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THE IMPACT OF AN ULTRA-TRAIL ON THE DYNAMIC OF CARDIAC, INFLAMMATORY, RENAL AND OXIDATIVE STRESS BIOLOGICAL MARKERS CORRELATED WITH ELECTROCARDIOGRAM AND ECHOCARDIOGRAM

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KEYWORDS
Ultratrail; endurance; cardiac; inflammation and oxidative stress biomarkers; electrocardiogram; Echocardiogram

ABSTRACT

The aim of this study was to describe the effects of a 64.2 km ultra-trail on the biomarkers of muscle damage, inflammation and oxidative stress, and compare the results observed with an ECG and an echocardiogram, both performed before and after the race.

Thirty-three ultra-trail volunteers (45.8 ± 8.7 years old) were enrolled in our study. Three blood tests were drawn from each runner, one just before (TPRE), one just after (TPOST) and the last 3 h after the end of the race (TPOST3h).

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All the markers increased. The maximum concentrations observed were at TPOST3h and were significant ($p<0.001$) for creatine kinase, creatine kinase isoform MB, high-sensitivity C-reactive protein, uric acid and for the ratio of reduced glutathione to oxidised glutathione. However, in the case of myoglobin, high-sensitive troponin T, N-terminal pro-brain natriuretic peptide, oxidised glutathione, myeloperoxidase, cystatin C and creatinine, the most significant increases were at TPOST ($p<0.001$). Modifications were observed in the medical imaging using echocardiography such as reduction of left ventricule end-sytolic and diastolic volumes and left ventricular global longitudinal strain. ECG showed electrical criteria for left ventricular hypertrophy and incomplete right bundle branch block after the race.

Endurance races cause significant physiological stress to the body that can be measured by the increase of different biomarkers. From a laboratory perspective, it is important to take into account the possible exercise performed previous to the testing to avoid a misinterpretation of the results. From a training perspective, due to these increases in biomarkers, it is recommended that runners wait at least 72 h after an ultra-trail before subsequent training. In addition a transient impairment of ventricular function due to dehydration were observed.

Introduction

Ultra marathons are defined as races covering a distance of more than 42.2 km [1]. In recent decades participation in 'ultra marathons' has become increasingly attractive to millions of non-professional endurance athletes worldwide, with competitors pushing their bodies to their physiological limit [2,3]. Prolonged strenuous exercise can alter normal physiological processes, it can induce severe muscle damage including to the heart, imbalances in fluid and electrolyte concentrations, changes in immune function, and increased inflammation [2].

Generally, the studies already conducted have been carried out on small and homogeneous groups of athletes. Furthermore, the majority of studies have only dealt with troponin release, only a few have considered the multi-markers combined with the imaging approach during and after an ultra-trail [4,5].

The aim of our study was to evaluate cardiac, inflammatory, renal and oxidative stress biomarkers while also using an electrocardiogram and an echocardiograph to support our biological results.

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Methods

After approval by the local Ethical Committee, 33 welltrained volunteer runners (Caucasian males, 45.8 ±8.7 years old, mean BMI: 22.8 ±2.2) were enrolled in our study. The average training load of the athletes was 55.4 ±22.0 km per week.

The study took place during the Ultra-tour of Liege (Belgium), a race of 64.2 km with a positive difference in height of 1400 m. They had no history of any disease, or specifically cardiac disease symptoms except one subject suffered from hypertension and one from an atrial septal defect (ASD). Six of the thirty three were taking medication: cardioaspirine for the ASD subject, candesartan for the case of hypertension and cetirizine, escitalopram, ciclosporine and etanercept in one other case. None were smokers.

The subjects' heart rate and blood pressure were taken just before the race (TPRE), just after (TPOST) and 3 h after the end of the race (TPOST3h).

All participants underwent surface 12-lead ECGs (Cardiovit AT-101, Schiller, Switzerland) that were acquired at a paper speed of 25 mm/s and a scale of 10 mm/mV before and immediately after the end of the race. All ECGs were individually reviewed by a cardiologist according to the guidelines [6].

