

Biomarkers of exercise-induced myocardial stress in relation to inflammatory and oxidative stress

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ABSTRACT

Increased concentrations of biomarkers reflecting myocardial stress such as cardiac troponin I and T and brain natriuretic peptide (BNP) have been observed following strenuous, long-lasting endurance exercise. The pathophysiological mechanisms are still not fully elucidated and the interpretations of increased post-exercise concentrations range from (i) evidence for exercise-induced myocardial damage to (ii) non-relevant spurious troponin elevations, presumably caused by assay imprecision or heterophilic antibodies.

Several lines of evidence suggest that inflammatory processes or oxidative stress could be involved in the rise of NT-proBNP and Troponin observed in critically ill patients with sepsis or burn injury. We tested the hypothesis that inflammatory or oxidative stress is also responsible for exercise-induced cardiomyocyte strain in a large cohort of triathletes following an Ironman triathlon. However, the post-race increase in cardiac troponin T and NT-proBNP was not associated with several markers of exercise-induced inflammation, oxidative stress or antioxidant vitamins.

Therefore, we clearly need more studies with other inflammatory markers and different designs to elucidate the scientific background for increases in myocardial stress markers following strenuous endurance events.

Key words: Troponin, exercise, myocardial damage, assay imprecision, acute coronary syndrome

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INTRODUCTION

After years of contradictory discussion, there is currently widespread consensus that the athlete's heart is a physiological adaptation of the human heart following years of endurance training [1;2]. In addition, regular physical exercise is undoubtedly associated with reduced cardiovascular morbidity and mortality [3;4].

Nevertheless, in the past decades, the diagnosis of acute exercise-induced minor myocardial damage was difficult due to the lack of cardiospecific markers. Creatine kinase (CK) is released following both skeletal and myocardial damage and also the determination of the relatively cardiospecific isoenzyme CK-MB does not always improve diagnostic specificity due to an increased expression of CK-MB in skeletal muscles of endurance athletes [5].

During recent years, assays for the determination of cardiac troponin T (cTnT) and cardiac troponin I (cTnI) as well as BNP or NT-proBNP have significantly improved the diagnostic power to detect subclinical cardiac damage and dysfunction [6-8].

It has been demonstrated that strenuous, long-lasting exercise is associated with an increase in biomarkers for myocardial stress such as cardiac troponins and B-type natriuretic peptide even in healthy trained subjects without signs for myocardial disease [9].

The reason why apparently healthy subjects exhibit increased concentrations of these biomarkers is not yet fully understood. Explanations include: Assay imprecision, heterophilic antibodies, sympathoadrenergic stimulation, structural or functional overload leading to temporal membrane leakage or definite cardiomyocyte death [9].

An important aspect that has not been considered extensively, comprises the notion that inflammatory processes are involved in the exercise-induced myocardial stress reaction and the increase in cardiac troponins and (NT-pro)BNP.

Several investigations have demonstrated that increased concentration of cytokines such as IL-6, TNF-alpha, IL-2 and IL-1 β acutely modulate cardiac function and may induce cardiomyocyte damage [10-12].

The effect of increased cytokine concentrations on myocardial function were firstly investigated in sepsis and burn injury. The secretion of cytokines following sepsis or burn injury altered the contractile responsiveness of the heart. In addition, critically ill patients showed increased concentrations of cardiac troponins and in some investigations also increased levels of (NT-pro)BNP.

Although the magnitude is much smaller, the exercise-induced inflammatory reaction resembles, at least in part, immunological processes observed during sepsis or severe trauma [13]. Therefore, there is a rational background to study the association between the inflammatory response following exercise and biomarkers for cardiomyocyte stress. In addition, measuring the course of oxidative stress markers and in particular the protective role of antioxidant vitamins may provide further information, as these factors have already been shown to be involved in the initiation and propagation of inflammatory mediated myocardial stress reactions [14].

In the present work, we summarize current knowledge on the association between strenuous physical exercise and the increase in biomarkers reflecting

myocardial stress (cardiac troponins and [NTpro] brain natriuretic peptide). In addition, we will present new data showing the association between exercise-induced inflammatory and oxidative stress/antioxidant vitamins levels and cardiac troponin T and NT-proBNP.

Cardiac troponin

Troponin is a contractile protein and comprises 5 percent of muscle proteins. The troponin complex consists of troponin I, troponin C, and troponin T. These proteins are located within the myofibrils of cardiac and skeletal muscle and are encoded by different genes. They have a key role in the regulation of the calcium-mediated muscle contraction through interaction of actin with myosin [15]. cTnC functions as a calcium-receptor while cTnI prevents the adenosine triphosphatase activity when bound to actin. Troponin T fixes the troponin complex to tropomyosin. cTnT and cTnI are heart-specific with a specific amino acid sequence that allows a clear immunological differentiation from skeletal muscle troponins. Therefore, cTnT and cTnI are highly sensitive and specific serum markers to detect myocardial damage in the presence of peripheral muscle damage [16]. After myocardial damage, troponins are released from cardiomyocytes and are detectable within 3–10 hours in peripheral blood [17]. Although cardiac troponins are correctly regarded as the most cardiac-specific markers for the detection of myocardial damage, several aspects have to be considered in the interpretation of study results. The most important point is related to assay performance, i.e. antibody specificity, susceptibility of the assay to certain analytical interferences, pre-analytical factors and assay imprecision [18]. The first generation cTnT assay showed a considerable cross reactivity with skeletal muscle troponin and also the second generation assay possessed only poor linearity due to the use of bovine cTnT as an assay standard material. Therefore, results from studies using the latter assays should be interpreted with caution and are therefore not considered. Although these problems have been solved by the 3rd generation cTnT assay, also this assay, together with the various cTnI assays, share the problem of imprecision at very low troponin concentrations. It has been claimed that due to the high precision of current assays, the so called background noise appears undetectable. Therefore, any elevation of cardiac troponins above the 99th percentile of concentrations found in apparently healthy subjects has been designated as indicative of myocardial damage. However, to assure a correct diagnostic classification, this decision limit must be measured with a total imprecision (coefficient of variation [CV]) of less or equal 10 % [18;19]. It has been shown that no cardiac troponin assay was able to achieve the 10 % CV recommendation at the 99th percentile reference limit defined by the manufacturer [18].

Although there is convincing evidence that troponin assays have tremendously improved the diagnostic and prognostic power of laboratory markers in the detection of suspected acute coronary syndromes, their impreciseness at the detection limit must be considered, particularly in the interpretation of comparatively small troponin elevations following exercise. Additionally, with all assays the post-test probability for the presence of a clinical condition after a positive test result strongly depends on the pre-test probability of a disease in the investigated

population. Thus, in symptomless highly trained endurance athletes the pre-test probability for myocardial damage following competition is very low and is only marginally increased by the result of a positive troponin test. In contrast, in chest pain patients a positive troponin result almost proves the presence of myocardial damage.

Concerning exercise-induced increases in cardiac troponins, it has to be mentioned that cardiac troponins are present in the myocyte, both in a cytosolic (cTnT 6%; cTnI 3%) and a structurally-bound protein pool [17;20;21]. Therefore, the release of troponins following myocardial injury could be explained by 2 mechanisms: 1. Minor myocardial injury induces a loss of integrity of the membrane that results in transient leakage of troponin from the cytosolic compartment. 2. When damage is further aggravated, the activation of proteolytic enzymes leads to disintegration of the contractile apparatus, with continuous release of troponins from the bound protein pool. However, from the present knowledge it cannot be definitively decided whether limited troponin release may be associated with reversible myocardial injury. The current common consensus is that an increase in cardiac troponin above the 99th percentile of the CV should be interpreted as a sign of myocardial necrosis [21-23]. Minor myocardial damage can be distinguished from major necrosis, i.e. myocardial infarction by the duration of troponin release from myocardial fibres. In such instances, small elevations of cardiac troponins above the 10% CV cut off level usually return within the reference limit within 24-36 hours. In contrast, after MI cardiac troponin concentration remain elevated for 5 days or longer [24].

