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# **Title:** Blood and urinary abnormalities induced during and after 24-hour continuous running: a case report

**Short title:** Long duration exercise and hemolysis

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**Abstract**

In this reported clinical case, a healthy and well-trained male subject (aged 37 years, maximal oxygen uptake ($VO_2max$) 64 ml·kg$^{-1}$·min$^{-1}$) ran for 23 hours and 35 minutes covering 160 km (6.7 km·h$^{-1}$ average running speed). The analysis of hematological and biochemical parameters 3 days before the event, just after termination of exercise, after 24 and 48 hours of recovery revealed important changes on muscle and liver function and hemolysis. Moreover, the analysis of urine sediments showed an increment of red and white blood cells filtrations, compatible with transient nephritis. After 48 hours most of these alterations were recovered. Physicians and health professionals who monitor such athletic events should be aware that these athletes could exhibit transient symptoms compatible with severe pathologies and diseases, although the genesis of these blood and urinary abnormalities are attributable to transient physiological adaptations rather to pathological status.

**Keywords:** ultra-endurance exercise; liver damage, exercise-induced hemolysis, kidney damage.

## Introduction

The number of people participating in long-duration endurance sports events, such as marathons or ultramarathons, has been considerably increased over the last decade, and accordingly, an increased number of research studies have been carried out in these circumstances[1]. In the present case report, we show the effects of systemic effects provoked by 24-hour continuous running in a healthy trained subject.

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## Case report

A healthy well-trained (maximal oxygen uptake ($VO_2max$) 64 $ml \cdot kg^{-1} \cdot min^{-1}$, determined by a maximal incremental running test performed three days before) male subject (aged 37 years) performed a 24-hour running challenge to collect funds for research in a rare disease. The subject ran indoors, from 5 p.m. to 5 p.m. of the next day in a motorized treadmill. The running speed was initially set to 10.2 $km \cdot h^{-1}$ (54% of his $VO_2max$). During the challenge, the subject was allowed to stop running 5 times for a period of 5 minutes. The total distance covered was 160 km, while the running total time was 23hours and 35 minutes, yielding an average velocity of 6.7 $km \cdot h^{-1}$. The subject was allowed to drink liquids *ad libitum* (6415 mL drunk). Body mass was recorded before and just after cessation of exercise (total weight loss 2.3 kg, 3.7 % body mass).

**Con formato:** Sin Superíndice / Subíndice

Blood samples were drawn 3 days before the challenge, upon termination of exercise, and 24 and 48 hours post-exercise for blood panel analysis with an automated hematological analyzer and for biochemical determinations. Urine samples were taken 10 minutes before, during running and 48 hours after cessation of exercise. Urine density and pH were determined and sediments were examined for presence of erythrocytes and leukocytes by light phase microscopy.

The 24-hours running exercise induced classical effects on the hemoglobin and hematocrit levels after exercise and the subsequent days (**Table 1**) that reflected plasma volume shift (i.e., immediately post-exercise -5.4%, 24hours post-exercise -18.4% and 48hours post-exercise 8.2%) and water loss exercise. Biomarkers of muscle and liver damage were deeply affected by the 24-hours running exercise (**Table 1**). Serum creatine kinase (CK) activity increased by a ~50fold, compared to pre-exercise values. Circulating urea values were also increased (2.3fold). Aspartate transaminase (AST) and alanine transaminase (ALT) increased by a ~10 and a ~4fold, respectively. All the aforementioned changes lasted for 48 hours (**Table 1**).Total bilirubin was increased importantly (~2fold) after exercise, along with increased serum iron and, accordingly, increased transferrin saturation. On subsequent days, iron and transferrin saturation returned to baseline levels while circulating ferritin increased (**Table 1**).No relevant effects were found on serum electrolytes or metabolic markers, probably reflecting a sufficient fluid balance and nutrient supply during the challenge. The analysis of urine sediments showed an important increase of filtrated erythrocytes (up to 70-80 RBC per field after 15h 40’ running) and leukocytes (up to 15-20 WBC per field after 18h 25’ running) (**Figure 1**).

