# Cardiac Troponin T

## Cardiac Troponin T: Only Small Molecules in Recreational Runners After Marathon Completion

### TO THE EDITOR:

Vigorous endurance-type exercise causes cardiac troponin T (cTnT)[1] elevations that often exceed the diagnostic cutoff value for acute myocardial infarction (AMI) (*1*). It remains to be investigated whether exercise-induced cTnT release reflects a physiological or pathological process. Our recent studies utilizing multiple protein analysis techniques allowed determination of circulating cTnT forms, and it appeared that these are different in acute vs chronic pathologies (*2*, *3*). In sera of AMI patients, cTnT is degraded from its intact form (estimated molecular weight by SDS-PAGE; $MW_{est}$, 40 kDa) to primary ($MW_{est}$, 29 kDa) and subsequent secondary ($MW_{est}$, 14–18 kDa) fragments in a time-dependent manner (*2*). Only secondary fragments were detected in sera of patients with end-stage renal disease (ESRD) (*3*). The objective of the current study was to determine the circulating cTnT form(s) in recreational runners after marathon completion, which might provide additional insight into the mechanism of cTnT release.

The study population was a subgroup of recreational marathon runners (n = 10) of the 2007 Maas marathon (42.2 km) study population (n = 85) (*4*). Prerace and postrace sera samples were collected, aliquoted, and stored at −80 °C until analysis. Only participants with a postrace high-sensitivity (hs)-cTnT concentration of ≥70 ng/L were eligible because of technical detection limitations of the gel filtration chromatography (GFC) setup. The study complied with the Declaration of Helsinki, was approved by the Ethical Committee (METC 06–4-042) of the Maastricht University Medical Center (Maastricht, the Netherlands), and all marathon runners provided consent before participation. Molecular cTnT forms in serum samples were separated in 2015 by GFC on a HiPrep™ 16/60 Sephacryl™ S-300 HR column (GE Healthcare) as previously described (*3*). Per sample loaded, fractions of 1.25 mL were kept on ice until hs-cTnT, albumin, and myoglobin concentration analysis (<1 h), which was performed in every other sample. Albumin and myoglobin served as internal retention markers with retention volumes ($V_R$, corrected for void volume) at 42.5 and 55 mL, respectively. Paired non-Gaussian distributed results were presented as median (interquartile range) and analyzed with the Wilcoxon matched-pairs test. A $P$ value of <0.05 was considered statistically significant.

Endurance-type exercise caused significant hs-cTnT concentration increases in marathon runners from prerace to postrace concentrations of 4.1 (2.4–5.6) ng/L and 32.6 (21.5–51.8) ng/L ($P < 0.001$), respectively. The hs-cTnT concentration in the GFC subpopulation increased from 3.9 (2.7–6.6) ng/L to 103.5 (86.1–132.1) ng/L ($P = 0.0059$), respectively. Postrace hs-cTnT elution profiles of the GFC subpopulation revealed exclusive presence of 1 peak at $V_R$ 50 mL (Fig. 1). There was no indication that long-term sample storage affected the molecular cTnT composition (data not shown).

The current study demonstrated significant hs-cTnT elevations in marathon runners after vigorous exercise, and GFC analysis, which is at the moment the most sensitive technology to study cTnT forms, revealed that this concerned only secondary cTnT fragments of which the N-terminal and C-terminal ends of the protein were cleaved off. Thus, the

[1] **Nonstandard abbreviations:** cTnT, cardiac troponin T; AMI, acute myocardial infarction; ESRD, end-stage renal disease; hs, high sensitivity; GFC, gel filtration chromatography; $V_R$, retention volume.