Brain natriuretic peptide

Plasma concentrations of brain natriuretic peptide (BNP) have evolved as a new diagnostic tool for detecting both diastolic and systolic myocardial dysfunction [25-33]. The precursor of BNP is ProBNP, which is cleaved upon stimulation into the biologically active form BNP and the N-terminal rest NT-proBNP. BNP has a vasodilatory, natriuretic and diuretic activity that explains much of its biological function, i.e. to diminish myocardial strain mainly by reducing preload. Both BNP (half-life 20 min) and NT-proBNP (half-life 2 h) can be determined by immunoassays in plasma. BNP as well as NT-proBNP have shown a high sensitivity and specificity for the diagnosis of acute and chronic heart failure [30;34-37].

In contrast to cardiac troponins that indicate a structural damage to myocardial cells, an increase in BNP is a biomarker for functional myocardial overload or heart failure. The name "brain" is based on the historic fact that it was first detected in brain cells. However, BNP is mainly secreted from myocardial cells situated in atrium and ventricle. Therefore, it has been claimed that „Brain“natriuretic peptide should be renamed in "B-type" natriuretic peptide. Other members of the cardiac natriuretic peptides are the atrial natriuretic peptide (ANP) as well as the c-type natriuretic peptide (CNP).

Main stimuli for an increased liberation of BNP include a rise in intravascular volume, particularly an increase in central venous pressure and both right and left ventricular dysfunction as evidenced by an increased enddiastolic filling pres-

sure [28;38-41]. Both resting values as well as the magnitude of exercise-induced rise in BNP have shown to be associated with the severeness of myocardial insufficiency [30].

Therefore, it was an interesting finding that apparently healthy endurance trained athletes showed post-exercise increases in NT-proBNP that were beyond the cut-off levels for heart failure. Short maximal bouts of exercise e.g. ergometry to exhaustion were not associated with a rise in NTProBNP [27;28;40;42]. In contrast, long-lasting endurance events such as marathon running or triathlon competitions induced marked increases in NT-proBNP. There seems to be a trend for higher concentrations with increased exercise duration and age of the participants [26;43].

Cardiac troponin and NT-proBNP following exercise in athletes

Table 1 summarizes the results of some investigations that have measured cardiac troponins (cTnI; 3rd generation cTnT) and BNP/NT-proBNP following exercise. From 551 subjects investigated, approx. 33 % (variation in dependence of tests applied) showed an increase in cardiac troponins following endurance exercise. In most subjects, the increase was below the 10 % CV cut off limit reported by Panteghini et al. [18], and therefore, these results should be classified as troponin negative. 15 % of subjects showed an increase in cardiac troponins above the 10% CV cut-off, but below the cut-off level usually used for the diagnosis of myocardial infarctions with ST-segment elevations. In 5 % of the athletes, an increase above the cut-off level for myocardial infarction was detectable. With the exception of one subject in the study of Neumayr et al. [44], all investigations with subsequent post-race troponin analyses found that troponin levels had returned to baseline levels after 24 hours. The lack of cardiac symptoms and normal findings in ECG-analysis or SPECT sestamibi myocardial imaging [45] in all subjects, underlines the difficulties in interpreting the troponin results. In addition, most studies did not correct for hemoconcentration or verified troponin increases by measuring troponin with an assay of a different manufacturer. No study excluded analytical interferences with the troponin assay applied as a possible cause of a positive test result.

Therefore, some scientists hold that due to multiple limitations of the studies published so far, increased concentrations in cardiac troponins in well trained symptomless athletes are not evidentiary for myocardial injury. Their main point of criticism is a poor control for hemoconcentration, lack of exclusion of false positive test results due to analytical interferences from particles, bubbles or heterophilic antibodies or other assay dependent interfering substances in the samples [46]

However, the opinion that an exercise-induced increase in cardiac troponins in well trained athletes above the 10 % CV at the 99th percentile of the reference range should be considered as (at least minor) myocardial injury, is gaining more attention. The viewpoint is further supported by findings from post-exercise echocardiographic measurements showing a decrease in ejection fraction, wall motion disturbances and impaired diastolic function following strenuous endurance exercise [47-49]. In the study of Rifai et al., the subject with the high-

Table 1. Cardiac Troponin T/I (cTnT/cTnI) and Brain natriuretic peptide (BNP) following strenuous exercise.
(Papers cited in core clinical/sportsmedical journals).

Author	Subjects	Age (y)	Setting	Main results: Troponin	Main results: BNP	Ref.
Shave, R.E.	8 trained men	33 ± 9	Laboratory setting; 50 mile cycle trial in either normobaric normoxia or normobaric hypoxia	1 subject with increase in cTnT following normobaric hypoxia (0.016 µg/l)	Not measured	[72]
König, D.	11 professional cyclists	27 ± 4	Strenuous stage of a professional cycling race (Tour de Suisse)	2 subjects with increase in cTnT following race (0.03µg/l/0.1 µg/l)	Increase in BNP from 47.5 to 75.3 pg/ml. Increase was most pronounced in older athletes	[73]
Shave, R.E.	7 trained female	44 ± 7	2-day strenuous Lowe Alpine Mountain Marathon	3 subjects with increase in cTnT following day 1 (0.013-0.044 µg/l); 2 subjects with increase in cTnT following day 2 (0.014µg/l/0.017 µg/l)	Not measured	[20]
Vidotto, C.	12 male runners 13 female runners		Half-marathon race	2 h postexercise: 4 females and 4 male runners with cTnI above 99 th perc.	Increase in NT-proBNP from 39 to 107 pg/ml immediately post exercise	[50]
Neumayr, G.	38 trained men	~ 35	Strenuous cycling marathon in high altitude	8% of subjects with CK/CK-MB ratio > 6 %; 13 subjects with increase in cTnI immediately after competition (0.9-4.9 µg/l), 3 subjects above AMI cut-off; 24 h after competition cTnI < 1.0 µg/l in 12 subjects; 1 subject with further increase from 1.3 to 4.0 µg/l after 24 h	Not measured	[44]
La Gerche, A.	15 trained men	29 ±10	Ironman distance triathlon	1 subject with increase in cTnI following triathlon (0.9 µg/l) ^b	Not measured	[74]
Middleton, N.	13 trained male runners 1 trained female runner	29±5	London marathon	9 subjects with increase in cTnT; 2 above 99 th perc.	Increase in NT-proBNP from 21.6 to 47.1 pg/ml	[51]
Rifai, N.	11 trained men and 12 trained women	33 ± 8 43 ± 14	Hawaii Ironman triathlon	2 subjects with increase in cTnI following triathlon (4.44/2.09 µg/L = > AMI cut-off) ^c	Not measured	[47]
Neilan, TG	41 trained men and 19 trained women	41 range: 21-65	Boston marathon	60 % with cTnT above 99 th perc. (>0.01 ng/ml); 40 % at or above AMI cut-off (>0.03 ng/ml)	Increase in NT-proBNP from 63 to 131 pg/ml. Positive correlation with post-race myocardial dysfunction	[49]
Shave, R.E.	26 trained men	41 ± 10	2-day strenuous Lowe Alpine Mountain Marathon	13 subjects with increase in cTnT following day 1 (< 0.035 µg/l); 1 subjects with increase in cTnT following day 2 (0.017 µg/l). All subjects with increase in cTnT on day 1 were negative following day 2	Not measured	[75]

Neumayr, G.	16 trained men	37 ± 8	Race Across the Alps	6 (1) subjects with increase in cTnI (cTnT) following the race; 4 subjects with increase in cTnI above the 99 th perc (0.7-5.1 µg/l); 3 subjects with cTnI above AMI cut-off; subject with the highest cTnI also showed cTnT above AMI cut-off (0.11 µg/l); cTnI returned to baseline in all subjects after 24 h	Not measured	[76]
Neumayr, G.	29 recreational cyclists	34 ± 8	Bike marathon race	27 % with increase in cTnT above 99 th perc. (0.043-0.224 mg/l)	Increase in NT-ProBNP from 28 to 278 ng/L	[52]
Shave R.E.	8 trained men	29 ± 9	30 min. downhill-running	No increase in cTnT	Not measured	[77]
Cleave, P.	64 trained men	n.p.	New Zealand Ironman distance triathlon	13 subjects with increase in cTnI above 10 % CV; in 5 subjects cTnI was > AMI cut-off;	Not measured	[78]
Scharhag, J.	20 male endurance trained athletes	36 ± 7	Endurance exercise with either 1 h at 75 % VO ₂ max or 3 h at 60 % VO ₂ max	Slight increase in cTnI without any increase in cTnT.	Increase in NT-ProBNP by 9 ng/l and 30 ng/l respectively. No pathological findings in echocardiography	[79]
Scharhag, J	105 endurance trained athletes	40 ± 8	Marathon (n=46), 100 km run (n=14), mountain bike marathon (n=45)	cTnI: 74 % above 99 th perc. cTnT: 47 % above 99 th perc.	77 % of athletes showed increase in NT-ProBNP above reference limit. Increase was not correlated with alterations in troponin	[53]
Siegel A.J.	82 trained men	47 ± 7	Boston marathon (serial testing of the same group after 5 subsequent marathon races)	1997: 1 subjects with post-race increase in cTnI; 1 subject positive pre-race TnId 1998-2000: Significant increase in cTnI from 0.022 to 0.144 µg/L and in cTnI from 0.009 to 0.027 µg/L; 2 subjects with increase in cTnI above AMI cut-off (1.23; 1 µg/l) 2001: insignificant increase in cTnI from 0.009 to 0.027 µg/L and in cTnT from < 0.01 to 0.041.	Not measured	[45]

