/mL. The plasma LPS concentration in 4 of the 30 runners (13.3%) met the criteria for mild endotoxaemia (plasma LPS concentration > 5 pg/mL⁵).

Plasma Cytokines Concentration

Mean \pm standard deviation (SD) of plasma IL-6 concentration increased from 9.2 ± 4.1 pg/mL before the race to 15.2 ± 5.3 pg/mL after the race ($P < 0.05$) (Fig. 3). Plasma IL-10 concentration before the race (6.4 ± 2.4 pg/mL) differed significantly from post-race concentration (9.6 ± 3.0 pg/mL) by 50.0% ($P < 0.05$) (Fig. 3). The

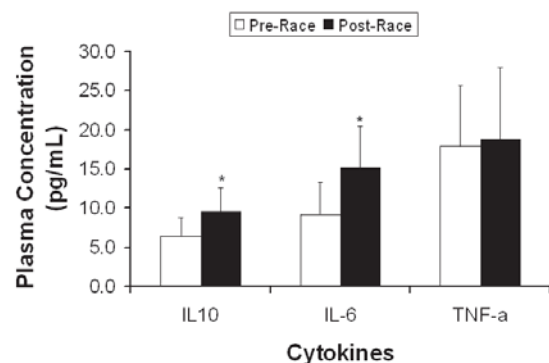


Fig. 3. Mean \pm SD plasma concentration of (a) IL-6, IL-10 and TNF- β , (b) IL-1 α and (c) IL-1 β before and after running 21 km. * $P < 0.05$ between pre- and post-race plasma concentrations.

Table 2. Relationship Between Exercise intensity, Fluid Balance, Peak Body Core Temperature and Post-race Plasma Lipopolysaccharide Concentration, and Between Percentage 100 Plus to Water Ratio Consumed During the 21-km Run and Post-race Leukocyte Subset Count

	Average percentage $\dot{V}O_2$ _{peak}	Average percentage HR _{max}	Race time	Peak body core temperature	Fluid balance		Post-race leukocyte	Post-race lymphocyte	Post-race granulocyte	Post-race monocyte
	% $\dot{V}O_2$ _{peak}	% HR _{max}	(min)	°C	kg		10 ⁹ cells/L	10 ⁹ cells/L	10 ⁹ cells/L	10 ⁹ cells/L
Post-race plasma lipopoly- saccharide concentration (pg/mL)						Percentage 100 Plus sports drink to water ratio				
r	-0.2	-0.15	0.27	-0.35	-0.05	r	0.02	-0.07	-0.1	-0.21
r ²	0.04	0.02	0.07	0.12	0	r ²	0	0.01	0.01	0.05
Sig. (2-tailed)	0.32	0.46	0.17	0.13	0.82	Sig. (2-tailed)	0.92	0.72	0.63	0.29

Correlation coefficient (r) and coefficient of determination (r²) between exercise intensity, fluid balance, peak body core temperature and post-race plasma lipopolysaccharide concentration, and between percentage of 100 Plus to water ratio ingested during the 21-km run and post-race leukocyte subset count.

concentration of plasma IL-1ra also increased significantly after the half-marathon, from 154.1 ± 0.4 pg/mL before the race to 189.8 ± 61.9 pg/mL after the race ($P < 0.05$) (Figure 3). However, running the half-marathon did not induce any significant changes in the plasma concentrations of both TNF- α (5.0%) and IL-1 β (-0.03%).

Influence of Tc, Fluid Balance and Exercise Intensity

No significant correlations was found between plasma LPS concentration and exercise intensity, fluid balance and Tc. Leukocyte and subset responses are also not correlated significantly with the different ratio of CE-WA drink mix (Table 2). There was also no significant difference in plasma LPS concentration between subjects in the upper and lower 50th percentile of Tc, fluid balance and exercise intensity.