A comprehensive 2 D transthoracic echocardiogram was performed in all participants before and immediately after the race using the Vivid cardiovascular ultrasound system (GE Healthcare, Little Chalfont, UK). All data obtained by echocardiography were analysed off-line with an EchoPAC workstation (GE Vingmed Ultrasound AS, Horten, Norway). Complete M-mode, 2 D, and Doppler evaluations were performed. Images were obtained in the parasternal and apical views. Systolic and diastolic left ventricular (LV) diameter and interventricular and posterior wall thickness were measured. LV end-systolic volume, LV end-diastolic volume, and left ventricular ejection fraction (LVEF) were calculated using the Simpson's biplane technique. Global longitudinal and circumferential strain and left atrial volume were also measured.

In all cases, blood samples were drawn at TPRE, TPOST and TPOST3h and collected in a heparinised or EDTA tube according to the analysis to be performed. The treatment of the blood samples was performed directly after the sampling to avoid confounding factors due to a bad pre-analytical phase. The markers chosen for measurement were:

- Cardiomyocyte and muscular injury markers: high
- sensitive (hs) troponin T (TnT), myoglobin (MYO), creatine kinase (CK), creatine kinase isoform MB (CK-MB) and high-sensitivity C-reactive protein (hs-CRP)
- Stretch biomarker: N-terminal pro-brain natriuretic peptide (NT-proBNP)

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- Inflammation and leucocyte activation markers: hs-CRP and myeloperoxidase (MPO)
- Renal function markers: creatinine (Cr), uric acid (UA) and cystatin C (CysC)
- Oxidative stress biomarkers: reduced and oxidised glutathione (GSH and GOX), lipid peroxide (POXL), superoxide dismutase (SOD), glutathione peroxidase (GPX), myeloperoxidase (MPO) and oxidised low-density lipoprotein (OLDL).

Three analysers were used to perform serial determinations: Cobas 8000 (Roche Diagnostics) for CK, CK MB, hsTnT, NT-proBNP, MYO, hs-CRP, UA, Cr and CysC; Cobas 6000 (Roche Diagnostics) for GSH, GOX, SOD and GPX (Randox reagent); Etimax 3000 (Diasorin) for MPO (Immundiagnostik reagent), OLDL (Mercodia reagent) and POXL (Biomedica reagent).

Data are presented as means and standard deviations. Normality was checked with the Shapiro-Wilks test and logarithm transformation was applied to normalise distributions.

Generalised linear mixed models (GLMM) were used to test the evolution of the parameters between time points. The Scheffe post hoc test was used for multiple comparisons. Correlations were calculated between biomarkers and also between ECG and echocardiographic parameters with cardiac biomarkers. Comparisons were considered significant at the 5% level ($p < 0.05$). Calculations were done in Statistica version 10.1.3.

Results

Baseline characteristics

Average race duration of the runners ranged from 06:27:22 h to 11:32:31 h.

Heart rate was significantly increased at TPOST compared with TPRE ($p < 0.00001$) and recovery values decreased slowly at TPOST3h but remained elevated compared to baseline values ($p < 0.01$).

Systolic blood pressure decreased significantly between TPRE and TPOST ($p < 0.01$) and remained stable during recovery ($p < 0.05$), while diastolic blood pressure was similar at all three points, only a slight decrease was observed over time (Table 1).

Pre/post race différences

In the Table 2, all the results observed at the different time points including the percentage of subjects exceeding the reference values are presented

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Table 1.

Heart rate (HR), Systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured at TPRE, TPOST and TPOST3h. *p*-Value between TPRE-TPOST and TPOST-TPOST3h are calculated.

	TPRE	TPOST	*p*-Value (TPRE–TPOST)	TPOST	TPOST3h	*p*-Value (TPOST–T POST3h)
HR	63 ± 11	91 ± 12	<0.001	91 ± 12	75 ± 12	0.000021
SBP	147 ± 20	118 ± 12	<0.001	118 ± 12	120 ± 16	0.545
DBP	86 ± 15	84 ± 11	0.643	84 ± 11	79 ± 10	0.074

HsTnT and MYO increased significantly from a minimum of 5 (TPRE) to a maximum of 160ng/L (TPOST) ($p < 0.0001$) and from 22 (TPRE) to 7898 pg/L (TPOST) ($p < 0.0001$) respectively before decreasing slowly but did not return to the baseline value.

