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Effects of endurance exercise on the urinary proteome analyzed by 2D-PAGE and Orbitrap mass spectrometry

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Abstract

Exercise-induced proteinuria is a well-known phenomenon and influencing parameters such as intensity and duration were studied extensively[1, 2]. While usually total protein or albumin was measured for determination of proteinuria, the investigation of qualitative changes of the urinary proteome may give insight into biochemical processes connected to strenuous physical exercise. The aim of the present study[3] was to search for qualitative and quantitative differences in the urinary proteome of athletes before and after endurance exercise by means of 2D-PAGE and to identify the altered proteins by nano-UPLC Orbitrap mass spectrometry. It shall serve as a starting point to build up individual 2D-PAGE protein maps of athletes.

Introduction

The protein composition of urine is basically determined in the kidneys by the permselectivity of the glomerular filter and different mechanisms of tubular reabsorption. While normally less than 150 mg of protein is excreted with the urine of a healthy human per day, different pathological conditions such as *diabetes mellitus* as well as strenuous exercise can lead to an abnormally high protein excretion called proteinuria. The alteration of the urinary proteome can originate from glomerular or tubular changes as well as from increased plasma concentrations of single proteins. Since the urinary proteome can therefore undergo quantitative and qualitative changes, the investigation of urinary proteins may give insight into biochemical processes connected to strenuous physical exercise or involved in the pathogenesis of certain diseases.

Materials and Methods

Urine samples were taken from three different groups of volunteers who gave their written consent to participate in the study: Control group samples (group “control”, C), endurance sport samples collected immediately after exercise (group “marathon”, M) and endurance sport samples without foregoing exercise (group “athletes at rest”, R).

Of each sample, 15 mL of urine were concentrated in centrifugal filters (cut-off 10 kDa) and prepared for 2D-PAGE by reduction and derivatization of cysteine residues. Isoelectric focussing was performed on two different IPG strips (7 cm, pH 3-6 and pH 5-8). IPG strips were applied to 8 cm 12 % Bis-Tris gels for SDS-PAGE and gels were stained with Coomassie Blue prior to evaluation by Image Master 2D Platinum software (GE Healthcare). Proteins differing in the investigated groups were digested with trypsin and identified by nano-UPLC Orbitrap mass spectrometry. For evalutation of the MS data, Proteome Discoverer 1.0 (Thermo Fisher) was used.

Results and Discussion

The analysis of the different urinary protein patterns led to the identification of six proteins which were elevated after a marathon run in comparison to a control group (Fig. 1).

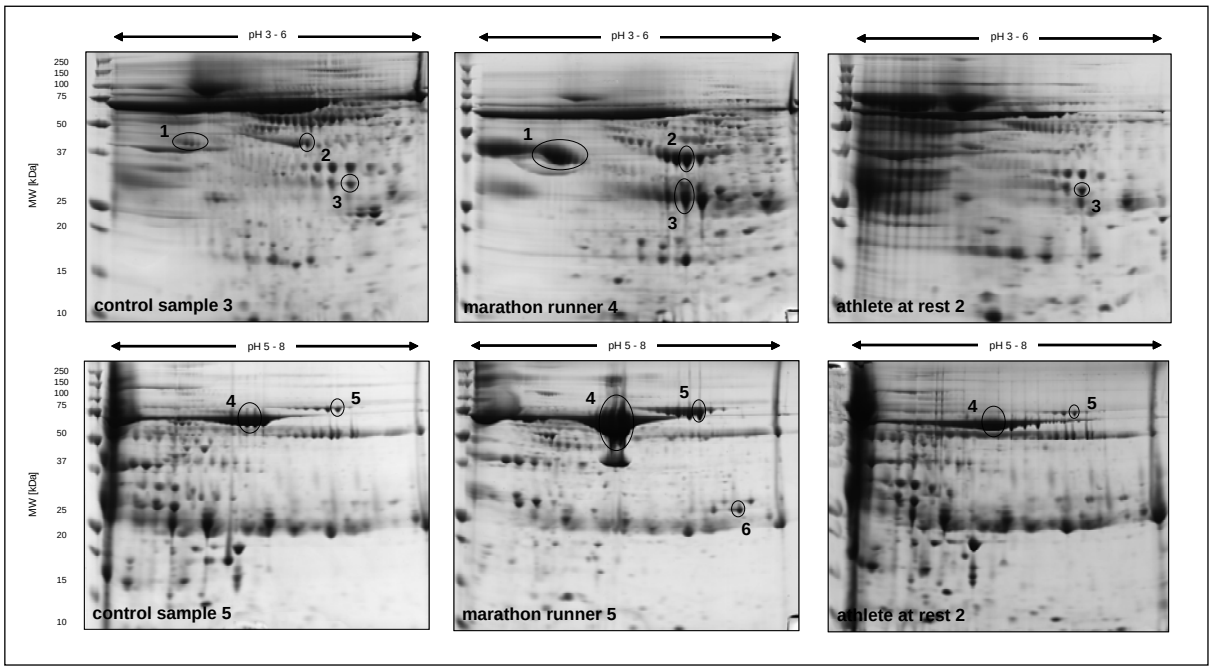

Fig.1. 2D-Gels of the different groups in two pH ranges. The encircled and numbered spots were applied to mass spectrometric identification; for identified proteins see Fig. 2.