LLD = Lower limit of detection; 99thperc = 99th percentile of the reference range; AMI = cut-off value for acute myocardial infarction
 cTnT = Cardiac Troponin T (3rd generation assay); CtnI = Cardiac Troponin I
 b = post-race examination were performed at a mean of 4.7 d after competition; c = cTnT also measured but not 3rd generation assay

est increase in cTnI also showed the highest number of hypokinetic myocardial wall segments [47].

It has been speculated that strenuous physical exercise above the individual strain tolerance could induce functional myocardial overload, energetical deprivation or increased liberation of intracellular Ca^{++} leading to a loss of membrane integrity resulting in transient leakage of troponin from the cytosolic compartment. If this transient process is fully reversible without any residuals, is likely – given the large number of endurance athletes without cardiomyopathy - but cannot yet be proven. The short duration (<24 h) of detectable troponin concentrations favors this explanation, because cardiomyocyte death with protein disintegration has shown to be associated with a much longer troponin release period (>5 days). Other hypotheses to explain increased troponin concentrations following strenuous long-lasting endurance exercise include inflammatory processes or free radical damage.

In most investigations, the increase in post-exercise NT-proBNP was more than 100 % from baseline [49-53]. There seems to be trend for higher post-exercise values with increasing age of the participants and duration of a strenuous endurance event [26;43]. Explanations for increased NT-proBNP concentrations following strenuous long-lasting endurance events include neurohumeral activation of BNP release as well as a temporary myocardial systolic and/or diastolic dysfunction involving both right and left ventricles due to mechanical or volume overload.

In addition, recent findings from experimental and clinical studies suggest that inflammatory or oxidative stress related processes may promote the release of BNP after endurance exercise [54].

Table 2

Antioxidant vitamins, muscular stress and inflammatory markers 2 days before (14.07), immediately after (16.07), 1 day after (17.07), 5 days after (21.07) and 19 days after the Ironman Triathlon. ** = $p < 0.01$ compared to baseline values

	14.07	16.07	17.07	21.07	04.08
Antioxidant vitamins					
Vitamin C ($\mu\text{mol/l}$)	66,6 \pm 13	102 \pm 26,6 **	68,2 \pm 13,2 **	80,9 \pm 15,2 **	82,8 \pm 14 **
Alpha-Tocopherol ($\mu\text{mol/l}$)	22,6 \pm 7,3	26,6 \pm 7 **	21,8 \pm 6	22,5 \pm 5,8	21,8 \pm 5,8
Muscle stress markers					
Creatine kinase (U/l)	120 \pm 76	1553 \pm 2003 **	5298 \pm 6240 **	457 \pm 808 **	168 \pm 248
Creatine kinase MB (U/l)	5 \pm 4	45 \pm 50 **	125 \pm 142 **	11 \pm 13 **	5 \pm 5
Myoglobin ($\mu\text{g/l}$)	50 \pm 13	1965 \pm 1321**	531 \pm 531	72 \pm 25	65 \pm 39
Inflammatory markers					
PMN-Elastase (ng/ml)	45,6 \pm 22,8	238 \pm 137 **	94,6 \pm 103 **	44,4 \pm 30,5	36 \pm 15,8 **
Myeloperoxidase ($\mu\text{g/l}$)	57,2 \pm 30,9	253 \pm 122 **	97 \pm 82 **	61 \pm 57,6	41,2 \pm 24,9 **

Cardiac function in relation to inflammatory/oxidative stress in the diseased state

The association between cardiac function and inflammatory markers can be divided into two main categories. On the one hand, increased levels of inflammatory cytokines (IL-6, TNF-alpha) and proteins of the APR (hsCRP) have been identified as subclinical markers of future heart failure. In 732 elderly Framingham Heart Study subjects without coronary heart disease, the risk to develop congestive heart failure was threefold when IL-6, TNF-alpha and hsCRP were elevated [55]. If the increase in inflammatory biomarkers reflects existing subclinical myocardial damage or is indicative of other inflammatory processes such as atherosclerotic plaque formation, cannot be answered conclusively [56]. In this context, the finding that the increase in CRP levels following Q-wave infarction seemed to be linked to the amount of myocardial tissue damage rather than the degree of pre-existing inflammation, merits further attention and evaluation [57]. First evidence suggests that high concentrations of inflammatory biomarkers are associated with a worsened prognosis following myocardial infarction or heart failure [57;58]. Most recently, it was found that plasma concentrations of myeloperoxidase (MPO) predict mortality following myocardial infarction [59]. Levels of MPO were also increased following strenuous physical exercise and the authors speculated on possible interactions between a rise in MPO and cardiac troponin and NT-proBNP [60].

On the other hand, apart from the prognostic aspect, it has been shown that increased concentration of cytokines such as IL-6, TNF-alpha, IL-2 and IL-1 β acutely modulate cardiac function and may induce cardiomyocyte damage [10-12].

The first studies showing the effect of increased cytokine concentrations on myocardial function were investigations of sepsis and burn injury. It could be demonstrated that the secretion of cytokines following sepsis or burn altered the contractile responsiveness of the beating heart or isolated myofibers. The acute effect is multilayered and involves different cellular mechanism such as nitric oxide (NO), sphingolipid mediators, alterations in intracellular Ca⁺⁺ handling and the arachidonic acid pathway [10;61]. Chronic effects are mediated by interstitial matrix remodelling mainly by the induction of specific matrix metalloproteinases leading to myocyte hypertrophy and myocardial fibrosis [62].

The rational background to study the association between the inflammatory response following exercise and biomarkers for cardiomyocyte stress is based on several similarities to the pathologic condition. Although the magnitude is much smaller, the exercise-induced inflammatory reaction resembles at least in part immunological processes observed during sepsis or severe trauma [13]. Moreover, despite the absence of myocardial infarction or coronary occlusion, several studies have shown that 15-25 % of critically ill patients exhibited increased concentrations of cTnI [63]. Positive correlation between troponin levels and left ventricular dysfunction have been found and also an increase in NT-proBNP has been reported by some investigators. However, the evidence that sepsis is always associated with an increase in BNP or NT-proBNP could not be ascertained [63].

Nevertheless, the data so far are sufficient to suggest a possible association between the exercise-induced cardiomyocyte stress reaction and inflammatory processes [54]. In addition, measuring markers of oxidative stress and the protective role of antioxidant vitamins may provide further information, as these factors have shown to be involved in the initiation and propagation of inflammatory mediated myocardial stress reactions [14].

Cardiac function in relation to inflammatory/oxidative stress following strenuous exercise

To test the hypothesis whether inflammatory/oxidative stress is involved in the cardiomyocyte stress reaction, we investigated related parameters in 42 well-trained healthy male triathletes (age 35.3 ± 7.0 yr, height: 180.6 ± 0.1 cm, weight: 75.1 ± 6.4 kg, BMI: 23.0 ± 1.2 kg/m², VO_2 peak 56.6 ± 6.2 mL•kg⁻¹•min⁻¹, training volume 10.7 ± 2.6 h•wk⁻¹) who participated in the 2006 Ironman in Austria.

The Ironman triathlon took place in Klagenfurt in July 2006 and consisted of 3.8 km swimming, 180 km cycling and 42.2 km running. The race took place under near optimal climatic conditions.

