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EVOLUTION OF THE SLOPES OF ST2 AND GALECTIN-3 DURING MARATHON AND ULTRATRAIL RUNNING COMPARED TO A CONTROL GROUP

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Abstract :

Background: Previous studies have suggested that exercising may induce cardiac damage. Galectin-3 (Gal-3) and soluble suppression of tumorigenicity 2 (ST2) are very interesting biomarkers for heart failure and myocardial fibrosis. We aimed to compare the kinetics of emerging fibrosis cardiac biomarkers as Gal-3 and ST-2 in endurance runners, and recreational runners before and after a running event represented by a marathon and an ultratrail event.

Methods: Blood samples were taken from 19 healthy non-elite marathon runners (42 km), 27 ultratour runners (67 km), and 14 recreational runners who represented the control group (10 km) just before the run (T0), just after (T1) and 3 h after (T2), in order to analyze Gal-3, ST2, hsTnT, NT-proBNP, CKMB and hsCRP. We compared the percentage of evolution and the slopes obtained from T0 to T1 (pT0T1) and from T1 to T2 (pT1T2), between the different groups of runners participating in three different races.

Results: Plasma cardiac biomarker concentrations increased significantly from baseline to immediately post-exercise and most of the time decreased over the subsequent 3-h period. For pT0T1 and pT1T2, the markers Gal-3 and ST2 showed a significant difference between types of run ($p<0.05$ and $p<0.0001$, respectively). During the recovery time, Gal-3 returned to the baseline values but not ST2 which continued to increase.

Conclusions: Gal-3 and ST2 are considered as a reflection of cardiac fibrosis and remodeling. The evolution of both was different, particularly after the recovery time. ST2 values exceeding cutoff values at any time.

Keywords :

Fibrosis cardiac biomarkers; galectin-3; long distance running; marathon; running; ST2; ultratour.

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Introduction

New cardiac biomarkers such as galectin-3 (Gal-3) and suppression of tumorigenicity 2 (ST2) have emerged in exploring cardiac fibrosis. These proteins participate in heart failure pathophysiology and each of their concentrations is increased in patients who exhibit cardiac remodeling and fibrosis, making them useful in monitoring disease progression [1]. Gal-3 is a member of the galectin protein family, found intracellularly and secreted into the extracellular environment through non-classic mechanisms [2, 3]. It is involved in numerous physiological and physiopathological mechanisms and high levels are an indicator of poor prognosis and mortality [4]. With respect to cardiology, Gal-3 is secreted by cardiac macrophages, which are activated during an inflammatory process. In the event of heart failure, where inflammation appears to play a key role, it is involved in the recruitment and proliferation of inflammatory cells and the activation of cardiac fibroblasts [3, 5]. It has a paracrine and endocrine action leading to secretion of pro-collagen and ultimately to cardiac fibrosis with the subsequent remodeling of the myocardium [5]. Determination of Gal-3 levels as a mean of cardiac prognosis was approved in early 2013 by the American College of Cardiology (AACC) and the American Heart Association (AHA) [6].

Previous studies have shown that taking part in intense and sustained exercise leads, in the long term, to the formation of cardiac fibroid deposits, which may be involved in cardiac arrhythmias [7, 8]. Gal-3 thus appears as an interesting cardiac biomarker for cardiovascular screening in athletes.

ST2, also known as soluble interleukin (IL)-1 receptor-like, has also a prominent role in cardiovascular disease as a marker of inflammation, tissue fibrosis, matrix remodeling and myocyte strain [9, 10].

ST2 has been proposed as the new gold standard biomarker for heart failure prognostication [11]. On the basis of all available data, the American Association of Clinical Chemistry and American Heart Association guidelines 2013 recommend the measurement of ST2 for additive risk stratification in patients with acute or chronic ambulatory heart failure [9, 11, 12].

Blood concentration of ST2 is increased in various diseases such as inflammatory diseases and heart diseases and is considered a valuable prognosis marker for both conditions. Nevertheless, ST2 lacks disease specificity and, therefore, is not a valuable marker for heart failure diagnosis [13]. The major source of circulating ST2 in healthy individuals and in patients with distinct diseases is currently not fully established. To the best of our knowledge, ST2 has been rarely studied in extended physical exertion studies.

The aim of our observational study was to measure and compare at three time points the dynamic slopes of Gal-3 and ST2 in three different populations of healthy subjects submitted to different intensive efforts. Three populations of subjects engaging in physical exercise were enrolled: marathon (MRT), ultratour runners (UT) and controls (CT) to see if cardiac fibrosis, from a biological point of view, is likely to be present.

