We then calculated the peak intensity (%) of the glycated peptides by the following equation:

\[
\frac{(\text{Ratio})}{(1 + \text{Ratio})} \times 100
\]

As shown in Fig. 1A, we obtained a highly linear calibration curve for the glycated hexapeptides over a wide concentration range (0–20%). We also obtained a highly linear calibration curve for HbA1c over a wide concentration range (Fig. 1B). As shown in Table 1, the peak intensity values for the two samples used to assess assay variability were highly reproducible (CVs ≤2%). The percentages of glycated peptide and HbA1c calculated on the basis of their respective calibration curves were similar. The causes of the small discrepancies between the values obtained with the two calibration curves should be identified by further experiments. Probable causes may include the different susceptibilities of glycated and nonglycated Hb to the enzyme, errors in mixing glycated and nonglycated materials, and impurities in the calibrators.

We previously reported a method that involved monitoring of both univalent and divalent ions (6, 7), whereas Kobold et al. (4) measured only divalent ions, and in the present report, we measured only univalent ions. Use of the expression (0.5 × peak area of doubly protonated ions + 1 × peak area of univalent ions) for each peptide to calculate the ratio of the peak intensities for both peptides improved the reproducibility of the ratio over that calculated with use of divalent ions only. However, in repeated experiments, the values obtained without use of labeled internal standards varied, depending on instrument conditions. The values reported here were fairly constant among assays on different days. Daily calibration is not necessary because the peak intensity ratios for labeled glycated and nonglycated peptides were highly reproducible. The peak intensity ratio for glycated/nonglycated peptides measured with the standard material, in which the concentration ratio of glycated/nonglycated was 1:10, remained constant with a mean (SD) value of 0.189 (0.007) and a CV of 1.2%.

We have used the proposed method to assess the HbA1c values in specimens that contain abnormal Hb. The method is convenient and reliable for determining HbA1c in such specimens.

We thank the IFCC Working Group for HbA1c Standardization for providing the calibrators.

References


**Table 1. Results of studies on intra- and interassay variability.**

<table>
<thead>
<tr>
<th>Peak intensity</th>
<th>Hexapeptide calibratorsa</th>
<th>Hb calibratorsb</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intraassay</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample I</td>
<td>4.377 0.087 2.0 10</td>
<td>4.414 4.171</td>
</tr>
<tr>
<td>Sample II</td>
<td>8.727 0.107 1.2 10</td>
<td>8.910 8.317</td>
</tr>
<tr>
<td><strong>Interassay</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample I</td>
<td>4.340 0.079 1.8 5</td>
<td>4.376 4.136</td>
</tr>
<tr>
<td>Sample II</td>
<td>8.811 0.125 1.4 5</td>
<td>8.996 8.397</td>
</tr>
</tbody>
</table>

a Number of analyses.

b Values were calculated with calibration curves constructed with synthetic hexapeptides.

c Values were calculated with calibration curves constructed with HbA1c solution.

Post-Race Kinetics of Cardiac Troponin T and I and N-Terminal Pro-Brain Natriuretic Peptide in Marathon Runners, Markus Herrmann,1,2 Jürgen Scharhag,2 Marina Miclea,6 Axel Urhausen,6 Wolfgang Herrmann,7 and Wilfried Kindermann2 (1 Department of Clinical Chemistry/Central Laboratory, University Hospital of Saarland, D-66421 Homburg/Saar, Germany; 2 Institute of Sports and Preventive Medicine, University of Saarland, Stadtwald, 66123 Saarbrücken, Germany; 6 author for correspondence: fax 49-6841-1623109, e-mail kchwher@uniklinik-saarland.de)

