Pseudonephritis is Associated with High Urinary Osmolality and High Specific Gravity in Adolescent Soccer Players

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The study aimed to evaluate the effect of exercise on urine sediment in adolescent soccer players. In 25 15-year-old (range 14.4–15.8 yrs) athletes, urinary protein, osmolality and cytology were analyzed by flow cytometry and automated dipstick analysis before (T₀), during (T₁), and after a match (T₂). All athletes had normal urine analysis and blood pressure at rest, tested before the start of the soccer season. Fifty-eight samples were collected (T₁₀: 20, T₁: 17, T₂: 21). Proteinuria was present in 20 of 38 samples collected after exercise. Proteinuria was associated with increased urinary osmolality (p < .001) and specific gravity (p < .001). Hyaline and granular casts were present in respectively 8 of 38 and 8 of 38 of the urinary samples after exercise. The presence of casts was associated with urine protein concentration, osmolality, and specific gravity. This was also the case for hematuria (25 of 38) and leucocyturia (9 of 38). Squamous epithelial cells were excreted in equal amounts to white and red blood cells. A notable proportion of adolescent athletes developed sediment abnormalities, which were associated with urinary osmolality and specific gravity.

A century ago, athlete’s proteinuria, hematuria, and the presence of casts were described in adult marathon runners (1,27). Fifty years later, the abnormalities, which are induced by exercise, were named athlete’s pseudonephritis (10). Essential

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for this diagnosis is the spontaneous reversibility of the abnormalities. This type of proteinuria has a half-time value of approximately 1 hr (10), and the hematuria disappears within 24 hr (12). In adults, hematuria and proteinuria after exercise have been noted in many reports (1,3,10,27), as were the presence hyaline and granular casts (12,13,14). Sport’s hematuria has been attributed to a glomerular source (8) as well as to exercise-induced bladder trauma (4,25). Intensive exercise induces redistribution of body fluids and renal challenges, especially when profuse sweating and insufficient rehydration disturbs the fluid balance. Urine concentration during sporting is influenced by fluid intake before and during the event and by fluid loss, mainly as sweat and urine. Until now, these phenomena received little attention in pediatric literature, and the real prevalence of athlete’s pseudonephritis in children has not been studied (20). This report describes the abnormalities occurring in male adolescents during a soccer match and examines the relationship between exercise, urinary specific gravity, and osmolality and urine composition.

Subjects and Methods

Two elite teams of the same age category belonging to the same soccer club, team A (n = 13) and team B (n = 12), were examined unexpectedly during a soccer game—after the warm-up period immediately before the match (T₀), at half time (T₁), and immediately after the match (T₂). Fluid intake from T₀ on was measured with individualized drinking bottles. Athletes were weighed before and after the match in their underwear. Urine was collected at T₀, T₁, and T₂ and was kept at 4°C until analysis the same afternoon. Players unable to void at least 7 ml, the minimum amount of urine necessary to perform a full analysis, on 2 of 3 sampling moments were excluded from the study. Sweat loss was estimated based on the change in body weight, corrected for drinking volume and urinary output.

All subjects underwent a yearly general medical examination. They were in excellent health and physical condition with a normal blood pressure and a normal urine analysis at rest before the start of the soccer season. They were annually instructed on healthy nutrition and advised to drink mineral water and/or sport drinks during the match and the breaks.

A match lasts 2 × 40 min, interrupted by a 10-min break. Group A played at 2 p.m. in cold weather (6°C) with icy rainfall and air humidity of 85%. Group B, played at 2 p.m. in warmer, sunny weather (17°C) and air humidity of 80%.

The specific gravity was determined on the Urисys 2400 analyzer (Roche). A specific gravity below 1,020 was considered as euhydration (24). The urine osmolality was measured with the Osmometer 3900 (Advanced Instruments). Urine sediments were analyzed by flow cytometry with a Sysmex UF-100, as described by Langlois et al. (15). They were analyzed for red blood cells (RBC), leucocyturia (WBC), squamous epithelial cells, hyaline casts, and granular casts. Microhematuria was defined as >3 RBC/μl (12), significant hematuria was defined as >50 RBC/μl (20), and leukocyturia as >25 WBC/μl. Samples were considered positive with the presence of >30/μl for squamous epithelial cells, >3/μl for hyaline casts, or >1/μl for granular casts. Proteinuria was analyzed by an automated biuret reaction on a Cobas Integra 800 Autoanalyser (Roche). Proteinuria was considered to be present when more than 0.12 mg/L protein was measured. Dipstick urine analysis was carried out before the flow cytometrical analysis using Combur 10 Test M strips and
an automated reflectance photometer (Urisys 2400). The strips had reagent pads for semiquantitative assessment of relative density, pH, leukocyte esterase, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, and hemoglobin/myoglobin.

