

WHAT DOES POST-EXERCISE PROTEINURIA TELL US ABOUT KIDNEYS?

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Abstract. Objectives. Post exercise proteinuria (PEP) is found in about 20–40% of sportsmen after intensive exercise. Urinary NGAL is a new marker of tubulointerstitial kidney damage. The relationship between PEP and uNGAL has not been defined yet. In presented study a resting uNGAL as a predictor of PEP was analyzed. The changes of albuminuria after exercise were monitored to estimate a frequency and range of PEP. **Methods.** 40 amateur healthy runners (mean age 36.65 ± 10.61 years) participating in 10-km run took part in the study. Before and after the competition urine was collected. NGAL, albumin and creatinine were subsequently measured in urine. uNGAL to creatinine ratio (NCR) and albumin to creatinine ratio (ACR) were calculated. **Results.** 28 participants (mean age 37.9 ± 11.46, 19 M, 9 F) with uNGAL below 15 ng/ml before competition were analyzed. The increase of ACR was observed in every case. Mean post-exercise ACR was 104.55 ± 123.1 mg/g and was significantly higher than pre-exercise ACR 6.33 ± 5.86 mg/g ($p < 0.0005$). The positive correlation was found between resting NCR and post-exercise ACR ($r = 0.60$, $p < 0.05$). **Conclusions.** Resting uNGAL positively correlated with PEP. The possible explanation of these findings is that persons with PEP had some early, occult tubulointerstitial kidney damage. It is speculated that those runners have higher risk of chronic kidney disease.

Key words: chronic kidney disease, albuminuria, health, running

Introduction

The kidneys play a pivotal role in the fluid and acid-base homeostasis of the human body (Curthoys, Moe, 2014). Due to renin and erythropoietin production and secretion, they regulate arterial blood pressure and hemoglobin levels, respectively. It is hard to imagine proper physical activity without normal kidney function. Indeed, fatigue and muscular weakness are the commonest symptoms of renal failure. (Bello, Kawar, El Kossi, El Nahas, 2010). During intensive exercise the kidneys struggle against dehydration and acidosis. Of prime importance is the proximal tubule, which contributes to fluid homeostasis by the reabsorption of water and solutes. The proximal

tubule is also a metabolic organ and plays an important role in gluconeogenesis, a process in which lactate is consumed and glucose is produced (Curthoys, Moe, 2014).

The kidneys experience some significant changes caused by high-intensity physical activity. During exercise a decrease in renal blood flow of up to 30–40% is observed (Junglee et al., 2012).

It is not surprising that some abnormalities in laboratory tests are found after exercise. Some of these, like post-exercise proteinuria (PEP), are thought to be physiological (Poortmans, Blommaert, Baptista, De Broe, Nouwen, 1997).

In the minority of sportsmen who have undertaken strenuous exercise, the glomerular filtration rate (GFR) decreases and consequently the creatinine level in the blood is increased. When this rise exceeds 0.3mg/dl, acute kidney injury (AKI) is diagnosed (Junglee et al., 2013) (Poortmans, Gulbis, De Bruyn, Baudry, Carpentier, 2013). A rise in “new markers of AKI”, such as neutrophil gelatinase-associated lipocalin (NGAL) was also found after physical exercise (Lippi et al., 2012). According to the literature, PEP reflects only functional changes (Poortmans, 1985). An increase in NGAL is observed in tubulointerstitial damage. Therefore, it is not clear whether any correlation between PEP and NGAL exists. This was studied only once and no correlation between PEP and post-exercise NGAL was found (Junglee et al., 2012). Urinary NGAL is a predictor of AKI and the progression of chronic kidney disease (CKD). In this study, we measured resting urinary NGAL in healthy persons and analyzed whether this marker can serve as a predictor of PEP.

Methods

The study population consists of 40 amateur runners (22 males (M) and 18 females (F)), who ran 10 km road race. The runners were approached via local amateur sports club between February and March 2013. All participants were healthy, active adults (mean age 36.65 ± 10.61 years), without kidney disease, hypertension or diabetes. Runners taking medicines, especially non-steroidal anti-inflammatory drugs, were excluded.

Exercise protocol

The study involved the collection of urine samples before and after the 10km road race. The 10 km races organized in Gdynia are among the most popular and prestige races in the Pomerania region and the study was performed during these events because they are a good occasion for participants to obtain season's or personal best results.

Biochemical analyses

Urine was collected within 30 minutes of starting the run (a sample at rest) and within 30 minutes after the event (an exercise sample). Samples for the measurement of albumin and creatinine were analyzed immediately. Samples for the measurement of urinary NGAL were frozen immediately and stored at -20°C . The samples were frozen for six months before analysis.

