binding on the Roche assay. Larger studies must be performed to confirm this finding.

In conclusion, the Abbott assay demonstrated the fewest false negative results >14 d postsymptom onset and the fewest false positive results. While the Roche assay detected more positive results earlier after onset of symptoms than the other assays, none of the assays demonstrated high enough clinical sensitivity before day 14 from symptom onset to diagnose acute infection. Nonetheless, the clinical performance between the Roche, Abbott, and EI SARS-CoV-2 assays are similar and can detect antibodies to SARS-CoV-2 in a majority of patients 14 d after the onset of symptoms.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

K.G. Hock, provision of study material or patients; N.M. Logsdon, statistical analysis, provision of study material or patients; C.W. Farnsworth, statistical analysis.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: A.M. Gronowski, *Clinical Chemistry*, AACC.

Consultant or Advisory Role: N.W. Anderson, Diasorin Molecular.

Stock Ownership: None declared.

Honoraria: None declared.

Research Funding: None declared.

Expert Testimony: None declared.

Patents: None declared.

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DOI: 10.1093/clinchem/hvaa132

Increases in High-Sensitivity Cardiac Troponin I in Athletes during a Long-Term Period of Routine Training Out of Competition

To the Editor:

Cardiac troponin I (cTnI) concentrations have been shown to increase in athletes after high-endurance exercise *(1)*. Different theories have been put forward to explain this phenomenon including cTnI release from the cytosol, increased myocyte membrane permeability, inflammation, and cardiac stress during exercise *(1)*. However, data on variation of cTnI in athletes during stable training conditions are lacking. We aimed to assess whether cTnI concentrations increase during stable training conditions, and, if so, the duration of the increased concentrations, the influence of high-intensity exercise, and the time since the last training session.

The study population consisted of 30 healthy athletes (15 males), aged 19–53, recruited from triathlon clubs. Inclusion criteria were: training more than 13 h per week, normal medical examination (no physical injury, cardiovascular, kidney, or other chronic disease) and normal routine laboratory test results, a normal stress test including electrocardiogram, and no hospitalization during the previous 4 weeks. High-endurance exercise was defined as training sessions with a heart rate (HR) >82% of the maximum HR. Eleven monthly samples were collected per athlete at 8–10 AM on weekdays under standardized preanalytical conditions (10 h fasting, no high-endurance exercise the previous 24 h and being out of competition the previous week). Subjects filled in a questionnaire at each visit including health status, medication, last competition, last training, and changes in diet. The study was approved by the Hospital Ethical Committee and written consent was obtained from all participants. Serum samples were stored at −80 °C with hs-cTnI measured in duplicate on an Atellica Solution system (Siemens Healthineers). All samples from each athlete were analyzed in the same analytical run. Internal quality control was performed at two levels (37 and 5486 ng/L; CV_A 3.9 and 2.7%, respectively) and the assay LoD was 1.3 ng/L. The 99th

Table 1. hs-cTnI results (ng/L) in three athletes[a].

Sex, age	Visit	Time since last exercise (h: min)	Type of exercise (within 24 h)	Duration (min)	Intensity[a]	hs-cTnI (ng/L)	Creatinine (mg/dL)
Female, 32 years	01	17:46	Cycle	90	Low	132.6	0.82
	02	16:38	Gym	85	Low	353.6	0.76
	03	16:16	Cycle	150	Medium	243.9	0.78
	04	>24:00	No exercise	-	-	114.1	0.83
	05	11:08	Cycle/swim	60/60	Medium/medium	316.1	0.78
	06	>24:00	No exercise	-	-	137.1	0.83
	07	>24:00	No exercise	-	-	282.6	0.79
	08	10:57	Run/swim	40/40	Medium/medium	1349.3	0.81
	09	12:38	Run	40	Medium	1267.1	0.76
	10	16:24	Swim	40	Low	645.7	0.81
	11	12:23	Gym	60	Low	589.9	0.73
Male, 18 years	01	12:54	Run/swim	Not recorded	Not recorded	586.6	0.84
	02	11:33	Swim	60	Medium	618.1	0.79
	03	14:36	Run	Not recorded	Not recorded	1285.1	0.81
	04	9:53	Swim	60	Not recorded	1372.3	0.77
	05	10:28	Swim	Not recorded	Not recorded	1479.6	0.87
	06	23:56	Cycle	Not recorded	Low	673.1	0.85
Male, 21 years	01	15:23	Run	20	Low	318.1	0.93
	02	10:49	Cycle/swim	45/45	Low/low	308.1	0.88
	03	11:50	Cycle	60	Low	628.8	0.93
	04	12:07	Swim	60	Medium	708.8	0.90
	05	10:52	Run/swim	60/60	Low/low	309.8	0.92
	06	13:47	Run	60	Low	249.2	1.02
	07	11:38	Run/swim	45/60	Low/low	522.5	0.90
	08	11:05	Run/swim	60/35	Low/low	2185.9	0.91
	09	>24:00	No exercise	-	-	735.9	0.89
	10	19:02	Run	45	Medium	1279.6	0.96