Statistical analyses were performed with IBM-SPSS 20. Results are shown as medians and minimum and maximum between brackets. As results had no normal distribution, nonparametric tests corrected for ties were used. The Wilcoxon sum rank test was used to analyze paired samples, the Mann-Whitney U test to evaluate differences between groups, and the Spearman rho to evaluate correlations. Distributions were analyzed with an $X^2$ or a Fisher’s exact test according to the number of athletes in each group.

The study was approved by the West-Flanders ethics committee. Athletes as well as their parents signed an informed consent at the start of the soccer season.

**Results**

**Athletes and Collected Samples**

Two teams of the same birth year (25 male athletes), belonging to the same soccer club, agreed to participate in the study after informed consent. The players had a median age of 15 years (range 14.4–15.8 years). None of the athletes had a medical history of renal disease. Two subjects were unable to void on two of the three sampling moments and two produced on one sampling moment only 5 ml of urine and were not able to void on another moment. Twenty-one athletes were included in the study. None of the athletes drank during the game, only during breaks. At the end of the match (from T0 on), median fluid intake was 252 ml (0–841 ml). The median weight loss was 0.7% (0.09–2.17%), and the calculated sweat loss was 596 (91–1327) ml.

Team B, playing during sunny weather, lost significantly more weight (1.2% [0.2–2.2%], $p = .019$), drank significantly more (332 ml [0–841 ml], $p = .02$), and had more sweat loss (939 ml [596–1,327 ml], $p = .001$) compared with team A (weight loss: 0.6% [0.09–1.0%]; drinking volume: 163 ml [0–267 ml]; sweat loss: 277 ml [91–790 ml]). There were, however, no significant differences in the urine analyses of the two teams. The weight loss was not associated to any of the urinary parameters.

The four dropouts were equally distributed over Team A (2) and Team B (2). They were not significantly different from the included athletes. Their median weight loss was 0.6% (0.3–0.8%), median drinking volume was 160 ml (50–350 ml), and their sweat loss was 387 ml (120–430 ml). They voided at different sampling moments (1: T0; 1: T1; 2:T2). The urinary osmolality (531 mOsm/kg [467–932 mOsm/kg]) and specific gravity (1026 [1,021–1,031]) of the single urinary sample collected from these athletes were all above the euhydration cutoff.

**Bacteria and Nitrites**

All samples were negative for nitrites and bacteria.

**Urine Composition After the Warm-up Period (T0)**

**Osmolality/Specific Gravity.** Ten minutes before the match (T0), 20 players were able to produce urine (one missing sample). The median osmolality was 266 mOsm/kg (110–1,233 mOsm/kg), and the specific gravity was 1,006 (1,003–1,033). Seven athletes (35%) started with a specific gravity above 1,020.
**Proteinuria.** After the warm-up at the start of the game (T0), 20% (4 of 20) of the boys had a urinary protein content that was slightly above the cutoff of 0.12 g/L (ranging from 0.16 to 0.4 g/L). However, previous tests at full rest had been negative.

**Haematuria.** The median amount of RBC at T0 was 1 (0–27) /μl, with four samples exceeding 3 RBC/μl.

**Leukocyturia.** The median amount of WBC at T0 was 1 (0–27) /μl, with only one sample exceeding 25 WBC/μl. At T0, no granular casts, abnormal amounts of hyaline casts (0 [0–1] /μl), or squamous epithelial cells (1 (0–6) /μl) were seen.

**Combined Abnormalities.** At T0, 20% (4 of 20) samples had one or more abnormalities, of which 1 sample was positive for protein as well as RBC and WBC.

**Urine Composition During (T1) and After the Match (T2)**

**Collected Samples.** At T1, 17 athletes were able to void a sufficient volume of urine (four missing samples) and 21 at T2.

