Serum Copeptin and Midregion Proadrenomedullin (MR-proADM) After an Ultramarathon

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Background: Although it is widely acknowledged that physical activity confers several health benefits, it remains uncertain whether strenuous and physically demanding exercise might determine biological effects that might turn to be ultimately unfavorable for health. Copeptin and midregion proadrenomedullin (MR-proADM) are emerging cardiovascular and stress biomarkers, but little is known about the influence of strenuous physical exercise on their concentrations. **Methods:** The present study was performed to investigate the variation of copeptin and MR-proADM, along with that of serum creatinine and estimated glomerular filtration rate before and after a 60 km ultramarathon in 16 healthy Caucasian males. **Results:** The serum concentrations of both copeptin and MR-proADM remarkably increased after the

60 km run, by 6.4 times (interquartile range (IQR), 2.710.4) and 2.3 times (IQR, 1.8–2.6), respectively. A highly significant correlation was observed between the increase of creatinine and MR-proADM, but not between serum creatinine and copeptin. The percentage of subjects exhibiting values above the upper limit of the reference range in male was 0% for both copeptin and MR-proADM before the ultramarathon, but increased to respectively 81 and 63% postexercise. **Conclusion:** The evidence that an ultramarathon causes a substantial increase of copeptin and MR-proADM raises doubts as to whether exhaustive exercise might be considered globally beneficial or even safe, especially in unfit or/and untrained population. *J. Clin. Lab. Anal.* 29:15–20, 2015. © 2014 Wiley Periodicals, Inc.

Key words: sport; physical exercise; marathon; copeptin; midregion proadrenomedullin

INTRODUCTION

It is widely acknowledged that physical activity and exercise confer several benefits for healthier lifestyle as well as in the growing battle against obesity, cardiovascular disease, and other chronic disorders such as diabetes, osteoporosis, decline of cognitive function, and cancer (1). The International Olympic Committee has recently endorsed recommendations advocating that all governments should encourage physical exercise and health promotion on global health and regional agency agendas (1). Although it is hence clear that sedentary behavior or an inadequate high-level physical exercise in unfit or/and un-

healthy population might be deleterious, it remains uncertain whether strenuous and physically demanding exercise might determine short- and long-term biological effects that might turn to be ultimately unfavorable for health

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(2). It has been recently observed that exhaustive exercise in physically fit and trained people is a healthy practice, which may even increase life expectancy (3). On the other hand, however, several sports modalities such as cycling and long-distance running (e.g., marathon and ultramarathon) have been associated with a remarkable increase in biomarkers of cardiac injury (4–7), as well as with radiological findings suggestive of myocardial necrosis and fibrosis (8). Oxidative stress is clearly involved in the pathophysiology of cardiac remodeling and heart failure as well as in skeletal muscle damage induced by exhaustive exercise (9). Exhaustive or acute physical exercise causes an increase in the generation of reactive oxygen species, and therefore raises the oxidative stress in muscle and in other organs, resulting in cell damage (10, 11).

Copeptin is the C-terminal portion of vasopressin, a 39 amino acid glycopeptide of unknown function, which is produced during cleavage of arginine vasopressin (AVP), also known as antidiuretic hormone (12). AVP is mainly released from the neurohypophysis to maintain water balance by the kidney, and thereby contributes to osmotic and cardiovascular homeostasis. Besides the clinical significance as a surrogate marker of AVP secretion, there is increasing evidence that the combined determination of copeptin with cardiac troponins in patients with chest pain might accelerate the diagnosis of acute coronary syndrome or acute myocardial infarction (AMI) (13–15), as well as mirror disease severity and help predict unfavorable outcomes in patients with chronic stable heart failure (16). Additional studies have also suggested that copeptin assessment may provide reliable prognostic information in patients after an AMI (17), as well as in those with chronic heart failure (18). Increased levels of copeptin might also predict an increased risk for diabetes independently of conventional risk factors (19). The prognostic value of copeptin has been established in other severe diseases such as sepsis, pneumonia, lower respiratory tract infections, and stroke (20). Most importantly, it was also recently demonstrated that copeptin concentration correlates with the individual stress level (21).

