EXPERT MEDICAL REPORT

Prepared by

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Date of report: October 5th 2017

Objective of the report:

Secondary analyses of urine specimens collected among athletes having participated to the Vancouver and Sotchi Winter Olympic Games

Prepared at the request of:

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Project Manager- Anti-doping re-analysis and research

Medical and Scientific Department

International Olympic Committee (IOC) asks

Lausanne, Switzerland

Signature: M. Burnier. Date: October 5-th, 2017

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Summary of conclusions

I have been asked to:

1. To determine reference values for various urinary analytes (Na, K, Cl, Ca, creatinine, urine density) coming from samples taken from top level athletes tested at the time of XXI Olympic Winter games in Vancouver.
2. To determine for each sample collected at the time of the XXII Olympic Winter Games and analyzed for the same analytes, if the values are within the reference values obtained from the control population at the XXI Olympic Winter Games and in agreement with data published.

In the presented analyses, I have assessed the distribution of urinary sodium, potassium, chloride and calcium concentrations in 250 samples for the XXI Olympic Winter games and calculated for each parameter: the mean ± standard deviation and upper and lower 95% confidence intervals, the median with the 5% and % percentiles. Statistical characteristics were done for women and men separately. For urinary creatinine, similar characteristics cannot be given because urinary creatinine depends on many factors including age, sex, body weight and urinary volume which are not available. Gravity was within the normal range of 1000 to 1035 in all samples.

Regarding samples of the XXII Olympic Winter Games, similar statistical analyses were performed but with the identification of potential true outliers defined as greater than the mean of Vancouver data + 3 standard deviations. With this approach, we identified 13 samples (of 5 men and 8 women) which are definitively out of range and even out of renal physiological possibilities suggesting strongly a manipulation of the samples, for ex. an addition of sodium chloride (NaCl).

Background information

Some urine samples collected during the XXII Olympic Winter Games in 2014 have allegedly been manipulated. This was reported in a previous extensive report published by Pr. Richard H. McLaren. One of the supposed manipulations was the addition of salt in urine samples to correct urine density.

The IOC decided to reanalyze a number of urine samples collected at the time of the XXII Olympic Winter Games and simultaneously to re-analyze urine samples collected during the XXI Olympic Winter Games in Vancouver to establish reference values among top level athletes with regard to each analytes.

The Services

The IOC asks for the following services:

1. To determine reference values for various urinary analytes (Na, K, Cl, Ca, creatinine, urine density) coming from samples taken from top level athletes tested at the time of XXI Olympic Winter games in Vancouver.
2. To determine for each sample collected at the time of the XXII Olympic Winter Games and analyzed for the same analytes, if the value are within the reference values obtained from the control population at the XXI Olympic Winter Games and in agreement with data published. In doing so, all necessary statistical analyses will be performed.
3. To summarize all findings in a full report which could be presented in front of a court.

Documents to be analyzed

The Service provider received one excel file containing laboratory values measured by the clinical laboratory of the Centre Hospitalier Universitaire Vaudois in Lausanne, Switzerland. The measured urinary parameters were: sodium (2 measurements), potassium, chloride, calcium, creatinine, and gravity measured using two methods (Atago and refractometer). There were 250 samples for the Vancouver Games and 230 samples for Sotchi. The samples from Vancouver concerned athletes from all around the World including Russians whereas the samples from Sotchi concerned only Russian athletes. The gender distribution was the following: for Vancouver, 135 women, 110 men and 5 samples without indications of sex, for Sotchi there were 141 men and 89 women. A mix of sports was represented in both groups.

The characteristics of the methods of measurement provided by the laboratory were:

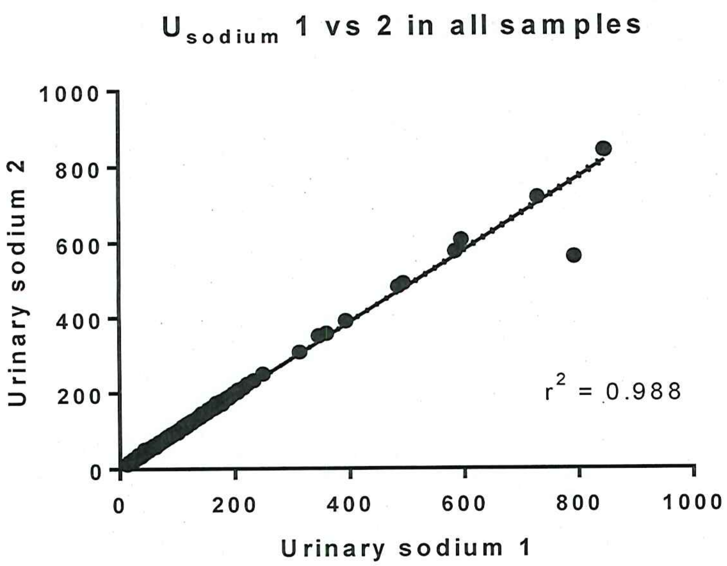
Parameter	Method	Measurement intervals
Urinary creatinine	Jaffé	250 – 55'000 umol/l
Urinary sodium	Potentiometry	10 – 350 mmol/l
Urinary potassium	Potentiometry	3 – 100 mmol/l
Urinary chloride	Chloridometry	10 -299 mmol/l
Urinary total calcium	NM-BAPTA	0.2 – 7.5 mmol/l

If the concentration was above the limit, the sample was diluted.
The CHUV laboratory had no LOD or LOQ limits.
Of note, there are no real reference values for these urinary methods.

There were two samples (2889100, 2890663) with insufficient volume and no sodium measurements. Eight measurements of chloride (2 in Vancouver and 6 in Sotchi) were below the limit of detection (<10 mmol/l) and 8 samples of Vancouver had urinary calcium values below the limit of detection (< 0.2 mmol/l).

We found an excellent correlation between the two measurements of sodium in the urine with a r^2 of 0.988 (Figure 1).

Figure 1: correlation between the 2 measurements of urinary sodium



We also found an excellent correlation between the measurements of specific gravity with two different methods (Figure 2).

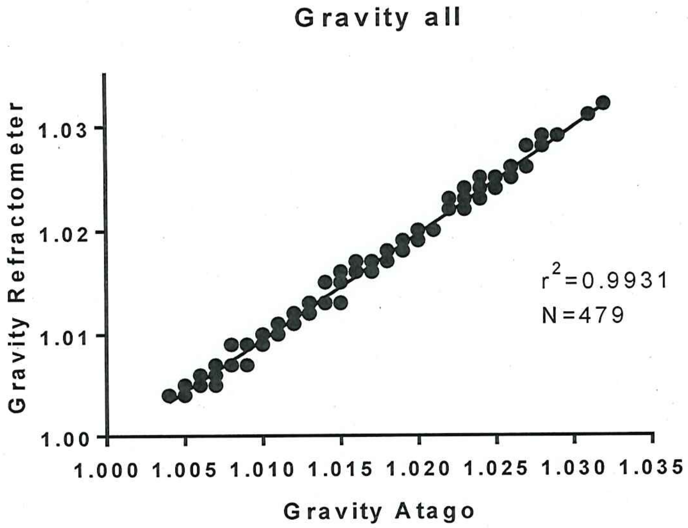


Figure 2: correlation between the 2 measurements of urinary gravity

Analyses performed

For each urinary parameter, the distribution of values was analyzed together with the mean, the maximum and minimum value, the standard deviation and the upper and lower 95% confidence interval. In addition, the median and the 5% and 75% and 95% percentiles were calculated.

Analyses were made separately for men and women and for the two sets of samples.

Correlations between parameters and corrections of urinary electrolytes concentrations by urinary creatinine (µmol/l) were also performed by gender and site.

At last, the availability of urinary sodium and potassium concentrations enabled to calculate urinary osmolarity according to the following formula:

$$Uosm = (U_{Na} + U_K) \times 2 + \text{glucosuria} + \text{urea}$$

In healthy subjects, glucosuria is zero and we used a fixed amount of urea for men (280 mmol/l) and women (180 mmol/l) based on the mean urinary urea concentrations observed in a random sample of the Swiss population (n=1500) (Glatz et al, 2017).

With these approaches, the goal was to determine the "apparently" normal range based on the Vancouver data and to identify potential outliers in the samples of XXII Olympic Winter Games.