**Osmolality/Specific Gravity.** The median osmolality at T1 was 293 (95–991) mOsm/kg and at T2, 473 (114–932) mOsm/kg. The median specific gravity was 1,005 (1,003–1,031) at T1 and 1,018 (1,003–1,031) at T2. There was no significant change over time in the osmolality or in the specific gravity. The number of athletes with a specific gravity above 1,020 was 7 of 17 (41%) at T1 and 9 of 21 (43%) at T2. Different urinary sediment abnormalities according to specific gravity above or below 1,020 are described in Table 1.

**Table 1 Pooled Sediment Changes in the Urinary Samples (N = 58) with Specific Gravity at or Above (n = 23) and Below 1,020 (n = 35)**

<table>
<thead>
<tr>
<th>Pooled urinary samples</th>
<th>Euhydrated specific gravity &lt;1,020, n = 35</th>
<th>Dehydrated specific gravity ≥1020, n = 23</th>
<th>C² test for the evaluated variable according to hydration status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria (&gt;1.12 mg/L)</td>
<td>no 27 (77%) 6 (35%)</td>
<td>yes 8 (23%) 15 (65%)</td>
<td>(p &lt; .0001)</td>
</tr>
<tr>
<td></td>
<td>yes 8 (23%) 15 (65%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;3 RBC/μl</td>
<td>no 25 (71%) 5 (18%)</td>
<td>yes 10 (29%) 19 (82%)</td>
<td>(p &lt; .0001)</td>
</tr>
<tr>
<td></td>
<td>yes 10 (29%) 19 (82%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;25 WBC/μl</td>
<td>no 33 (94%) 15 (65%)</td>
<td>yes 2 (6%) 8 (35%)</td>
<td>(p = .012)</td>
</tr>
<tr>
<td></td>
<td>yes 2 (6%) 8 (35%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;30 SEC/μl</td>
<td>no 34 (97%) 16 (70%)</td>
<td>yes 1 (3%) 7 (10%)</td>
<td>(p = .019)</td>
</tr>
<tr>
<td></td>
<td>yes 1 (3%) 7 (10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;3 HC/μl</td>
<td>no 34 (97%) 16 (70%)</td>
<td>yes 1 (3%) 7 (30%)</td>
<td>(p = .002)</td>
</tr>
<tr>
<td></td>
<td>yes 1 (3%) 7 (30%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1 GC/μl</td>
<td>no 34 (97%) 16 (70%)</td>
<td>yes 1 (3%) 7 (30%)</td>
<td>(p = .002)</td>
</tr>
<tr>
<td></td>
<td>yes 1 (3%) 7 (30%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. Significance of the differences between euhydrated and dehydrated samples is calculated by chi square or Fisher’s exact test is given as two sided p-value. Abbreviations: RBC = red blood cells; WBC = white blood cells; SEC = squamous epithelial cells; HC = hyaline casts; GC = granular casts.
Proteinuria. Protein losses after exercise were 0.11 (0–1.73) g/L at T1 and 0.21 (0–1.83) g/L at T2. The proteinuria increased significantly over time (p = .011; Figure 1). Proteinuria defined as protein concentration > 0.12 g/L, was present in 20 out of 38 (52%) collected samples after exercise (T1: 53% [9 of 17], T2: 52% [11 of 21]).

Proteinuria was associated to urine osmolality (T1: p < .0001, rs = 0.805; T2: p = .001, rs = 0.661) as well as to specific gravity (T1: p = .002, rs = 0.681; T2: p = .001, rs = 0.659).

Sediment Abnormalities Over Time. The number of RBC (p = .003), WBC (p = .003), squamous epithelial cells (p = .001) and hyaline casts (p = .034) urine increased significantly over time. Seven athletes develop granular casts.

Haematuria. The median RBC loss was 5 (0–200) RBC/μl at T1 and 5 (1–392) RBC/μl at T2. There was a significant association with urinary osmolality (T1 p = .022; T2 p < .0001) as well as with specific gravity (T1: p = .021; T2: p < .0001). Haematuria (after exercise), defined as >3 RBC/μl, was present in 66% (25 of

Figure 1 — Box plots of proteinuria (g/L) at the different evaluation points (start [T0], half-time [T1], and at the end of the soccer game [T2]) divided according to hydration status. Euhydration defined by specific gravity below 1,020, and hypohydration defined by specific gravity above 1,020.
38) of the samples (T1 65% [11 of 17]; T2 66% [14 of 21]). Half (5 of 10) of these had RBC counts of >50 RBC/μl.