## Discussion

The data displayed in this case report are of great interest for physicians and health professionals to know the possible physiopathological implications induced by prolonged exercise as well as the effects on blood and urinary analytical determinations.

Serum CK activity, a widely used marker of skeletal muscle damage[2], was highly increased, thereby reflecting presence of ruptured skeletal muscle cells, although, the exercise-induced hemolysis could account for some of the CK increment.

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The 24-hours running exercise caused an overload of hepatic function that probably resulted on liver damage (**Table 1**). More concern arouses that, after 48 hours of exercise cessation, the damage on liver tissue is still present as the elevated transaminases levels at this time-point show (**Table 1**).

Another worrying finding is the important hemolysis induced by this kind of challenge (**Table 1**), probably due to mechanical damage on red blood cells by foot strike during running[3] along with alterations on erythrocyte cytoskeleton proteins[4]. Yet the subject's iron metabolism seemed to be able to deal with the abrupt exercise-induced increase in iron serum levels, as shown by the increased levels of ferritin after 24 and 48 hours. However, bilirubin was still increased after 48-hours, which could imply sustained mild hemolysis throughout the recovery period and concomitant liver damage induced by the unconjugated bilirubin form.

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Previous studies have shown the effects of ultra-long running exercises on muscle and liver damage and hemolysis. After a 200-km running race(23-34 hours), AST increased by a ~15fold and ALT by a~4fold[5], which is in agreement with the elevations found here (**Table 1**). Robach *et al*[6] showed the hemolytic effect of a mountain ultra-marathon (37.6±5.9 hours), with total bilirubin concentration increasing by a ~3fold after exercise. Severe rhabdomyolysis (~245fold increases in serum CK activity), along with high increases in AST (~50fold) and ALT (~13fold), ,were found after a 246 km race (33.3±0.5 hours)[7].

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Last but not at least, it was described the "*athletic nephritis*" as the transient nephritis resulting from strenuous exercise which disappear within hours to days after the end of exercise[8]. It increases by increasing intensity and duration of exercise. Previous studies have suggested acute kidney injury induced by prolonged exercise[9,10], however this is the first case report showing the urinary changes induced by such prolonged effort through analysis of urine sediment (**Figure 1**).

As this case clearly reflects, ultra-long running exercise can provoke a transient systemic imbalance that results on severe muscle and liver biomarkers changes along with hemolysis and transient nephritis. Physicians and health professionals who monitor such athletic events should be aware that these athletes could exhibit transient symptoms compatible with severe pathologies and diseases, although the genesis of these blood and urinary abnormalities are attributable to transient physiological adaptations rather to pathological status.

## Conflict of interests

None of the authors have any conflict of interest.

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## Table and Figure Captions

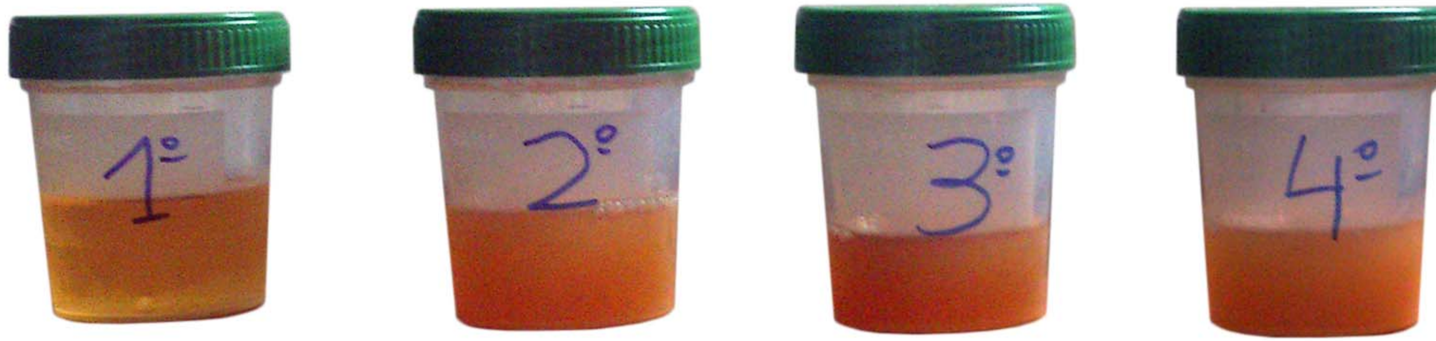
**Table 1.** Blood count, muscle and liver damage, iron metabolism, electrolytes and metabolic biomarkers before and after a 24-hours continuous running exercise.