The definition of "true" outliers was: any value above the mean+3 SD of the Vancouver set of samples. Possible outliers were those with values above the mean + 2SD of the Vancouver group. Some possible outliers were also looked for based on the coherence of laboratory values for example sodium, potassium and chloride concentrations and urinary creatinine or gravity or osmolarity in accordance with renal physiology.

Results

1. Urinary sodium concentrations

In male athletes participating in the XXI Olympic Winter Games in Vancouver, the mean urinary sodium excretion was 95.4 ± 49.37 mmol/l (mean ±SD) and a maximal value of 250 and a minimal value of 12 mmol/l. In women, the mean was 67.39 ± 40.88 mmol, a maximal value at 180 and a minimal value at 11 mmol/l.

According to these values outliers would be defined as urinary sodium concentrations >243 mmol/l for men (>99th percentile) and >190 mmol/l for women considering a comparable mean urinary sodium concentration in the groups.

Figure 3 and figure 4 below show the distribution of urinary sodium concentrations in the samples of Vancouver for men and women.

Figures 3

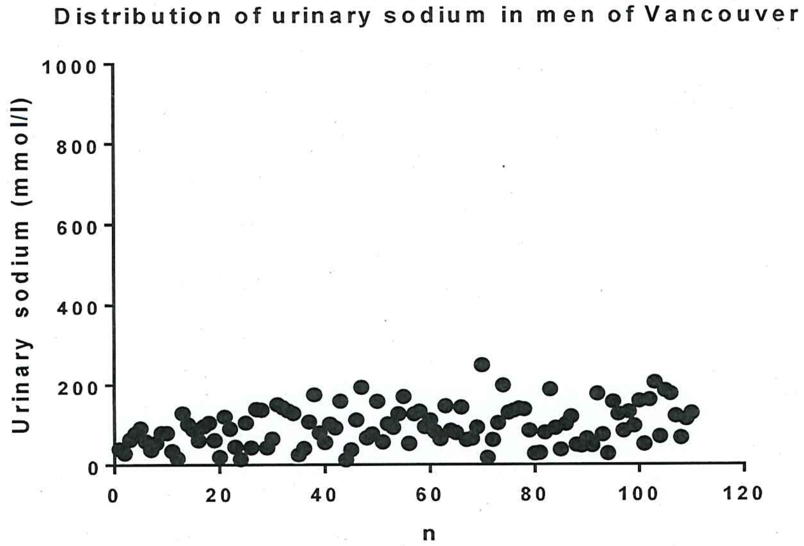
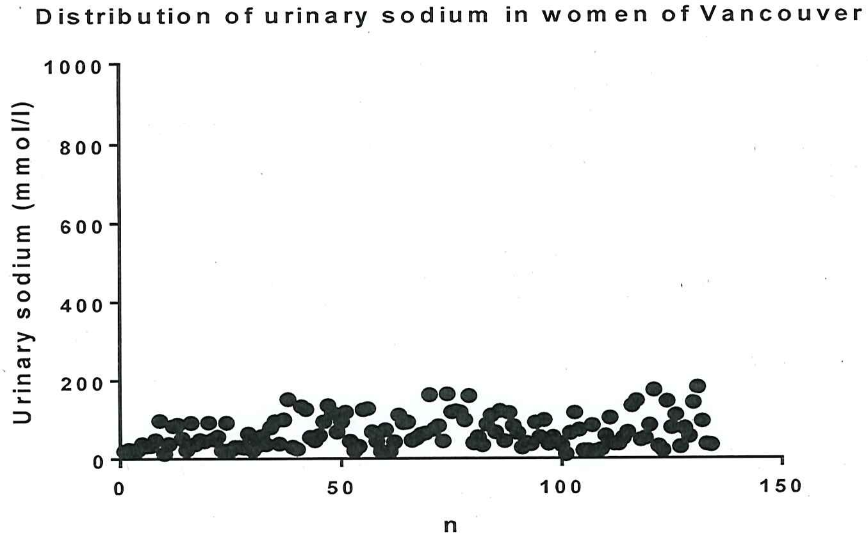
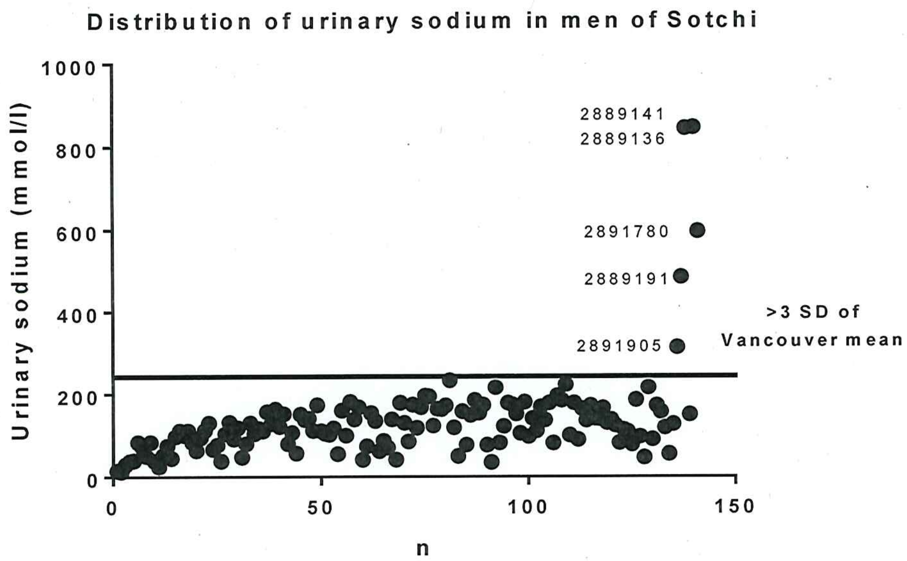


Figure 4



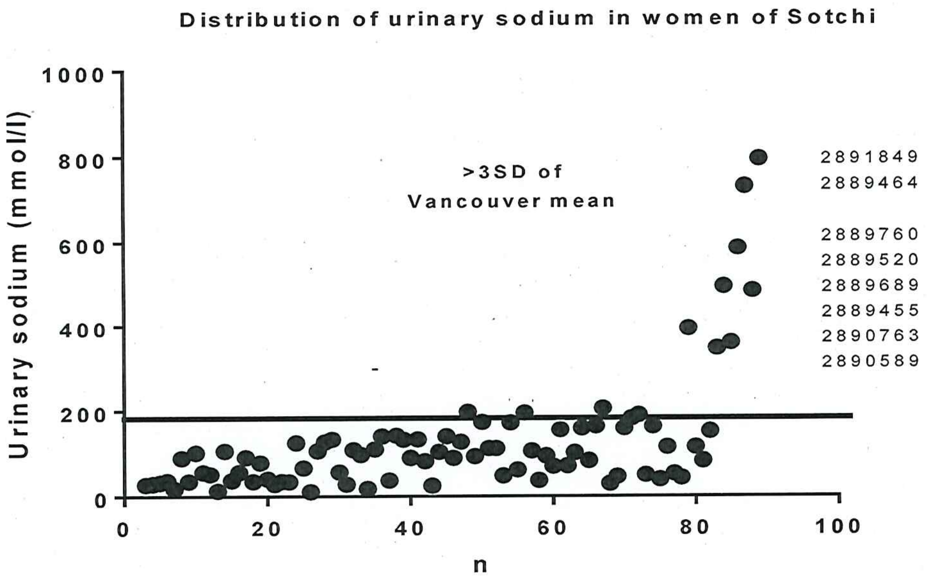
In samples of men of the Sotchi group, mean urinary sodium concentration were 135.0 ± 111.48 mmol/l, a maximal at 843 mmol/l and a minimal value at 12 mmol/l. The distribution is shown in Figure 5 below. Five samples were above 3 SD of the mean of Vancouver samples.

Figure 5



In women of the Sotchi Games, mean urinary sodium concentration was 126.66 ± 131.98 mmol/l, a max of 719 and a min at 11 mmol/l. The distribution of values is shown in Figure 6. Eight samples had values greater than 3 SD from the mean of Vancouver samples.

