

Low markers of muscle damage and inflammation following a 3-day trail run

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Abstract

Objectives. To investigate the effect of a 3-day trail run on markers of muscle damage and inflammation in recreational runners.

Main outcome measures. Pre- and post-stage and 24-hour and 72-hour post-race concentrations of serum creatine phosphokinase (CPK), high sensitivity C-reactive Protein (hsCRP), cortisol, cardiac Troponin T (cTnT), and osmolality (sOsm) as well as urinary myoglobin (uMb), changes in body mass, delayed onset muscle soreness (DOMS) and thigh circumference (TC) were measured. Continuous recordings of heart rate (HR) and intestinal temperature (T_{intest}) were made throughout each stage.

Results. Heart rate ranged between 77% and 83% age-predicted maximum (APmax) and T_{intest} between 36.1 and 40.2°C during the three stages. Significant rises in mean serum CPK, hsCRP, sOsm and blood neutrophil count reached peak concentrations of 1488U/l, 8.91mg/l, 298mosm/l and $10.21 \cdot 10^9/l$ ($p < 0.001$), respectively. No evidence of elevations in uMb and cTnT were detected. The stage-induced increments in DOMS correlated positively with CPK, $r = 0.71$; 95% CI [0.62, 0.78], TC decreased significantly post S1_{post} and S2_{post} ($p < 0.05$) and a maximum mean body mass loss of 3.09% ($\pm 1.04\%$) occurred during S2.

Conclusion. Three consecutive days of 95-km trail running resulted in low markers of muscle damage and inflammation, despite the maintenance of a heart rate above 77% APmax, T_{intest} rising above 39°C and mean body mass decrement of $> 2.0\%$.

Introduction

Trail running events are becoming increasingly popular with amateur athletes.¹ These are generally regarded as more strenuous than road running due to the nature of the trails, which can involve diverse challenges including single track paths on steep ascends and descends in mountains, crossing rivers and running along grasslands and through forests.² Although physiological response to single-day trail running has been assessed,^{1,4} the cumulative effects of multi-day trail running on markers of muscle damage and inflammation have not yet been reported.

Prolonged endurance exercise causes muscle damage that initiates an inflammatory response and subsequent remodelling of muscle.⁵ The extent of this damage is augmented by increases in exercise intensity, the eccentric component of contraction,⁶⁻⁸ heat stress index and dehydration.³ The greater contractile load per unit in muscles of the lower limb, as they contract eccentrically during

downhill running,⁸ has been associated with increased mechanical damage to the muscle fibres, resulting in muscle membrane leakage and elevated concentrations of circulating muscle enzymes and proteins.⁹ Systemic markers of inflammation also rise^{5,7} and swelling, decreased mobility and delayed-onset muscle soreness (DOMS) are common.^{5,6} The presence of myoglobin in the urine has been reported in severe cases.⁵

Although the direct cause-and-effect relationship between dehydration and hyperthermia is currently contentious,¹⁰ it has been reported that these augment exercise-induced muscle damage,^{3,4} detrimentally affect performance and pacing during trail running and increase post-exercise DOMS.^{3,4,11} Cleary *et al.*¹¹ reported an association between dehydration and hyperthermia and attributed an increase in muscle damage to the increased degradation of muscle proteins with elevated deep-muscle temperature.

The aims of the study were therefore to determine effects of a multiday trail run on the markers of muscle damage and inflammation in experienced recreational runners, measuring serum and urinary levels of selected skeletal muscle, cardiac and hepatic proteins in association with changes in red and white blood cell and serum cortisol concentrations before and after every stage and at 24 hours post-race (24PR) and 72 hours post-race (72PR). A further aim was to assess the possible effect of dehydration and hyperthermia on the markers of muscle damage and inflammation.

It was hypothesised that the three consecutive days of trail running would result in elevations of systemic and urinary markers of skeletal muscle damage and inflammation that are higher than previously reported during road running events of similar duration, and that the muscle damage and inflammation would be augmented by hyperthermia and dehydration.

Method

Ethical clearance

This 8-day observational cohort study took place during a 3-day trail run and for 5 days following completion of the Three Cranes Trail Run, at Karkloof, KwaZulu-Natal, South Africa on 25 - 27 February 2011.