NT-proBNP, CK and CK-MB concentrations increased significantly ($p < 0.0001$) from 6 (TPRE) to 929 ng/L(TPOST), from 61 (TPRE) to 13256UI/L (TPOST), and from 2 (TPRE) to 253 pg/L (TPOST), respectively and then continued to increase slightly between TPOST and TPOST3h. Only one runner showed a CK-MB: CK ratio higher than 5% at TPOST3h.

Hs-CRP increased significantly ($p < 0.0001$) over time (90-fold between TPRE and TPOST3h, from a min 0.2 to a max 17 mg/L).

CR and CysC increased significantly from TPRE to TPOST ($p < 0.0001$) before decreasing slowly without a return to the baseline at TPOST3h. UA increased significantly over time, at TPOST3h it was still increasing ($p < 0.0001$). UA increased significantly between TPRE and TPOST ($p < 0.0001$) but continued to rise between TPOST and TPOST3h ($p < 0.0001$).

Most of the oxidative stress biomarkers did not show any significant differences between the three time points excepted for POXL and MPO. POXL decreased significantly ($p < 0.0001$) over time. MPO increased significantly ($p < 0.0001$) at TPOST before decreasing at TPOST3h without a return to the baseline. The GSH: GOX ratio (GRO) increased two-fold between TPRE and TPOST3h, it increased over time but not significantly.

About hydration, there is a significant decrease of 2.8 ±1.3 kg ($p < 0.0001$) in the body mass of the runners between TPRE and TPOST. During the 3 h of recovery, the weight increased significantly of 1.77 ± 1.10 kg ($p < 0.0001$) but remained significantly lower of 0.95 ± 1.32 kg ($p = 0.0012$) compared to TPRE.

Concerning the ECG characteristics of the participants, no sign of atrial fibrillation or any other arrhythmias, including atrio-ventricular conduction abnormalities and ectopic beats, were found in the runners. There were no abnormalities of ST-T waves aspects and of QT interval duration. Six had electrical criteria for left ventricular hypertrophy. Incomplete right bundle branch block was observed in 14 participants in

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association with left anterior bundle block in one. No correlation was found between ECG characteristics and cardiac biomarkers (hsTnT, NT-proBNP, CK and CK-MB).

In the Table 3, all the echocardiographic data are presented. Echocardiographic parameters recorded before racing were within normal ranges. No participant demonstrated features of left ventricular hypertrophy nor a dilated left ventricle. Echocardiography performed after the race demonstrated significantly reduced left ventricle end-diastolic volume (from 127.1 ±33.2 to 91.3 ± 20.3 mL, $p = 0.001$) and end-systolic volume (from 46.6 ±16.1 to 26.6 ± 5.8 mL, p = 0.001). Left ventricular global longitudinal strain was significantly reduced after the racing (from 21.0 ± 2.6 to 18.8 ± 2.5%, p = 0.036).

Biomarker correlation

A correlation was found between some biomarkers such as CK-CKMB and CK-TnThs. These correlations observed between CK-CKMB ($r = 0.81$, $p < 0.0001$) and CK-TnThs ($r = 0.36$, $p = 0.037$). Correlation analysis was performed between the echocardiographic parameters, hsTnT and NT-proBNP showing only a positive correlation between NT-proBNP and the left ventricular global longitudinal strain at TPOST ($r = 0.618$, $p < 0.05$).

We observed a correlation between the duration of the race and increase in CK and CKMB ($r = -0.50$, $p = 0.0012$ and $r = -0.67$, $p = 0.0002$ respectively).

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Table 2.