Annually there are cases of sudden cardiac death during and after marathon races (1–3), which has caused athletes and physicians to frequently ask whether marathon running damages the heart. Modern laboratory analyses, such as tests for cardiac troponin T and I (cTnT and cTnI) and N-terminal pro-brain natriuretic peptide (NT-proBNP), provide additional information about cardiac cell damage and wall stress with high sensitivity and specificity (4–7). Previous studies have investigated cTnT and cTnI in runners, cyclists, and triathletes (8–16), but the results are controversial, mainly because the assays were first- (cTnI) or second-generation (cTnT) troponin assays and the cutoff points were inconsistent. In the present study we investigated cTnT and cTnI during a marathon race with third- (cTnT) and second-generation
(cTnI) assays, as well as NT-proBNP. We hypothesized that marathon running may change cardiac troponin concentrations and that increased troponin concentrations are possibly associated with an increased mechanical load on the myocardium, exemplified by NT-proBNP.

We investigated 46 randomly selected participants (40 males and 6 females) of the Mainz Marathon 2002 (Germany) with a mean (SD) age of 40 (7) years, a mean height of 178 (7) cm, a mean weight of 73 (9) kg, and a mean body mass index (BMI) of 23.1 (1.9). The mean running time was 239 (34) min. To allow coverage of the whole spectrum of participating runners, there were no particular exclusion criteria. All participants filled in a questionnaire to register health status, cardiovascular risk factors, and training volume. The analysis of these questionnaires revealed that runners had performed regular endurance training over the past 7 (6) years. Cardiovascular risk factors and diseases were distributed as follows: acute myocardial infarction (AMI) in the family (n = 12), smokers (n = 3), ex-smokers (n = 1), mild hypercholes-
terolemia (n = 2), hypertension (n = 1), gestational diabetes (n = 1), nonasthmatic allergies (n = 11), and mild asthma (n = 1). Medicaments were used by six runners: oral contraceptives (n = 2), jodide (n = 1), antiallergics (n = 2), and antihypertensives (n = 1).

Venous blood samples for the determination of cTnT, cTnI, and NT-proBNP were taken before, 15 min, and 3 h after the race. In addition, in nine athletes, a fourth sample was taken on the next morning (24 h post-race). For the measurement of cTnT and NT-proBNP, we used Roche Diagnostics methods on an automated analyzer (Elecsys 2010). cTnI was assayed with the AccuTnI method (Beckman Coulter) on an Access analyzer. The troponin results were interpreted according to the guidelines of the European Society of Cardiology and the American College of Cardiology (17, 18). The upper reference limits (URLs) for the cardiac troponins corresponded to the 99th percentiles in healthy controls and were 0.010 and 0.040 μg/L for cTnT and cTnI, respectively. The cutoffs for AMI were the lowest troponin concentrations above the URL that gave a CV of 10%; for cTnT and cTnI, these were 0.030 and 0.060 μg/L, respectively. The URLs for men and women (<50 years) for the NT-proBNP method (Roche Diagnostics) are 88 and 153 ng/L (manufacturer’s information from the package insert; data not published).

Values are expressed as the mean (SD) for variables that followed a gaussian distribution and as the median (range) for non-gaussian-distributed variables. Gaussian distribution was tested with a Kolmogorov–Smirnov test. For statistical calculations, cTnT concentrations <0.010 μg/L (detection limit) were set as 0.000 μg/L. Changes in non-gaussian-distributed variables were tested with the Friedman test. Medians and mean values were compared using the Wilcoxon test for paired samples or the Student t-test. The Spearman correlation coefficient was calculated for the relationship between laboratory data and the anamnestic variables. P <0.05 was considered statistically significant. For multiple comparisons, the Bonferroni correction procedure was used.