Following approval by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal, subjects gave written consent after having been informed of the experimental procedures.

Subjects

Twenty-one apparently healthy subjects, who met the inclusion criteria (age: ≤ 50 and an average training distance of 60 km per week)



Fig. 1. Selected images of the running terrain.

and did not use chemical stimulants, were accepted into the study. Nineteen (6 males, 13 females) completed all three stages of the race and 15 runners (4 males, 11 females) completed all within- and post-race assessments.

Setting

The Three Cranes trail run, over 3 days and a total distance of 95 km, was divided into 3 consecutive stages comprising 29.3, 37.9 and 27.8 km, starting and finishing each day at the same base camp. Athletes were accommodated in a race village and full catering was provided for the duration of the race, including at the aid stations along the route. The routes consisted of gravel and forestry roads, narrow rocky

mountain footpaths and grassy jeep track. Elevation gains reached 1 020, 1 226 and 680 m, while elevation losses were recorded at 1 021, 1 231 and 687 m during S1 (Stage 1), S2 (Stage 2) and S3 (Stage 3) respectively (Table 1). Selected images of the running terrain are presented in Fig. 1.

Baseline measurements

Following race registration the afternoon before the race, basic anthropometric measurements were recorded, including body mass (kg), stature (cm) in bathing suits without shoes, thigh circumference (TC) (measured 15 cm above the superior border of the patella) and four-site skinfold (supra-iliac, subscapular, biceps and triceps) for the

Table 1. Elevation changes (m) and ambient temperature ranges during the three stages of the trail run

	Elevation gain	Elevation loss	Ambient temperature range (°C)
Day 1	1 020	1 021	11.5 - 21.7
Day 2	1 226	1 231	12.4 - 22.8
Day 3	680	678	12.1 - 21.2
Total	2 926	2 930	

determination of % body fat.¹² A pre-race questionnaire detailing the athletes' running and racing experience, training terrain and health status was also completed.

Daily protocol

Pre stage

The subjects presented themselves to a designated testing area 30 - 90 minutes before the start of the stage, handing in a first early-morning urine sample. TC was measured, venous blood sampling was conducted in the seated position and resting heart rate (HR) and blood pressure (BP) were recorded after a 3 - 5-minute period of relaxation. A simple pre-stage questionnaire including a rating of the degree of muscle soreness they were experiencing, was completed and the subjects were asked to keep a record of their fluid intake and urine output during the stage. After breakfast and final voiding of bladders, body mass (measured in running attire without shoes), was taken within 5 minutes prior to the start of the event.

Within stage

Environmental conditions and temperature were supplied on the hour by a meteorological station located 9.5 km from the base camp. Heart rate was recorded using a polar HR monitor (Polar Electro OY, Finland) at 5-minute intervals and % age-predicted maximum (APmax) was determined according to the formula, $220 - \text{age}$.¹⁴

A subsample of 12 athletes volunteered to ingest the *Cor-Temp* disposable tablets, containing temperature sensors (HQ Inc, Palmetto, FL), at least 3 hours prior to the start of each stage. The HR and intestinal temperature (T_{intest}) data are part of a more detailed study focussing on the relationship between T_{intest} , HR and hydration status.¹³

Post stage

The subjects proceeded directly to the designated testing area where BP, mass and TC were measured within 3 - 5 minutes, blood and urine samples were taken and a short DOMS and post-stage questionnaire providing details regarding the use of non-steroidal anti-inflammatory drugs (NSAIDs) and muscle soreness, were completed. In available athletes (n=10), a further measurement of TC was taken 4 hours after completion of S1 and S2.

The same protocol was followed pre- and post-stage on the 3 days of the race.

Post race

At 24PR and 72PR, participants presented for further blood/urine sampling, BP, HR and anthropometric measurements. They were also requested to complete a DOMS questionnaire for the 5 days following the race, using a five-point Likert scale, and to return this together with a general post-race questionnaire, following completion of the study.

