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Comparison of conventional and highly-sensitive troponin I measurement in ultra-marathon runners

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Abstract Cardiac troponins are a mainstay in the diagnostic approach of patients with suspected acute coronary syndrome. Along with other causes of cardiac injury, strenuous aerobic exercise is an important source of troponin leakage from myocardium. Due to recent immunoassays development, there is no information on variation of highly-sensitive (HS) troponin I (TnI) in ultra-marathon runners. We studied 15 healthy trained Caucasian athletes before and immediately after completion of a 60 km, ultra-marathon. TnI was measured with both the conventional AccuTnI and the novel HS-AccuTnI immunoassays. At the end of the ultra-marathon the concentration of HS-AccuTnI significantly increased from the baseline value (19.2 ± 4.2 vs. 5.2 ± 0.8 ng/l; $P = 0.001$). The number of athletes displaying HS-AccuTnI values exceeding the 99th percentile of the reference limit was 2 (13%) pre-exercise, increasing significantly to 12 (80%; $P < 0.001$) post-exercise. Measurable value of AccuTnI were found in 1 (7%) and 12 (80%; $P < 0.001$) athletes pre- and post exercise, respectively. All AccuTnI values were below the 99th percentile reference limit pre-exercise, whereas this cut-off was overcome in 20% of athletes, post-exercise. These results suggest that the myocardium release of TnI during strenuous aerobic exercise mirrors that of troponin T. Moreover, the improved sensitivity of the HS-AccuTnI over the conventional assay makes it more suited for detecting even minor elevations of TnI in blood.

Keywords Sport · Physical exercise · Marathon · Troponin · Cardiac biomarkers

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Introduction

Cardiac troponins are the cornerstone as well as the biochemical gold standards in the diagnostic approach of patients with suspected acute coronary syndrome (ACS) [1]. Reliable evidence also attests that their measurement provides reliable predictive and prognostic information on cardiovascular outcomes and overall mortality [2]. Routine troponin testing for has been first introduced in the early 2000s, when the consensus document published by the European Society of Cardiology (ESC), the American College of Cardiology (ACC), and the American Heart Association made specific recommendations that an increased cardiac troponin value should lead to a diagnosis of non-ST-segment elevation MI [3]. The ACC and the ESC, along with the National Academy of Clinical Biochemistry, further recommended the use of a decision limit for myocardial injury established at the 99th percentile of the reference population [4]. It was finally advocated that the optimal precision of the assay at the 99th percentile of the reference limit should be established at a total imprecision [i.e., coefficient of variation (CV)] lower than 10% [5].

Due to the compelling request for better assays with improved clinical sensitivity and lower imprecision, the analytical performance of troponin testing has remarkably improved since the early 2000s, and the diagnostic threshold

for myocardial injury has been contextually lowered. In particular, the advent of the so-called "highly-sensitive" (HS) immunoassays was allowed to measure ng/l of cardiac troponins as compared with μg/l of the former conventional methods [6]. Troponin immunoassays are thus currently classified according to the percentage of samples obtained from healthy individuals displaying values that are actually measurable below the 99th percentile, i.e., ≤50% for conventional immunoassay, from 50% to ≤75% for first-generation HS-immunoassays, from 75% to ≤95% for second-generation HS-immunoassays and >95% for third-generation HS-immunoassays [7]. Although the remarkable improvement of analytical sensitivity over this past decade has undeniably enhanced the overall diagnostic performance, clinicians are now facing emerging challenges for the interpretation of analytically significant, but clinically questionable troponin elevations above the 99th percentile.

Along with well-established causes of myocardial injury such as ischemia, myocarditis, trauma and sepsis, significant elevations of troponin in blood have been reported in patients suffering from atrial fibrillation, hypertension, systolic dysfunction, impaired renal function, or undergoing chemotherapy [8]. Another important source of troponin leakage from myocardium is strenuous aerobic exercise [9–11], since troponin values exceeding the 99th percentile of the reference limit have been frequently observed in endurance athletes, especially after marathon and ultra-marathon run. In a recent critical review of the literature, the burden of troponin increase after long distance running (i.e., marathon and ultra-marathon) varied widely. When troponin was assessed with conventional immunoassays, post-marathon values exceeding the cut-off were observed in 8–100% of cases for troponin T (TnT), and in 32–92% of cases for troponin I (TnI), respectively [12]. As regards ultra-marathon running, the percentage of increases of TnT measured with conventional immunoassays was paradoxically lower than that recorded after the marathon, varying from zero to 20% [12]. Due to the recent introduction in the market, only three studies have however assessed the prevalence of troponin elevation measured with HS-immunoassays after marathon running [13–15], and only one after ultra-marathon running [16]. Moreover, all of them have assessed HS-TnT, so that information on the variation of HS-TnI after strenuous endurance exercise are lacking to the best of our knowledge. Therefore, the aim of this study was to compare post-exercise values of TnI as assessed with both a conventional and a HS-immunoassay in 15 athletes immediately after a 60-km, ultra-marathon.