Subjects did not take any medication or antioxidant supplementation (more than 100% of RDA) in the 6 wk before the race until the end of the study.

METHODS

Blood samples were taken 2 days (d) before the race, immediately (within 20 minutes), 1 d, 5 d and 19 d after the race. The samples were immediately cooled to 4°C and the serum separated at 3000 rpm for 20 min at 4°C within 4 hours. Aliquots were frozen at -80°C until analysis. Whole blood was taken for the haematological profile; post-exercise concentrations of each parameter were corrected for exercise-induced alterations in plasma volume.

Cardiac troponin T (cTnT) and NTproBNP were determined using electrochemiluminescence technology employed within the Modular analytics E170 analyzer (Roche Diagnostics, Lewes, Sussex, UK). The oxidative stress markers malondialdehyde (MDA) and conjugated dienes (CD) were determined both by HPLC using the method of Wong [64] and Banni [65], respectively.

Concentrations of alpha-tocopherol (α -Toc) were analyzed using HPLC according to the method of Jakob and Elmadfa [66]. Ascorbic acid (Vit-C) was detected photometrically by the method of Denson and Bowers [67]. Plasma creatine kinase (CK) and creatine kinase MB (CKMB) activity was measured using an automatic analyzer (Vitros DT 60 II module; ortho-clinical diagnostics, Germany). Plasma interleukin 6 (IL-6) was determined by the Quantikine HS Immunoassay kit (R&D Systems GmbH, Wiesbaden, Germany). Concentrations of high sensitive C-reactive protein (hsCRP) and myoglobin were analyzed nephelometrically (Dade Behring, Marburg, Germany) Myeloperoxidase (MPO) concentrations were measured in plasma using the immunodiagnostik MPO ELISA kit (Immundiagnostik AG, Bensheim, Germany), Polymorphonuclear elastase (PMNelas) was determined using a quantitative enzyme immunoassay (Milena Biotec GmbH, Bad Nauheim, Germany).

RESULTS

Figures 1-6 show the pronounced myocardial and pro-oxidative stress response as well as the course of IL-6 as major stimulator and hsCRP as one of the main constituents of the acute phase response (APR) following the ironman triathlon. With the exception of one athletes who had slightly elevated cTnT at baseline (0.014 µg/l), all participants had normal values for cTnT before the competition. Immediately following the triathlon, cTnT increased above the 99th percentage of CV in 57 % of athletes; 3 of them were above the cut-off value for myocardial infarction (MI) (> 0.1 µg/ml). The day after the competition, cTnT was above the 99th percentage of CV in 3 subjects but clearly beyond the cut-off levels for MI in all athletes.

NT-proBNP increased considerably after the triathlon and all but 2 athletes showed NT-proBNP above the upper reference limit (URL) for beginning heart failure. In addition, on the day after the race, 60 % of athletes exhibited NT-proBNP levels above the URL. 5 days following the competition NT-proBNP was still elevated but had returned below the URL except for one athlete who had already shown increased concentrations at baseline.

Previous results concerning the course of cTnT and NT-proBNP in endurance events have already been discussed (see Tab. 1). The increase in cTnT in the present study was relatively high but not unusual, whereas the increase in NT-proBNP above 500 pg/ml was higher than in most previous investigations. However, the duration of exercise in most studies that have measured NT-proBNP was shorter (e.g. marathon or half-marathon [50;51] than an Ironman triathlon.

The high stress that the athletes were exposed is also reflected by the course of the other stress parameters investigated. Following the triathlon, MDA and CD as oxidative stress markers increased significantly. Furthermore, IL-6 and hsCRP as components of the APR as well as PMNelas and MPO as markers of the cellular inflammatory response rose significantly.

In addition, the triathlon induced a high exercise-induced stress reaction of peripheral muscle cells as indicated by the increase in myoglobin, CK and CKMB, although the latter could also origin from cardiomyocytes. As shown in most previous investigations, plasma concentrations of vitamin C and alpha-tocopherol increased following exercise [68].

There was no significant correlation between cTnT and NT-proBNP immediately after the competition. The strong correlation ($r^2 = 0.77$) that was observed between these parameters the day after the race was based on only three subjects and should not be considered as representative.

As shown in tab. 3 and 4, there was no correlation between cTnT or NT-proBNP and any parameter reflecting inflammatory (IL-6, hsCRP, MPO, PMNelas) or oxidative stress (MDA, CD). In addition, serum concentrations of antioxidative vitamins (Vit-C, α -Toc) were not associated with cTnT and NT-proBNP, respectively. Parameters reflecting peripheral muscle stress (Myo, CK, CKMB) were positively correlated with cTnT following the race.

DISCUSSION

The most important finding of this investigation was that cTnT and NT-proBNP were not related to markers of inflammatory or oxidative stress. The considerable

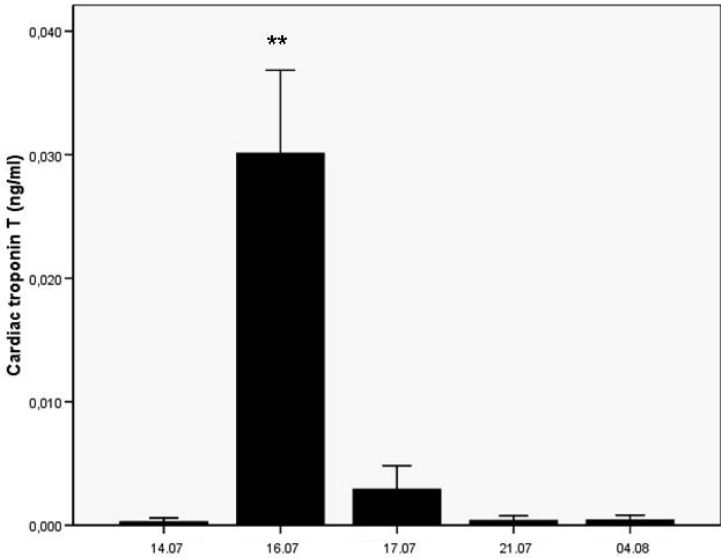


Fig 1. Cardiac troponin T (cTnT) 2 days before (14.07), immediately after (16.07), 1 day after (17.07), 5 days after (21.07) and 19 days after the Ironman Triathlon. ** = $p < 0.01$ compared to baseline values

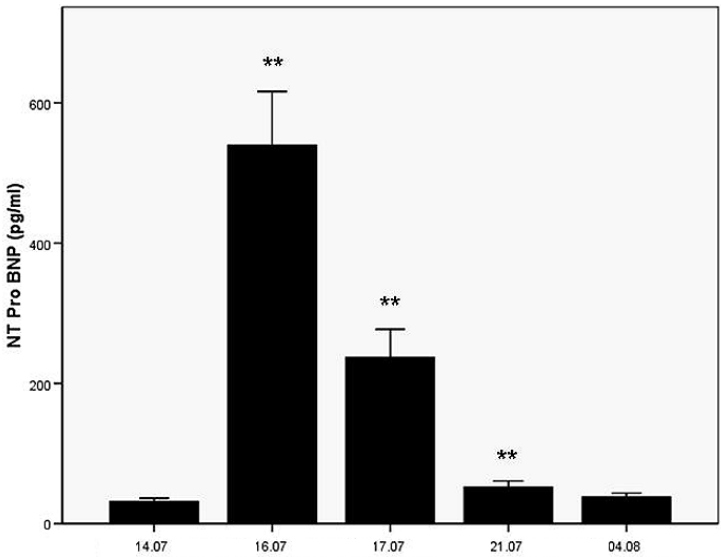


Fig 2. NT-ProBNP 2 days before (14.07), immediately after (16.07), 1 day after (17.07), 5 days after (21.07) and 19 days after the Ironman Triathlon. ** = $p < 0.01$ compared to baseline values