Figure 6



The summary of statistics are shown in the table below (Table 1)

	Men Vancouver	**Women Vancouver**	**Men Sotchi**	**Women Sotchi**
Number of values	110	134	141	87
Minimum	12	11	13	11
25% Percentile	58.5	35.5	82	43
Median	**91**	**56**	**119**	**94**
75% Percentile	130.5	94.25	163	142
Maximum	249	182	**847**	**793**
1% Percentile	12.22	11.7	38	20.8
99% Percentile	244.3	179.6	221.3	490
Mean	**95.58**	**68**	**135**	**129.6**
Std. Deviation	**49.41**	**41.06**	**111.6**	**143.6**
Std. Error of Mean	4.711	3.547	9.398	15.39
Lower 95% CI of mean	86.24	60.98	116.5	98.96
Upper 95% CI of mean	104.9	75.02	153.6	160.2

Urinary sodium concentrations corrected by the creatinine

As measured urinary sodium concentrations are highly dependent on the concentration of the urines, it is recommended to correct the values by the urinary creatinine concentration in order to cancel the volume effect. For this reason, the ratio of $U_{Na}/U_{creatinine}$ has been calculated and the distribution of this parameter has been analyzed.

Of note, there are no well-defined ranges for urinary creatinine concentrations based on spot urines as this depends on sex, age, muscle mass, weight and urine concentrations. Reference values have been published for 24 hour urine collections (Forni et al 2015)

Figure 7 below shows the correlation between urinary sodium and urinary creatinine in the samples of Vancouver (men and women pooled).

The correlation was significant: r2 was 0.400 for a n of 250 samples (p<0.001). The correlation shows that that the higher the urinary creatinine concentration, the higher the urinary sodium concentration.

Urinary sodium vs urinary creatinine in Vancouver samples

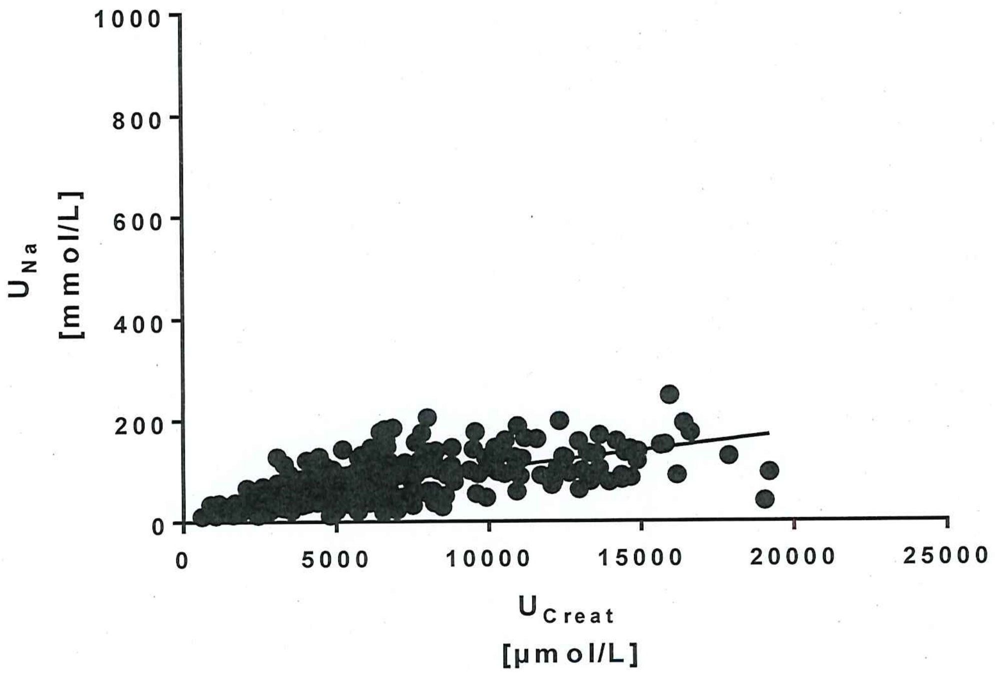
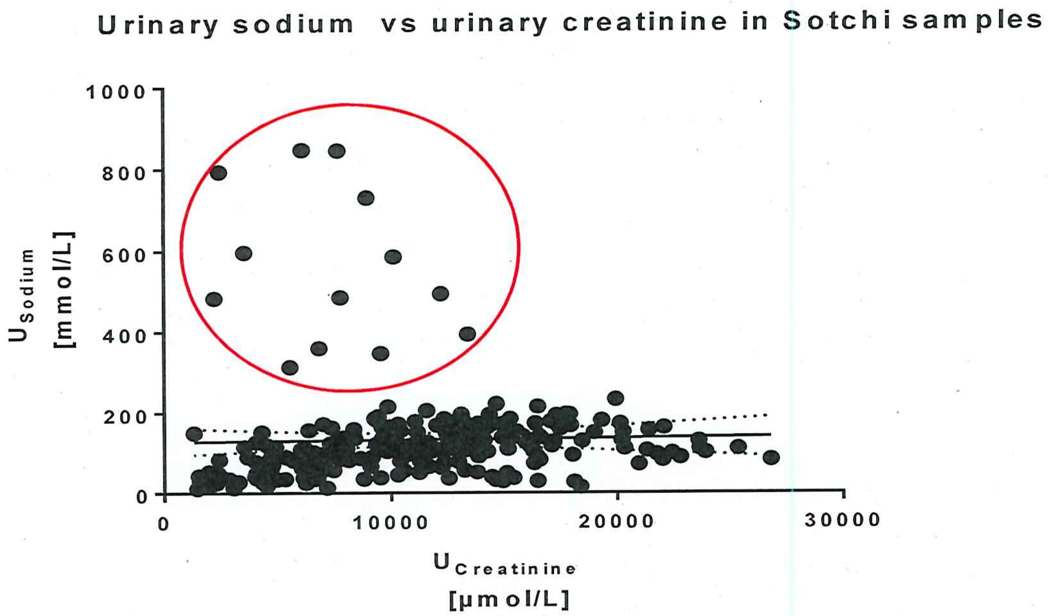
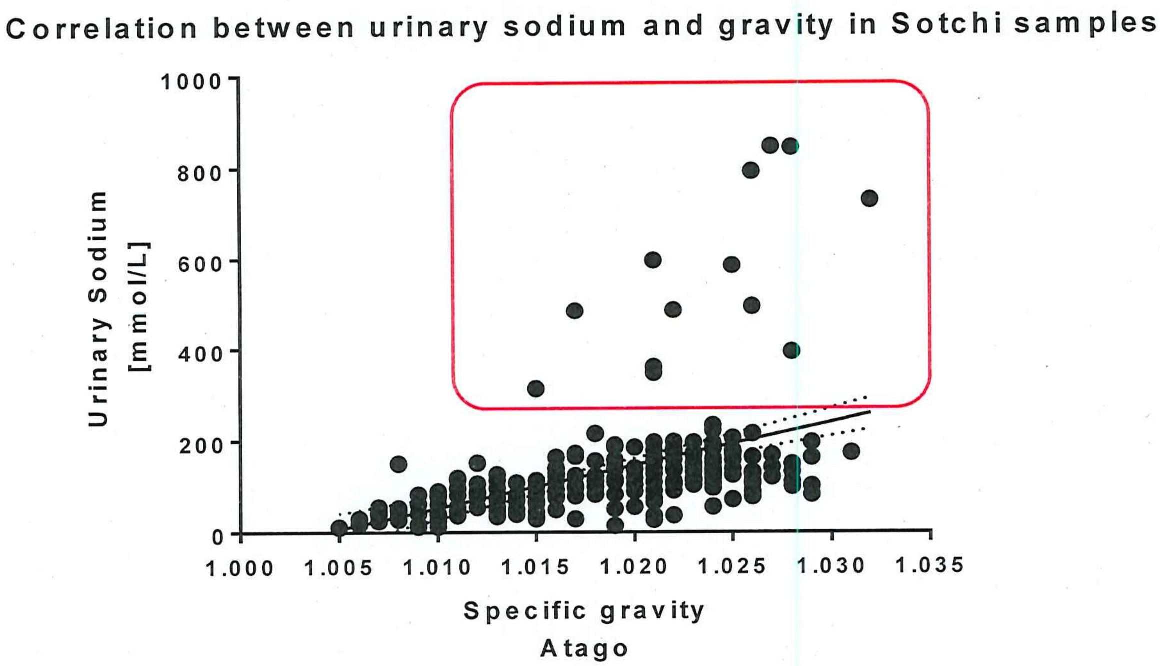


Figure 8 below shows the same correlation for the samples of Sotchi (men and women together). As shown in the figure, the 13 outliers identified in figures 5 and 6 are clearly recognized again (red circle) indicating that the high urinary sodium concentration is not explained by very concentrated urines as they occur in rather non-concentrated urines.