Measurement of urinary albumin, creatinine and lipocalin-2/NGAL

Urinary albumin was measured by an immunoturbidimetric assay (ALBT2, Roche Diagnostics GmbH Mannheim for USA). Urinary creatinine was measured by a kinetic colorimetric assay (CREA, Roche Diagnostics GmbH Mannheim for USA). Urine concentrations of human lipocalin-2/NGAL were measured using

the ELISA method (Quantikine High Sensitivity Human by R&D Systems, Minneapolis, Minn., USA), according to the manufacturer's protocol. Read absorbance measurements were made using a plate reader Chromate 4,300 USA at a wavelength of $\lambda = 450$ nm. Minimum detectable concentrations were determined by the manufacturer as 0.012 ng/ml. The intra- and inter-assay coefficients for NAGL were 4.4% and 5.6%.

ACR and NCR calculations

The albumin-to-creatinine ratio (ACR, mg/g) and uNGAL-to-creatinine ratio (NCR, $\mu\text{g/g}$) were calculated. The definitions of albuminuria are shown in Table 1 (Mattix, Hsu, Shaykevich, Curhan, 2002).

Table 1. Definitions of albuminuria (Mattix et al., 2001)

	Spot urine		
	Albumin mg/l	sex	ACR mg/g
Normal albuminuria (formerly normoalbuminuria)	<20	M	<17
		F	<25
High albuminuria (formerly microalbuminuria)	20–200	M	17–250
		F	25–355
Very high albuminuria (formerly macroalbuminuria)	>200	M	>250
		F	>355

Abbreviations. ACR - albumin to creatinine ratio (mg/g) , M – male, F – female.

The normal values for NCR are not established.

Statistical analysis

We used Statistica 10 software (StatSoft, Poland) for the analysis. As the Shapiro-Wilk test showed that the distributions of ACR and NCR were significantly different from normal ($p < 0.05$), we used a non-parametric Mann-Whitney test and Spearman's rank correlation test for the statistical analysis. A p -value < 0.05 was considered statistically significant.

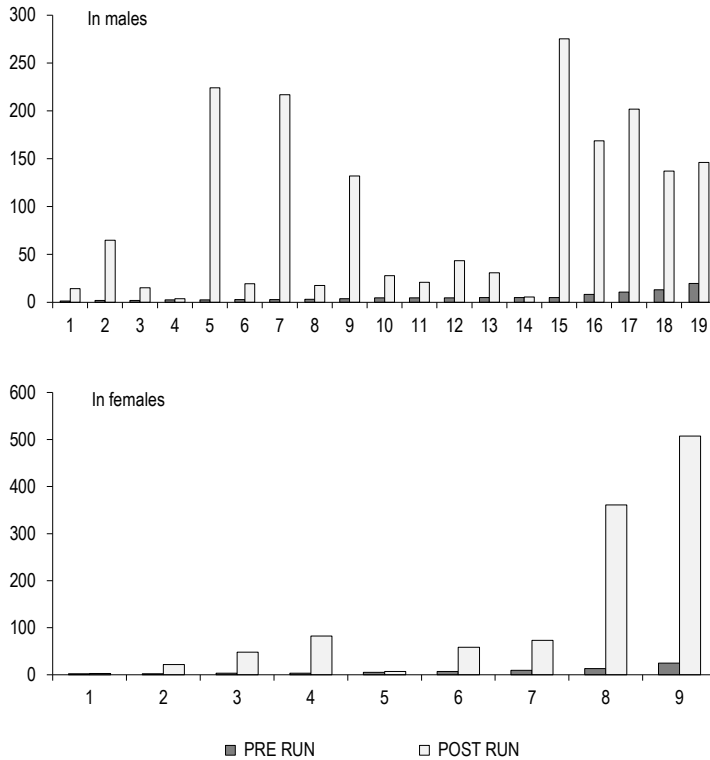
Ethics

The authors declare that the experiments reported in the manuscript were performed in accordance with the ethical standards of the Helsinki Declaration. All participants provided written consent and ethical approval was provided by the Medical University of Gdansk ethics committee (approval nr NKBBN/171/2013).

Results

The aim of the study was to examine if uNGAL in healthy persons is a predictor of PEP. uNGAL indicates tubular kidney damage and also neutrophil activation (Helmersson-Karlqvist et al., 2013). Therefore, only the runners with a resting uNGAL of below 15ng/ml were analyzed to exclude persons with an occult infection. 28 runners (19 males, 9 females), mean age mean age 37.9 ± 11.46 years were analyzed. Pre-exercise ACR was normal in everyone (mean 6.33 ± 5.86 , range 1.22–24.6 mg/g). An increase in ACR was observed in every case to mean $104.55 (\pm 123.1$

mg/g). This increase was statistically significant ($p < 0.0005$). The range of post-exercise ACR was very wide from 2.89 to 507.11 mg/g. The increase in ACR varied from 1.15 to 83.49 (mean 18.3 ± 20.91) fold (see Figure 1).



