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Evaluation of sources of low and high creatinine concentrations in drug-screening urine samples

Harald Ertl, Jürgen Hartleb

Labor Lademannbogen MVZ GmbH, D-22339 Hamburg, Germany

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Abstract

Aim: Abnormally low urine creatinine values can be explained by dilution. In contrast, the reasons for high values are less apparent. Potential reasons are a good compliance, the reduced thirst of opiate addicts as well as supplementation with creatine. The objective of this study was to evaluate sources of low and high creatinine concentrations.

Methods: We evaluated 1978 samples sent to our lab for drug screening. In addition to drug and creatinine analysis the concentrations of urea, uric acid as well as phosphate were determined. Furthermore, we tested urine from human subjects consuming creatine.

Results and Discussion: Compared to data from medical samples, the bar chart of the drug-screening samples showed a broader shape, a higher mean creatinine, higher frequency of values < 0.3 g/l (10.0 % vs. 6.0 %, respectively), and higher frequency of values > 2.5 g/l (12.4 % vs. 3.7 %, respectively). All samples with creatinine < 0.3 g/l had urea, uric acid and phosphate values below their lower reference values, except for 4 samples in which urea was slightly above the lower reference. All low values of creatinine were most likely due to dilution. None of the samples with high creatinine (> 2.5 g/l) had urea, uric acid and phosphate below their lower reference, which would indicate an *in vivo* creatine consumption. Some samples showed an average creatinine although urea, uric acid and phosphate were below their lower reference (17 samples > 0.8 g/l creatinine). To analyze whether these results may be explained by supplementation of creatine, a data subset including all 'normal' data was generated (creatinine > 0.3 but < 2.5 g/l). Within this subset the correlation between creatinine and urea, uric acid or phosphate was examined. As a result, the constellation found in 17 samples can be explained by normal variation and therefore is not a clear hint for adulteration. The experiments with oral supplementation of creatine resulted in clear pattern: consumption of creatine, whether as a commercial product or as 60 g pure creatine, did not alter the creatinine concentration. Each attempted dilution together with *in vivo* creatine supplementation resulted in diluted values of all measured parameters. The only adulteration effect found was the more yellowish colour of the diluted urine samples, which is due to the vitamin B2 (riboflavin) co-administered with the creatine.

Conclusion: All samples with creatinine < 0.3 g/l were most likely due to dilution, because urea, uric acid and phosphate were also very low. All samples with creatinine > 2.5 g/l had also high concentrations of urea, uric acid and phosphate and therefore most likely were not adulterated (not elevated due to *in vivo* creatine consumption). The cause of samples with average creatinine, but urea, uric acid and phosphate below their lower reference remains unclear. A single *in vivo* consumption of creatine did not cause such a constellation in our *in vivo* experiments. Medical reasons as well as a chronic *in vivo* consumption of creatine remain as possible causes.

1. Introduction

Abnormally low urine creatinine values, usually defined as creatinine < 0.3 or < 0.2 g/l, can be explained by *in vivo* dilution (drinking of water). In contrast, the reasons for high values > 2.5 g/l are less apparent. Potential reasons are a good compliance of the probands, the reduced thirst of opiate addicts as well as supplementation with creatine [1]. The objective of this study was to evaluate potential sources of low and high creatinine concentrations in drug screen urine samples.

2. Material and Methods

2.1. Statistical data evaluation

We evaluated 1978 samples sent to our lab for drug screening. Approximately 75 % of the samples were not collected with direct observation, whereas about 25 % were collected either with direct observation or by using a marker system for identifying the urine identity. Urine adulteration by dilution *together with* creatine consumption is most likely when samples were collected with direct observation or by using a marker system, because in these cases it is much more difficult to give clean urine from another person.
In addition to drug and creatinine analysis the concentrations of urea, uric acid as well as phosphate were determined (all of them using Roche® reagents, creatinine Jaffé method, Roche Cobas Integra 800®). The results of the drug analysis were not evaluated in this study.

2.2. In vivo creatine supplementation

Furthermore, we tested urine obtained directly from human subjects consuming creatine. A commercially available product ("Clear Machine" with "Extra Creatine Bag") as well as 60 g pure creatine (from a pharmacy) were used for two different experiments. According to the "guidelines of use" available together with the product, water was consumed at the same time (0.5 litre) and 2-3 hours afterwards (1.0 litre) to reach a reasonable dilution of the urine. The goal of the dilution clearly is to fall below the cutoff limits of a drug screening analysis. The commercial product beside creatine mainly contains starch, sugars, artificial flavour as well as vitamin B2 (riboflavin), the latter for masking the diluted urine with a yellowish colour. For comparison also a third experiment without creatine supplementation but with maximum *in vivo* dilution was performed (drinking of 2.0 litres of water in 2 hours without giving urine during this time).

3. Results and Discussion

3.1. Statistical data evaluation

Compared to data from medical samples (Fig. 1), the bar chart of the drugscreening samples showed a broader shape, a higher mean creatinine, higher frequency of values < 0.3 g/l (10.0 % vs. 6.0 %, respectively), and higher frequency of values > 2.5 g/l (12.4 % vs. 3.7 %, respectively).