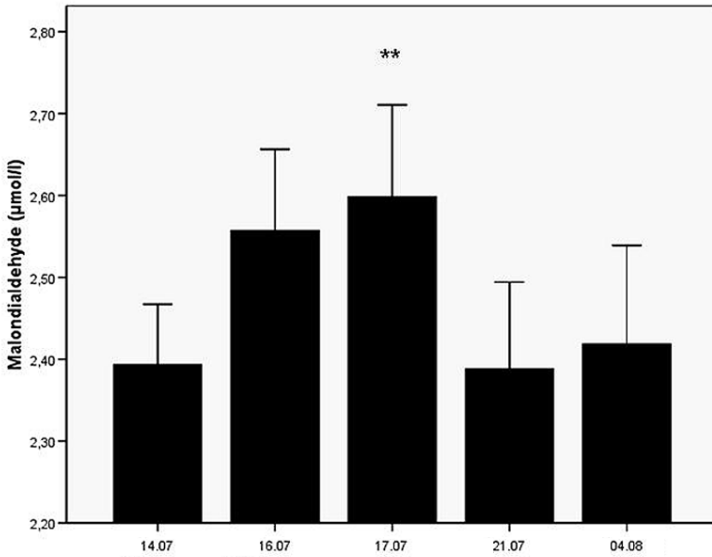


Fig 3. Malodialdehyde (MDA) 2 days before (14.07), immediately after (16.07), 1 day after (17.07), 5 days after (21.07) and 19 days after the Iron-man Triathlon. ** = $p < 0.01$ compared to baseline values

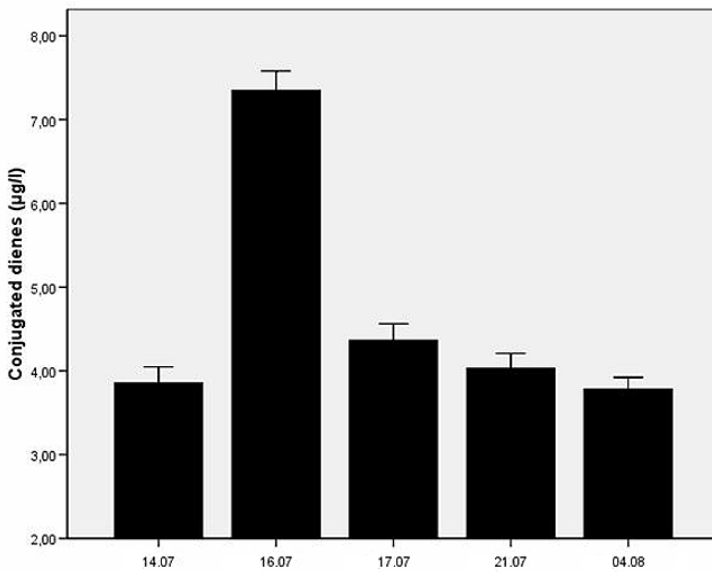


Fig 4. Conjugated dienes (CD) 2 days before (14.07), immediately after (16.07), 1 day after (17.07), 5 days after (21.07) and 19 days after the Iron-man Triathlon. ** = $p < 0.01$ compared to baseline values

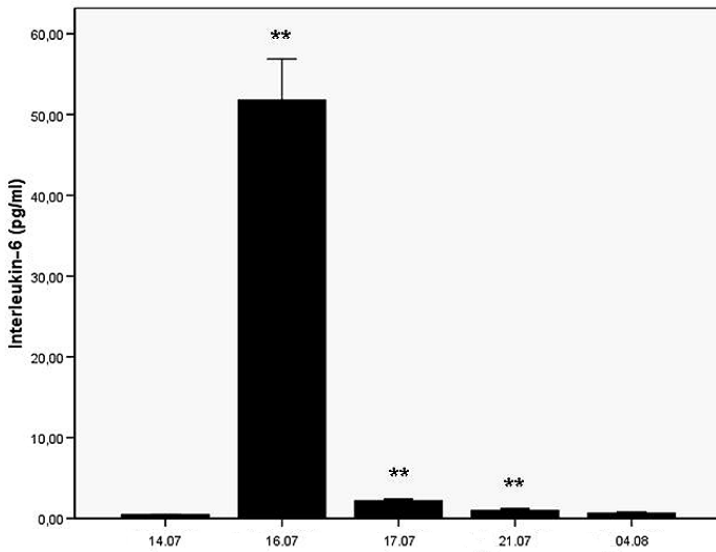


Fig 5. Interleukin 6 (IL-6) 2 days before (14.07), immediately after (16.07), 1 day after (17.07), 5 days after (21.07) and 19 days after the Ironman Triathlon.

** = $p < 0.01$ compared to baseline values

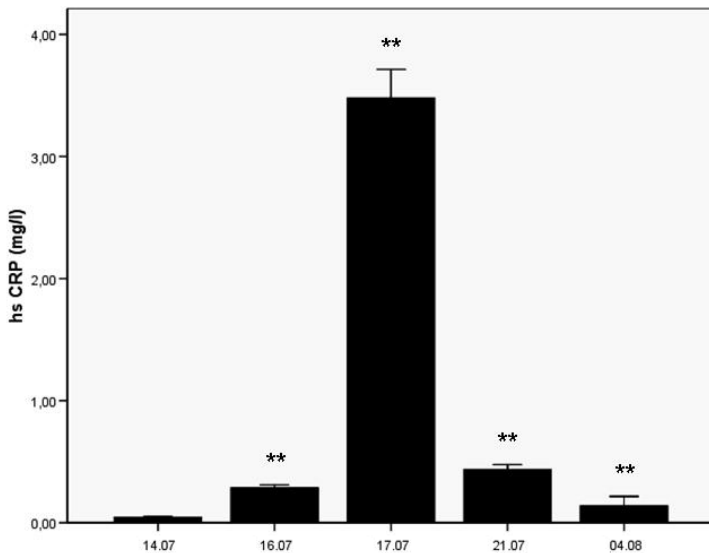


Fig 6. high sensitive C-reactive protein (hsCRP) 2 days before (14.07), immediately after (16.07), 1 day after (17.07), 5 days after (21.07) and 19 days after the Ironman Triathlon.

** = $p < 0.01$ compared to baseline values

increase in each parameter investigated reflects the significant stress that was induced by this ultra-endurance event. Compared with previous investigations, the magnitude of the individual stress response in this study was comparable or even higher [9;69-71]. The positive correlation between markers of skeletal muscle damage (CKMB was far below the ratio suggesting myocardial damage) and cTnT directly after the race is not clear. According to present knowledge, the

Table 3 Correlation between cardiac troponin T (cTnT), NTproBNP and investigated parameters immediately after the ironman triathlon

	cTnT 16.07	NT-proBNP 16.07
	Coefficient of correlation	Coefficient of correlation
Age (y) -,200	,191	
peak oxygen consumption (ml O ₂ /kg/min)	,238	-,267
Training hours/week (h)	,155	-,100
Finishertime (h)	-,198	,199
Malondialdehyd 16.07 (μmol/l)	-,246	-,026
Conjugated dienes 16.07 (μg/l)	,035	-,030
Vitamin C 16.07 (μmol/l)	-,228	,050
alpha-Tocopherole 16.07 (μmol/l)	,186	-,080
Creatinkinase 16.07 (U/l)	,436(**)	,101
Creatinkinase MB 16.07 (U/l)	,451(**)	,063
Myoglobin 16.07 (μg/l)	,396(**)	-,062
Interleukin_6 16.07 (pg/ml)	,101	,274
hsCRP 16.07 (mg/l)	,205	,270
PMN Elastase 16.07 (ng/ml)	-,038	-,234
Myeloperoxidase 16.07 (μg/l)	,057	-,248

* Correlation is significant (p< 0.05)

** Correlation is highly significant (p< 0.01)

Table 4 Correlation between cardiac troponin T (cTnT), NTproBNP and investigated parameters one day after the ironman triathlon

	cTnT 17.07	NT-proBNP 17.07
	Coefficient of correlation	Coefficient of correlation
Age (y)	,327(*)	-,017
peak oxygen consumption (ml O ₂ /kg/min)	-,147	,009
Training hours/week (h)	-,102	,038
Finishertime (h)	,136	-,099
Malondialdehyd 17.07 (μmol/l)	,138	,154
Conjugated dienes 17.07 (μg/l)	,189	-,021
Vitamin C 17.07 (μmol/l)	-,201	-,123
alpha-Tocopherole 17.07 (μmol/l)	-,095	-,219
Creatinkinase 17.07 (U/l)	-,082	,095
Creatinkinase MB 17.07 (U/l)	-,022	,147
Myoglobin 17.07 (μg/l)	,040	,086
Interleukin_6 17.07 (pg/ml)	-,109	,105
hsCRP 17.07 (mg/l)	-,072	,048
PMN Elastase 17.07 (ng/ml)	,259	,239
Myeloperoxidase 17.07 (μg/l)	-,242	-,281

* Correlation is significant (p< 0.05)

third generation assay of troponin shows no cross-reactivity with skeletal muscle troponin. Therefore, it could be suggested that the mechanisms responsible for peripheral muscle damage are at least partially identical to those responsible for cardiomyocyte stress.