Discussion

This study investigated the responses of plasma LPS and pro- and anti-inflammatory cytokines during a 21-km race in a warm and humid environment. The relationships between plasma LPS concentration and exercise intensity, fluid balance and heat strain, and between CE to WA drink mix ingested and leukocyte subset responses were also investigated. Average plasma LPS concentration increased by 31.6% after the race, and the highest post-race plasma LPS concentration was 7.5 pg/mL. The concentrations of pro-inflammatory cytokines (IL-1 β and TNF- α) did not change significantly during the race. In contrast, significant increases in anti-inflammatory cytokines (IL-10, 50.0% and IL-1ra, 23.2%) and inflammatory responsive cytokine (IL-6, 65.2%) were observed after the race. We also observed significant increases in granulocyte (119.5%) and leukocyte (66.2%) counts and a significant decrease in lymphocyte

count (25%) after the race, and these changes in leukocyte subset counts are not influenced by the CE-WA drink ratio ingested.

The leukocyte subset responses observed in our study are consistent with those reported in other studies. In our runners, mean granulocyte count increased by 119.5% and leukocyte count increased by 66.2%, whereas lymphocyte count decreased by 25%. These changes in immune cell counts are consistent with the post-exercise immune cell responses reported in laboratory studies^{7,8,30} and following an ultra-marathon.³¹ Post-exercise lymphopenia have been attributed to the redistribution of lymphocytes to the extravascular compartments, due to the concurrent increase in plasma cortisol concentration.^{8,32} Cortisol inhibits the movement of lymphocytes into the circulation and promote the shifting of lymphocytes from the circulation to other lymphoid compartments.^{8,32} It appears that running 21 km in warm and humid conditions induces a similar magnitude of immune response compared to running up to 2.5 h in the laboratory^{7,8,30} and a marathon in cooler conditions.³¹ These results suggest that immune responses after exercise may not respond linearly with exercise volume.

To the best of our knowledge, this is the first study to investigate the effects of CE-WA drink mix ratio ingested on immune responses during endurance exercise. Earlier studies have compared the effects of drinking either CE or WA drinks on immune cell responses during 2 to 3 h of endurance exercise in the laboratory environment.^{7,8,23} During exercise, carbohydrate intake during exercise mitigates leukocytosis and lymphopenia through the inhibition of cortisol secretion by the hypothalamus-pituitary-adrenal axis.²⁴ Compared with drinking water, ingesting CE drinks during exercise attenuated the magnitude of leukocytosis (28%-59%) and lymphopenia

(5%-25%)^{6,8,23} after exercise. However, most endurance runners would normally drink a mixture of WA or CE drinks, and not WA or CE drink alone, during endurance races. Our results suggest that ingesting a mixture of WA with CE drink does not mitigate post-exercise leukocytosis and lymphopenia. The correlation between the leukocyte subset counts and the ratio of CE-WA drink mix ingested is not significant. There is also no significant difference in immune cell count between subjects in the upper and lower 50th percentile of CE-WA drink mix ratio. These results suggest that the moderating effects of CE-drinks on leukocyte subset responses through the cortisol pathway may be diminished when consumed with water. The findings from laboratory studies which showed that ingesting CE drink during intense exercise is able to moderate post-exercise leukocytosis and lymphopenia^{6,8,23} may not be applicable in race performances. This disparity between field and laboratory results can be attributed mainly to differences in fluid intake profile of laboratory subjects and runners in endurance races.

Running 21 km in a warm and humid climate led to a 31.6% increase in plasma LPS concentration. In our study, mean plasma LPS concentration increased from 1.9 pg/mL before the race to 2.5 pg/mL after the race. Except for 2 runners in whom we did not detect any LPS in the plasma, the other 28 runners had plasma LPS concentrations ranging from 0.05 to 7.5 pg/mL. These concentrations of plasma LPS are similar to those reported in a triathlon (5-15 pg/mL)¹⁶ and in a full marathon (5-13 pg/mL).⁵ Taken together, these results suggest that in race conditions, plasma LPS concentration may not be differentiated by work volumes. In a non-pathological state, the concentration of LPS in the blood may reach equilibrium during endurance exercise. This equilibrium in LPS concentration may reflect the balance between exercise-induced influx of LPS from the gut into the circulation and the responses of the reticuloendothelial system in the liver and the anti-LPS antibodies in the circulation in removing LPS from the body.³³⁻³⁵