Haematological analysis and anthropometric measurements

Each measurement was carried out by the same researcher for all subjects and at each time point. Venous blood samples were drawn from the antecubital fossa, with subjects in the seated position, within 5 - 15 minutes of completing the stage. Blood samples for the assessment of full blood count (FBC) and serum osmolality (sOsm) and urine samples were stored at 4°C and transported to a commercial pathology laboratory. Complete blood counts were measured on an Advia-120 Hematology Analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) and included erythrocyte indices and differential leukocyte counts. Both urine and serum osmolality were measured by freezing-point depression, using a Kyoto Daiichi osmostat, OM 6020 (Japan). Urine samples were also assessed for myoglobin (uMb) and specific gravity using the refractive index method on a Beyer Test Strip.

Further aliquots of serum, separated by centrifugation @ 3 000 rpm and stored in dry ice were transferred to an -80°C ultrafreezer or transported to a commercial pathology laboratory for analysis of creatine phosphokinase (CPK), cortisol, cardiac troponin T (cTnT) and high sensitivity C-reactive protein (hsCRP) concentrations.

Statistical analyses

Data are presented as mean \pm standard deviation (SD). The significance of the accumulative time-dependent stage-induced changes from pre-race ($S1_{\text{pre}}$) to post race ($S1_{\text{post}}$, $S2_{\text{post}}$, $S3_{\text{post}}$), as well as recovery rates were assessed comparing $S2_{\text{pre}}$, $S3_{\text{pre}}$, 24PR and 72PR to baseline ($S1_{\text{pre}}$), $S2_{\text{pre}}$ and $S3_{\text{pre}}$ were assessed for the entire group using repeated measures one way analysis of variance. The time point of the significant differences was confirmed using a Tukey *post hoc* analysis.

Comparisons between NSAID users and non-users were conducted using independent Student's *t*-tests. Pearson's product moment coefficient of correlation, with a confidence interval (CI) of 95%, was used to test the relationship between the changes in measured outcomes including CPK, neutrophil concentrations, hsCRP and serum cortisol.

All statistical calculations were performed using SPSS, version 18 (SPSS Inc., Chicago, USA). Level of significance was set at $p < 0.05$.

Results

Environmental conditions

Temperature recorded on the hour during the three stages of the race ranged from 11.5°C to 22.8°C (Table 1). It did not rain, maximum wind speed recorded was 2.8 m/s and the relative humidity ranged from 54% to 97%.

Table 2. Mean \pm SD baseline physical characteristics of subjects (n=19)

Variable	Mean \pm SD
Age (years)	39.3 \pm 7
Height (cm)	169.0 \pm 10
Mass (kg)	65.8 \pm 12
% body fat	21.7 \pm 4
Resting heart rate (bpm)	56.9 \pm 5
Systolic blood pressure (mmHg)	124.7 \pm 7
Diastolic blood pressure (mmHg)	81.8 \pm 7

Table 3. Training status and performance characteristics of athletes (n=19)

Characteristics	Mean±SD	Range
Running experience		
Number of years	12.4±8.1	2 - 27
Number of competitive endurance events	136.3±55.6	18 - 500
Weekly training distance (kilometres per week)	65.9±20.1	12.5 - 105
Number of days per week on different training terrains		
Hills	1.4±0.8	1 - 4
Off road incl. forest /trail/beach	1.7±1.5	0 - 6
Road	3.9±1.3	0 - 5
Race time (hour:minute:second)		
Stage 1	4:04:31±25:54	3:06:06 - 5:22:48
Stage 2	5:39:12±25:31	4:14:56 - 7:46:27
Stage 3	3:14:15±21:06	2:38:38 - 6:51:50
Average heart rate (beats per minute)		
Stage 1	150.8±21.3	73 - 191
Stage 2	140.7±22.5	60 - 186
Stage 3	138.5±23.3	75 - 198
Mean as %APmax*		
Stage 1	83±8.8	71 - 112
Stage 2	78±7.8	55 - 105
Stage 3	77±8.1	63 - 105

Data presented as mean (±SD) and range.
*Age-predicted maximum (220-age).

Subjects

As is shown in Tables 2 and 3, athletes ranged from 25 to 50 years of age, their weekly training distance averaged 65.9±20.1km per week for 12.4 years (range 2 - 27 years) and they presented without abnormalities in their vital signs. Of the 19 subjects, 12 used NSAIDs, including aspirin, ibuprofen and diclofenac.