[a] All three athletes displayed consistently high troponin concentrations (>sex-specific 99th percentile) over the duration of the study (monthly collections). Intensity of training was defined according to the percentage of maximum HR: low (68–74%), medium (75–82%), and high (>83%).

percentiles were 39 and 54 ng/L for females and males, respectively. Statistical analyses included Mann–Whitney and Pearson tests to compare hs-cTnI values in samples taken following nonhigh-endurance exercise during the previous 24 h in samples collected after a rest period. The correlation between hs-cTnI and the elapsed time since the last exercise was also examined.

In total, 292 blood samples were collected. Median hs-cTnI concentrations (interquartile range) were 13 (7–46) and 5 (3–21) ng/L for male and female athletes, respectively. Four female athletes had a minimum of one hs-cTnI value above the sex-specific 99th percentile comprising in total 18% of results in females ($n = 146$). Seven male athletes had a minimum of 1 hs-cTnI value >99th percentile, with 22% of total results above the 99th percentile ($n = 146$). No exercise in the previous 24 h was reported for 136 samplings, and in 95% of these samples cTnI was measurable. 97% of samples drawn after exercise the previous 24 h were measurable ($n = 156$), with 29% (45 samples from 5 male and 3 female athletes) being above the sex-

specific 99th percentile, compared to 9% of the samples when the athletes had refrained from exercise the prior 24 h (13 samples from 3 male and 3 female athletes, out of 136). Mean (SD) time from the last exercise session was 15(5) h.

We observed a significant difference in hs-cTnI concentrations between samples from participants who had reported exercise 24 h before sampling (14 ng/L) and those who had rested (5 ng/L), $P < 0.01$. For participants who had exercised the previous 24 h, a negative correlation was observed between cTnI and hours since the last exercise session ($r = -0.2$, $P < 0.01$). Previous studies have shown that hs-cTnI concentrations follow a curve that peaks about 5–6 h postexercise, returning to the basal value within 24 h (*2*, *3*). This pattern is different from that observed in a MI event (*4*). Furthermore, the increases in cTnI appear related to the intensity of the exercise (*5*). To our knowledge, there are not any studies that describe cTnI values in athletes monitored when out of competition and where high-endurance exercise the day before sampling was avoided. We observed concentrations over the 99th percentile sporadically for a third of the included athletes. Three athletes, 2 males and 1 female (18, 21, and 32 years old, respectively), had high hs-cTnI values consistently during the study, with no discernable pattern between hs-cTnI concentrations and the type, duration, and intensity of exercise prior to samplings (Table 1). The athletes did not report any heart-related symptoms, but further studies, such as angiogram, to rule out silent ischemia were not performed.

In summary, hs-cTnI values above the 99th percentile were observed in athletes under stable training conditions. The performance of moderate exercise in the time frame of 24 h before sampling was directly related to hs-cTnI concentration, and hs-cTnI concentrations were also inversely related to the elapsed time since the last exercise session. These results should be taken into account when interpreting cTnI measurements of athletes in an emergency context.

Nonstandard Abbreviations: cTnI, cardiac troponin I; HR, heart rate; hs-cTnI, high-sensitivity cardiac troponin I.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

J. Díaz-Garzón, statistical analysis; A. Buno Soto, statistical analysis, provision of study material or patients.

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

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DOI: 10.1093/clinchem/hvaa129

High Speed Centrifugation Before Frozen Storage of Plasma Is Critical for Quantitative Analysis of Mitochondrial-Derived Cell-Free DNA

To the Editor:

There is growing interest in the use of circulating mitochondrial-derived cell-free DNA (cmtDNA) in molecular diagnostics. The abundance of mitochondrial DNA, in comparison to nuclear DNA, makes the qualitative and quantitative analysis of cmtDNA an attractive prospect. The identification of cmtDNA as a 'damage associated molecular pattern' that may augment disease processes, strengthens the importance of research into cmtDNA physiology (*1*).

The importance of preanalytical sample preparation and storage