Adrenomedullin (ADM) is a 52 amino acid vasoactive peptide that plays an important role in microcirculation and endothelial dysfunction. It is mainly expressed from the adrenal medulla and adipose tissue, as well as from endothelial and vascular smooth muscle cells, immune cells, and several endocrine glands (22). The precursor pre-proADM, which is transcriptionally induced by insulin, hypoxia, and several inflammatory stimuli, is further activated to ADM after cleavage of another biologically active peptide known as proADM N-terminal 20 peptide (PAMP) and two peptides flanking ADM, that is, the midregional part of proADM (MR-proADM 45–92) and the carboxy-terminus of the molecule (proADM 153–185). Recent evidence attests that MR-proADM is an

independent predictor for events in patients with symptomatic coronary artery disease (23), chronic heart failure (24), stroke (25), and other severe disorders such as amyloidosis (26), sepsis (27), community-acquired pneumonia (28), and acute dyspnea (29), even if the causal contribution of MR-proADM to the pathogenesis of these pathologies remains unclear.

Although both copeptin and MR-proADM can hence be considered emerging cardiovascular biomarkers, little is known about the influence of strenuous physical exercise on their concentrations, especially on that of the latter peptide. The present study was performed to investigate the value of copeptin and MR-proADM as biochemical markers of cellular damage after high-level physical exercise. For this purpose, we measured copeptin, MR-proADM, serum creatinine, and estimated glomerular filtration rate (EGFR) before and after a 60 km ultramarathon run.

MATERIALS AND METHODS

Study Design

The population study consisted of 16 healthy Caucasian males (mean age: 41 years, range: 34–50 years), who had been engaged in specific endurance training for at least 4 years (mean training regimen: 248 ± 32 min/week; maximal oxygen uptake, VO_2 : 65 ± 2 ml/kg/min). The athletes concluded a 60 km ultramarathon equipped with a heart rate (HR) monitor at $82 \pm 4\%$ $\text{VO}_{2\text{max}}$. The percentage was calculated according to VO_2/HR relationship assessed with incremental test. None of the subjects suffered from acute or chronic diseases, reported regular or recent medication intake, including antioxidants and nicotine. Strenuous exercise was also precluded for at least 36 h before this study. The ultramarathon began at 08:00 A.M. and was run on a hilly and demanding course, on a cloudy and partially rainy day, with temperatures ranging from 6 to 8°C and humidity from 54 to 87%. Blood samples were collected at fast, 20 min before the participants warmed up (pre), and 10 min after the end of the ultramarathon (post). All subjects gave an informed consent for being tested, and the study was carried out in agreement with the Declaration of Helsinki and under the terms of all relevant local legislation. Blood was collected in vacuum tubes containing no additives (Becton-Dickinson, Oxford, UK), separated by centrifugation at $3,000 \times g$ within 30 min from venipuncture and stored at -70°C .

Laboratory Measurements

Serum creatinine was measured by Jaffe, rate-blanked, and compensated assay on a Beckman Coulter AU5800,

employing proprietary reagents (Beckman Coulter Inc., Brea, CA). The kidney function was also defined by EGFR, according to the formula developed and validated in the Modification of Diet in Renal Disease (MDRD) study, and further modified by Levey et al. for methods traceable to the serum creatinine reference system (30). The resulting equation is as follows: $EGFR = 175 \times (\text{serum creatinine}^{-1.154}) \times (\text{age}^{-0.203}) \times 1.212$ (if black) $\times 0.742$ (if female), and it does not require a body weight variable because it normalizes GFR for a standard body surface area of 1.73 m². The lower limit of the reference range for this formula is conventionally fixed at <60 ml/min/1.73 m².