**Figure 1.** Urine sediment analysis data from samples taken before, during running and after 48 hours recovery. RBC/HPF: red blood cells per high power field. WBC/HPF white blood cells per high power field.

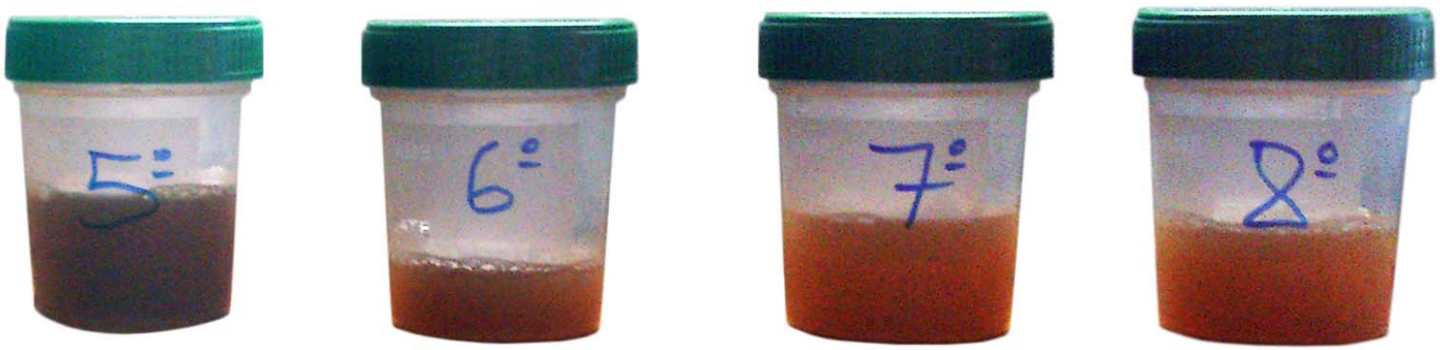
**Table 1.** Blood count, biochemical muscle and liver markers, iron metabolism, electrolytes and metabolic biomarkers before and after continuous running exercise.