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Materials and methods

POPULATION

Plasma samples for biomarker analysis were drawn from three different populations of males: (1) CT: 14 subjects engaged in less than 2 h of sport per week and whose effort for the study consisted of 1 h of running at a speed at the limit of their capacity (75% VO_{2max}), the training level and training duration of this control group corresponds to the general recommendation for weekly exercise, called "control group" in this study. Briefly, participants started exercising on a treadmill speed of 8 km h^{-1}. The speed was subsequently increased by 2 km h^{-1} every 3 min until exhaustion was reached. Oxygen uptake (VO_2), minute ventilation (VE), respiratory exchange ratio (RER) (Ergostick, Geratherm Respiratory, Bad Kissingen, Germany) and heart rate (Polar Belt, Polar, NY, USA) were measured. The test was stopped when the participant could not maintain the required pace or had reached voluntary exhaustion. The criteria used to assess VO_{2max} were an RER ≥1.10, a heart rate in excess of 90% of the age-predicted maximum (i.e. 220 – age) and identification of a VO_{2max} plateau (<150 mL/min increase despite a further velocity increase); (2) MRT: 19 subjects who trained regularly and whose effort consisted of about 4 h of running (Maasmarathon – 42,195 km); (3) UT: 27 subjects who trained regularly and whose effort consisted of about 8 h of running (Ultratour de Liège – 67 km) with a difference in altitude of 1500 m). Blood samples were obtained at three time points: T0: prior to running; T1: immediately after the run; T2: 3 h after finishing. T1 corresponds to the time needed for the subjects to complete their run, respectively 1 h for CT, 4 h for MRT and 8 h for UT. T2 corresponds to T1 + 3 h, i.e. 4 h, 7 h and 11 h for CT, MRT and UT, respectively.

The present study was approved by the Ethics Committee of the University of Liège (Belgium).

BIOMARKER ANALYSES

Gal-3 was measured with the VIDAS® Galectin-3 kit (BioMérieux, Marcy l'Etoile, France, REF 411191) by an enzyme-linked fluorescence assay. The limit of detection and quantification were 2.4 ng/mL and 3.3 ng/mL, respectively.

According to the risk classification proposed for heart failure patients [20, 21], a Gal-3 level below or equal to 17.8 ng/mL corresponds to low risk, a level between 17.9 and 25.9 ng/mL to intermediate risk, and a level above 25.9 ng/mL to high risk. The level of ST2 was determined by an ELISA (Presage® ST2 Assay kit – Critical Diagnostics, San Diego, CA, USA, REF BC-1065).

The ST2 analysis cutoff of 35 ng/mL was selected by choosing a Presage ST2 Assay concentration value above the 90th and below the 95th percentile of a reference healthy group. The limit of detection was 1.8 ng/mL and of quantification was 2.4 ng/mL.

We also measured cardiac and inflammatory biomarkers such as hsTnT (REF 05092744190), NT-proBNP, creatin kinase isoform MB (CKMB, REF 05894808190), highly sensitive C-reactive protein

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(hsCRP, REF 04628918190) were measured on a Roche Cobas instrument according to the manufacturer's instructions.

STATISTICAL ANALYSIS

Results are presented as means with standard deviation (SD) for each time and each group. All biomarkers except Gal-3 were log-transformed to normalize their distribution.

For each subject and each biomarker, the slopes pT0T1, between T0 and T1, and pT1T2, between T1 and T2, were calculated as follows:

$$\mathrm{p}T0T1(X) = \frac{X(T1) - X(T0)}{time(T1) - time(T0)}$$

$$\mathrm{p}T1T2(X) = \frac{X(T2) - X(T1)}{time(T2) - time(T1)}$$

where T is the time of measurement and X is the biomarker. For each type of run and each bio-marker, the slope between T0 and T1 and the slope between T1 and T2 were tested by the paired Student t-test.

The slopes pT0T1 and pT1T2 were compared between types of run by the analysis of variance (ANOVA) and Scheffé's *post-hoc* test. The results were considered significant at $p < 0.05$. Calculations were done with SAS version 9.4 (SAS Institute, Cary, NC, USA) and the figures were drawn with R version 3.5.

Results

The demographic characteristics and running experience of the runners are described in Table 1. All biomarkers in the three groups of subjects at T0, T1 and T2 are displayed (means with SD) in Table 2. Figures 1 and 2 provide a visual comparison of the values and their variations for Gal-3 and ST2. MRT showed the greatest variations and CT showed the smallest variations.