Biomarkerds (abbreviation and units)	Reference range values	Time point (mean ± SD) TPRE	% exceeding the RV at TPRE	TPOST	% exceeding the RV at TPOST	*p*-Value TPRE TPOST	TPOST3h	% exceeding the RV at TPOST3h	*p*-Value TPOST-TPOST3h	*p*-Value overal evolution
Creatinin (CR)(mg/L)	7.2–11.8	9.14 ± 1.05	3.20%	12.46 ± 2.09	61.30%	<0.0001	11.03 ± 2.02	35.50%	<0.0001	<0.0001
Cystatin C (CysC)(mg/L)	0.59–1.04	0.81 ± 0.1	0%	1.01 ± 0.19	35.50%	ns	0.83 ± 0.16	16%	0.0358	0.0125
Uric acid (UA)(UI/L)	<70	54.66 ± 9.45	9.70%	71.58 ± 14.87	61.30%	<0.0001	74.03 ± 12.57	76%	<0.0001	<0.0001
C-reactive protein high sensitivity (CRP hs) (mg/L)	<1 mg/L: low risk 1–3 mg/L: moderate risk >3mg/L: high risk	0.57 ± 0.41	0.00%	2.03 ± 2.0	26%	<0.0001	3.5 ± 3.11	32%	<0.0001	<0.0001
Creatine-kinase CK (UI/L)	30–175	171.67 ± 109.21	38.70%	1915.82 ± 2185.32	100%	<0.0001				
2386.11 ± 2719.9	100%	ns	<0.0001							
Creatine-kinase isoform MB (Muscle Brain) (CKMB) (ug/L)	0–6	4.27 ± 2.13	19.40%	29.05 ± 37.76	100%	<0.0001	36.85 ± 47.18	100%	ns	<0.0001
Creatine-kinase MB / Creatine-kinase Muscle ratio (MBR)	0–5	2.72 ± 0.84	0%	1.75 ± 0.83	0%	<0.0001	1.8 ± 0.92	4%	ns	<0.0001
Myoglobin (MYO) (µg/L)	28–72	40.62 ± 14.67	3.20%	2216.43 ± 1769.99	100%	<0.0001				
1610.13 ± 1424.18	100%	0.0163	<0.0001							
Troponin T High sensitive (TNT hs)(ng/L)	<14	1 ± 1	0%	30 ± 30	68%	<0.0001	20 ± 20	60%	0.0315	<0.0001
N-terminal prohormone of brain natriurtetic peptide (NT-proBNP)(ng/L)	<103	40.84 ± 33.57	3.20%	301.25 ± 208.1	93.50%	<0.0001	290.66 ± 197.09	96%	ns	<0.0001
Reduced glutathione (GSH) (µmol/L)	717–1110	882.88 ± 151.86	6.50%	919.79 ± 143.96	9.70%	ns	934.67 ± 161.81	12%	ns	0.0263
Oxidised glutathione (GOX) (µmol/L)	<10	7.9 ± 9.72	25.80%	9.55 ± 12.79	25.80%	ns	6.14 ± 7.02	24%	ns	ns
Reduced/Oxidised glutathione ratio (GRO)	111–747	271.31 ± 264.91	44.80%	314.75 ± 290.28	46.40%	ns	369.14 ± 301.28	41.70%	ns	ns
Glutathione peroxydase (GPX) (UI/g HgB)	20–56	58.41 ± 17.02	45.20%	57.78 ± 16.65	45.20%	ns	57.54 ± 17.67	36%	ns	ns
Superoxyde dismutase (SOD) (UI/g HgB)	785–1570	1618.78 ± 147.76	64.5	1585.48 ± 174.07	54.80%	ns	1557.34 ± 142.6	56%	ns	ns
Lipidic peroxyde (POXL) (µmol/L)	<432	308.09 ± 211.81	22.60%	207.55 ± 163.26	6.50%	<0.0001	198.44 ± 172.06	4%	ns	<0.0001
Oxidised LDL (OLDL) (U/L)	28–70	46.37 ± 10.44	0%	46.31 ± 10.53	3.20%	ns	43.39 ± 9.87	4%	ns	ns
Myeloperoxydase (MPO) (ng/mL)	<55	29.84 ± 17.27	6.50%	43.38 ± 21.03	12.90%	<0.0001	36.2 ± 18.81	8%	<0.0001	<0.0001

Legend. Summarise of all the results (abbreviation- units- mean ± SD) for the markers measured with the percentage exceeding the reference values (RV) at each time point and the *p*-value between TPRE and TPOST and between TPOST and TPOST3h (recovery).