Serum copeptin and ADM were assayed by Time-Resolved Amplified Kryptat Emission (TRACE) on Kryptor (Thermo Scientific B.R.A.H.M.S, Germany). According to the manufacturer's specification, the intra- and interassay imprecision of copeptin immunoassay at concentrations of 20 and 50 pmol/l are 6–12% and 8–13%, respectively. The analytical detection limit, the 97.5% percentile of the male reference range as well as the cut-off of copeptin in the diagnostics of cardiovascular disorders and AMI have been set at 4.8, 19.1 pmol/l and 14 pmol/l, respectively (13). According to the manufacturer's specification, the intra- and interassay imprecision of MR-proADM immunoassay at concentrations ranging from 0.5 to 6 nmol/l are comprised between 4 and 11%. The analytical detection limit and 95% percentile of the reference range are 0.05 and 0.52 nmol/l, respectively.

Statistical Analysis

The Wilcoxon signed-rank test and the χ^2 test (for categorical variables) were used to evaluate the significance of exercise-induced variations. Data with a nonnormal distribution were normalized using a logarithmic transformation prior to analysis. Statistical analyses were carried out with Analyse-it for Excel software (Analyse-it Software, Leeds, UK) and the level of statistical significance was set at $P < 0.05$. Data are presented as the median and interquartile range (IQR). The postmarathon results have also been adjusted for plasma volume change, according to the formula of Dill and Costill, as described elsewhere (31).

RESULTS

All the athletes completed successfully the ultramarathon, with no clinically meaningful symptoms. The main results of this study are shown in Table 1. The serum concentration of creatine significantly increased by 31% (IQR, 13–58%) after the run, whereas the EGFR decreased by the same extent. The serum concentrations of both copeptin and MR-proADM remarkably increased

TABLE 1. Variation of Serum Creatinine, EGFR, Copeptin, and MR-proADM After a 60 km Ultramarathon Run in 16 Male Athletes

	Prerun	Postrun	<i>P</i>
Creatinine (μmol/l)	68 (60–76)	92 (72–114)	<0.001
EGFR (ml/min/1.73 m ²)	111 (98–127)	80 (61–106)	<0.001
Copeptin (pmol/l)	5.9 (5.0–10.3)	40.6 (20.3–100.2)	<0.001
MR-proADM (nmol/l)	0.29 (0.27–0.34)	0.68 (0.51–0.80)	<0.001

after the 60 km run, by 6.4 times (IQR, 2.7–10.4) and 2.3 times (IQR, 1.8–2.6; Fig. 1), respectively. A highly significant correlation was observed between the delta increase (i.e., the ratio between the post- and prerun value) of serum creatinine and MR-proADM ($r = 0.680$; $P = 0.004$), but not between serum creatinine and copeptin ($r = -0.064$; $P = 0.81$; Fig. 2). Finally, the percentage of subjects exhibiting values above the upper limit of the reference range in male was 0% for both copeptin and MR-proADM before the ultramarathon, but increased to respectively 13 (81%) and 10 (63%) postexercise.

DISCUSSION

There is only one study that has assessed the variation of copeptin after short-term exercise, two after long-distance endurance exercise, whereas none has investigated the variation of MR-proADM after exercise to the best of our knowledge. Maeder et al. originally studied 161 patients undergoing cardiopulmonary exercise testing (CPET) with upright symptom-limited cycle ergometer tests (mean exercise time of 6.3 min and increments of 10–25 W/min) (32), and observed a significant increase (i.e., median increase of 59%) of copeptin concentration from rest to peak at exercise. Interestingly, the percent change in copeptin was independently associated with maximal work rate as well as with the previous use of several cardiac medications by virtue of their surrogate status for the presence of coronary artery disease or previous AMI. Hew-Butler et al. reported that copeptin levels significantly increased during and at the end of extreme distance runs despite decreases in plasma Na²⁺, thus suggesting the existence of a nonosmotic vasopressin stimulation during this strenuous exercise activity (33). Bürge et al. also investigated 50 male ultramarathoners undergoing a 100 km ultramarathon and found that serum creatinine increased by nearly 30% after the exercise (34), whereas plasma copeptin concentration remarkably increased by nearly 12 times, and hence to a greater extent as that observed in our study (i.e., 6.4 times) (35). Interestingly, the change in copeptin was not associated with the number of urinations, urine excretion, or fluid intake during the race. This is consistent with our findings, showing