Table 3 summarizes the number (n) of subjects (in %) above the reference values for each biomarker for each run.

At T0, we noticed that 100% of runners showed Gal-3 levels below the lowest reference value (17.8 ng/mL) but for ST2, we had already observed concentrations above the cut-off values (<35 ng/mL) according to the different training group.

At T1, 9M (47.4%) showed Gal-3 concentration above 25.9 ng/mL while, respectively, 12 UT (44.4%) showed Gal-3 concentrations between 17.8 and 25.9 ng/mL and 4 UT (14.8%) showed Gal-3 concentrations higher than 25.9 ng/mL. A majority of MRT and UT (>80%) showed ST2 values higher than the reference values.

At T2, no subject exceeded the 25.9 ng/mL cutoff for Gal-3 but 11 MRT (57.9%) and five UT (18.5%) remained within the "17.9–25.9 ng/mL" range (including the four runners who showed a high-

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risk level at T1). A majority (>80%) of CT had ST2 values higher than the reference values. The totality of MRT and UT runners showed ST2 values higher than reference values.

Table 1: Characteristics of the 57 runners.

Variable	CT (n=14)	MRT (n=19)	UT (n=27)
Age, years	37.7±3.43	42.0±7.98	45.3±9.91
BMI, kg/m²	23.8±2.50	23.6±1.76	23.4±2.80
Height, cm	178.9±8.1	178.1±7.2	178.2±7.5
Weight, kg	75.9±8.1	75.0±8.5	74.8±10.4
Weekly training, h/week	–<2 h	5 h 48±2 h 32	6 h 43±2 h 43
Number of races of this type already run	–	7.4±17.5	5.7±5.0
Pace/km		5 min 28 s±39 s	7 min 36 s±1 min 08 s
Race performance	1 h	3 h 50 min 48 s±27 min30 s	8 h 07 min 39 s±1 h 12 min 48 s

CT, controls; MRT, marathon; UT, ultratour. Mean±SD.

Table 2: Characteristics of the cardiac biomarkers with respect to type of run and time.

Variable	Time	CT		MRT		UT	
		n	Mean±SD	n	Mean±SD	n	Mean±SD
Gal-3, ng/mL	T0	14	10.8±2.63	19	9.23±2.56	27	9.39±2.94
	T1	14	14.1±3.38	19	23.9±5.59	27	19.8±4.39
	T2	14	13.4±2.85	19	17.6±3.67	27	15.2±2.69
CKMB, µg/L	T0	14	2.32±1.06	18	4.64±1.48	27	4.59±2.22
	T1	14	2.69±1.17	18	9.21±9.87	27	30.4±41.5
	T2	14	3.13±1.30	18	11.8±10.2	27	36.9±47.2
hsCRP, mg/L	T0	14	0.82±0.96	19	2.27±5.72	27	0.57±0.40
	T1	14	0.88±0.95	19	2.01±4.68	27	1.64±1.50
	T2	14	0.88±0.95	19	2.79±5.40	27	3.39±3.18
NT-proBNP, ng/L	T0	14	34.3±19.3	19	31.1±25.3	27	46.4±34.7
	T1	14	43.0±21.8	19	107±82.6	27	325±220
	T2	14	40.9±21.3	19	91.1±63.2	27	291±197
ST2, ng/mL	T0	14	37.9±12.1	19	42.9±21.1	27	34.0±15.2
	T1	14	40.5±12.1	19	69.7±34.4	27	91.1±53.6
	T2	14	46.8±13.7	19	115±48.2	27	112±67.2
hsTnT, ng/L	T0	14	6.29±3.71	19	5.68±1.64	27	5.22±0.80
	T1	14	5.86±1.99	19	38.1±34.7	27	32.7±29.9
	T2	14	11.9±8.28	19	35.5±26.6	27	22.1±18.5

NT-proBNP; CKMB, creatin kinase isoform MB; hsCRP, highly sensitive C-reactive protein; CT, controls; MRT, marathon; UT, ultratour. Mean±SD.

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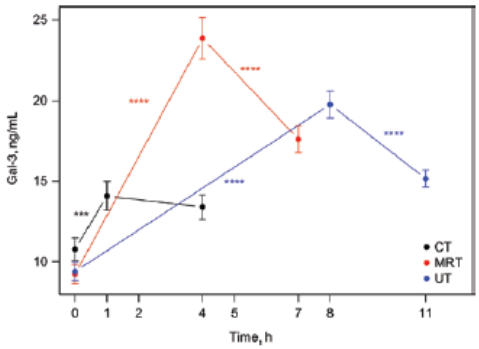

Figure 1: Evolution of the Gal-3 with respect to type of running exercise (* <0.05; ** <0.01; *** <0.001; **** <0.0001).