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Table 3.

	TPRE	TPOST	*p*-Value
Left atrial volume (mL)	72.7 ± 16.0	63.0 ± 12.0	0.100
Left ventricular diastolic diameter (mm)	53.2 ± 6.9	50.5 ± 5.5	0.256
Left ventricular systolic diameter (mm)	34.4 ± 6.0	33.2 ± 5.6	0.568
Diastolic septal thickness (mm)	10.6 ± 1.4	11.6 ± 1.8	0.094
Systolic septal thickness (mm)	14.0 ± 2.4	14.6 ± 2.3	0.488
Diastolic posterieur wall thickness (mm)	10.2 ± 1.6	10.7 ± 1.9	0.398
Systolic posterior wall thickness (mm)	14.9 ± 2.3	15.4 ± 2.7	0.585
Diastolic left ventricular volume (mL)	127.1 ± 33.2	91.3 ± 20.3	0.001
Systolic left ventricular volume (mL)	46.6 ± 16.1	26.6 ± 5.8	0.001
Left ventricular mass (g)	208.4 ± 79.8	219.2 ± 75.0	0.708
Left ventricular circumferential strain (%)	16.5 ± 5.5	17.0 ± 4.1	0.775
Left ventricular global longitudinal strain (%)	21.0 ± 2.6	18.8 ± 2.5	0.036

Legend. Echocardiographie data taken at TPRE and TPOST and the p-value between the two measurements

Discussion

This study investigated the effects on the biomarkers for cardiac and renal function, inflammation and oxidative stress of strenuous exercise in an ultra-trail of 64.2 km with a positive difference in height of 1400 m. An ECG and echocardiogram were also performed in order to explore correlations with the different studied biomarkers and thereby to correlate the impact of this race on the heart.

TPRE data, although obtained in an 'at-rest' state, may have been influenced by the effects of daily training. In our study some subjects had pre-race values higher than the mean of the other runners and, notably, had results above the upper laboratory reference values. Some of these subjects presented the highest values after the race, as is the case for NT-proBNP.

Multiple studies have reported elevations in cardiac biomarkers following a race such as a marathon and established as being from a myocardial source, such as troponins and B-natriuretic peptide concentrations. This has been further supported by data demonstrating strong associations between elevated cardiac biomarkers and the post-race development of abnormalities shown on 2-dimensional echocardiograms [7,8]. At the Boston Marathon more than 60% of asymptomatic runners had measurable increases in TnT levels, with 40% at or above the decision limit for acute myocardial necrosis. These changes were inversely related to training experience [9]. In our study we found up to 68% of the results above the decision limit at TPOST3h, so our findings are comparable to previous research.

Some other studies have demonstrated that subject characteristics, exercise parameters and hydration status may be related to the exercise-induced increase in cardiac tropnonins, among others [10]. However, no correlation with the training experience was found in our study: this corresponds with the study of

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Khodaee et al. [11].

The degree of TnT release seemed to somewhat parallel the degree of endurance exercise [9]. In our study the most significant increase was at 160ng/L at TPOST, and was observed for the subject with the highest number of running km per week among our participants.

NT-proBNP has been shown to clinically increase in patients with heart failure whose myocardial functions have declined by myocardial infarction or chronic hypertension, serving as an indicator showing stress within the myocardial walls caused by myocardial pressure overload [12].

A thought-provoking fact in our study is that NT-proBNP values significantly increased after prolonged strenuous exercise in healthy individuals, as also shown in a study of a 100 km ultra marathon [13]. The increase of NT-proBNP was greater than that of patients corresponding to the New York Heart Association class II to III. From this standpoint it is possible to consider that the elevated NT-proBNP level observed in this study could have some clinical significance and does not solely represent numerical data. Finally, 96% of the runners exceeded the laboratory reference values at TPOST3h and 93.5% at TPOST, as already reported with 77% of the participants in a marathon exceeded the reference limit of NT-proBNP [14,15]. Previous research has reported that NT-proBNP is related to the exercise time [16-20]. Natriuretic peptide is significantly increased in response to not only short-term exercise but also endurance exercise [13,21]. Another hypothesis is that the increase of natriuretic peptide may be related to the exercise-induced physiological endocrine response to the myocardial stretch [16,18]. The increase of natriuretic peptides (NPs) may be related to transient myocardial wall stress, metabolic effects on cardiomyocytes or exercise-induced neuroendocrine response to the myocardial stretch [17,18].