Materials and methods

The population study consisted of 15 healthy trained Caucasian males, (mean age: 41 years, range: 34–50 years), who had been engaged in specific endurance training for 3–10 years (mean training regimen: 242 ± 35 min/week; maximal oxygen uptake, VO2: 64 ± 2 ml/kg/min). The VO2 max was assessed before the study by cycle ergometric incremental test exercise. The athletes performed a 60 km, ultra-marathon equipped with a heart rate (HR) monitor at 82 ± 4% VO2 max. The percentage was calculated on the basis of the VO2/HR relationship determined during the incremental test. None of the athletes had acute or chronic diseases, or reported intake of medications, including antioxidants or nicotine. Strenuous exercise was also avoided 36–48 h before the trial. The run started at 08:00 A.M. and developed on a hilly and demanding course, on a cloudy and partially rainy day, with a temperature from 6 to 8°C, and humidity from 54 to 87%. Blood samples were collected after an overnight fast, 20 min before the participants warmed up ("pre"), and within 10 min after completion of the trial ("post"). All subjects gave an informed consent for being tested and the study was approved by the local Academic ethical committee. Blood was collected in vacuum tubes containing no additives (Becton–Dickinson, Oxford, UK), immediately separated by centrifugation at 3,000×*g*, and kept stored at −70°C until measurement. TnI was measured with both the conventional AccuTnI and the novel HS-AccuTnI immunoassays, on an Access 2 (Beckman Coulter Inc., Brea CA, USA). The conventional AccuTnI method is characterized by a 99th percentile reference limit, an optimal imprecision (i.e., concentration corresponding to 10% CV), a limit of detection (LOD) and a limit of blank (LOB) of 34, 49, 10 and 10 ng/l [17]. The 99th percentile reference limit, the optimal imprecision, LOD and LOB of the novel HS-AccuTnI immunoassay are 8.6, 8.6, 2.1 and 1.0 ng/l [18]. The same instrument and reagent lots were used throughout the study.

The Wilcoxon Signed-Rank test and the χ^2 test (for categorical variables) were used to evaluate the significance of exercise-induced variations. Data with a non-normal distribution were normalized using a logarithmic transformation prior to analysis. Statistical analyses were performed using the statistical package SPSS version 12.0 (SPSS, Chicago, IL) and the level of statistical significance was set at $P < 0.05$. Data are shown as mean ± standard error of the mean (SEM).

Results

All the athletes completed the ultra-marathon successfully, and without symptoms. The main results of this investigation are synthesized in Fig. 1. All pre- and post-exercise samples had measurable values (i.e., ≥LOD) with the HS-AccuTnI. At the end of the ultra-marathon the concentration of HS-AccuTnI significantly increased from the