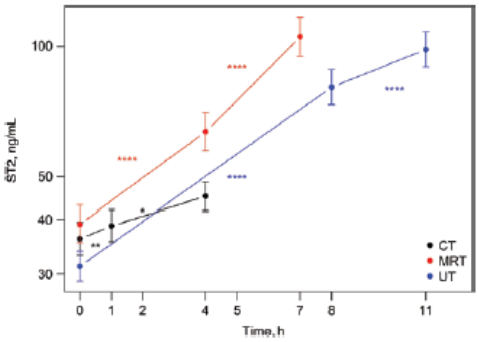

Figure 2: Evolution of the ST2 with respect to type of running exercise (* <0.05; ** <0.01; *** <0.001; **** <0.0001).

Table 4 shows the slopes between T0 and T1 (pT0T1) and between T1 and T2 (pT1T2) for the tested cardiac and inflammation biomarkers according to the type of run.

For Gal-3, the increase per hour of running (pT0T1) was significantly lower for UT than for CT and MRT ($p < 0.0001$). Over the first 3 h post-run, the Gal-3 level significantly dropped in MRT and UT ($p < 0.0001$) but remained at the same level in the CT ($p = 0.27$).

The ST2 increase per hour was significant in all groups and differed in the three groups during the run ($p = 0.031$). After the end of the run, ST2 continued to increase, most quickly among marathon runners ($p < 0.0001$). Between T0 and T1, the increase was different between each group ($p = 0.031$). The rise was significant within each group $p = 0.0010$, $p < 0.0001$ and $p < 0.0001$ for CT, MRT and UT, respectively. Between T1 and T2, the increase inside the group ($p = 0.013$, $p < 0.0001$ and $p < 0.0001$ for CT, MRT and UT, respectively) was significant. A more significant increase in the MRT group in comparison with CT and UT was observed ($p < 0.001$).

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Discussion

Evidence has established the link between high levels of physical activity, regular exercise training and the reduced long-term risk of various chronic diseases, including cardiovascular disease (CVD) and all-cause mortality [14].

Table 3: Number of subjects (in %) above the reference values for each biomarker for each run.

Reference values	Gal-3, ng/mL		CKMB, µg/L	hsCRP, mg/mL	NT-proBNP, ng/L	sST2, ng/mL	hsTnT, ng/L
	17.8–25.9	>25.9	>4.5	>6.0	>105.0	>35.0	>14.0
CT (n = 14)							
T0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (71.4)	1 (7.1)
T1	0 (0.0)	0 (0.0)	2 (14.3)	0 (0.0)	1 (7.1)	10 (71.4)	1 (7.1)
T2	0 (0.0)	0 (0.0)	1 (7.1)	0 (0.0)	1 (7.1)	13 (92.9)	7 (50.0)
MRT (n = 19)							
T0	0 (0.0)	0 (0.0)	11 (57.9)	2 (10.5)	1 (5.3)	12 (63.2)	0 (0.0)
T1	0 (0.0)	9 (47.4)	17 (89.5)	2 (10.5)	4 (21.1)	17 (89.5)	16 (84.2)
T2	11 (57.9)	0 (0.0)	19 (100.0)	2 (10.5)	6 (31.6)	19 (100.0)	18 (94.7)
UT (n = 27)							
T0	0 (0.0)	0 (0.0)	25 (92.6)	0 (0.0)	0 (0.0)	11 (40.7)	0 (0.0)
T1	12 (44.4)	4 (14.8)	17 (63.0)	2 (7.4)	26 (96.3)	26 (96.3)	22 (81.5)
T2	5 (18.5)	0 (0.0)	27 (100.0)	3 (11.1)	26 (96.3)	27 (100.0)	17 (63.0)

NT-proBNP; CKMB, creatin kinase isoform MB; hsCRP, highly sensitive C-reactive protein; CT, controls; MRT, marathon; UT, ultratour.

Table 4: Comparison of slopes between T0 and T1 and between T1 and T2 for cardiac and inflammation biomarkers with respect to type of run.