The same samples of Sotchi appear as outliers when urinary sodium is plotted against urinary gravity as shown below **(Figure 9).** No such case is found in Vancouver samples (see supplemental figure)

Figure 9



2. Urinary potassium concentrations

In Vancouver samples, mean urinary potassium concentrations were 48.7 ± 32.2 mmol/l in men and 46.76 ± 32.2 mmol/l in women. Therefore, means were very similar in men and women. In men 4 samples were greater than the 95% percentile and in women, 5 samples were above the 95% percentile. Three of them were considered as true outliers (>99.95%). The distribution in men and women are presented in figures 10 and 11. The line represents the 95% percentile

Figures 10

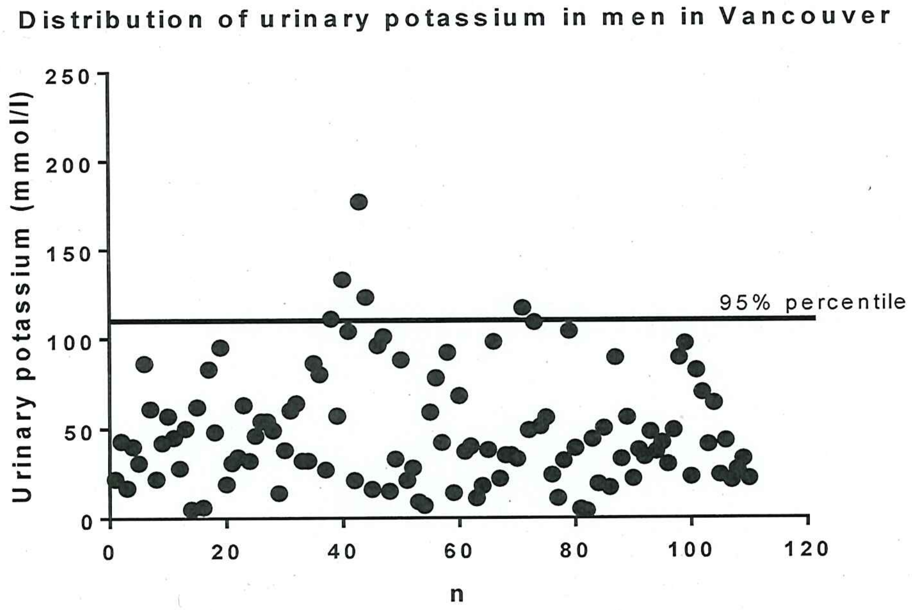
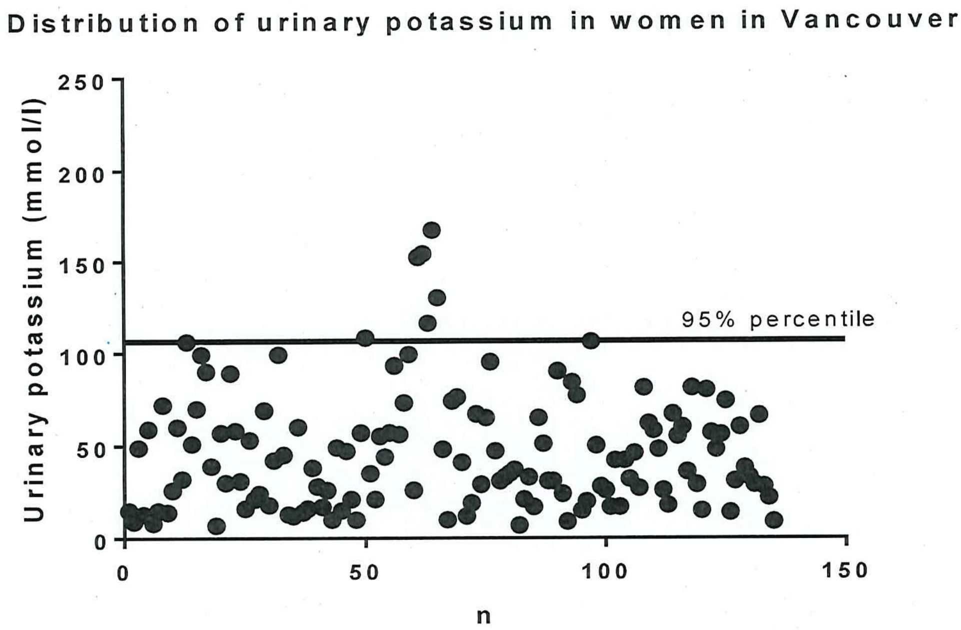


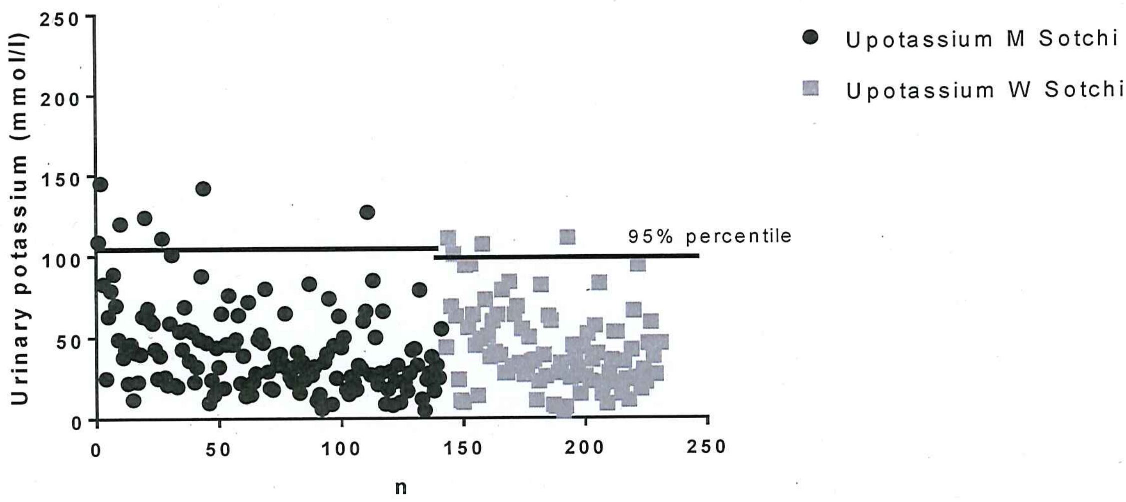
Figure 11



In samples of Sotchi, mean urinary potassium concentrations were 42.88±28.0 mmol/l in men, and 43.4±25.9 mmol/l in women. The distribution is shown in Figure 12

Figure 12

Distribution of urinary potassium in men and women in Sotchi



A summary of statistics on urinary potassium concentrations are shown in the **Table 2** below:

	Men Vancouver	**Women Vancouver**	**Men Sotchi**	**Women Sotchi**
Number of values	110	135	141	88
Minimum	4	7	5	4
25% Percentile	24	21	23	25
Median	**40.5**	**39**	**35**	**38**
75% Percentile	63.25	62	55	59.75
Maximum	177	167	145	111
5% Percentile	8.1	9.8	10.1	8.45
95% Percentile	109.9	106.4	108.2	98.3
Mean	**48.75**	**46.76**	**42.88**	**43.48**
Std. Deviation	**32.19**	**32.19**	**28.04**	**25.94**
Std. Error of Mean	3.069	2.771	2.361	2.765
Lower 95% CI of mean	42.67	41.28	38.21	37.98
Upper 95% CI of mean	54.84	52.24	47.55	48.97

In summary, there are no significant outliers in the samples of Sotchi. In the samples of Vancouver, there are a few samples with very high urinary potassium concentrations which are rather unusual and not well explained.