Animal studies or investigations with critically ill patients have shown an association between the release of inflammatory cytokines and markers of functional and structural myocardial stress [54]. In addition, free radicals, oxidative stress and antioxidant vitamins as protective factors have been shown to be involved in the regulation of myocardial stress [14].

Although the inflammatory stress was decisively smaller than during sepsis or burn injury, the absence of any association suggests that the parameters we have chosen were not involved in the initiation of functional or structural cardiomyocyte stress in this design. In addition, plasma levels of antioxidant vitamins did not show a negative association in terms of having an attenuating effect.

However, although the numbers of athletes investigated was relatively high, we acknowledge the limitations of the correlational analysis. The intriguing hypothesis and the results so far from subjects with sepsis, burn injury or myocardial dysfunction should increase our effort to further investigate the (patho)physiological cause and effect of the exercise-induced increase in cardiac troponins and (NTpro)BNP. For the future, it seems particularly important to consider other cytokines such as TNF-alpha or IL-2 as these two have also shown modulatory effects of cardiac function [65].

CONCLUSION

Strenuous long-lasting endurance exercise is associated with an increase in biomarkers of structural (cardiac troponins) and functional (BNP/NT-proBNP) myocardial stress. While usually, the increase was rather modest, some apparently healthy athletes without cardiovascular diseases showed post-exercise concentrations of these markers within the range of myocardial infarction or congestive heart failure. Scientific interpretations for these findings range from methodological problems or assay imprecision, temporal impairment of membrane integrity to clear evidence for myocardial tissue damage. Several pathomechanisms were made responsible for the increase in cardiac troponin and BNP such as mechanical and functional overload, adrenergic stimulation as well as free radical damage and inflammatory processes. However, inflammatory or oxidative stress related parameters investigated in the present study were not associated with CTnT or NT-proBNP concentrations.

The question if or when physical exercise may become harmful to cardiac myocytes or may even result in long-term impairment of myocardial function is of particular importance for all athletes. Therefore, there is a reasonable background to claim for more studies investigating the cause and effect of the exercise-induced myocardial stress reaction. By considering the association between myocardial dysfunction during sepsis or burn injury, there is a rationale to further investigate this problem with the inclusion of other inflammatory parameters than in the present investigation.

Nevertheless, an increase in cardiac troponins or NT-proBNP, particularly above the cut-off limit for myocardial infarction or heart failure, respectively, should always result in comprehensive cardiological examination to rule out underlying cardiovascular diseases.

REFERENCES

1. Keul,J., H.H.Dickhuth, M.Lehmann, and J.Staiger. The athlete's heart - haemodynamics and structure. *Int. J. Sports Med.* 3 (Suppl 1): 33-43, 1982
2. Spirito,P., A.Pelliccia, M.A.Proschan, M.Granata, A.Spataro, P.Bellone, G.Caselli, A.Biffi, C.Vecchio, and B.J.Maron. Morphology of the "athlete's heart" assessed by echocardiography in 947 elite athletes representing 27 sports. *Am. J. Cardiol.* 74: 802-806, 1994
3. Jolliffe,J., K.Rees, R.S.Taylor, D.Thompson, N.B.Oldridge, and S.Ebrahim, 2002. Exercise-based rehabilitation for coronary heart disease. *The Cochrane Library*, Issue 1, Oxford, pp. 1-25.
4. Després,J.P., B.Lamarche, C.Bouchard, A.Tremblay, and D.Prud'Homme. Exercise and the prevention of dyslipidemia and coronary heart disease. *Int. J. Obes. Relat. Metab. Disord.* 19 Suppl 4: S45-51, 1995
5. Apple,F.S. and P.A.Tesch. CK and LD isozymes in human single muscle fibers in trained athletes. *J. Appl. Physiol* 66: 2717-2720, 1989
6. Apple,F.S., A.H.Wu, and A.S.Jaffe. European Society of Cardiology and American College of Cardiology guidelines for redefinition of myocardial infarction: how to use existing assays clinically and for clinical trials. *Am. Heart J.* 144: 981-986, 2002
7. Mair,J.. Cardiac troponin I and troponin T: are enzymes still relevant as cardiac markers? *Clin. Chim. Acta* 257: 99-115, 1997
8. Shapiro,B.P., H.H.Chen, J.C.Burnett, Jr., and M.M.Redfield. Use of plasma brain natriuretic peptide concentration to aid in the diagnosis of heart failure. *Mayo Clin. Proc.* 78: 481-486, 2003
9. König, D., Schumacher, Y. O., Berg, A., Dickhut, H. H., and Mair, J. Cardiac troponins in the assessment of myocardial injury following exercise in athletes. *Am J Med Sports.* 6;4:177-182 2004.
10. Prabhu,S.D.. Cytokine-induced modulation of cardiac function. *Circ. Res.* 95: 1140-1153, 2004
11. Horton,J.W., D.L.Maass, J.White, and B.Sanders. Myocardial inflammatory responses to sepsis complicated by previous burn injury. *Surg. Infect. (Larchmt.)* 4: 363-377, 2003
12. Maass,D.L., J.White, and J.W.Horton. IL-1beta and IL-6 act synergistically with TNF-alpha to alter cardiac contractile function after burn trauma. *Shock* 18: 360-366, 2002
13. Northoff,H., S.Enkel, and Ch.Weinstock. Exercise, injury, and immune function. *Exerc Immunol Rev* 1: 1-25, 1995
14. Horton,J.W., D.J.White, D.L.Maass, D.P.Hybki, S.Haudek, and B.Giroir. Antioxidant vitamin therapy alters burn trauma-mediated cardiac NF-kappaB activation and cardiomyocyte cytokine secretion. *J. Trauma* 50: 397-406, 2001
15. Mair,J., F.Dienstl, and B.Puschendorf. Cardiac troponin T in the diagnosis of myocardial injury. *Crit Rev. Clin. Lab Sci.* 29: 31-57, 1992