Variable[d]	Slope	CT			MRT			UT		p-Value
		n	Mean ± SD	p-Value[e]	n	Mean ± SD	p-Value[e]	n	Mean ± SD	p-Value[e]	
Gal-3, ng/mLh	T0T1	14	3.31 ± 2.42[a]	0.0002	19	3.66 ± 1.42[b]	<0.0001	27	1.30 ± 0.47[a,b]	<0.0001	<0.0001
	T1T2	14	−0.23 ± 0.74[a,b]	0.27	19	−2.08 ± 1.25[a]	<0.0001	27	−1.54 ± 0.92[b]	<0.0001	<0.0001
CKMB, µg/Lh	T0T1	14	0.15 ± 0.18	0.010	17	0.12 ± 0.14	0.0017	27	0.21 ± 0.11	<0.0001	0.12
	T1T2	14	0.05 ± 0.07	0.016	17	0.09 ± 0.09	0.0005	27	0.06 ± 0.04	<0.0001	0.18
hsCRP, mg/Lh	T0T1	14	0.09 ± 0.31	0.28	19	−0.00 ± 0.04[a]	0.91	27	0.12 ± 0.08[a]	<0.0001	0.036
	T1T2	14	−0.00 ± 0.12[a,b]	0.88	19	0.24 ± 0.19[a]	<0.0001	27	0.25 ± 0.15[b]	<0.0001	<0.0001
NT-proBNP, ng/Lh	T0T1	14	0.26 ± 0.38	0.024	19	0.31 ± 0.16	<0.0001	27	0.25 ± 0.07	<0.0001	0.64
	T1T2	14	−0.02 ± 0.06	0.26	19	−0.05 ± 0.08	0.017	27	−0.03 ± 0.05	0.0034	0.45
sST2, ng/mLh	T0T1	14	0.07 ± 0.06	0.0010	19	0.12 ± 0.08	<0.0001	27	0.12 ± 0.05	<0.0001	0.031
	T1T2	14	0.05 ± 0.07[a]	0.013	19	0.17 ± 0.10[a,b]	<0.0001	27	0.07 ± 0.05[b]	<0.0001	<0.0001
hsTNT, ng/Lh	T0T1	14	−0.03 ± 0.46[a,b]	0.84	19	0.40 ± 0.18[a,c]	<0.0001	27	0.19 ± 0.09[b,c]	<0.0001	<0.0001
	T1T2	14	0.17 ± 0.19[a,b]	0.0048	19	0.00 ± 0.08[a,c]	0.84	27	−0.13 ± 0.06[b,c]	<0.0001	<0.0001

Values having the same superscript letter ([a], [b] or [c]) are significantly different according to Scheffé's *post hoc* test. [d]Except for Gal-3, slopes were calculated on the logarithm. [e]Slopes between T0 and T1 and between T1 and T2 were tested by the paired Student t-test for each type of run. NT-proBNP; CKMB, creatin kinase isoform MB; hsCRP, highly sensitive C-reactive protein; Gal-3, galectin-3; ST2, soluble suppression of tumorigenicity 2.

Mortality due to cardiac events during or after exercise has been described in diverse previous studies. The death incidence linked to physical exercise is more important among elderly people than among younger ones and non-regular practitioners [15]. However, in recent years and decades, running has increased and particularly for races longer than the classical

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marathon distance (42.195 km) which have experienced a boom and so have the potential cardiac events [16].

Intensive running is characterized by a multi-organ stress highlighted by the huge increase of muscular cytolysis together with cardiac and inflammation biomarkers [17].

In our study, we observed a significant increase of the necrosis, stretch and inflammation biomarkers. It was already demonstrated that the release of cardiac biomarkers could be transient and thus reversible [18, 19], as was also observed in our study. Whether regular repetition of intensive exercise might lead, in the longer term, to fibrosis and heart failure remains to be determined. In this regard, ST2 and Gal-3, involved in the hormonal dysregulation known to occur in heart failure, could provide great insight as they also increase under these conditions, we focused then on these fibrosis biomarkers. As high plasma values of Gal-3 and ST2 are considered as a risk predictor in the general population and for patients with heart failure, we expected low baseline values of those biomarkers in runners [7, 9]. Furthermore, a recent systematic review highlights an increased prevalence of myocardial fibrosis in endurance athletes [20]. This finding is new and may suggest that the accumulation of acute prolonged, high-intensity exercise induces detrimental adaptations to cardiac tissue possibly reflected in an acute change in cardiac biomarkers. In this case, ST2 and Gal-3 could bring interesting information.