3. Urinary chloride concentrations

The analyses for urinary chloride concentrations were similar to those performed with sodium. Again 13 samples of marked outliers were identified which corresponded to those with elevated urinary sodium concentrations.

Figure 13

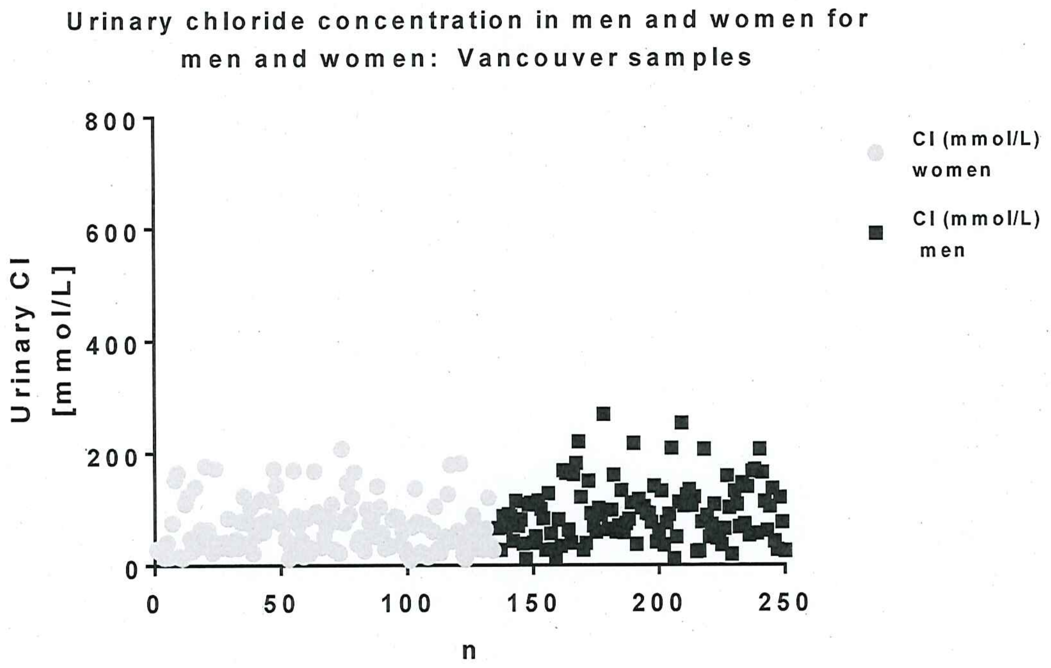
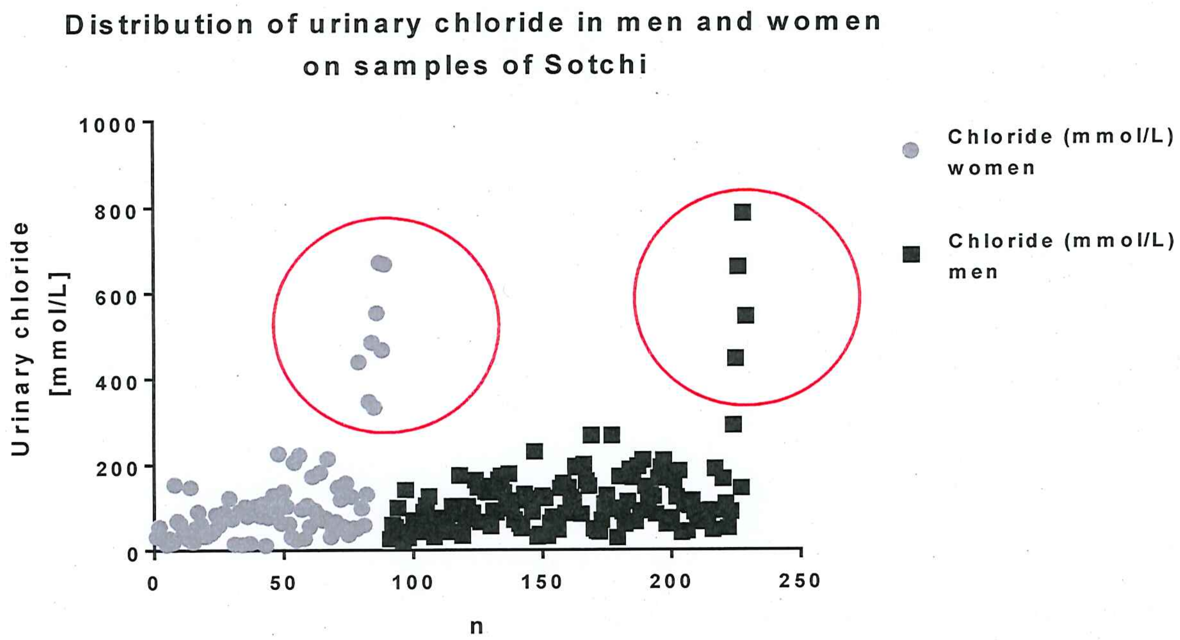


Figure 14



The summary of statistics for urinary chloride concentrations are shown in the **Table 3** below.

Table 3	**Vancouver women**	**Vancouver men**	**Sotchi women**	**Sotchi men**
Number of values	133	114	85	139
Minimum	10	10	12	23
25% Percentile	31	52.5	42.5	63
Median	**59**	**87**	**81**	**97**
75% Percentile	87.5	121	128.5	147
Maximum	208	270	**672**	**784**
5% Percentile	15	24.75	16.3	33
95% Percentile	170.6	207.5	480.6	268
Mean	**68.13**	**93.36**	**120.3**	**120.8**
Std. Deviation	**46.21**	**53.88**	**136.8**	**102.7**
Std. Error of Mean	4.007	5.046	14.84	8.71
Lower 95% CI of mean	60.2	83.36	90.77	103.6
Upper 95% CI of mean	76.05	103.4	149.8	138
KS normality test				
KS distance	0.1196	0.07543	0.2476	0.1712
P value	<0.0001	>0.1000	<0.0001	<0.0001
Passed normality test (alpha=0.05)	No	Yes	No	No

4. Urinary calcium concentrations

For urinary calcium concentrations, the distribution of values and the ratio of urinary Ca/creatinine were calculated for men and women.

Overall, no major deviations were observed and both samples groups were rather similar as shown in figures 15 and 16 below. Yet, statistically, 10 samples of the Vancouver group were considered as possible outliers (>99percentile) and none in the Sotchi group. When corrected for urinary creatinine, some samples remain above the 95 or 99% percentile among the Sotchi samples but it is difficult to consider them as outliers as these subjects may have had an hypercalciuria which is quite common feature in the population