| | Pre Exercise | Post Exercise | | |
|---|---|---|---|---|
| | | 0 hours | 24 hours | 48 hours |
| Hemoglobin (g/dL) | 14.6 | 15.2 (104%) | 16.8 (115%) | 14.0 (96%) |
| Hematocrit (%) | 40.9 | 41.7 (102%) | 44.5 (109%) | 38.7 (95%) |
| RBC ($10^6$/µL) | 4.79 | 4.94 (103%) | 5.21 (109%) | 4.56 (95%) |
| MCV (fL) | 85 | 84 (99%) | 85 (100%) | 85 (100%) |
| MCH (pg) | 30.5 | 30.72 (101%) | 32.27 (106%) | 30.64 (100%) |
| MCHC (g/dL) | 35.8 | 36.38 (102%) | 37.84 (106%) | 36.07 (101%) |
| RDW (%) | 12.8 | 12.05 (94%) | 12.46 (97%) | 12.5 (98%) |
| Platelet Count ($10^6$/µL) | 181 | 129 (71%) | 148 (82%) | 133 (73%) |
| Platelet Volume (fL) | 8.2 | 10.0 (122%) | 9.3 (113%) | 9.2 (112%) |
| Leukocytes ($10^3$/µL) | 4.2 | 8.5 (202%) | 5.1 (121%) | 4.3 (102%) |
| Neutrophils ($10^3$/µL) | 2.0 | 4.4 (224%) | 2.5 (127%) | 2.1 (107%) |
| Lymphocytes ($10^3$/µL) | 1.6 | 3.0 (182%) | 1.8 (112%) | 1.5 (89%) |
| Creatine-kinase activity (U/L) | 170 | 8824 (5191%) | 5339 (3141%) | 3381 (1989%) |
| AST (U/L) | 25 | 241 (964%) | 236 (944%) | 196 (784%) |
| ALT (U/L) | 14 | 56 (400%) | 68 (486%) | 70 (500%) |
| GGT (U/L) | 18 | 18 (100%) | 15 (83%) | 16 (89%) |
| Uric acid (mg/dL) | 6.1 | 6.3 (103%) | 5.8 (95%) | 5.6 (92%) |
| Urea (mg/dL) | 30 | 69 (230%) | 57 (190%) | 41 (137%) |
| Creatinine (mg/dL) | 0.7 | 1 (143%) | 0.8 (114%) | 0.7 (100%) |
| Bilirrubin (mg/dL) | 0.63 | 1.47 (233%) | 1.27 (202%) | 0.96 (152%) |
| Iron (µg/dL) | 70 | 124 (177%) | 76 (109%) | 76 (109%) |
| Ferritin (ng/mL) | 136.3 | 162.7 (119%) | 196.4 (144%) | 189 (139%) |
| Transferrin (mg/dL) | 260.8 | 267.9 (103%) | 220.4 (85%) | 225.7 (87%) |
| Transferrin saturation (%) | 21.5 | 37 (172%) | 27.6 (128%) | 26.9 (125%) |
| Ca (mg/dL) | 9.99 | 9.95 (99%) | 9.17 892%) | 9.36 (94%) |
| $Na^+$ (mEq/L) | 139 | 138.9 (99%) | 139.1 (100%) | 138.9 (99%) |
| $K^+$ (mEq/L) | 4.6 | 3.8 (83%) | 4 (87%) | 4.1 (89%) |
| $Mg^{2+}$ (mg/dL) | 1.8 | 2 (111%) | 2 (111%) | 2 (111%) |
| Glucose (mg/dL) | 89 | 96 (107%) | 95 (107%) | 82 (92%) |
| Triglycerides (mg/dL) | 42 | 52 (124%) | 33 (79%) | 57 (136%) |
| Total cholesterol (mg/dL) | 150 | 142 (95%) | 135 (90%) | 128 (85%) |
| HDL-cholesterol (mg/dL) | 58 | 59 (102%) | 66 (114%) | 62 (107%) |
| LDL-cholesterol (mg/dL) | 84 | 73 (87%) | 62 (74%) | 55 (66%) |
| Total proteins (g/dL) | 9.1 | 8.3 (91 %) | 7.8 (86%) | 7.6 (84%) |

Data are expressed as absolute values and as (percentage of pre-exercise values). AST: aspartate transaminase, ALT: alanine transaminase, GGT: gamma-glutamyltransferase, HDL: high-density lipoprotein cholesterol, LDL: low-density lipoprotein, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, MCV: mean corpuscular volume, RBC: red blood cell, RDW: red blood cell distribution width.




| Time | 10’ pre | 4h 25’ running | 6h 12’ running | 9h 15’ running | 13h 15’ runnin |
|---|---|---|---|---|---|
| Density (g/cm$^3$) | 1.010 | 1.015 | 1.025 | 1.025 | 1.020 |
| pH | 7.0 | 6.0 | 5.0 | 5.0 | 5.0 |
| RBC/HPF (count) | 0-2 | 0-2 | 4-6 | 60-70 | 60-70 |
| WBC/HPF (count) | 0-2 | 0-2 | 1-2 | 6-8 | 7-9 |




| Time | 15h 40’ running | 18h 25’ running | 19h 55’ running | 20h 55’ running | 48h post |
|---|---|---|---|---|---|
| Density (g/cm$^3$) | 1.025 | 1.025 | 1.025 | 1.025 | 1.005 |
| pH | 5.0 | 5.0 | 5.0 | 5.0 | 7.0 |
| RBC/HPF (count) | 70-80 | 60-70 | 70-80 | 50-60 | 0-2 |
| WBC/HPF (count) | 10-15 | 15-20 | 6-8 | 4-6 | 0-2 |