The hematocrit and hemoglobin levels at all three time points to correct for possible post exercise dehydration have been determined. There was an average 5% increase in hemoglobin concentration between the preexercise sample and the sample drawn immediately after the exercise in athletes. Hemoglobin levels in the sample drawn 3 h post-exercise were comparable to pre-run levels indicating increase in values due to the dehydration effect directly after the run. This was not seen in the CT group after 1-h running. When corrected for hemoglobin concentration cardiac biomarker levels were not significantly different.

Our results show that baseline levels of biomarkers were higher in the training groups compared to the CT group. Indeed, long-term training effect can possibly cause heart muscle enlargement leading to higher baseline values as already shown by others [21–25].

All three groups had low baseline values for Gal-3 whereas those for ST2 were already above the cutoff point, regardless of the training status. Remarkably also for the CT group in which low values were expected. The

cutoff point for ST2 might therefore be questioned in this regard. It was also noted that some of the participants in the group practiced amateur jogging for less than 2 h a week. It thus seems that training for MRT, UT and CT does not affect resting levels for Gal-3 in opposition to ST2. Different kinetics are observed in each biomarker, therefore a detailed discussion is individually required.

These variations might be justified on the basis of various mechanisms involved in sustained effort.

Gal-3 is released during differentiation of monocytes into macrophages and is involved in many processes during the acute inflammatory response. It has been identified as a causal

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factor in the development of fibrosis of the heart (and other organs). Thus, Gal-3 is involved in the systolic and diastolic dysfunction [26].

The average basal level of Gal-3 was similar in all subjects and within the normal range (<17.8 ng/mL). This is not surprising as all the subjects were apparently healthy men, showing no signs of chronic heart failure or other pathologies involving an increase in Gal-3. The hypothesis that athletes training several times per week have a higher basal rate because their metabolism does not return to normal rate was not confirmed by this study for Gal-3. Nevertheless, it was the case for ST2 [27]. Our findings do confirm, however, the increase in Gal-3 after intense effort as it was demonstrated in other studies [27, 28].

The increase in average Gal-3 levels observed just after the effort and the decrease observed over the first 3 h post-effort were significant in the three groups. This suggests that the exercise must be sufficiently sustained in order to significantly alter the Gal-3 levels. The greatest increase and the greatest subsequent decrease were observed in the group of MRT runners. In the UT group, they were significantly smaller but still high in comparison with CT subjects. This may appear surprising because the UT runners had run twice as long, although the intensity of the run was inferior to that of the MRT runners. The MRT runners took about 4 h to run 42 km, while the UT runners took 8 h to run 67 km. Therefore, the increase in Gal-3 could be more correlated to the intensity than to the duration of the effort produced. Although Gal-3 levels are clinically determined in cardiology, it should be considered that Gal-3 is expressed ubiquitously in humans; it could suffer from a lack of cardiospecificity [3]. In mice, it has been demonstrated that Gal-3 increase during endurance exercise originates primarily from skeletal muscle [27]. This increase, together with the IL-6 increase and leukocytes, could be part of the well-known inflammatory response to exercise [7].

Moreover, a momentary increase in Gal-3 does not appear to lead to heart problems. The development of cardiac fibrosis requires a certain length of time. It definitely correlates with a chronically above-normal level of Gal-3, causing continuous stimulation of fibrocytes.

Although it is known as a very stable marker [29], we also observed that different individuals showed different rates of recovery after the effort. In some cases, the Gal-3 level dropped rapidly towards the basal level, while in others, this occurred more slowly. It would be interesting to understand why such differences exist and what they could lead to. A slower reduction in the Gal-3 level means the individual is exposed to high concentrations of Gal-3 for a longer time, leading to eventual consequences for the heart. We have observed some correlations between Gal-3 and other markers in response to intense physical activity. Of course, special care should be taken about such results and conclusions because differences between marathoners and ultratour runners could be due either to the exercise imposed or to their respective training regimens and also for CT runners who lacked training and performed a much lighter exercise.

By contrast, ST2 measurement provides a strong serologic overview of the cumulative myocardial fibrotic process [9].

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The effect of repeated exercise may increase ST2 concentration in the blood due to an acute rise in cardiac load. According to the systematic review of Van de Schoor et al. [20], the prevalence of myocardial fibrosis was strongly associated with the cumulative lifelong exercise dose.

In our study, the ST2 is already substantially higher at the baseline perhaps due to accumulation and could be a best predictor of cardiac fibrosis development. Data from Bayes-Genis et al. indicates that for every 10 ng/mL increase in ST2, there is around 20% increase in event risk [9].