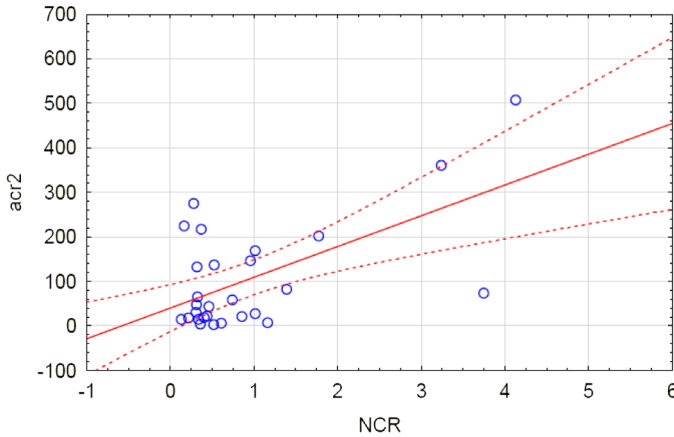
Abbreviations. ACR – albumin to creatinine ratio (mg/g).

Figure 1. The pre- and post-exercise ACR in males and females

After the competition, 21 runners had high or very high albuminuria (see Figure 1). The mean resting uNGAL was 6.03 ± 2.99 ng/ml (range 1.67–11.89), and resting NCR 0.93 ± 1.06 μ g/g (range 0.13–4.13).

Resting ACR correlated positively with resting NCR ($r = 0.68$, $p < 0.05$) and a post-exercise ACR ($r = 0.67$, $p < 0.05$). A positive correlation was found between resting NCR and post-exercise ACR ($r = 0.60$, $p < 0.05$) (Figure 2).

There was no correlation between post-exercise ACR and BMI. There was no correlation between post-exercise ACR and age. There were no significant differences between the sexes. The runners with very higher albuminuria obtained better results in the race, but without statistical significance.



95% confidence level.

Figure 2. The correlation between resting NCR and post-exercise ACR

Discussion

A post-exercise rise of albuminuria was expected in this study and was observed in every case (Figure 1). It gave us the confidence that the intensity and duration of exercise was chosen well and the method of estimating PEP was sensitive enough to find even slight changes in protein loss. After exercise, ACR increased 18.3 times. There was a great variability in the ACR increase between participants (Figure 1). This prompted a question about factors causing such huge differences. The main aim of this study was to analyze uNGAL at rest as a predictor of PEP. Post-exercise uNGAL was not analyzed because a significant increase in uNGAL after a 10 km run was not expected. In previous studies, a rise in uNGAL was observed only after strenuous exercise (Lippi et al., 2012).

In this study the resting urinary NGAL-to-creatinine ratio correlated positively with resting albuminuria (ACR). This was not surprising because both are risk factors for nephropathies and cardiovascular diseases (CVD) (Helmerson-Karlqvist et al., 2013; Abdallah et al., 2013; Hasegawa et al., 2015). Resting NCR also correlated positively with post-exercise albuminuria. In other words, a marker of tubulointerstitial damage (uNGAL) predicts a physiological condition – PEP. This prompted another question: Is it possible that PEP is a kind of “kidney stress test” and unmasks some very early kidney damage?

In healthy persons, the kidneys filter approximately 180 liters of plasma containing 7.2 kg of albumin every day and over 99.999% of albumin is retained by the combined action of selective filtration and tubular reuptake (Johnstone, Holzman, 2006). In the rest, only 5–10 mg of the 7,200,000 mg of albumin flowing in plasma through the kidneys is lost in urine. In healthy humans, no protein is observed in a routine dipstick examination of urine. Proteinuria is one of the most common findings after exercise. It was first reported in urine studies of Boston marathon runners in 1941 (McCullough et al., 2011), and the increase in albumin excretion after exercise was first described in 1978 (Viberti, Jarrett, McCartney, Keen, 1978). Exercise is known to be the most common factor affecting albuminuria, alongside fever (Miller et al., 2009). PEP is a common finding and occurs in 20–30% of sportspeople. The frequency

of PEP depends on the type of exercise and the method of estimation. In several classic papers by Poortmans (Poortmans et al., 1997), it was revealed that PEP is related to the absolute intensity of exercise. Poortmans speculated that PEP is caused mainly by enhanced glomerular membrane permeability, because albumin was the main protein lost during exercise (Poortmans et al., 1997). However, why this “physiological” proteinuria occurs and why it varies greatly between healthy persons was not fully established. Hemodynamic changes in kidney vessels was put forward as an explanation, because during exercise renal blood flow and hydrostatic pressure in glomeruli are increased.