Figure 15

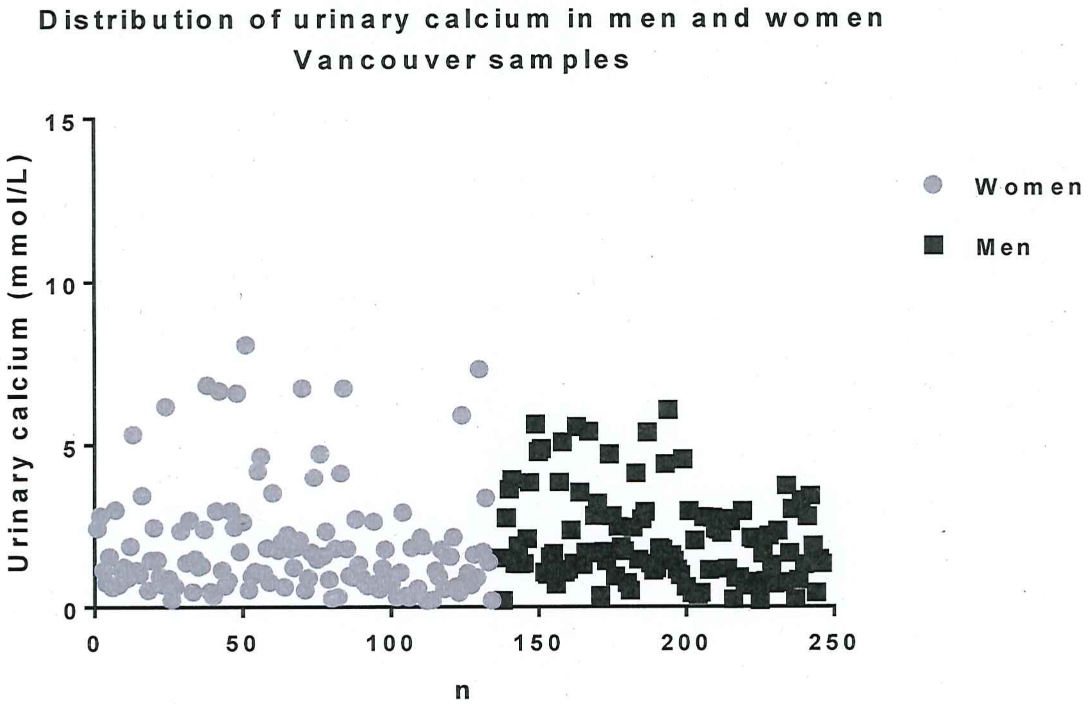


Figure 16

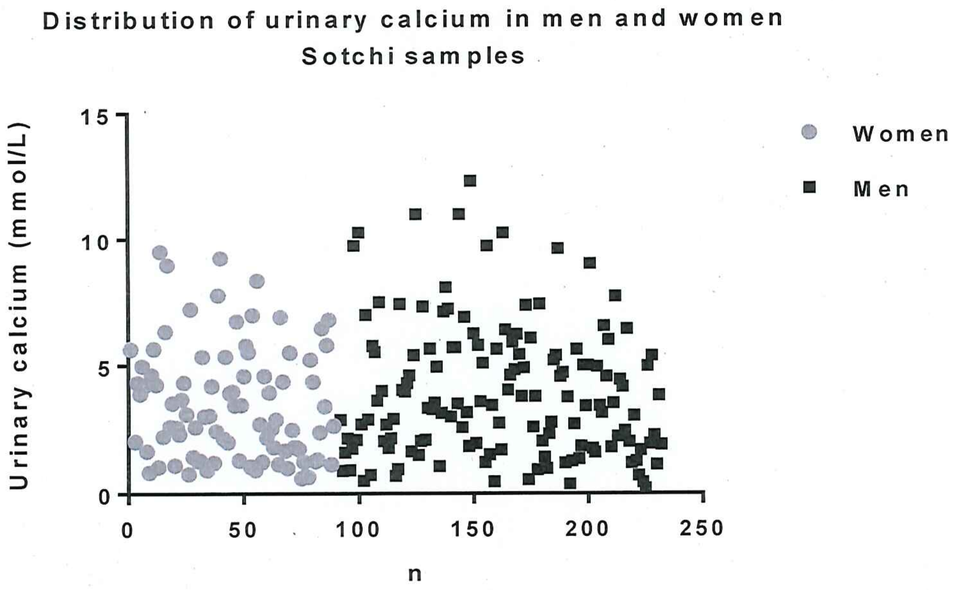


Figure 17

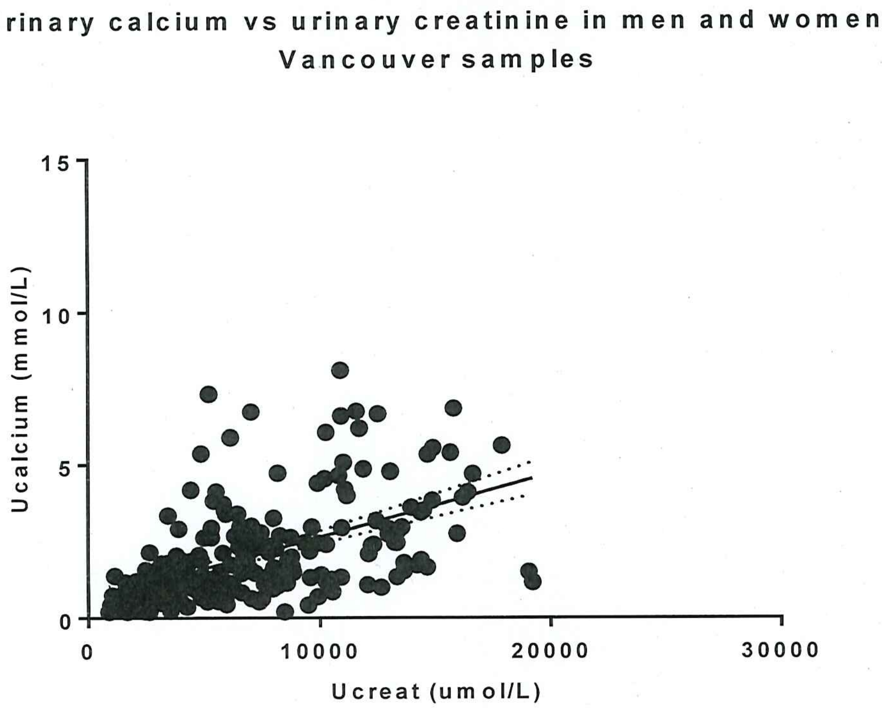
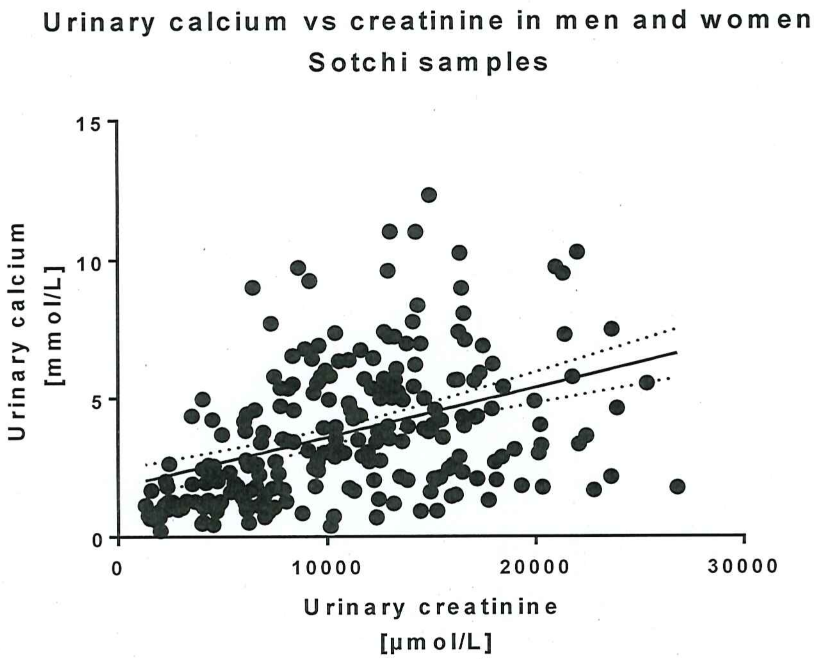


Figure 18



Summary of statistics of urinary calcium concentrations (Table 4)

	Vancouver women	Vancouver men	Sotchi women	Sotchi men
Number of values	128	108	88	141
Minimum	0.2	0.2	0.61	0.21
25% Percentile	0.7375	1.063	1.505	1.84
Median	**1.3**	**1.675**	**2.96**	**3.44**
75% Percentile	2.355	2.795	4.9	5.58
Maximum	8.07	6.05	9.52	12.32
5% Percentile	0.3045	0.3545	0.807	0.692
95% Percentile	6.619	5.23	8.095	9.719
Mean	**1.876**	**2.082**	**3.477**	**3.936**
Std. Deviation	**1.702**	**1.402**	**2.235**	**2.598**
Std. Error of Mean	0.1504	0.1349	0.2383	0.2188
Lower 95% CI of mean	1.578	1.815	3.003	3.503
Upper 95% CI of mean	2.173	2.35	3.95	4.368
95% CI of median				
Actual confidence level	95.84%	95.72%	95.78%	95.71%
Lower confidence limit	1.07	1.41	2.4	2.88
Upper confidence limit	1.61	2.1	3.93	4.04
Sum	240.1	224.9	306	555
KS normality test				
KS distance	0.1974	0.1315	0.1232	0.08635
P value	<0.0001	<0.0001	0.0021	0.0119
Passed normality test (alpha=0.05)?	No	No	No	No

5. Urinary gravity

The distribution of urinary gravity in the two set of samples is shown in figures 19 and 20 below. The normal values range between 1'000 and 1'035 depending on the state of hydration. The distribution is similar in both groups and comparable in men and women. The mean value is significantly higher in Sotchi samples (1019 vs 1013, p<0.001). At the 0.5% level, there were no outliers identified on the two distributions.