**Leukocyturia.** The median amount of WBC in the urine at T1 was 6 (0–85) WBC/μl and 7 (0–220) WBC/μl at T2. leukocyturia, defined as >25 WBC/μl urine was present in 24% (9/38; T1 17% [3 of 17]; T2 29% [6 of 21]). Dipstick WBC-esterase, present in neutrophils, was negative in 88% (8/9) of WBC positive samples, suggesting the presence of other WBC types. The number of WBC in urine was also associated to urine osmolality (T1: \(p = .004\); T2: \(p = .001\)) and to specific gravity (T1: \(p = .007\); T2: \(p = .002\)).

**Squamous Epithelial Cells.** The median amount squamous epithelial cells was 3 (0–92) /μl at T1 and 4 (0–119) /μl at T2. Presence of high amounts of (>30/ μl) squamous epithelial cells was present in 21% (8/38) of the samples after exercise. The squamous epithelial cell count increased significantly with urinary osmolality (T1: \(p = .002\); T2: \(p = .011\)), with specific gravity (T1: \(p = .002\); T2: \(p = .006\)) and was also associated to the voided volume (T1: \(p = .0001\); T2: \(p = .002\)).

**Hyaline Casts.** The number of hyaline casts/μl was 0 (0–30) /μl at T1 and 0 (0–40) /μl at T2. They were present in a higher concentration than the standard reference (3/μl) in 21% (8/38) of the urine samples during the match. There was a significant association of the amount of hyaline casts and proteinuria (T1: \(p < 0.005\); T2: \(p < .001\)) urinary osmolality (T1: \(p = .001\); T2: \(p = .002\)) and specific gravity (T1: \(p = .002\); T2: \(p = .01\)).

**Granular Casts.** The amount of granular casts detected in the urine samples after exercise was 0 (0–5)/μl at T1 and 0 (0–8)/μl at T2. The presence of granular casts was associated to the urinary osmolality (T1: \(p = .014\); T2: \(p = .02\)) as well as to specific gravity (T1: \(p = .003\); T2: \(p = .017\)).

**Combined Abnormalities.** At T1 65% (11 of 17) of urine samples had at least 1 abnormality. Eight (8/11, 73%) samples displayed multiple abnormalities. At T2, this was the case for 76% (16 of 21) of which 12 of 16 (75%) displayed multiple abnormalities.

**Discussion**

This study describes the urinary abnormalities in trained adolescents induced by the efforts of a soccer game. The two teams were very comparable due to the narrow age group and identical training but played under different weather conditions. The observed weight loss (0.7% [0.09–2.17%]) was within the reported weight loss in adult soccer players (26). Adolescents playing in warmer weather drink more, sweat more, and lose more weight than those playing in cooler weather conditions, as is expected (6). Adolescents do not compensate sufficiently for the increased losses when playing in warm weather. The data of the urinary analysis were pooled, as there were no significant differences between the two groups, and the urinary abnormalities were not associated to the weight loss.

At the start of the game, at least 30% of the athletes started with a suboptimal hydration as their specific gravity exceeded 1,020 (24). Although specific gravity might not be the ideal way to evaluate hydration status, it is the only feasible
method on the soccer field. The compliance to previous instructions on prehydration and drinking during the game is very heterogeneous and frequently insufficient in these adolescents. Over time, we did not observe a significant change in specific gravity or urinary osmolality, which could be the result of the delayed change of these urinary markers in case of acute dehydration (24).

As in adults, half of our population develops proteinuria, and two thirds also have hematuria, granular and hyaline casts (12,13). Apparently, adolescent athletes with normal urine analysis and blood pressure at rest have the same risk for the development of athlete’s pseudonephritis as adult athletes (20).