CK is a marker that is clinically expressed in myocardial infarction, and is also expressed in muscular injury during extreme conditions [22]. CK reflects cellular necrosis and tissue damage after acute or chronic injury of the working muscles [23]. CK levels can then increase as a result of prolonged strenuous physical exercise, particularly following triathlons, marathons, and ultra marathons. The CK level in serum rises during the event and remains subsequently elevated during the first 24 h-48h [24]. This could therefore explain the continued elevation that we observed again after TPOST. It is interesting to note that the majority of the athletes were asymptomatic and required no major medical attention. Therefore, in clinical settings, these elevated CK levels can be monitored with clinical symptoms [25]. The correlations observed between CK-CKMB and CK-TnThs plead for an increase perhaps more of muscular origin than from myocardial damage origins. We observed also a link between the duration of the race and an increase in some biomarkers such as CK and CKMB. For these last ones, the slower the runners were the less the increase of the CK and CKMB was high.

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Correspondingly, MYO, like CK, is considered to be a marker of acute or chronic muscular damage and cell necrosis [26].Previous studies have shown that in cases of exercise, an increase of CK and MYO of up to four-fold can be observed and a slight increase for TnT. Activation of CK may be associated with symptoms such as pain, fatigue, and a decline in muscular strength during high-intensity, long-distance exercise due to damaged skeletal muscles, as observed in our study [27].

In our study we also observed a significant increase of these biomarkers at the end of the race. This observation confirms the strenuous exercise-induced muscle damage and non-specific inflammatory response (highlighted by the increase of CRP concentration) that has already been shown in other studies on long distance running [28-30].

Noakes reported that the increase in CK after longduration exercise is more closely related to the duration of exercise rather than the intensity [30].

In our study, the CK-MB: CK total ratio only reached the level of 5 (cut-off for positive cardiac origin) for one subject, indicating that the release is more likely to be related to muscular damage and not from the myocardium muscle, except for one individual runner who also had one of the highest levels of TnThs (57 mg/L).

The muscle damage following intense prolonged exercise, as in our study, is a consequence of both metabolic and mechanical factors closely related to the duration of exercise rather than to the intensity [31].

Marked elevations of serum levels of CK-MB isoenzyme (up to 21 times the upper limit of normal) in marathon runners during the post-race period were first demonstrated many years ago [9]. While skeletal rhabdomyolysis does appear to be the principal source of CK-MB, another lesion that should be considered in this setting is ischaemic injury to individual myocardial fibres; such injury may cause substantial leakage of CK-MB isoenzyme and may be reversible or irreversible (cardiac myocytolysis). Cardiac myocytolysis may be defined as disintegration of individual myocardial fibres unaccompanied by an inflammatory response. Ischaemia resulting in myocytolysis may involve randomly distributed myocardial fibres during a strenuous exercise [32].

Hs-CRP is a cardiovascular risk factor but also an inflammation marker. If we had decided to use it as a cardiovascular risk factor it would have been challenging because 16% of the runners at TPOST already presented concentrations above the 1 mg/L mark and so could have been considered as at moderate risk. At TPOST, 58% were at moderate risk and 26% at high risk and finally, at TPOST3h 32% at high risk. The hypothesis that it is more a reflection of the inflammation than of a cardiovascular risk is the most probable, but this hypothesis cannot be rejected as other cardiac biomarkers also increased in parallel.

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Yoon et al. showed in their study that the increase in CRPs reflects the inflammatory response to injury in the working muscles, indicating that muscular damage has more impact than cardiac damage [12].