Of the 21 subjects who initially agreed to participate in the study, one subject (male) withdrew after S1 due to an ankle injury and another (female) after S2 due to medical reasons. The baseline physical characteristics of the remaining 19 subjects are provided in Table 2. Four subjects were however unable to provide blood samples at 24PR and 72PR.

Intensity of effort

The mean ±SD and range of time spent completing each stage and average HR on the run, are given in Table 3. Total average running time of the athletes was 12h57±2h51.

Markers of muscle damage and inflammation

As shown in Table 4, these included a significant increase in circulating neutrophil concentrations ($p<0.001$) which peaked at 10.21 ± 1.54

$10^9/l$ at $S1_{post}$, serum CPK and hsCRP which peaked at $S3_{post}$ at $1488\pm1053U/l$ ($p\leq0.001$) and $8.91\pm6.63mg/l$ ($p\leq0.001$), respectively. cTnT and uMb were undetected in all samples throughout the 3-day event.

An exercise-induced increase in serum cortisol concentration was only detected following $S2_{post}$. TC decreased significantly from 54.1 ± 4.4 cm at $S1_{pre}$ to 51.8 ± 3.9 cm at $S1_{post}$ ($p<0.001$) and returned to the pre-race measurement of 54.1 ± 4.0 cm at 24PR. DOMS ranged from 4.8 ± 1.6 , 5.6 ± 1.8 and 5.1 ± 1.1 at $S1_{post}$, $S2_{post}$ and $S3_{post}$ respectively, and decreased to 1.73 ± 1.3 at 24PR.

Significant positive correlations were evident between blood neutrophil concentrations and serum CPK, $r=0.27$, 95% CI [0.11, 0.41], serum CPK and hsCRP concentrations, $r=0.50$, 95% CI [0.29, 0.66] and DOMS and CPK, $r=0.71$, 95% CI [0.62, 0.78].

Dehydration, intestinal temperature (T_{intest}), HR and muscle damage

The mean % body mass loss for the entire group (n=19) during the three stages was 2.9 ± 0.7 , 3.1 ± 0.8 and 1.9 ± 0.9 , while the mean sOsm (n=19) increased from 288.9 ± 4.8 to 293.7 ± 5.7 ($p=0.003$), 288.4 ± 6.4 to 295.6 ± 6.0 ($p=0.003$) and 292.2 ± 4.1 to 295.0 ± 5.6 ($p=0.006$) mOsm/kg, during S1, S2 and S3, respectively. When the pooled data for each stage were compared (n=51), the paired post-pre changes in sOsm correlated inversely with the changes in % body mass, $r=-0.36$, 95% CI [-0.57,-0.094].

The pooled data examining the relationship between the change of sOsm and change in serum CPK for the three stages (n=57) revealed an insignificant positive correlation ($r=0.034$, 95% CI [-0.228, 0.291]).

The maximum T_{intest} ranged between $38.3^\circ C$ and $40.2^\circ C$ and only exceeded $40^\circ C$ in two of the 12 athletes monitored (Table 5). The relationship between change in T_{intest} and serum CPK was insignificant ($p>0.05$) for the 11 individuals from whom complete sets of data were available ($r=0.24$, 95% CI [-0.42, 0.734]).

Users of NSAIDs

The 12 athletes who used NSAIDs had maximum serum CPK and hsCRP concentrations of 1332 ± 943.5 U/l and 8.58 ± 6.7 mg/l at $S3_{post}$ and the non-users 1754 ± 1251.3 U/l and 9.47 ± 7.0 mg/l, with no significant difference between the groups ($p=0.456$; 0.788). The neutrophil count reached a maximum of 9.95 ± 2.1 and 9.75 ± 0.4 $10^9/l$, respectively, for users and non-users ($p=0.82$). There was also no significant difference between NSAID users and non-users in terms of serum cortisol, post race DOMS scores, running times, TC or sOsm ($p>0.05$).