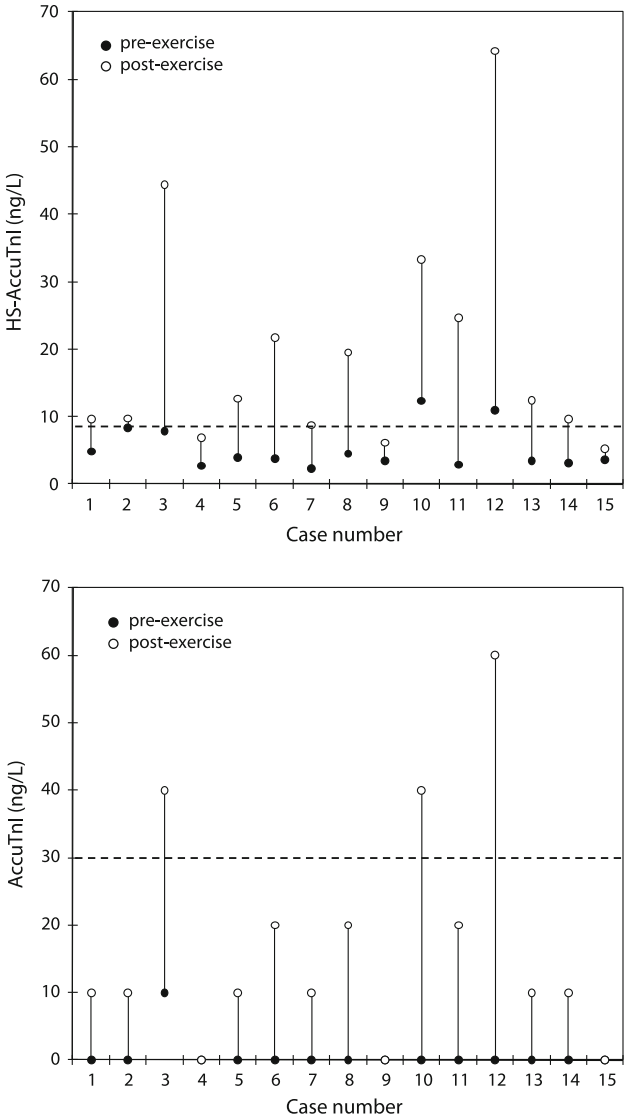

Fig. 1 Variation of conventional (AccuTnI) and highly sensitive troponin I (HS-AccuTnI) after a 60 km, ultra-marathon run in 15 male recreational athletes. The *dotted line* is drawn at the 99th percentile of the reference limit of AccuTnI (i.e., 34 ng/l) and HS-AccuTnI (i.e., 8.6 ng/l) immunoassays. *Filled circle* pre-exercise, *empty circle* post-exercise

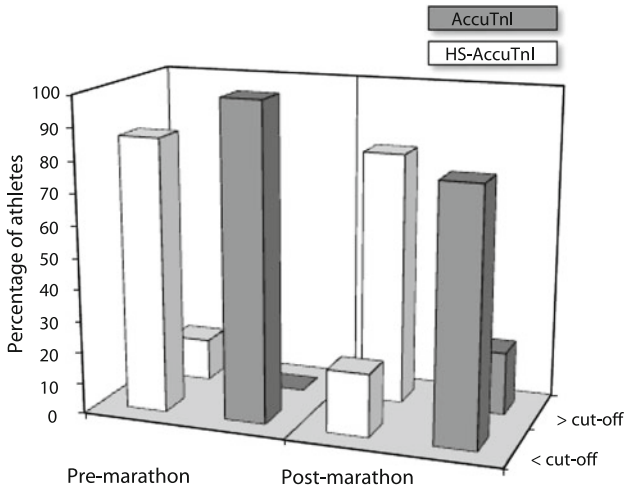

Fig. 2 Frequency of athletes with conventional (AccuTnI) and highly sensitive troponin I (HS-AccuTnI) values below and above the 99th percentile cut-off of the reference limit of AccuTnI (i.e., 34 ng/l) and HS-AccuTnI (i.e., 8.6 ng/l) immunoassays

baseline value (19.2 ± 4.2 vs. 5.2 ± 0.8 ng/l; p = 0.001). The number of athletes displaying HS-AccuTnI values exceeding the 99th percentile of the reference limit (i.e., 8.6 ng/l) was 2 (13%) pre-exercise, increasing significantly to 12 (80%; $P < 0.001$) post-exercise (Fig. 2). Measurable value of AccuTnI were found in 1 (7%) and 12 (80%; $P < 0.001$) athletes pre- and post exercise, respectively. Nevertheless, all AccuTnI values were below the 99th percentile reference limit (i.e., 34 ng/l) pre-exercise, whereas this cut-off was overcome in 3 out of the 15 athletes (20%) at the end of the ultra-marathon ($P = 0.096$).

Discussion

There are no other biomarkers that currently perform better than troponins in the diagnostic approach to the ACS. The remarkable diagnostic performance is supported by the high sensitivity, which approximates 100% after 12 h from the onset of symptoms, as well as by their absolute cardiospecificity, since the current immunoassays only react with cardiac isoforms [1, 19]. The advent of the new generation of HS-immunoassays has further magnified their diagnostic sensitivity, producing additional advantages, but also emerging challenges. The ability to detect even minor releases of troponin from the myocardium has made it possible to obtain measurable concentration of this biomarker in the healthy population and, even more importantly, to identify early releases not attributable to the physiological renewal of the cardiomyocytes [19], thereby extending their clinical application to most cardiac pathologies. On the other hand, such an increased sensitivity has however complicated the diagnostic reasoning and the differential diagnosis in patients with suspected ACS, since myocardial sufferance due to a variety of causes other than ischemia or coronary heart disease should be troubleshot in patients with modest increases of troponin concentration, especially at admission in the emergency department. These sources of elevation include also aerobic exercise.