The proteinuria of exercise, well characterized by Poortmans and Jeanloz (23), was shown to be of a mixed type. It seems to be induced by ischemic insults to the filtration barrier in the glomeruli of the kidney cortex in combination with insufficiency of tubular resorption of leaked protein (7,22). A 75% drop of renal flow during exercise is a result of renal prostaglandin secretion (7). This drop in renal flow is also influenced by a combination of sympathetic nervous activity, release of catecholamine substances, plasma angiotensin II, and vasopressin concentrations (10,18). Further on, exercise was shown to increase the kidney endothelin-1 production, which is a potent vasoconstrictor, and to decrease the nitric oxide production, a vasodilator (17). The observed proteinuria (up to a maximum of 2.54 mg/min/1.73 m²) exceeded the amounts observed during a Marathon (0.083–0.313 mg/min) in adults (3,19).

Why the urinary abnormalities of exercise occur only in about 50% of the investigated players remains an open question. Differences of individual physical condition could play a role, though this was not obvious from training records. Regular endurance training diminishes the splanchnic and renal blood flow reduction (18). Improved physical condition contributes to a decreased norepinephrine response of renal vasculature and results in an increased kidney blood flow during exercise. Besides the protecting effects on the kidney, the increased splanchnic blood flow of well-trained athletes might also confer benefits for the carbohydrate metabolism during exercise (18). Further, as proteinuria was associated to urinary osmolality and specific gravity, hydration status could also influence the occurrence of pseudonephritis (11).

The prevalence of hematuria in literature was shown to be variable according to the type of sport: 20% in marathon runners, 55% in football players, and 80% in swimmers (17). Rapid resolution is an important feature of sports induced hematuria, whether gross or microscopic (4,9,10,14,19). Different origins have been identified for this hematuria. Combined etiologies are probably involved such as: renal ischemia, hypoxic damage to the kidney, release of hemolyzing factors, bladder and/or kidney trauma, foot-strike hemolysis, nonsteroidal anti-inflammatory drugs, dehydration, increased circulation rate, myoglobin release, and peroxidation of red blood cells (12). Reid et al. (25) described predominantly nonglomerular bleeding in marathon runners in case of short distance running whereas glomerular bleeding was associated to greater distances. As about 30% of the RBC positive samples were also positive for squamous epithelial cells, this could indicate a traumatic origin of the RBC due to bladder contusions as demonstrated by Blacklock (4). The significant association of both RBC and squamous epithelial cells with urinary volume and volume drank during the match sustains this hypothesis. In this study, no RBC morphology tests were performed, therefore the origin of the RBC is not investigated.
On the other hand, the combination of different anomalies in more than half of the samples with hematuria (WBC, proteinuria, hyaline casts and glomerular casts), plead indirectly for at least in part a glomerular origin of the RBC in these samples. Haematuria has been associated to severity and duration of the physical stress (10). As the actual time played was not recorded, this could not be analyzed in this study.

Although self-limiting, the repeated RBC loss through the urine can be a contributing factor to the anemia of the athlete (12). Search for exercise induced hematuria including RBC morphology should be the first step to take when evaluating athlete’s anemia (8, 12,16). Gross hematuria can be provoked to confirm the cause-effect relationship between exercise and hematuria (21).

The presence of, in majority esterase negative WBC in urine points also to an inflammatory reaction not driven by neutrophils in the kidney. As a 1:1 RBC-WBC ratio is observed, simple leaking at glomerular level cannot be the explanation of this observation.

The results plead for the maintenance of a normal diuresis, low specific gravity, and a low urinary osmolality during exercise. All observed urinary abnormalities were associated to urinary osmolality and specific gravity (11,20). Although children are stimulated to participate in physical activities, insufficient attention is given to hydration before and during the exercise. Dehydration has, however, proved to reduce performance and accuracy (2,5) in children and adult athletes, and coaches should be sensitive for this aspect.

There are limitations to this study. Although the narrow age range, one sport type, and a single gender of the studied group makes comparison between athletes easy, generalization to other sports, age categories, and gender is not possible. Moreover, it would have been better to evaluate the same athlete group under the different weather conditions to evaluate the impact of climate changes. Further on, the future studies should start before the warm-up period, include data on the time played, and also collect urinary samples after 24–48 hr to be sure about the diagnosis of exercise-related abnormalities. Estimation of the weight loss remains tricky in growing children. A basal weight should therefore be measured in the week before the exercise. More studies are needed investigating hydration and sediment abnormalities induced by exercise in trained as well as untrained children. An intervention study optimizing hydration could enlighten whether these abnormalities are preventable. In general, a better follow-up of all sporting children is necessary.

References