Discussion

Evidence of muscle damage and inflammation

The results of the present study indicate that very little muscle damage and inflammation occurred during 3 days of trail running despite athletes running for a total average of 12h57 at an average HR of 77 - 83% APmax (Table 3). The serum CPK concentration, which increased progressively to reach peak concentrations at $S3_{post}$, indicated only a mild cumulative effect of muscle damage during the race, which rejects the original hypothesis. Furthermore, the changes in neutrophil count, serum cortisol and hsCRP concentrations and DOMS also confirm low levels of inflammation and a rapid recovery. Most athletes in our study had no muscle soreness at 72PR, which correlated with the CPK concentration that had dropped close to the clinical upper limit of normal by 72PR.¹⁵ The consistently low release of muscle proteins into the bloodstream in all 19 subjects, which was also not accompanied

Table 4. Mean±SD white and red blood cell indices and markers of muscle damage and inflammatory response before and after every stage and at 24PR and 72PR

Variable	Stage 1		Stage 2		Stage 3		24PR	72PR
	Pre	Post	Pre	Post	Pre	Post		
Red blood cells (10 ¹² /l)	4.7	4.7	4.5	4.6	4.4	4.4	4.1	4.3
Haemoglobin (g/dl)	±0.5	±0.5	±0.4	±0.5	±0.4	±0.4	±0.4	±0.4
Haematocrit (%)	14.4	14.3	13.7	14.1	13.2	13.3	12.7	13.2
White blood cells (10 ⁹ /l)	±1.3	±1.2	±1.0	±1.2	±0.9	±1.1	±0.9	±1.2
Neutrophils (10 ⁹ /l)	42.4	42.1	40.2	41.1	39.9	39.5	38.1	40.5
Lymphocytes (10 ⁹ /l)	±3.9	±3.3	±2.9	±3.2	±2.7	±2.9	±2.5	±3.4
Eosinophils (10 ⁹ /l)	6.0	12.8*	6.5	12.6**	7.1	9.7***	6.7	6.5
Basophils (10 ⁹ /l)	1.2	±1.7	±1.3	±2.0	±1.3	±2.2	±1.2	±1.5
CPK (U/l)	2.9	10.2*	3.4	9.7**	3.7	7.3***	3.6	4.2
hsCRP (mg/l)	±0.7	±1.5	±1.0	±1.9	±1.0	±2.1	±1.0	±1.4
Cortisol (nmol/l)	2.1	1.4	2.2	1.73	2.4	1.5	2.1	1.6
uMb (mcg/ml)	±0.5	±0.5	±0.6	±0.6	±0.7	±0.4	±0.6	±0.5
cTnT (µg/l)	0.3	0.1	0.2	0.1	0.3	0.1	0.2	0.1
Thigh circum (cm)	±0.2	±0.1	±0.2	±0.1	±0.2	±0.1	±0.2	±0.1
	0.04	0.1	0.0	0.06	0.05	0.0	0.04	0.03
	±0.02	±0.03	±0.02	±0.05	±0.02	±0.02	±0.02	±0.01
	116.5	275.4*	419.8	971.6**	953.7	1488***	595.6*	201.9*
	±54.6	±105.9	±212.6	±534.2	±579.3	±1053	±361.4	±111.3
	0.7	-	-	-	-	8.9*	6.6*	2.0*
	±0.5	-	-	-	-	±6.6	±6.2	±1.7
	759.1	779.1	729.2	934.9**	646.8	583.2	-	-
	±154.8	±233.3	±134.1	±216.9	±112.4	±213.2	-	-
	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
	-	-	-	-	<0.01	<0.01	<0.01	-
	54.1	51.8*	54.1	53.3	53.4	53.8	54.1	53.5
	±4.4	±3.9	±4.6	±4.6	±4.4	±4.2	±3.9	±3.8

*v. S1pre, p<0.001
**v. S2 pre, p<0.001
***v. S3 pre, p<0.001
uMb=urinary myoglobin; cTnT=cardiac troponin T; n/d=not detected.

by elevation in cTnT and uMb in this study, confirms a profile of low degrees of muscle damage. Further evidence is the fact that TC was not significantly elevated at any post-stage or post-race time-point, but was reduced after S1 (p<0.001), confirming previous findings of reduced swelling and a post-race decrease in muscle mass.¹⁶

The low systemic markers of muscle damage and inflammation, when compared with previous findings following the Comrades Marathon¹⁶ confirm the findings of Millet *et al.*¹ who, in their study on the neuromuscular consequences of extreme running in a 166 km mountain ultra-marathon, reported that post-race serum concentrations of CPK, hsCRP and neutrophils were lower than those measured after a road race with similar finishing times.¹ These researchers attributed their findings of low concentrations of systemic markers of muscle damage and inflammation to the relatively soft underfoot surfaces and to the athletes frequently being forced to walk, jump and climb due to the technical demands of the terrain.