Fig. 1. Variation of copeptin and MR-proADM after a 60 km ultramarathon run in 16 male athletes.

that the postexercise increase of serum copeptin was not related to that of serum creatinine, thereby suggesting that the variation of this marker is not related to renal function and/or fluid homeostasis, but might be attributable to other factors such as stressor events, which activate the hypothalamic–pituitary–adrenal axis (20,21). AVP is synthesized in the hypothalamus essentially in response to high osmolality and arterial hypovolaemia but is also substantially released in the context of the stress response, which might thereby include strenuous exercise as well (20).

The significant association between variation of serum creatinine and MR-proADM suggests that renal func-

Fig. 2. Linear regression analysis and Spearman's correlation coefficient (r) between the delta increase (i.e., the ratio between the post- and prerun value) of creatinine and copeptin, and creatinine and MR-proADM.

tion and hypovolaemia might have partially contributed to its variation. A systemic increase in ADM typically reflects an overflow from local sites of production and action, mostly caused by endothelial damage (e.g., in chronic heart failure and septic shock) rather than from physiological secretion by the adrenal gland (22). In certain conditions increased plasma ADM has, in fact, a hormonal function, causing a general decrease in vascular resistance and a fall in blood pressure (22), whereas the hormonal effects of vasopressin include vasoconstriction,

stimulation of release of adrenocorticotrophic hormone, and prolonged activation of vasopressin receptors, which mediate antidiuretic effects and myocardium hypertrophy (17). In the same group of athletes, we have previously measured troponin I with both a highly sensitive and a contemporary-sensitive troponin I immunoassays, observing that the values of these biomarkers significantly increased after the trial. Nevertheless, the diagnostic cut-off was exceeded in a minority of cases (i.e., 20%) and the overall increase was limited, thus compatible with transitory and reversible myocardial ischemia rather than attributable to myocardiocyte necrosis (7). As such, the variation of both copeptin and MR-proADM is likely due to the cardiac stress evoked by the strenuous aerobic exercise, rather than due to necrotic effects, including myocardial damage.

Regardless of the underlying causes supporting the increase of both copeptin and MR-proADM, the results of this study have clinical implications. In agreement with previous studies, the concentration of copeptin, as well as that of MR-proADM, is remarkably increased after long-distance running. Therefore, the hypothesis of an exercise-induced increase of both copeptin and MR-proADM should be considered before using these biomarkers for assessment of cardiovascular risk, myocardial injury, and cellular damage. Then, the evidence that a 60 km run causes a clinically meaningful increase of cardiac troponins (7), but also of copeptin and MR-proADM, raises several doubts as to whether exhaustive exercise might be considered globally beneficial or even safe, especially in unfit or/and untrained population. It is also worthwhile mentioning that the increase of both peptides can play a pathophysiological role, promoting unfavorable health effects.

All these factors support the evidence of a strong association of both biomarkers with adverse health outcomes throughout a wide spectrum of clinical conditions. Copeptin and MR-proADM should be considered as innovative biomarkers suitable for application in clinical and sports medicine, since it can be safely used to rule out cellular damage in patients who had undergone recent exhaustive exercise. Although further studies are needed to determine the exact role of these peptides, our results open the possibility to use these peripheral signals as possible biomarkers of exercise-induced damage and stress.

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