While the plasma protein albumin (Spot 4) is a known marker for a glomerular dysfunction, alpha-1-microglobulin (Spot 3) indicates an impaired function of the proximal tubule. By contrast, orosomucoid 1 (Spot 1) is an acute phase protein whose expression is generally increased in response to various stress stimuli. Zinc-alpha-2-glycoprotein 1 (Spot 2) is a protein produced and secreted by adipocytes of white and brown fat tissue and supposed to have a stimulatory effect on lipolysis. Transferrin (Spot 5) is a protein of the blood plasma responsible for iron delivery, whose synthesis rate depends on the iron status of a person. Finally, carbonic anhydrase 1 (Spot 6) is an enzyme only expressed in erythrocytes where it mediates the rehydration of carbon dioxide to bicarbonate. For this reason, this protein is only found in urine if an increased lysis of erythrocytes takes place.

All the detected proteins seem to be linked to physiological changes resulting from endurance exercise such as an increased fat metabolism to fulfil the energy demand during a marathon run or the destruction of erythrocytes due to mechanical, oxidative or osmotic stress (Fig. 2).

Table 1. Identified proteins and possible causes for their increased urinary excretion

Elevated proteins after strenuous physical exercise			
Spot	**Identified protein**	**Possible renal alteration**	**Possible metabolic alteration**
1	Orosomucoid 1	Increased glomerular permeability	Tissue injury
2	Zinc-alpha-2-glycoprotein 1	Increased glomerular permeability	Increased lipolysis
3	Alpha-1-microglobulin	Tubular dysfunction	-
4	Albumin	Increased glomerular permeability	-
5	Transferrin	Increased glomerular permeability	Iron deficiency
6	Carbonic anhydrase 1	-	Hemolysis

Moreover, the quantitative measurement of the urinary protein to creatinine ratio as a parameter for the determination of proteinuria showed in nine out of ten marathon samples a markedly increased protein excretion up to 72.9 mg protein per mmol creatinine, which is defined as proteinuria (Fig. 3).

Compared to the samples of marathon runners, the 2D-PAGE profiles of athletes at rest did not differ from those of control samples. According to former studies[4], these findings show that the qualitative and quantitative changes of the urinary proteome in the course of exercise are only of temporary duration.

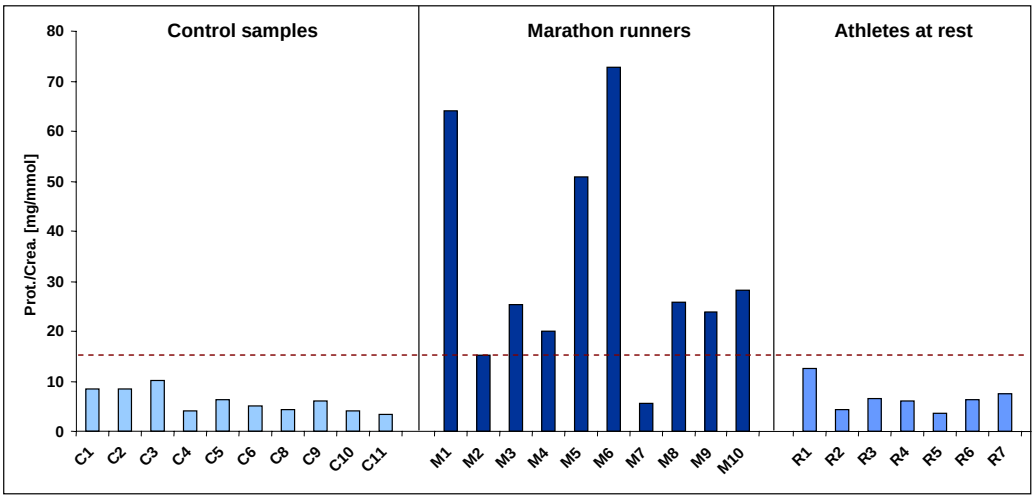

Fig. 2. Protein to creatinine ratios of the different samples. The dashed line indicates the limit for proteinuria (15 mg/mmol)

This study can serve as a starting point to build up individual 2D-PAGE protein maps of athletes, which may lead to a physiological monitoring system for athletes in training and competition and to a complementation of the blood passport in doping control.

Acknowledgements

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