During extensive exercise-induced muscle damage myoglobin may be released into the urine and be indicative of exertional rhabdomyolysis and possible risk of renal failure.⁸ Clarkson⁹ however reported that exertional muscle damage in healthy athletes can cause profound serum CPK elevations without renal impairment. In our study the absence of uMb was confirmed by the relatively low increases in systemic neutrophil, serum CPK and hsCRP concentrations.

In this study we suspect that although the primary factor which reduced the amount of repetitive and eccentric unidirectional stress encountered during the race was most probably the underfoot surfaces, the majority of which were primarily soft, large fluctuations in the pace of running and varied muscle recruitment patterns over the different terrains may also have played a role.

The positive correlation between DOMS scores and CPK concentrations supports the findings of Nieman *et al.*² who, in their study on 60 participants in the 160 km 1-day Western States Endurance Trail Run in the Sierra Nevada Mountains in northern

Table 5. Individual T_{intest} and associated HR, changes in hydration status and peak serum CPK concentration ($n=12$)

Subject Number	T_{intest}			Max		Max%		$\Delta sOsm$ (mOsm/kg)	Δ Body Mass % (U/l)	Max serum CPK
	Max	Min	Mean	HR	Mean HR	APmax	HR*			
1	39.2	36.6	38.5	176	167	100	95	-3	-3.4	#
2	38.7	37.2	38.3	161	148	94	87	-6	-2.9	772
3	39.4	37.3	38.7	164	153	88	82	13	-2.7	1562
4	39.6	36.8	38.7	193	144	112	83	-6	-4.1	1057
5	39.2	35.2	37.4	168	154	95	87	10	-2.8	1523
6	39.8	36.1	37.7	168	160	92	87	7	-3.1	4478
7	38.7	36.9	38.2	152	140	89	82	11	-3.0	772
8	39.2	37.3	38.1	180	159	95	72	-23	-2.3	1198
9	40.2	37.1	38.0	181	144	102	81	-5	-3.3	1076
10	38.9	37.4	38.4	156	150	84	81	5	-3.2	1089
11	38.3	35.8	37.4	146	135	84	78	6	-2.8	1057
12	40.1	37.3	38.5	171	149	90	79	7	-2.3	1151
Mean	39.3	36.8	38.2	168	150.3	93.8	82.8	4.17	2.99	1430
SD	0.6	0.7	0.4	13.4	9.0	8.0	5.8	9.42	0.49	1042

T_{intest} =intestinal temperature; max=maximum; min=minimum; HR=heart rate; bpm=beats per minute; SD=standard deviation; CPK=creatine phosphokinase.
¹⁴ age-predicted maximum heart rate
[#] subject withdrew after completing S1.

California, showed that there were significant associations between CPK, muscle soreness and the cytokines, interleukin (IL)-6, IL-10, IL-1ra (receptor antagonist), granulocyte colony-stimulating factor and macrophage inflammatory protein 1 β .

Systemic markers of cardiac damage

The effect of prolonged strenuous exercise on systemic cardiac markers of damage has been studied extensively,¹⁸⁻²⁰ with evidence of transient elevations during and immediately after exercise, which return to normal within 3 days in healthy athletes.^{18,19} These temporary elevations have been hypothesised to be due to myocardial stress and reversible cardiomyocyte membrane damage.^{18,19} Exercise is known to cause an increased myocardial oxygen demand and cardiac troponin turnover in all athletes,¹⁸ which might be linked to tachyarrhythmias and sudden cardiac death, when associated with prolonged increases (>3 days) in cTnT concentrations above 0.05 μ g/l.¹⁸ At no stage during our study were increased cTnT concentrations measured, supporting the attenuated increase in serum CPK concentration and absent uMb values as well as the lower concentration of serum cortisol despite maintenance of an intensity of effort which fluctuated from 63 to 112% APmax. It is possible that serum cTnT also did not increase due to the variation in HR (60 - 220bpm) that occurred during this race, which may have stimulated the cardiac muscle at irregular intervals and possibly reduced myocardial stress by permitting periods of recovery.