Forty-five athletes finished the marathon without complaints. One participant suffered from exercise-induced, non-asthmatic dyspnea. A previous pulmonologic examination excluded organic disease. All laboratory data are summarized in Fig. 1. Pre-race cTnT was below the URL in all runners. At 15 min and 3 h post-race, the median cTnT was 0.006 (0.000–0.103) μg/L and 0.000 (0.000–0.174) μg/L, respectively; 23 athletes had cTnT concentrations above the URL at 15 min and 18 at 3 h post-race. At both time points, eight athletes had cTnT above the AMI cutoff. At 24 h post-race, all tested athletes were cTnT-negative. Athletes with post-race cTnT concentrations above the URL were divided in those with a transient (decreasing cTnT at 3 h post-race) and a prolonged (increasing cTnT at 3 h post-race) cTnT increase. The two groups did not differ in age, BMI, training volume, or running time.

Median pre-race cTnI was 0.020 (0.010–0.050) μg/L. At 15 min and 3 h post-race, cTnI was 0.050 (0.010–0.360) μg/L and 0.070 (0.020–0.930) μg/L, respectively. Twenty-seven athletes had cTnI concentrations above the URL at 15 min post-race, with 17 athletes having concentrations above the AMI cutoff. At 3 h post-race, 33 athletes had cTnI concentrations above the URL, with 27 having concentrations above the AMI cutoff. At 24 h post-race, one of nine tested athletes had increased cTnI. In contrast to cTnT, kinetics for cTnI at 15 min and 3 h post-race did not differ. Referring to the URL and the cutoffs for AMI, we found discrepant cTnT and cTnI results in 14 and 19 athletes, respectively, at 15 min and 3 h post-race. A result was defined discrepant if cTnT and cTnI results in the same athlete were not consistently above or below the URL or the cutoff for AMI. Most but not all of these discrepant results were near the URL. Although these discrepancies corresponded to relatively small deviations, we can state that the correlation between cTnT and cTnI is still not optimal, especially at low concentrations.

Pre-race NT-proBNP was 44 (17–144) ng/L. At 15 min and 3 h post-race, NT-proBNP was significantly increased: 137 (40–953) ng/L and 123 (44–550) ng/L, respectively. In 38 runners, post-race NT-proBNP concentrations exceeded the URL and were comparable to values found in AMI, unstable angina pectoris, and other cardiac diseases (19). At 24 h post-race, median NT-proBNP had decreased to 82 (52–194) ng/L in the tested athletes. Between 15 min and 3 h post-race, NT-proBNP had decreased in 30 runners (transient increase), whereas it had increased or not changed in 16 runners (prolonged increase). The runners with a transient or prolonged NT-proBNP increase were not the same as those with a transient or prolonged cTnT increase. Post-race cTnT was significantly correlated with cTnI (at 15 min post-race, r = 0.8; P <0.001; at 3 h post-race, r = 0.78; P <0.001). NT-proBNP, age, BMI, running time, and training were not associated with cTnT or cTnI concentrations after the race. NT-proBNP at 3 h post-race was weakly correlated
with the running time ($r = 0.5; P < 0.001$) and the weekly amount of training ($r = -0.3; P < 0.05$).

The present results demonstrate that increased cTnT, cTnI, and NT-proBNP can frequently be found in recreational runners after a marathon race. Increased cTnT and cTnI after a marathon are most probably of cardiac origin and not influenced by peripheral muscle damage (9, 20, 21). The cTnT and cTnI values above the URL must be divided in those above and below the cutoffs for AMI (22). Recent studies have shown that under certain circumstances, increased cTnT values below the AMI cutoff are associated with an increased risk of death (19, 23) and should not be ignored. We can therefore affirm that increased post-exercise cTnT and cTnI can not be excluded as relevant cardiac risks. However, no evidence is available to clarify the mechanism of release of cardiac biomarkers after a marathon race. Until the clinical impact of increased cTnT and cTnI after endurance exercise is clarified, we recommend that affected athletes should undergo further cardiologic investigation, including a stress test.

The change in NT-proBNP was independent of the increases in cTnT and cTnI, which is in contrast to our hypothesis. If NT-proBNP represents myocardial wall stress, increases in cTnT and cTnI are independent of mechanical load during exercise. However, no outcome studies have been reported in these athletes.

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References