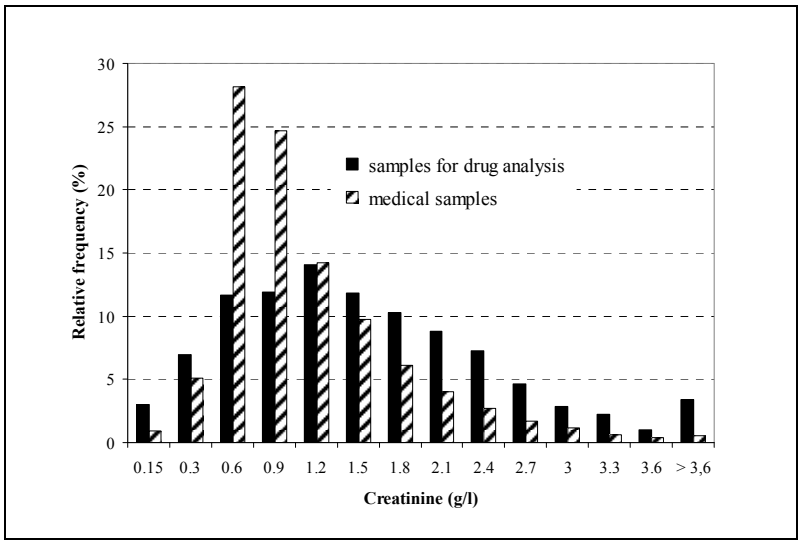


Fig. 1. Frequency distribution of creatinine in drug screening samples (N = 1978) compared to frequency distribution data from medical samples (N = 17015) (our own data).

All samples with creatinine < 0.3 g/l had urea, uric acid and phosphate values below their lower reference values, except for 4 samples among which urea was slightly above the lower reference. All low values of creatinine were most likely due to dilution.

None of the samples with high creatinine (> 2.5 g/l) had urea, uric acid and phosphate below their lower reference, which would indicate an *in vivo* creatine consumption. All samples with creatinine > 2.5 g/l had high concentrations of urea, uric acid and phosphate.

Some samples showed an average creatinine, whereas urea, uric acid and phosphate were below their lower reference (17 samples > 0.8 g/l creatinine). This inconsistency might be caused by *in vivo* creatine consumption.

To analyze whether these results may be explained by supplementation of creatine, a data subset including all 'normal' data was generated (creatinine > 0.3 but < 2.5 g/l / N=1533). Within this subset the correlation between creatinine and urea, uric acid or phosphate was examined. The data for the strongest correlation, which occurs between creatinine and urea, is shown in Fig. 2. The other correlations were even more weak.

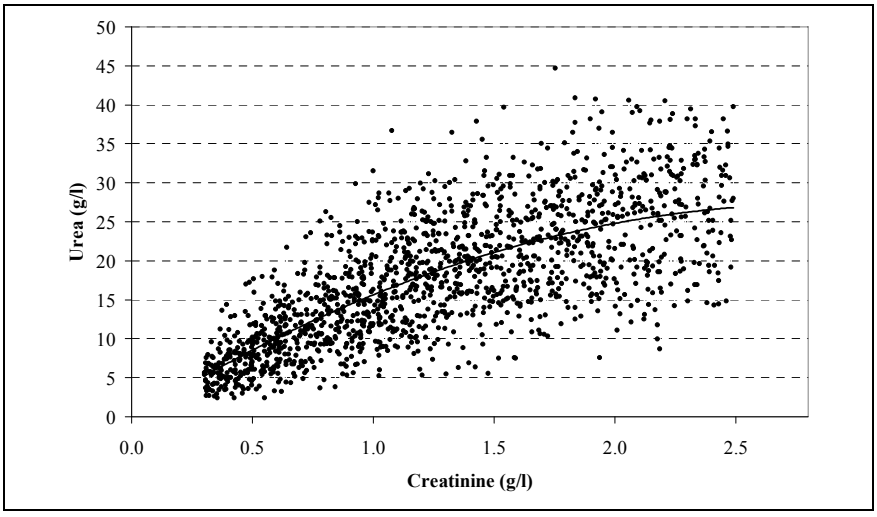


Fig. 2. Correlation between creatinine and urea (N = 1533).

Taking into account the wide variation within the dataset used for correlation (creatinine and urea, uric acid or phosphate) it becomes clear that a urine with urea, uric acid and phosphate below their lower reference value can be explained by normal variation and therefore is not a strong hint for adulteration.