Users of NSAIDs

Both NSAID users and non-users were included in this study following recent findings that although markers of muscle inflammation are changed by NSAID usage, degree of muscle damage is unaffected.^{21,22} Nieman *et al.*² reported that NSAID users did not have reduced race times, muscle damage or DOMS, while Friden and Lieber⁶ reported

that administration of NSAIDs after eccentric exercise resulted in a short-term benefit of pain relief, but a long-term detrimental effect on muscle adaptation, inhibiting protein synthesis by suppressing the inflammatory reaction. Paulsen *et al.*²² also indicated that although NSAIDs inhibited prostaglandin synthesis and local and systemic responses, they did not affect actual markers of muscle damage. In this study there was however no statistical difference in the measured markers of muscle damage or inflammatory response between NSAID users and non-users.

Dehydration, intestinal temperature (T_{intest}), HR and evidence of muscle damage

Although some athletes in our study experienced up to 4% body mass loss and others, on occasion, raced at a HR of more than 100% APmax (Table 5), these athletes did not present with clinical signs of dehydration, severe hyperthermia or increased muscle damage as reflected by changes in sOsm, $T_{intest} >40^{\circ}C$ or changes in serum CPK concentration, respectively.

As the statistically significant ($p<0.05$) inverse correlation between % change in body mass and post-pre change in sOsm was low ($r=-0.365$), sOsm, widely reported golden marker of hydration status,²³ was used to quantitate changes in hydration status.

The correlation between hydration status and systemic markers of muscle damage, as reflected by stage-induced changes in sOsm and serum CPK concentrations, although statistically significant, was low. Hence it cannot be concluded from the 51 sets of paired data reported in this study that hydration status has an overriding effect on systemic markers of muscle damage.

In the 12 individuals in whom continuous recordings of T_{intest} were recorded (Table 5), the correlation between race-induced changes in T_{intest} and systemic markers of muscle damage was also low and statistically insignificant. The data provided in this study, although

based on a relatively small sample size, do not provide any support for the suggestion that rises in core body temperature exacerbate muscle damage.

Conclusion

The relatively low post-race concentrations of systemic and urinary markers of muscle damage and inflammation,⁵ when compared with those reported following road running events of similar duration,¹⁵ are attributed to softer underfoot surfaces, large fluctuations in pace of running and varied muscle recruitment patterns over the widely differing terrains.¹ The sporadic increases in intensity of effort, rises in T_{intest} , substantial body mass loss and increases in serum osmolality during the event, did not confirm previous suggestions^{3,4,11} that thermal and hydration status is directly related to the degree of muscle damage.

It would be of interest to investigate the impact of pre-race preparation on markers of muscle damage and inflammatory response found following this multi-day trail running event and to control the nutritional and fluid intake in future field work on multiday trail running.

Acknowledgements

Special thanks are extended to:

- Ms Heidi Mocke and the Wildlands Conservation Trust for permitting the collection of these data at the 2011 Three Cranes Challenge multiday trail run and for providing the research team with accommodation in the 'race village' located in the Bushwillow Campsite. They are also thanked for provision of the topographical data re the altitude gains and deficits during the race.
- Ergonomics Technologies, Pretoria and the MRC Research Unit for Exercise Science and Sports Medicine for the loan of *Cor Temp* data recorders which made the continuous monitoring of HR and T_{intest} possible.
- Professor AJ McKune and Mr D Naicker for their support with the field side collection of data reported in this paper.
- Ampath Laboratories for its assistance with phlebotomy and chemical pathology.
- The SA Weather Office for provision of precise data regarding the environmental conditions during the different stages of the race.
- Asokaran Rajh for assistance with graphic artwork.

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