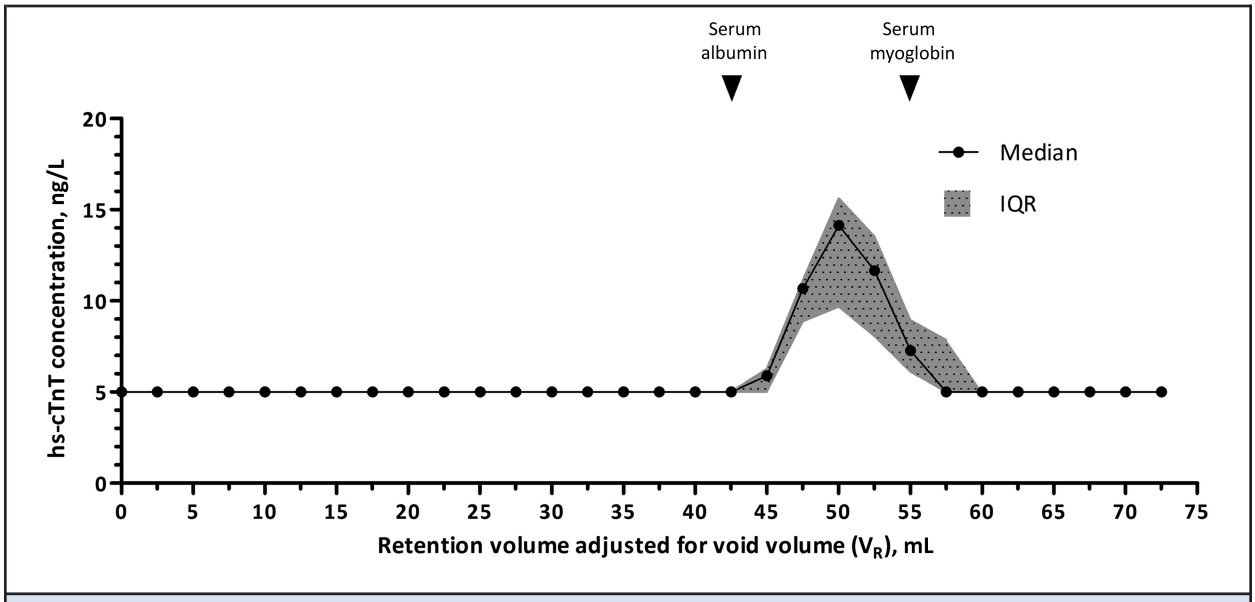


**Fig. 1. GFC elution profiles of postrace serum samples from our marathon runner subpopulation (n = 10).** Data of hs-cTnT concentrations are presented as median (black) and interquartile range (IQR; gray). Albumin and myoglobin served as internal retention markers.

circulating molecular cTnT form in marathon runners postrace is identical to the form seen in patients with ESRD and different than observed in patients with AMI.

It is most likely that postrace hs-cTnT elevations reflect a physiological mechanism: first, as exercise leads to decreased cardiovascular mortality (*1*) and second, as we found cardiac troponin elevations in the majority (hs-cTnT, 86%; cTnI, 74%; data not shown) of marathon runners (*4*). There are multiple physiological explanations for exercise-induced cTnT release, such as increased membrane permeability because of reactive oxygen species production, physiologic remodeling of the myocardium, increased cardiac workload, or a relatively new theory called "bleb" formations, which are cell membrane buds caused by transient ischemia (*1*). Cardiovascular stress in patients with ESRD is of chronic origin and associated with abnormal left ventricular morphologies and overall higher cardiovascular risk (*3*). Additionally, recent work from our group indicated that it is unlikely that impaired renal elimination is the main cause of chronic cTnT elevations (*5*). Other factors, such as (subclinical) myocardial injury, might explain the persistently increased cTnT levels in patients with ESRD (*5*). So, as in patients with AMI, cardiac stress is a common characteristic in marathon runners and patients with ESRD. According to the present data, both populations are seen to have small circulating cTnT molecules, in contrast with patients with AMI, in whom predominantly bigger cTnT molecules are observed in the acute phase. Whether differences in treatment and/or (patho)physiology are of any influence remains to be elucidated.

In conclusion, postrace hs-cTnT serum concentrations in recreational marathon runners exceeded the diagnostic threshold for AMI and solely existed of secondary cTnT fragments ($MW_{est}$, 14–18 kDa). This molecular cTnT form seems identical to its shape in patients suffering from ESRD and differs from patients with AMI.