Significant research has already shown that acute bouts of exercise have the potential to trigger a transitory increase in Reactive Oxygen Species generation and shift the redox balance in normotoxic and hypoxic environments [33,34]. We observed an increase in the GSH level 3 h after exercise, this is supported by the study of Feifei Li et al. where they also observed an increase in the GSH level [35]. Repeated exposure to increased ROS from long-term exercise in well-trained marathon runners may lead to an upregulation in anti-oxidant defense capacity and an associated shift in the redox balance, thus providing adaptive protection during acute bouts of exercise, supported by the SOD concentrations that were above the upper limit of the laboratory reference point even at TPRE in more than 64.5% of the runners. It has been postulated that the significant increase in the GSH level during exercise represents a part of the self-protecting activation in humans [36]. For oxidised LDL, it is not surprising that no increase was seen as a long induction period is required in order to observe a change, and in this case it was too short to have an impact on the oxidation of the LDL.

MPO is an inflammation marker as well as a marker of the activation of neutrophils during intense physical effort [37], accordingly MPO increased significantly in our study. These increases could be explained by the production of oxygen species during exercise.

Considering hydration, Cr, CysC and UA increased at TPOST and stayed higher at TPOST3h for UA, while Cr and CysC decreased during the recovery time, this could be related to the renal function, or to déhydration. During an ultra-marathon all runners expérience déhydration with a decrease in body mass [38]. The greatest body weight loss occurs in the first hours of running [39]. Therefore, ultra marathoners have to drink large amounts of fluids during a race to avoid dehydration in terms of body weight loss [40]. In our study, we observed a significant decrease in the body weight of the runners between the beginning and the end of the race. During the 3 h of recovery, the body weight increased significantly but remained significantly lower compared to before the race. Changes in body weight provide a sample and accurate index to estimate hydration status during exercise [41]. According other studies, body weight reduction are well tolerated during ultramarathon [42] but could have a negative effect on the renal function [43].

Indeed, dehydration may contribute to decrease renal perfusion, compounded the risk for acute kidney injury during ultramarathon [44].

During this type of running, damage to the kidney with an impaired renal function is quite often observed [45]. Indeed, the prevalence of an acute kidney injury in ultra marathon running is nearly 50% of all runners [44].

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From a pathophysiological point of view, the skeletal muscle damage leads to an influx of muscle proteins into the bloodstream e.g. myoglobin. In certain circumstances, such as pronounced dehydration or heat, there may be a marked accumulation in the kidney with consequent kidney damage [22]. In our case, we can therefore understand the brief decrease of the renal function, which finally rapidly returned to the normal level [22,46,47].

Global longitudinal strain (GLS) is considered to be an effective parameter for quantifying left ventricular function [48,49] more sensitive than LVEF assessed by a 2 D echocardiogram. The participants in our study had some degree of left ventricular dysfunction, as reflected by a significant reduction of GLS after racing. Interestingly, the decrease of left ventricular performance, even slight, correlated to the NT-ProBNP level, a marker of left ventricular dysfunction. Post-race reduction of left ventricular volumes could be related to dehydration as shown by the kinetics of Cr and CysC and by the body weight loss observed between the beginning and the end of the race.

Our study has several limitations. Firstly, it was an observational study, so we are unable to make a definitive statement on the causality of the abnormal results. Secondly, it is possible that we did not take into account some unknown confounders. Finally, every runner is unique, so our results cannot be fully extrapolated to other races, especially due to the current variation of possible running distances, the speed, the altitude change etc.

Conclusions

Running 64 km is a tremendous challenge for the entire organism. There is evidence that endurance exercise has beneficial health effects. However, longterm excessive exercise may causes significant changes in the different biomarkers studied in the continuously growing cohort of middle age amateur runners. Moreover, our findings reflect the potential differential individual impact of ultra-endurance training and racing, and provide new insights into such races with multiple stressor contributions. Because the trend is to return to normal values after the recovery time for most of the biomarkers studied, the response of the organism seems physiological, without consequences even in the short term. Unfortunately, it was difficult to obtain a further blood test and so difficult to be sure that all the biomarkers returned to baselines in the days following the race. For the laboratory, it is needed to take into account the possible exercise previously performed to a blood test to avoid a misinterpretation of the results. Finally, it would seem to be prudent to advise the middle-aged or older endurance athlete about the risk of very intensive exercise.

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