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A proteomic study of plasma protein changes under extreme physical stress

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ABSTRACT

The Spartathlon race (brisk walking a distance of 246 km in less than 36 h) was employed as a model of severe physical stress to investigate proteomic alterations in the plasma of athletes at the start (Athens) and finish (Sparta) of the race, as well as 48 h after the race (Post). The athletes' plasma was analyzed by 2D gel electrophoresis (2-DE) and the differentially expressed proteins were identified by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS). The ProteoSeek™ Albumin/IgG removal kit and the ProteoMiner™ enrichment kit were utilized to detect medium- and low-abundance proteins, whose expression may be masked due to high-abundance proteins. Our results were confirmed by Western blot and biochemical analyses. Overall fifty-two proteins were differentially expressed between the starting point, the finishing line and two days after the end of the race. Of these, thirty proteins were involved in inflammation, while the rest concerned anti-oxidation, anti-coagulation and iron and vitamin D transport. These results indicate that prolonged physical stress affects circulating stress-related proteins, which might be employed as biomarkers of stress-related diseases.

Biological significance
The current study employed the Spartathlon, as a model of prolonged endurance exercise, to identify and isolate putative biomarkers of inflammation under extreme physical stress conditions. These protein quantitative variations may pave the way to exploration and

Abbreviations: A1AG1, α-1-acid glycoprotein; A2M, α-2-macroglobulin; A1AT, α-1-antitrypsin; ApoA-I, Apolipoprotein A-I; APOH, β-2-glycoprotein; CERU, ceruloplasmin; CD5L, CD5 antigen-like precursor; CFAB, complement factor B; CFAH, complement factor H; COPD, chronic obstructive pulmonary disease; CO3, complement component 3; C1S, complement component 1S; CRP, C reactive protein; FIBA, fibrinogen alpha chain; FR, free radical; HPT, haptoglobin; HSP7C, heat shock cognate 71 kDa protein; HEMO, hemopexin; IGHA1, Ig alpha-1 chain C region; IGKC, Ig kappa chain C region; ITIH4, Inter-α-trypsin inhibitor H4; HMWK, kininogen-1; MIF-1, Macrophage Migration Inhibitory Factor; MBL, Manose Binding Lectin; PON1, paraoxinase 1; PAI-1, plasminogen activator inhibitor 1; ROS, reactive oxygen species; SAA, Serum Amyloid A; SAMP, Serum Amyloid P Component; THRB, prothrombin; TM, thrombomodulin; TTHY, Prealbumin/Transthyretin; VTDB, vitamin D binding protein; VTNC, vitronectin

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understanding of stress-related physiological processes, the stress response itself and diseases whose onset appears to be linked to stress.

1. Introduction

Any sporting event involving running or walking longer than the traditional Marathon length of 42 km is considered an ultramarathon. There are two types of ultramarathons: those that cover a specific distance (50 km, 100 km, 50mi and 100mi), and those that require the athlete to run as far as possible within a specified length of time (6, 12, and 24 h to 3, 6 and 10 days). Other races include double marathons, 24-hour races and multi-day races.

The "Spartathlon", one of the most demanding and grueling sporting events, is an annual ultramarathon foot race that takes place in Greece (Fig. 1). Athletes must run the 246 km distance from Athens to Sparta within 36 h, with ambient daily temperatures between 5 and 36 °C and mean daytime relative humidity between 60 and 85% [1,2]. Only a third of the runners complete the course. The physical strain associated with the "Spartathlon" renders it an ideal study model of long-term severe physical stress [3,4]. The runners endure dramatic systemic and inflammatory changes, as their immune system functions intensively to cope with heart and skeletal muscle and other organ damage secondary to excessive physical strain [5,6]. Depending on the nature, degree and duration of the physical activity, the different cell types of the body suffer varying levels of oxidative stress [1]. The latter is due to production of excessive levels of cytotoxic oxidants, including oxygen and nitric oxide free radicals and an imbalance between the production of reactive oxygen species (ROS) and the ability of the anti-oxidant/detoxifying system to prevent and repair the resultant damage [7]. Keap1–Nrf2 [Kelch-likeECH-associated protein 1–nuclear factor (erythroid-derived2)-like2] is the major regulator of cytoprotective responses to oxidative and electrophilic stress that ameliorates traumatic and oxidative stress by coordinating the antioxidant and anti-inflammatory response [8]. Under normal conditions, Nrf2 is present at very low levels in the cellular cytoplasm due to an interaction with Keap1, an ubiquitin ligase adaptor protein that targets Nrf2 for degradation. But under oxidative stress conditions, Keap 1 is less effective, allowing Nrf2 to enter the nucleus and initiate the transcription of cytoprotective, antioxidative genes and their proteins, which can balance high ROS levels [9].

Physical activity increases free radical (FR) production and taxes the available antioxidant mechanisms, especially during periods of intensive training. One of the best-known FR is ROS, which are chemically reactive molecules containing oxygen normally produced during aerobic metabolism [10].

Fig. 1 – "Spartathlon", a 246 km foot race.