Figure 19

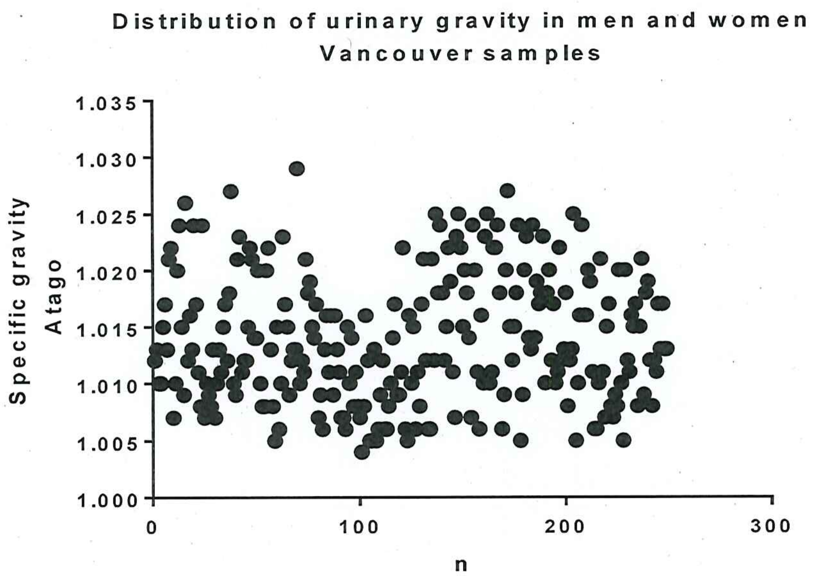
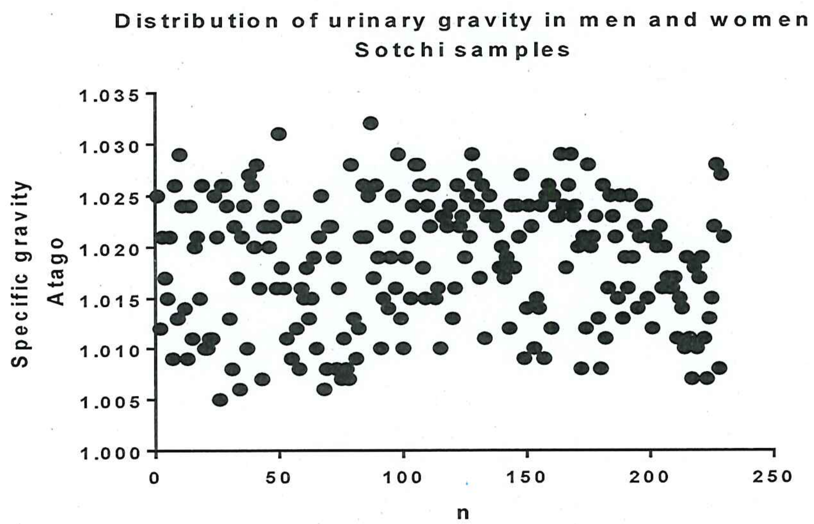


Figure 20



6. Correlation between urinary gravity and calculated urinary osmolarity

As mentioned earlier, urinary osmolarity can be calculated based on urinary sodium, potassium, glucose and urea. Glucosuria is usually negative in healthy subjects. Urinary urea concentrations have been fixed based on previous epidemiological data at 280 mmol/L for men and 180 mmol/l for women, which is probably an underestimation in athletes (Glatz et al, 2017). The figures below show the correlations between urinary gravity and calculated osmolarity in the two sample sets.

As shown in Figure 21, with the samples collected in Vancouver, there is an excellent correlation between the calculated osmolarity and urinary gravity with in women (r^2 =0.79, n=134, p<0.001). A similar correlation was found in men (r^2=0.736, n=115, p<0.001)

Figure 21

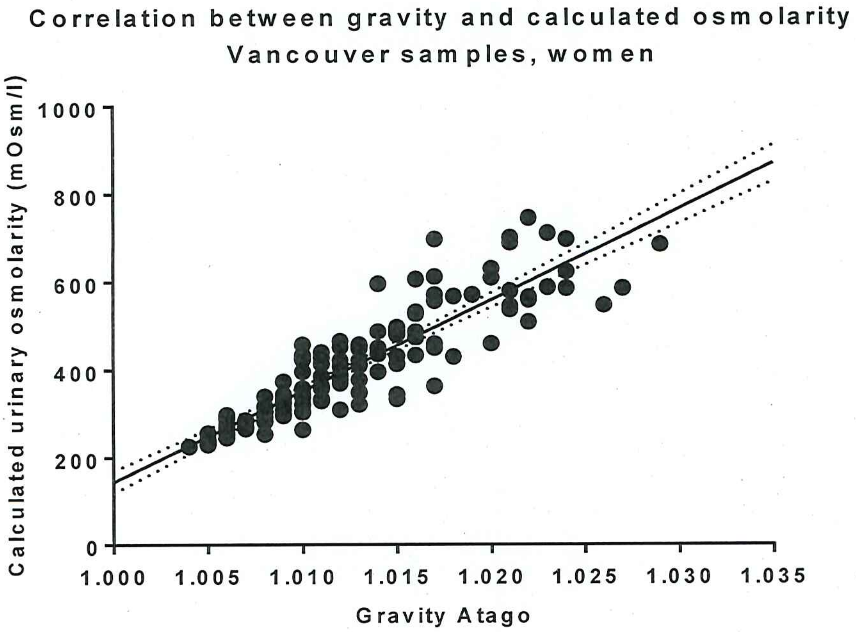
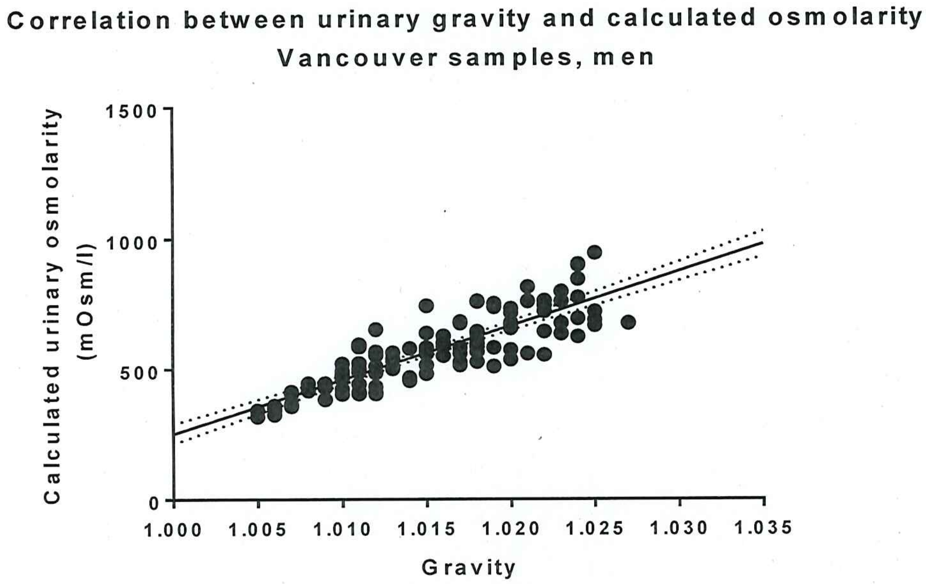


Figure 22



However, this was not the case with samples collected in Sotchi which shows calculated osmolarities way above the physiological capacity of the kidney to concentrate (max osmolarity in humans of 1200 mOsm/L) (Sands et al, 2014). Moreover, clear outliers are found with very high osmolarities in urines with a low gravity which suggests a discrepancy. Statistically, no correlation was found in women (r^2= 0.007) and a weak correlation was found in men (r^2=0.268)