We found ST2 levels to be higher in the UT group especially at the end of the race than in the MRT and CT. The observation done about Gal-3 increase seemingly correlating more with the intensity than with the duration of the effort was not valid with ST2 as it was higher on average among UT. Same kinetics were observed for ST2 and Gal-3 between T0 and T1 but in opposition to Gal-3, ST2 continued to rise between T1 and T2. It thus seems that more exercise correlates with a higher ST2 concentration.

Regarding Gal-3 and ST2, different kinetics were observed across time.

Several hypotheses could reflect the effect such as:

(a) Experience: UT runners are usually more experienced, some adaptation phenomenon could influence the release of the biomarkers.
(b) Length of the race: as already observed for other cardiac necrosis and stretch biomarkers in marathon orcycling study [30, 31], the time with the highest release was usually at around 5 h after the beginning of the race. Even if the results were normalized for comparison according to the time, the same intervals were not sampled between the different races and this could suppose a limitation for our study as any intermediate peak could have been missed.
(c) From a cardiological point of view, there are two interesting ST2 isoforms: soluble ST2, measured in our study, and ST2 Ligand (ST2L), presenting two different roles. When bound to IL-33, ST2L confers an inhibitory effect on the inflammatory response and provide a cardioprotective effect from excessive stress. On the other hand, ST2, the soluble form, may neutralize this protective effect. The elevation of ST2 in our study could involve cardiac stress due to the loss of the cardioprotective effect.
(d) To objective the biomarker increase, it is important to bear in mind that there is an equilibrium between the marker secretion and elimination. Perhaps in the case of ST2, the elimination ratio does not go with the secretion and so, we observed an increase. But this hypothesis could be possible at short terms but not in long terms. Indeed, the ST2 was already above the reference value at baseline. MRI imaging could provide interesting information to correlate ST2 levels to the fibrosis induced by the accumulation of exercise.

Conclusions

The average Gal-3 levels of the subjects in each group evolved like the average levels of the other markers except for ST2. ST2 exhibited a unique profile as it continued to increase after the recovery time point. As sudden cardiac death (SCD) among athletes has been impossible to

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predict and as cardiac fibrosis could be involved in the onset of SCD providing complementary information to the other well established biomarkers, the emergence of the novel cardiac biomarkers, Gal-3 and ST2, now offer the opportunity to explore which athletes may be most at risk of experiencing SCD.

A perspective could be to complement our study with MRI imaging. It could serve as a proof to know if it is really cardiac fibrosis or inflammation.

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REFERENCES

1. Wu AH, Wians F, Jaffe A. Biological variation of galectin-3 and soluble ST2 for chronic heart failure: implication on interpretation of test results. Am Heart J 2013;165:995–9.
2. Dumic J, Dabelic S, Flögel M. Galectin-3: an open-ended story. Biochim Biophys Acta – Gen Subj 2006;1760:616–35.
3. Gruson D, Ko G. Galectins testing: new promises for the diagnosis and risk stratification of chronic diseases? Clin Biochem 2012;45:719–26.
4. de Boer RA, van Veldhuisen DJ, Gansevoort RT, Muller Kobold AC, van Gilst WH, Hillege HL, et al. The fibrosis marker galectin-3 and outcome in the general population. J Intern Med 2012;272:55–64.
5. Hrynchyshyn N, Jourdain P, Desnos M, Diebold B, Funck F. Galectin-3: a new biomarker for the diagnosis, analysis and prognosis of acute and chronic heart failure. Arch Cardiovasc Dis 2013;106:541–6.
6. Gupta A, Ghimire G, Hage FG. Guidelines in review: 2013 ACCF/ AHA Guideline for the Management of Heart Failure. J Nucl Cardiol 2014;21:397–9.
7. Hättasch R, Spethmann S, de Boer RA, Ruifrok WP, Schattke S, Wagner M, et al. Galectin-3 increase in endurance athletes. Eur J Prev Cardiol 2014;21:1192–9.
8. Trivax JE, Franklin BA, Goldstein JA, Chinnaiyan KM, Gallagher MJ, DeJong AT, et al. Acute cardiac effects of marathon running. J Appl Physiol 2010;108:1148–53.
9. Peñafiel J, Lupón J, Zamora E, Urrutia A, Vila J, de Antonio M, et al. Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification. J Am Coll Cardiol 2013;63:158–66.
10. Miñana G, Núñez J, Bayés-Genís A, Revuelta-López E, Ríos-Navarro C, Núñez E, et al. ST2 and left ventricular remodeling after ST-segment elevation myocardial infarction: a cardiac magnetic resonance study. Int J Cardiol 2018;270:336–42.
11. Bayes-Genis A, Zhang Y, Ky B. ST2 and patient prognosis in chronic heart failure. Am J Cardiol 2015;115:64B–9.
12. Bayes-Genis A, De Antonio M, Vila J, Peñafiel J, Galán A, Barallat J, et al. Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification: ST2 versus galectin-3. J Am Coll Cardiol 2014;63:158–66.
13. Benjamin Dieplinger TM. Soluble ST2 in heart failure. Heart Fail Clin 2018;14:41–8.
14. Lavie CJ, Lee DC, Sui X, Arena R, O'Keefe JH, Church TS, et al. Effects of running on chronic diseases and cardiovascular and all-cause mortality. Mayo Clin Proc 2015;90:1541–52.