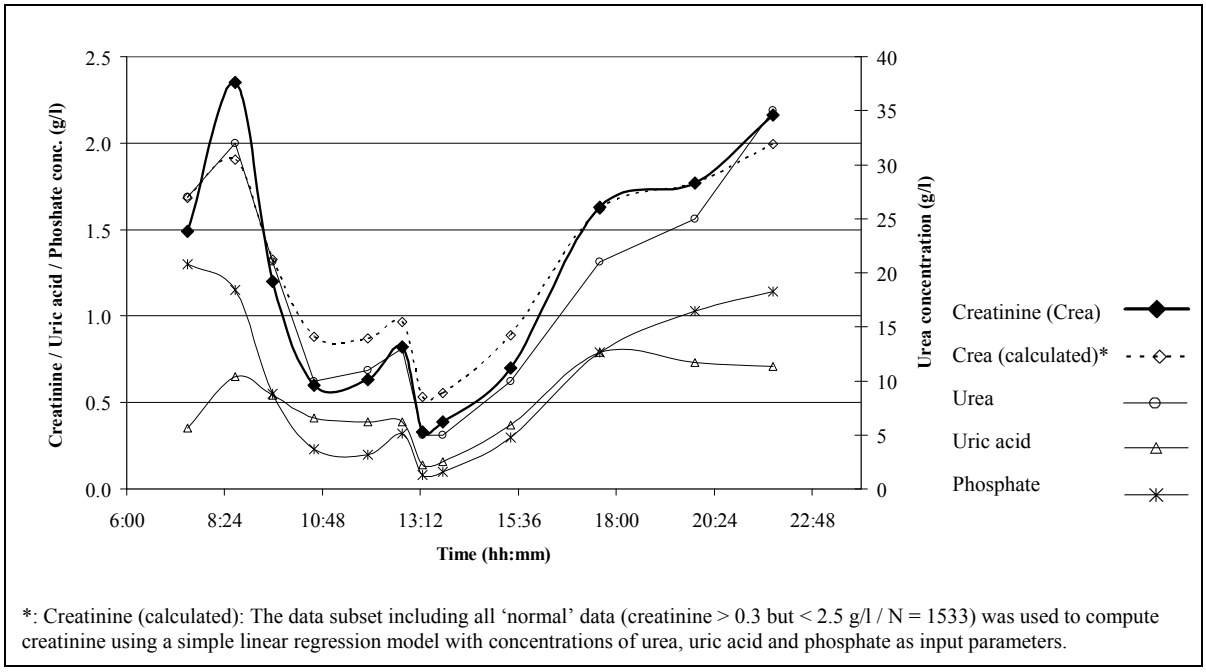
3.2. *In vivo* creatine supplementation

The analysis of the urine samples from subjects with oral supplementation of creatine resulted in clear pattern: The creatine consumption, whether using a commercial product (Fig. 3) or 60 g pure creatine, in no case altered the creatinine concentration (detected by Jaffé method). Each attempted dilution together with *in vivo* creatine supplementation resulted in diluted values of creatinine, urea, uric acid and phosphate.

Minimum creatinine concentrations were 0.33 g/l after "Clear Machine" and 0.43 g/l after applying 60 g pure creatine, as a result of a very effective dilution guideline with respect to the 0.3 g/l creatinine cutoff. The experiment with maximum dilution resulted in clearly suspicious creatinine concentrations of ≥ 0.1 g/l.

The only adulteration effect found was a yellowish colour of the diluted urine samples, which is due to the vitamin B2 (riboflavin) co-administered with the creatine. However, the shade of colour of riboflavin is quite different from the natural yellow colour of urine and therefore may be more suspicious then helpful in urine adulteration.

The urine samples collected 2-3 hours after creatine consumption had creatine concentrations greater than the maximum solubility (14 g/l in water at +25°C). Creatine obviously is excreted mainly unchanged. Using the enzymatic method of creatinine determination, false high creatinine concentrations would have been detected, because this method has the same sensitivity for creatine as for creatinine [2].



*: Creatinine (calculated): The data subset including all 'normal' data (creatinine > 0.3 but < 2.5 g/l / N = 1533) was used to compute creatinine using a simple linear regression model with concentrations of urea, uric acid and phosphate as input parameters.

Fig. 3. Concentrations of creatinine, urea, uric acid and phosphate prior to, during (9:00 to 12:00) and after administration of "Clear Machine" with "Extra Creatine Bag".

4. Conclusion

Our dataset of drug-screening urine samples showed a typical creatinine distribution as compared to the literature, e.g. [1]. The additional determination of urea, uric acid as well as phosphate concentrations together with the *in vivo* experiments gave valuable results:

All samples with creatinine < 0.3 g/l were most likely due to dilution, because urea, uric acid and phosphate were also very low. All samples with creatinine > 2.5 g/l had also high concentrations of urea, uric acid and phosphate and therefore most likely were not adulterated, especially not elevated due to *in vivo* creatine consumption.

The cause of samples with average creatinine, but urea, uric acid and phosphate below their lower reference remains unknown. A single *in vivo* consumption of creatine as evaluated in our *in vivo* experiments did not cause such a constellation. Medical reasons as well as a chronic *in vivo* consumption of creatine remain as possible causes.

5. References

[1] Arndt T. Urin-Kreatininkonzentration: Kenngröße zur Prüfung auf Probenverwertbarkeit? Teil 2. Auswertung von ca. 20.000 Kreatinin-Analysen im Rahmen des Drogenscreenings. Toxichem Krimtech 2007;74(3):155-158

[2] Degel F. Immunchemische Tests im Urin zur Verlaufsbeurteilung von Drogenabusus. Deutsches Ärzteblatt 2004;101(17):A1168-1170