We did not find any effects of exercise intensity, Tc and fluid balance on plasma LPS concentration. Our results contradicted the findings of Pals et al²⁰ who reported that the permeability of the gut is increased with higher intensity exercise, which may promote LPS translocation from the gut into the circulation.¹¹ Pal and colleagues did not measure circulating LPS, but determined changes in small intestinal permeability using urinary excretion ratio of lactulose-to-rhamnose, where lactulose (a disaccharide) is normally not capable of crossing the small intestinal barrier, due to its large molecular size. Both sugars were ingested as a solution before the exercise. In addition, plasma concentration of LPS is not influenced by the influx of LPS

alone, but also by the removal of LPS from the body by the reticuloendothelial system in the liver and anti-LPS antibodies in the circulation.³³⁻³⁵ Therefore, the influence of exercise intensity on gut permeability alone (LPS influx) cannot fully account for plasma LPS concentration during intense exercise. Our results suggest that both fluid balance and heat strain are also unlikely to play significant roles in promoting endotoxaemia during a 21-km race. The role of dehydration and heat strain on plasma LPS translocation was first suggested by Moseley and Gisolfi¹¹ as possible mechanisms for promoting the translocation of gut-LPS into the circulation and endotoxaemia during exercise but these mechanisms have not been proven. Previous studies only reported increased gut permeability with hyperthermia in animals²¹ and with fluid restriction in humans,¹⁸ using fluorescent labelled dextran and urinary excretion ratio of lactulose-to-rhamnose as probes, respectively. As mentioned above, the post-race LPS concentration is a result of both LPS influx and clearance. Hence, the expected relationships are not observed.

The concentrations of pro-inflammatory cytokines, IL-1 β and TNF- α , did not change significantly during the half-marathon. In contrast, the concentration of IL-6, an inflammatory responsive cytokine increased by 1.7-fold. The concentrations of anti-inflammatory cytokines, IL-10 and IL-1ra, also increased significantly by 1.5-fold and 1.2-fold respectively during the race. Our results are consistent with the cytokine profiles in runners after a 6 h endurance race, where plasma concentrations of IL-1 β and TNF- α did not change significantly before the race, which was coupled with significant increases in plasma IL-6 (3.9-fold) and IL-1ra (4.7-fold) concentrations.³⁶ The greater responses of IL-6, IL-10 and IL-1ra could have inhibited the increase in IL-1 β and TNF- α in both our half-marathon runners and the runners in the 6 h race. These results suggest that under non-pathological conditions, where the exercise stress is well-tolerated, the anti-inflammatory pathways may prevail over the inflammatory pathway. This suggestion is supported by the cytokine profile of runners who completed a 160-km race.³¹ In these runners, the greater magnitudes of increase in the plasma concentrations of IL-6 (48.6-fold), IL-10 (51.8-fold) and IL-1ra (4.7-fold) were observed along with a small increase in TNF- α (1.1-fold). The relatively lower magnitude of change in the individual cytokines in our runners, compared with runners in the 6 h and 160-km endurance races, can be attributed to the difference in exercise volume. Taken together, these results suggest that cytokine response is associated with exercise volume and that the anti-inflammatory cytokines are able to cope with responses of the inflammatory cytokines in endurance races when the exercise volume is well-tolerated by the athletes.

Conclusion

Our results showed that mild endotoxaemia could occur in well-trained athletes after running 21 km under warm and humid conditions. The increase in plasma concentration of LPS is not associated with exercise intensity, fluid balance or heat strain. Under these conditions, the anti-inflammatory cytokines are able to suppress the increase in pro-inflammatory cytokines. Our results also showed, for the first time, that ingesting a mixture of CE drink and WA, does not have a moderating effect on post-exercise leukocytosis and lymphopenia.

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