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16. Chapelle, J.P. Cardiac troponin I and troponin T: recent players in the field of myocardial markers. *Clin. Chem. Lab Med.* 37: 11-20, 1999
17. Mair, J., B.Thome-Kromer, I.Wagner, P.Lechleitner, F.Dienstl, B.Puschendorf, and G.Michel. Concentration time courses of troponin and myosin subunits after acute myocardial infarction. *Coron. Artery Dis.* 5: 865-872, 1994
18. Panteghini, M., F.Pagani, K.T.Yeo, F.S.Apple, R.H.Christenson, F.Dati, J.Mair, J.Ravkilde, and A.H.Wu. Evaluation of imprecision for cardiac troponin assays at low-range concentrations. *Clin. Chem.* 50: 327-332, 2004
19. Wu, A.H., Y.J.Feng, R.Moore, F.S.Apple, P.H.McPherson, K.F.Buechler, and G.Bodor. Characterization of cardiac troponin subunit release into serum after acute myocardial infarction and comparison of assays for troponin T and I. American Association for Clinical Chemistry Subcommittee on cTnI Standardization. *Clin. Chem.* 44: 1198-1208, 1998
20. Shave, R.E., E.Dawson, P.G.Whyte, K.George, D.Ball, C.D.Gaze, and P.Collinson. Cardiac troponin T in female athletes during a two-day mountain marathon. *Scott. Med. J.* 48: 41-42, 2003
21. Ooi, D.S., P.A.Isotalo, and J.P.Veinot. Correlation of antemortem serum creatine kinase, creatine kinase-MB, troponin I, and troponin T with cardiac pathology. *Clin. Chem.* 46: 338-344, 2000
22. Piper, H.M., P.Schwartz, R.Spahr, J.F.Hutter, and P.G.Spieckermann. Early enzyme release from myocardial cells is not due to irreversible cell damage. *J. Mol. Cell Cardiol.* 16: 385-388, 1984
23. Ishikawa, Y., J.E.Saffitz, T.L.Mealman, A.M.Grace, and R.Roberts. Reversible myocardial ischemic injury is not associated with increased creatine kinase activity in plasma. *Clin. Chem.* 43: 467-475, 1997
24. Wu, A.H., F.S.Apple, W.B.Gibler, R.L.Jesse, M.M.Warshaw, and R.Valdes, Jr.. National Academy of Clinical Biochemistry Standards of Laboratory Practice: recommendations for the use of cardiac markers in coronary artery diseases. *Clin. Chem.* 45: 1104-1121, 1999
25. Yousufuddin, M., M.Y.Henein, M.Flather, D.Wang, W.Shamim, C.O'Sullivan, M.Kemp, E.Kazzam, N.R.Banner, M.Amrani, and A.J.Coats. Incremental importance of peak-exercise plasma levels of endothelin-1 and natriuretic peptides in chronic heart failure. *J. Cardiovasc. Pharmacol.* 38: 468-473, 2001
26. Ohba, H., H.Takada, H.Musha, J.Nagashima, N.Mori, T.Awaya, K.Omiya, and M.Murayama. Effects of prolonged strenuous exercise on plasma levels of atrial natriuretic peptide and brain natriuretic peptide in healthy men. *Am. Heart J.* 141: 751-758, 2001
27. Nicholson, S., M.Richards, E.Espiner, G.Nicholls, and T.Yandle. Atrial and brain natriuretic peptide response to exercise in patients with ischaemic heart disease. *Clin. Exp. Pharmacol. Physiol* 20: 535-540, 1993
28. McNairy, M., N.Gardetto, P.Clopton, A.Garcia, P.Krishnaswamy, R.Kazanegra, M.Ziegler, and A.S.Maisel. Stability of B-type natriuretic peptide levels during exercise in patients with congestive heart failure: implications for outpatient monitoring with B-type natriuretic peptide. *Am. Heart J.* 143: 406-411, 2002
29. Marumoto, K., M.Hamada, M.Aburaya, and K.Hiwada. Augmented secretion of atrial and brain natriuretic peptides during dynamic exercise in patients with old myocardial infarction. *Jpn. Circ. J.* 59: 715-724, 1995

30. Kruger,S., J.Graf, D.Kunz, T.Stickel, P.Hanrath, and U.Janssens. brain natriuretic peptide levels predict functional capacity in patients with chronic heart failure. *J. Am. Coll. Cardiol.* 40: 718-722, 2002
31. Kohno,M., K.Yasunari, K.Yokokawa, T.Horio, H.Kano, M.Minami, M.Ikeda, and J.Yoshikawa. Plasma brain natriuretic peptide during ergometric exercise in hypertensive patients with left ventricular hypertrophy. *Metabolism* 45: 1326-1329, 1996
32. Huang,W.S., M.S.Lee, H.W.Perng, S.P.Yang, S.W.Kuo, and H.D.Chang. Circulating brain natriuretic peptide values in healthy men before and after exercise. *Metabolism* 51: 1423-1426, 2002
33. Friedl,W., J.Mair, S.Thomas, M.Pichler, and B.Puschendorf. Relationship between natriuretic peptides and hemodynamics in patients with heart failure at rest and after ergometric exercise. *Clin. Chim. Acta* 281: 121-126, 1999
34. Clerico,A., M.Fontana, L.Zyw, C.Passino, and M.Emdin. Comparison of the diagnostic accuracy of brain natriuretic peptide (BNP) and the N-terminal part of the propeptide of BNP immunoassays in chronic and acute heart failure: a systematic review. *Clin. Chem.* 53: 813-822, 2007
35. Matsumoto,A., Y.Hirata, S.Momomura, E.Suzuki, I.Yokoyama, M.Sata, Y.Ohtani, and T.Serizawa. Effects of exercise on plasma level of brain natriuretic peptide in congestive heart failure with and without left ventricular dysfunction. *Am. Heart J.* 129: 139-145, 1995
36. Clarkson,P.B., N.M.Wheeldon, R.J.MacFadyen, S.D.Pringle, and T.M.MacDonald. Effects of brain natriuretic peptide on exercise hemodynamics and neurohormones in isolated diastolic heart failure. *Circulation* 93: 2037-2042, 1996
37. Bettencourt,P., A.Ferreira, P.Dias, J.Pimenta, F.Frões, L.Martins, and M.Cerqueira-Gomes. Predictors of prognosis in patients with stable mild to moderate heart failure. *J. Card Fail.* 6: 306-313, 2000
38. Sagnella, G. A. Measurement and importance of plasma brain natriuretic peptide and related peptides. *Ann Clin Biochem* 38, 83-93. 2001.
39. Kato,M., T.Kinugawa, K.Ogino, A.Endo, S.Osaki, O.Igawa, I.Hisatome, and C.Shigemasa. Augmented response in plasma brain natriuretic peptide to dynamic exercise in patients with left ventricular dysfunction and congestive heart failure. *J. Intern. Med.* 248: 309-315, 2000
40. Steele,I.C., G.McDowell, A.Moore, N.P.Campbell, C.Shaw, K.D.Buchanan, and D.P.Nicholls. Responses of atrial natriuretic peptide and brain natriuretic peptide to exercise in patients with chronic heart failure and normal control subjects. *Eur. J. Clin. Invest* 27: 270-276, 1997
41. Maisel,A.S., P.Krishnaswamy, R.M.Nowak, J.McCord, J.E.Hollander, P.Duc, T.Omland, A.B.Storrow, W.T.Abraham, A.H.Wu, P.Clopton, P.G.Steg, A.Westheim, C.W.Knudsen, A.Perez, R.Kazanegra, H.C.Herrmann, and P.A.McCullough. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. *N. Engl. J. Med.* 347: 161-167, 2002
42. Barletta,G., L.Stefani, R.Del Bene, C.Fronzaroli, S.Vecchiarino, C.Lazzeri, F.Fantini, and G.La Villa. Effects of exercise on natriuretic peptides and cardiac function in man. *Int. J. Cardiol.* 65: 217-225, 1998
43. König,D., Y.O.Schumacher, L.Heinrich, A.Schmid, A.Berg, and H.H.Dickhuth. Myocardial stress after competitive exercise in professional road cyclists. *Med. Sci. Sports Exerc.* 35: 1679-1683, 2003