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# Interference of Hemoglobin $A_{1c}$ Due to Hemoglobin Franklin Park

## TO THE EDITOR:

Hemoglobin (Hb)[1] $A_{1c}$ (*1*) can be measured by cation-exchange HPLC, which separates hemoglobin fractions, including some Hb variants, based on the overall charge on the molecule. In populations for whom there is a high prevalence of homozygous sickle disease, it is advisable to routinely use to routinely use a separation technique such as HPLC because this allows identification of the HbS homozygous state (*2*). Sickle cell disease is marked by a shortened red cell life span, so clinical laboratories should not issue $HbA_{1c}$ results for cases of sickle disease. $HbA_{1c}$ immunoassays can be readily adapted to run on standard chemistry analyzers, but they have the disadvantage of not detecting the lack of HbA in cases of homozygous sickle cell disease or HbSC compound heterozygotes. However, immunoassays can provide an accurate $HbA_{1c}$ result in the presence of most Hb variants (*3*).

Here we describe a case in which ion-exchange chromatography detected a rare asymptomatic Hb variant that interfered with $HbA_{1c}$ determination.

$HbA_{1c}$ quantification was carried out using EDTA-anticoagulated blood on a Bio-Rad Variant II HPLC analyzer (Bio-Rad Laboratories) using the short 3-min $HbA_{1c}$ program. The *y* axis was scaled to the calculated $HbA_{1c}$ value. The range was set to 20% to include all expected $HbA_{1c}$ results. A pro-

[1] **Nonstandard abbreviations:** Hb, hemoglobin; $LA_{1c}$, labile $A_{1c}$.
[2] **Human Genes:** *HBB*, hemoglobin subunit beta.

prietary Bio-Rad algorithm, utilizing a 2-point calibration, is used to report the percentage of $HbA_{1c}$ results anchored to IFCC standard materials. If the software does not detect $HbA_{1c}$, then the *y* axis defaults to a voltage measurement. In addition, a second ion-exchange HPLC system was used—the Bio-Rad D-10 Dual System—and run in the extended (6 min) or $F/A_2$ β-thalassemia mode.

Gel electrophoresis was performed on a SPIFE 3000 system (Helena Laboratories) using spun, washed red cell pellets. Globin genotyping was carried out at Boston Medical Center using the residual washed whole blood pellet. The globin genes were amplified by PCR; the amplicons were purified; and nucleotide sequencing was performed using the ABI Big Dye Terminator Cycle Sequencing kit (Applied BioSystems) and an ABI 3730.

The specimen in question derived from an 82-year-old African-American woman who was not anemic and had normal red cell indices. The abnormality was brought to our attention because of the presence of a large variant (41%) with an overall abnormal elution profile. Typically, $HbA_{1a}$, $HbA_{1b}$, labile $A_{1c}$ ($LA_{1c}$), and $HbA_{1c}$ elute at 0.22, 0.3, 0.71, and 0.94 min, respectively, on the Variant II analyzer. This broad, highly atypical variant eluted at 0.6 to 1.2 min with a peak at 0.73 min and was labeled as $LA_{1c}$ by the software. $HbA_{1c}$ was not recognized in the elution profile, and as a result, the software defaulted the *y* axis to voltage. However, analysis on the Bio-Rad D10 extended $F/A_2$ program gave an $HbA_{1c}$ reading of 51%. Alkaline gel electrophoresis revealed no abnormalities (not shown). The acid gel showed a variant cathodal band and HbA. The chromatographic and electrophoretic behavior of this variant is summarized in Fig. 1.

This variant was further characterized by globin gene analysis as Hb Franklin Park (*4*) β codon 2 CAC>AAC or His2Asn; *HBB*[2]: c.7C>A. The original description of Hb Franklin Park is Histidine on codon 2 changed to Asparagine, due to CAT>AAT. In this case, the mutation is CAC>AAC. Both AAT and AAC code for Asparagine. To our knowledge, this is the first time this variant has been associated with an abnormal profile on ion-exchange chromatography leading to interference with $HbA_{1c}$ determination.

In Hb Raleigh, the N-terminal valine is replaced by an alanine, which is acetylated and not glycated (*5*). The acetylation also leads to the loss of positive charge, resulting in an earlier elution from a cation-exchange column. In Hb Franklin Park, the β-chain N-terminal valine (amino acid 1) remains unchanged, but the second amino acid histidine (positively charged) is replaced by asparagine (polar, but uncharged). This substitution would also result in the net loss of a positive charge near the N terminus.