Although physical activity is widely advocated in the primary and secondary prevention of cardiovascular disease [20], strenuous aerobic exercise is known to produce transitory abnormalities in systolic and diastolic function, especially in non-competitive athletes [21–23], mirrored by

modest elevation of both TnI and TnT, which are completely reversible after 36–48 h post-exercise. The release of both troponins from the myocardium has been here attributed to a transitory, ischemia-dependent shedding of blebs (also defined "bubbles") from the plasma membrane, which are then released in the circulation along with free cytosolic proteins, including TnI and TnT [10, 12]. This process is reversible and does not typically lead to necrosis of the cardiomyocyte, as demonstrated with cardiovascular magnetic resonance or late gadolinium enhancement techniques [24, 25].

Several studies have previously investigated the kinetics of cardiac troponins in marathon and ultra-marathon runners, providing different outcomes. This heterogeneity is mostly attributable to the analytical sensitivity of the assay, the choice of the 99th percentile reference limit, the distance of the run, as well as to the training status of the athletes. The three studies that have investigated post-exercise troponin variations in marathon runners, and the one that has measured this biomarker in ultra-marathon runners, have all used HS-TnT. Giannitsis et al. found cases with constantly low concentrations without changes, as well as cases with a early rise of Hs-TnT after a continuous 216 km race [16]. The prevalence of post-marathon values exceeding the 99th percentile of the reference limit of the Hs-TnT immunoassay has also been reported to vary between 86 and 94% across three studies [13–15]. No information is however available on HS-TnI in marathon and ultra-marathon runners, to the best of our knowledge. The analytical performance of the novel HS-AccuTnI is definitely better than the conventional AccuTnI, and globally comparable to that of HS-TnT. In fact, although the 99th percentile of the reference limit of conventional TnT and AccuTnI immunoassays are both ~ 30 ng/l, that of the novel HS-AccuTnI is 8.6 ng/l, versus 13 ng/l of HS-TnT. Considering that the molecular weights of TnI and TnT are 23.8 and 35.4 kDa respectively [1], the corresponding 99th percentile cut-offs are thereby 0.36 pmol/l for HS-AccuTnI, and 0.39 pmol/l for HS-TnT.

The measurement of HS-AccuTnI has allowed to provide novel and more accurate information about the variation of this biomarker after a 60 km run. Taken together, our results clearly show that all athletes had measurable values of TnI pre- and post-exercise, but the frequency of values exceeding the 99th percentile of the reference limit remarkably increased from 13 to 80% immediately after completion of the trial. In comparison, the troponin values measured with the conventional AccuTnI immunoassay only increased in a minority of athletes (i.e., 20%), post-exercise. These findings might have some important implications.

First we confirm previous findings on variation of Hs-TnT in marathon runners, inasmuch as we found a rather similar prevalence of post-exercise HS-AccuTnI values exceeding the 99th percentile cut-off (i.e., 80 vs. 86–94%) [12]. This suggests that the kinetics of release of both troponins from the myocardium during strenuous aerobic exercise is comparable, so that both biomarkers may be used interchangeably to monitor the athletes during or immediately after the run, for both clinical and experimental reasons. We have also demonstrated that the improved sensitivity of this HS-immunoassay makes it suitable to detect even minor and probably clinically negligible elevations of TnI, as compared with the conventional method. In analogy with the vast majority of previous studies on marathon and ultra-marathon runners [12], we had no access to the athletes for obtaining further sampling at different time points after the run. This is understandable, since a 60 km run is extremely stressful and it seemed unreasonable and even unethical to force the athletes to undergo additional testing. Nevertheless, the evidence that HS-AccuTnI was early and remarkably increased after the 60 km run suggests that strenuous aerobic exercise should be considered among the various causes that can generate significant elevations of this biomarker other than ACS. As regards the potential pathogenetic mechanism, it is conceivable that strenuous exercise promotes the generation of free radicals and favors membrane permeability, thereby causing a reversible myocardial injury with leakage of troponin from the cytosolic cellular pool [26]. Although the clinical impact of this exercise-induced leakage of intracellular troponins has not been fully defined so far, we agree with Mingels et al. [13], who suggested that athletes with a remarkable post-exercise troponin increase should not be strictly monitored in the lack of any clinical symptom of irreversible myocardial ischemia.

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