In recent years our understanding of the pathology of proteinuria has changed. In a “classical” model of kidney function, virtually no protein was filtered through glomerular membrane, except for a scarce amount of low-molecular-weight proteins (LMWP). The increase in LMWP excretion was thought to be typical for tubule dysfunction and albuminuria for glomerular dysfunction. It is now known that a huge nephrotic-range amount of albumin is filtered in glomeruli and it is only because of the tubular function that this protein is not observed in urine. Russo et al. (2007) showed that renal albumin filtration is 50 times greater than previously measured. This means that the proximal tubule is essential to prevent nephrotic range proteinuria (Russo et al., 2007).

It is possible that PEP is caused by tubular dysfunction. We know that albumin is reabsorbed mainly in the proximal tubule, and this tubule has some functions which are crucial in exercise (such as gluconeogenesis, acid-base homeostasis, reabsorption of water and sodium) (Curthoys, Moe, 2014). We may speculate that during exercise tubules have other important functions and are not trying to reabsorb albumin. During short-term exercise, it is better for the organism to regulate the water and electrolyte balance than to reabsorb every albumin from the filtrate.

This “tubular theory” of PEP might also explain the huge difference in the range of PEP in runners (Figure 1). Such a difference could not be explained by an increase in hydrostatic pressure in the glomerular vessels because blood flow could not differ so greatly between healthy persons. On the other hand, it is possible that exercise somehow “switches off” tubular reabsorption of albumin and at one moment, albuminuria can rise from the minimal to nephrotic range.

There is increasing interest in so-called new markers of AKI (Vanmassenhove, Vanholder, Nagler, Van Biesen, 2013). They help in an early diagnosis of AKI, but also are predictors of CKD progression and CVD events (Helmerson-Karlqvist et al., 2013; Abdallah et al., 2013; Hasegawa et al., 2015). One of the most commonly used is NGAL (Hasegawa et al., 2015). NGAL is a member of the lipocalin protein family that is produced in epithelial cells and neutrophils (Wu et al., 2013). This early marker of tubulointerstitial injury is used in clinical practice as well as in experimental studies. It identifies renal damage rather than renal dysfunction (Singer et al., 2013). So far, there have only been very few studies concerning the usefulness of new markers of AKI in exercise. In a large study of 425 runners, AKI was diagnosed in approximately 40% of marathon runners on the basis of elevated creatinine serum level (a rise of Cr \geq 0.3mg/dl). In the same group, significant elevation of urine uNGAL was observed (McCullough et al., 2011). uNGAL and serum NGAL increased after a 60-minute downhill, muscle-damaging run (Junglee et al., 2012; Junglee et al., 2013). In another study, uNGAL increased 7.7-fold after a 60-kilometer ultramarathon (Lippi et al., 2012).

Unlike albuminuria, which seems to be a marker of intensive exercise, new markers of AKI are typical for long, exhausting exercise. However, uNGAL as a predictor of PEP has not yet been studied. Although increased albuminuria in rest is a predictor of renal and CVD events, the post-exercise increase in albuminuria is thought to be

a physiological phenomenon. One fascinating possibility is that this benign condition occurring during exercise can unmask some diseases at their early, treatable stages.

ACR significantly increases in diabetics after exercise (Vanmassenhove et al., 2013), so it was suggested that post-exercise albuminuria unmasks early stages of diabetic nephropathy (DN) (Vanmassenhove et al., 2013; Di Paolo et al., 2007; Kim et al., 2012). The importance of PEP in patients with newly diagnosed type 1 diabetes is not established (Zmyslowska et al., 2007), but yet values are significantly higher in uncontrolled diabetes compared to those with good metabolic control (Agarwal, Thanvi, Vachhani, Kochar, Rastogi, 1998).

Conclusions

In the healthy population studied, both resting ACR and uNGAL were within normal values. There was an increase in ACR in every case and a huge variability in post-exercise ACR was found. What difference between runners is responsible for such significant variability?

It is not due to the intensity of the exercise; the distance was the same and all runners were amateurs. Nor is it because of some known co-morbidities, as the study population was healthy.

The answer must be in the kidney itself. Our hypothesis is that at one moment during exercise, the proximal tubule stops reabsorbing albumin. Such a condition probably lasts a very short time. It is well known that PEP disappears a few hours after exercise (Poortmans, 1984).

The possible explanation why runners differ so greatly is that some had mild tubulointerstitial damage and a lower capacity to reabsorb albumin. The positive correlation between uNGAL and ACR corresponds with this theory. Another explanation is that in some runners the proximal tubule stops reabsorbing albumin earlier, because it has to reabsorb water and electrolytes more intensively in runners who are dehydrated or exhausted. Those runners with albuminuria should probably drink more and consume more glucose before exercise.

In medicine, we are looking for screening tests that help us in early diagnosis. In sport, on the other hand, we are interested in simple tests which help us in more efficient training.

PEP seems to be a simple test which uncovers early tubulointerstitial kidney damage.

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