We have no other methods for routinely determining $HbA_{1c}$ in our diagnostic laboratory. However, boronate affinity chromatography would be the method of choice to determine HbA1c in this instance. Because the amino acid substitution in Hb Franklin Park is adjacent to the β N terminus, immunoassays would be expected to produce an inaccurate result (*3*).

If an early-eluting hemoglobin is encountered in the clinical laboratory, physicians should be advised to assess the state of glycemic control of that patient by means of boronate affinity chromatography.

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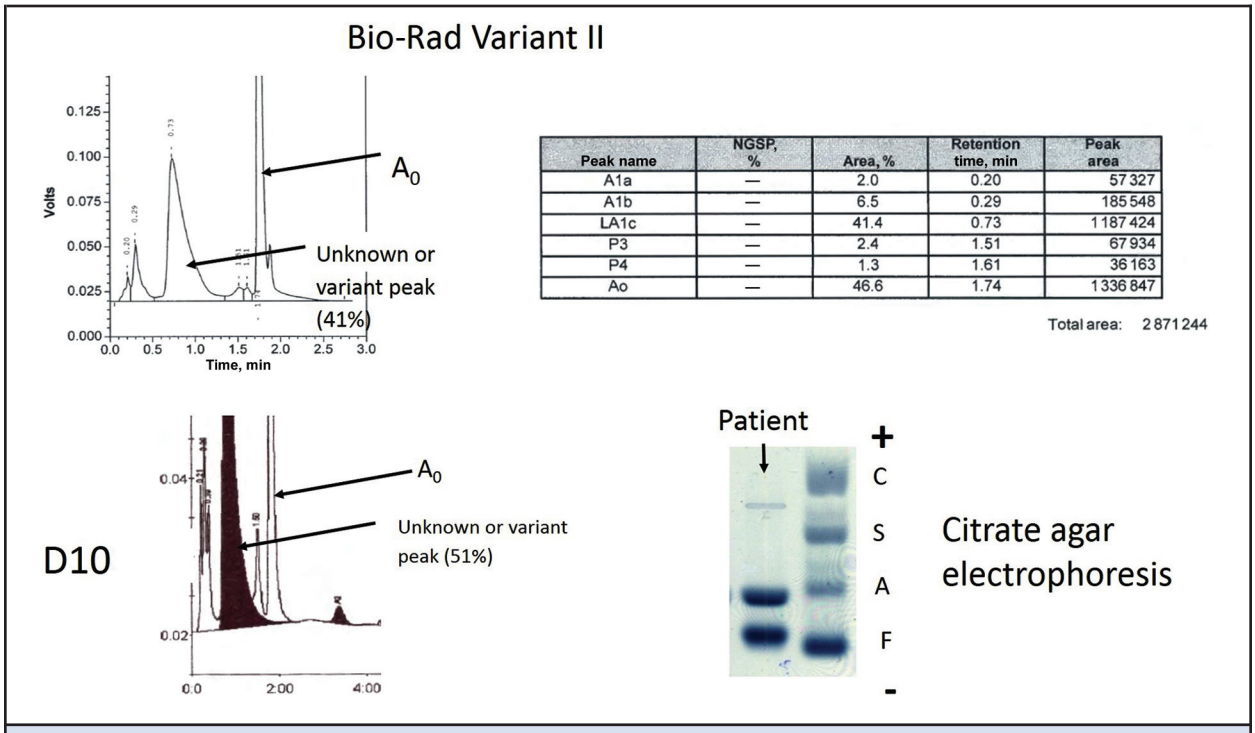


**Fig. 1. The cation-exchange HPLC (Bio-Rad Variant II) depicts the short $HbA_{1c}$ program.**

The software has designated the variant peak (41%) as $LA_{1c}$. The *y* axis depicts volts only. An alternative HPLC (Bio-Rad D10, extended mode) shows an apparent $A_{1c}$ of >50%. Acid citrate gel electrophoresis reveals a variant close, but slightly anodal, to HbF. NGSP, National Glycohemoglobin Standardization Program.

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