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44. Neumayr,G., H.Gaenger, R.Pfister, W.Sturm, S.P.Schwarzacher, G.Eibl, G.Mitterbauer, and H.Hoertnagl. Plasma levels of cardiac troponin I after prolonged strenuous endurance exercise. *Am. J. Cardiol.* 87: 369-71, A10, 2001
45. Siegel,A.J., E.L.Lewandrowski, K.Y.Chun, M.B.Sholar, A.J.Fischman, and K.B.Lewandrowski. Changes in cardiac markers including B-natriuretic peptide in runners after the Boston marathon. *Am. J. Cardiol.* 88: 920-923, 2001
46. Ng,S.M., P.Krishnaswamy, R.Morrissey, P.Clopton, R.Fitzgerald, and A.S.Maisel. Mitigation of the clinical significance of spurious elevations of cardiac troponin I in settings of coronary ischemia using serial testing of multiple cardiac markers. *Am. J. Cardiol.* 87: 994-999, 2001
47. Rifai,N., P.S.Douglas, M.O'Toole, E.Rimm, and G.S.Ginsburg. Cardiac troponin T and I, echocardiographic [correction of electrocardiographic] wall motion analyses, and ejection fractions in athletes participating in the Hawaii Ironman Triathlon. *Am. J. Cardiol.* 83: 1085-1089, 1999
48. Whyte,G.P., K.George, S.Sharma, S.Lumley, P.Gates, K.Prasad, and W.J.McKenna. Cardiac fatigue following prolonged endurance exercise of differing distances. *Med. Sci. Sports Exerc.* 32: 1067-1072, 2000
49. Neilan,T.G., J.L.Januzzi, E.Lee-Lewandrowski, T.T.Ton-Nu, D.M.Yoerger, D.S.Jassal, K.B.Lewandrowski, A.J.Siegel, J.E.Marshall, P.S.Douglas, D.Lawlor, M.H.Picard, and M.J.Wood. Myocardial injury and ventricular dysfunction related to training levels among nonelite participants in the Boston marathon. *Circulation* 114: 2325-2333, 2006
50. Vidotto,C., H.Tschan, J.Atamaniuk, R.Pokan, N.Bachl, and M.M.Muller. Responses of N-terminal pro-brain natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI) to competitive endurance exercise in recreational athletes. *Int. J. Sports Med.* 26: 645-650, 2005
51. Middleton,N., R.Shave, K.George, G.Whyte, J.Forster, D.Oxborough, D.Gaze, and P.Collinson. Novel application of flow propagation velocity and ischaemia-modified albumin in analysis of postexercise cardiac function in man. *Exp. Physiol* 91: 511-519, 2006
52. Neumayr,G., R.Pfister, G.Mitterbauer, G.Eibl, and H.Hoertnagl. Effect of competitive marathon cycling on plasma N-terminal pro-brain natriuretic peptide and cardiac troponin T in healthy recreational cyclists. *Am. J. Cardiol.* 96: 732-735, 2005
53. Scharhag,J., M.Herrmann, A.Urhausen, M.Haschke, W.Herrmann, and W.Kindermann. Independent elevations of N-terminal pro-brain natriuretic peptide and cardiac troponins in endurance athletes after prolonged strenuous exercise. *Am. Heart J.* 150: 1128-1134, 2005
54. McLachlan,C. and P.Mossop. Are elevations of N-terminal probrain natriuretic peptide in endurance athletes after prolonged strenuous exercise due to systemic inflammatory cytokines? *Am. Heart J.* 152: e1, 2006
55. Vasan,R.S., L.M.Sullivan, R.Roubenoff, C.A.Dinarello, T.Harris, E.J.Benjamin, D.B.Sawyer, D.Levy, P.W.Wilson, and R.B.D'Agostino. Inflammatory markers and risk of heart failure in elderly subjects without prior myocardial infarction: the Framingham Heart Study. *Circulation* 107: 1486-1491, 2003
56. Lowe,G.D.. The relationship between infection, inflammation, and cardiovascular disease: an overview. *Ann. Periodontol.* 6: 1-8, 2001
57. Brunetti,N.D., M.Correale, P.L.Pellegrino, A.Cuculo, and M.D.Biase. Acute phase proteins in patients with acute coronary syndrome: Correlations with diagnosis, clinical features, and angiographic findings. *Eur. J. Intern. Med.* 18: 109-117, 2007

58. Yip,H.K., C.L.Hang, C.Y.Fang, Y.K.Hsieh, C.H.Yang, W.C.Hung, and C.J.Wu. Level of high-sensitivity C-reactive protein is predictive of 30-day outcomes in patients with acute myocardial infarction undergoing primary coronary intervention. *Chest* 127: 803-808, 2005
59. Mocatta,T.J., A.P.Pilbrow, V.A.Cameron, R.Senthilmohan, C.M.Frampton, A.M.Richards, and C.C.Winterbourn. Plasma concentrations of myeloperoxidase predict mortality after myocardial infarction. *J. Am. Coll. Cardiol.* 49: 1993-2000, 2007
60. Melanson,S.E., S.M.Green, M.J.Wood, T.G.Neilan, and E.L.Lewandrowski. Elevation of myeloperoxidase in conjunction with cardiac-specific markers after marathon running. *Am. J. Clin. Pathol.* 126: 888-893, 2006
61. White,D.J., D.L.Maass, B.Sanders, and J.W.Horton. Cardiomyocyte intracellular calcium and cardiac dysfunction after burn trauma. *Crit Care Med.* 30: 14-22, 2002
62. Bradham,W.S., B.Bozkurt, H.Gunasinghe, D.Mann, and F.G.Spinala. Tumor necrosis factor-alpha and myocardial remodeling in progression of heart failure: a current perspective. *Cardiovasc. Res.* 53: 822-830, 2002
63. Maeder,M., T.Fehr, H.Rickli, and P.Ammann. Sepsis-associated myocardial dysfunction: diagnostic and prognostic impact of cardiac troponins and natriuretic peptides. *Chest* 129: 1349-1366, 2006
64. Wong,S.H., J.A.Knight, S.M.Hopfer, O.Zaharia, C.N.Leach, Jr., and F.W.Sunderman, Jr. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clin. Chem.* 33: 214-220, 1987
65. Banni,S., L.Lucchi, A.Baraldi, B.Botti, G.Cappelli, F.Corongiu, M.A.Dessi, A.Tomasi, and E.Lusvardi. No direct evidence of increased lipid peroxidation in hemodialysis patients. *Nephron* 72: 177-183, 1996
66. Elmadfa,I., C.Bartens, E.Jakob, and J.Konig. Nutritional status and the immune system: fat-soluble vitamins and other nutrients. *Bibl. Nutr. Dieta* 136-141, 1994
67. DENSON,K.W. and E.F.BOWERS. The determination of ascorbic acid in white blood cells. A comparison of W.B.C. ascorbic acid and phenolic acid excretion in elderly patients. *Clin. Sci.* 21: 157-162, 1961
68. Evans,W.J. Vitamin E, vitamin C, and exercise. *Am. J. Clin. Nutr.* 72: 647S-652S, 2000
69. König,D., K.H.Wagner, I.Elmadfa, and A.Berg. Exercise and oxidative stress: Significance of antioxidants with special reference to inflammatory, muscular and systemic stress markers. *Exerc Immunol Rev* 7: 108-133, 2001
70. La Gerche,A., K.A.Connelly, D.J.Mooney, A.I.Macisaac, and D.L.Prior. Biochemical and functional abnormalities of left and right ventricular function following ultra-endurance exercise. *Heart*, 2007
71. Tulloh,L., D.Robinson, A.Patel, A.Ware, C.Prendergast, D.Sullivan, and L.Pressley. Raised troponin T and echocardiographic abnormalities after prolonged strenuous exercise-the Australian Ironman Triathlon. *Br. J. Sports Med.* 40: 605-609, 2006
72. Shave,R.E., E.Dawson, G.Whyte, K.George, D.Gaze, and P.Collinson. Effect of prolonged exercise in a hypoxic environment on cardiac function and cardiac troponin T. *Br. J. Sports Med.* 38: 86-88, 2004
73. König,D., Y.O.Schumacher, L.Heinrich, A.Schmid, A.Berg, and H.H.Dickhuth. Myocardial stress after competitive exercise in professional road cyclists. *Med. Sci. Sports Exerc.* 35: 1679-1683, 2003

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74. La Gerche, A., A. Boyle, A. M. Wilson, and D. L. Prior. No evidence of sustained myocardial injury following an ironman distance triathlon. *Int. J. Sports Med.* 25: 45-49, 2004
75. Shave, R. E., E. Dawson, G. Whyte, K. George, D. Ball, D. C. Gaze, and P. O. Collinson. Evidence of Exercise-Induced Cardiac Dysfunction and Elevated cTnT in Separate Cohorts Competing in an Ultra-Endurance Mountain Marathon Race. *Int. J. Sports Med.* 23: 489-494, 2002
76. Neumayr, G., R. Pfister, G. Mitterbauer, A. Maurer, H. Gaenger, W. Sturm, and H. Hoertnagl. Effect of the "Race Across The Alps" in elite cyclists on plasma cardiac troponins I and T. *Am. J. Cardiol.* 89: 484-486, 2002
77. Shave, R., E. Dawson, G. Whyte, K. George, D. Ball, P. Collinson, and D. Gaze. The cardiospecificity of the third-generation cTnT assay after exercise-induced muscle damage. *Med. Sci. Sports Exerc.* 34: 651-654, 2002
78. Cleave, P., T. D. Boswell, D. B. Speedy, and D. R. Boswell. Plasma cardiac troponin concentrations after extreme exercise. *Clin. Chem.* 47: 608-610, 2001
79. Scharhag, J., A. Urhausen, G. Schneider, M. Herrmann, K. Schumacher, M. Haschke, A. Krieg, T. Meyer, W. Herrmann, and W. Kindermann. Reproducibility and clinical significance of exercise-induced increases in cardiac troponins and N-terminal pro brain natriuretic peptide in endurance athletes. *Eur. J. Cardiovasc. Prev. Rehabil.* 13: 388-397, 2006