Figure 23

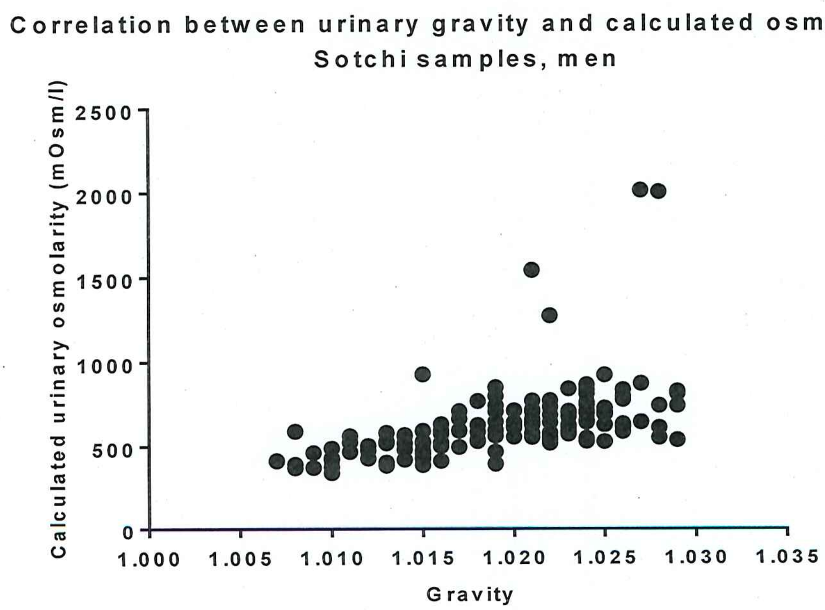
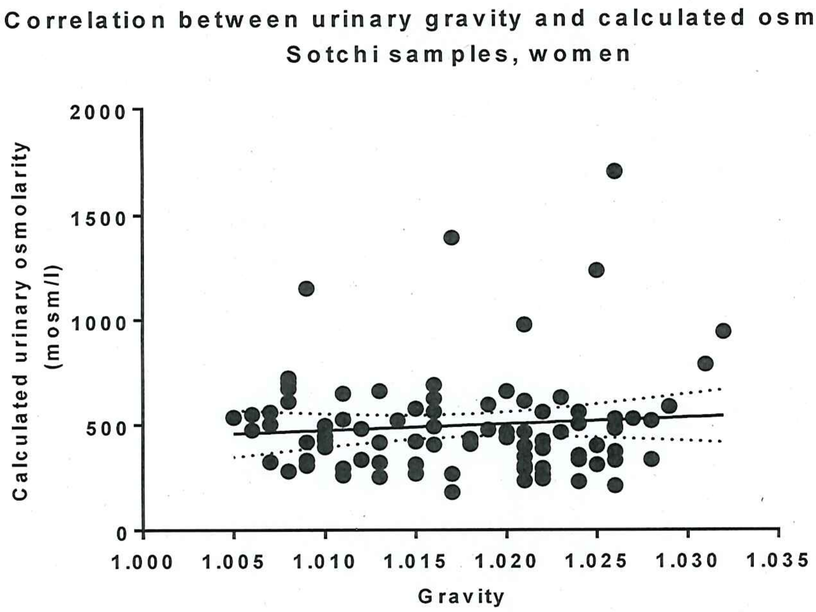


Figure 24



7. Evaluation of the results

The analyses of urinary electrolytes concentrations in spot urines of male and female athletes having participated in the XXI and XXII Olympic Winter Games show the following patterns:

Regarding urinary **sodium** concentrations, the values measured in Vancouver samples are relatively homogeneous and without clear outliers. All values are physiologically plausible. In contrast, among the samples collected in Sotchi, 13 samples were completely out of range and above 3 standard deviations from the mean of Vancouver samples but also above 2 standard deviations of the mean of Sotchi samples. These very high sodium concentrations are quasi incompatible with a normal sodium intake in humans.

Thus, for example, a concentration of 845 mmol/L is equivalent to 49.7 grams of sodium chloride in one liter (subject Nr 2889136). If this subject urinates only 500 ml in 24h, which is very unlikely, this would correspond to a sodium intake of about 25 g/d. If he urinates 1000 ml, the intake would be about 50 g of NaCl/day. However, one has to consider that the 49.7g NaCl /L were excreted in urines containing only 7'666 µmol/L creatinine. In a normal male athletes, the 24h urinary creatinine excretion is about 20'000 µmol/day. Thus, the amount of salt should be multiplied by 2.6 (20'000/7'666) leading to a daily sodium intake of 65 g NaCl/day if the subject urinates 500 ml and 130 g NaCl if he urinates 1000 ml/24h. These figures are not realistic and strongly suggest that sodium has been added in the following samples, even though in some areas of Russia and central Asia, very high sodium intakes have been reported in the range of 15 to 20 g NaCl/d (Powels et al, 2013).

The hypothesis of a NaCl addition in the 13 samples is further supported by the observation that very high concentrations of chloride were also found in these samples (Figure 14) but not calcium or potassium which often follow sodium if the high sodium concentration is due to a high food consumption.

At last, when calculating urinary osmolarity and comparing the values with the urinary gravity, one finds a perfect correlation with the samples collected in Vancouver (fig 21 and 22) and weaker correlations and clear outliers among the samples collected in Sotchi (Fig 23 and 24). At last, some of the calculated osmolarity (based on sodium and potassium) are clearly above the physiological capacity to concentrate urines, i.e. > 1200 mOsm/l (Sands et al, 2014).

The suspect samples are the following:
2889141, 2889136, 2891780, 2889191, 2891905 in men
2891849, 2889464, 2889520, 2889689, 2889455, 2890763, 2890589, 2889760 in women.

The sport categories concerned by these very high sodium values were bobsleigh for all men (n=5), ice hockey for 4 women, skiing for 3 women and biathlon for one women. Of note, among male bobsleigh athletes tested in Sotchi, 18 samples had normal urinary sodium concentrations.

Regarding urinary potassium and calcium concentrations in urine, the following comments can be made: urinary potassium concentrations were relatively homogeneous. However, both in the samples collected in Vancouver and in Sotchi, high potassium concentrations (>95 percentile) were sometimes measured with values of potassium greater than that of sodium. They were usually measured in very concentrated urines. They may reflect a secondary hyperaldosteronism due to some dehydration after the efforts although it is rather uncommon that potassium concentrations are so high.

Concerning urinary calcium concentrations, values were homogeneous in both groups of samples without any indications of outliers. Some elevated urinary calcium excretion may be observed in subjects with hypercalciuria, which is a common feature in the population.

Limits of the study:

There are of course some limits to these analyses. An important one is the absence of body weight which would have allowed calculating more precisely the expected amount of urinary creatinine for each subject and hence evaluating the sodium intake based on the spot urine.

8. Conclusion

With these analyses one has been able to characterize the distribution of urinary electrolytes in a large population of athletes having participated to Olympic Winter Games. These distributions and statistical characteristics may eventually serve as reference values for the future.

In addition, comparing the distributions of the XXI and the XXII Winter Games enabled identifying 13 outlying samples for which there is a very high suspicion of manipulation with an addition of external sodium chloride to the samples.

9. References:

Forni Ogna V, Ogna A, Vuistiner P, Pruijm M, Ponte B, Ackermann D, Gabutti L, Vakilzadeh N, Mohaupt M, Martin PY, Guessous I, Péchère-Bertschi A, Paccaud F, Bochud M, Burnier M. New anthropometry-based age- and sex-specific reference values for urinary 24-hour creatinine excretion based on the adult Swiss population. Swiss Survey on Salt Group. BMC Med. 2015; 27;13: 40

Glatz N, Chappuis A, Conen D, Erne P, Péchère-Bertschi A, Guessous I, Forni V, Gabutti L, Muggli F, Gallino A, Hayoz D, Binet I, Suter P, Paccaud F, Bochud M, Burnier M. Associations of sodium, potassium and protein intake with blood pressure and hypertension in Switzerland. Swiss Med Wkly. 2017; 147: w14411.

Sands JM, Layton HE. Advances in understanding the urine-concentrating mechanism. Annu Rev Physiol. 2014; 76:387-409.

Powles J, Fahimi S, Micha R, Khatibzadeh S, Shi P, Ezzati M, Engell RE, Lim SS, Danaei G, Mozaffarian D; Global Burden of Diseases Nutrition and Chronic Diseases Expert Group (NutriCoDE).Global, regional and national sodium intakes in 1990 and 2010: a systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. BMJ Open. 2013; 3(12):e003733.