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Status : Postprint (Author's version)

15. Le Goff C, Laurent T, Kaux J-F, Chapelle J-P. Intense physical exercise related to the emergent generation of cardio-vascular risk markers: a review. Biol Sport 2012;29:11–6.
16. Knechtle B, Nikolaidis PT. Physiology and pathophysiology in ultra-marathon running. Front Physiol 2018;9:634.
17. Perk J, De Backer G, Gohlke H, Graham I, Reiner Ž, Verschuren M, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). Eur Heart J 2012;33:1635–701.
18. Baker P, Davies SL, Larkin J, Moult D, Benton S, Roberts A, et al. Changes to the cardiac biomarkers of non-elite athletes completing the 2009 London Marathon. Emerg Med J 2014;31:374–9.
19. Salvagno GL, Schena F, Gelati M, Danese E, Cervellin G, Guidi GC, et al. The concentration of high-sensitivity troponin I, galectin-3 and NT-proBNP substantially increase after a 60-km ultramarathon. Clin Chem Lab Med 2014;52:267–72.
20. Van de Schoor FR, Aengevaeren VL, Hopman MT, Oxborugh DL, George KP, Thompson PD. LJMU research online. J Appl Sport Psychol 2015;27:216–34.
21. Legaz-Arrese A, George K, Carranza-García LE, Munguía-Izquierdo D, Moros-García T, Serrano-Ostáriz E. The impact of exercise intensity on the release of cardiac biomarkers in marathon runners. Eur J Appl Physiol 2011;111:2961–7.
22. Legaz-Arrese A, López-Laval I, George K, Puente-Lanzarote JJ, Mayolas-Pi C, Serrano-Ostáriz E, et al. Impact of an endurance training program on exercise-induced cardiac biomarker release. Am J Physiol Circ Physiol 2015;308:H913–20.
23. Fallon KE, Sivyer G, Sivyer K, Dare A. The biochemistry of runners in a 1600 km ultramarathon. Br J Sports Med 1999;33:264–9.
24. Siegel AJ, Silverman LM, Holman BL. Elevated creatine kinase MB isoenzyme levels in marathon runners. Normal myocardial scintigrams suggest noncardiac source. J Am Med Assoc 1981;246:2049–51.
25. Siegel AJ, Januzzi J, Sluss P, Lee-Lewandrowski E, Wood M, Shirey T, et al. Cardiac biomarkers, electrolytes, and other analytes in collapsed marathon runners. Am J Clin Pathol 2008;129:948–51.
26. Meijers WC, van der Velde AR, de Boer RA. ST2 and Galectin-3: ready for prime time? eJIFCC 2016;27:238–52.
27. Spethmann S, de Boer RA, Hättasch R, Knebel F, Schimke I, Borges AC, et al. Galectin-3 increase in endurance athletes. Eur J Prev Cardiol 2013;21:1192–9.
28. Salvagno GL, Schena F, Gelati M, Danese E, Cervellin G, Guidi GC, et al. The concentration of high-sensitivity troponin I, galectin-3 and NT-proBNP substantially increase after a 60-km ultramarathon. Clin Chem Lab Med 2014;52:267–72.
29. Milting H, Ellinghaus P, Seewald M, Cakar H, Bohms B, Kassner A, et al. Plasma biomarkers of myocardial fibrosis and remodeling in terminal heart failure patients supported by mechanical circulatory support devices. J Hear Lung Transplant 2008;27:589–96.
30. Le Goff C, Kaux J-F, Goffaux SB, Cavalier E. Cardiac biomarkers and cycling race. J Sport Sci Med 2015;14:475–6.
31. Le Goff C, Lennartz L, Vranken L, Kaux J-F, Cavalier E. Comparison of cardiac biomarker dynamics in marathon, semi-marathon and untrained runners: what is the impact on results interpretation? J Lab Precis Med 2019;4:6.