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Influence of creatine intake upon biochemical parameters in urine

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Abstract

Creatine (CR) is greatly used by athletes at any level, and its use is made commonplace. CR doesn't belong to the IOC list of forbidden substances, because its effects are still much debated. So far, the use of CR has shown only secondary side effects (gain in body mass, cramps).

The goal of this study is to investigate the impact of CR high dose intake on different biochemical parameters in urine and in serum (especially CR and creatinine). To enable the analysis of these parameters, we developed a technique to quantify CR and creatinine (CRN). Several effects of CR supplementation were shown : First, 20g CR per day during 5 days doesn't influence seric and urinary CRN concentrations (CRN concentrations in urine being widely used to measure renal function and urine concentration, it was important to make sure that CR intake didn't influence CRN excretion). Secondly, a great increase was noticed in CR concentration following CR supplementation (urinary concentrations decreased to physiological concentrations about 24 hours after the intake and seric concentration about 9 hours after the intake). An increase in urine specific gravity following CR intake was also observed.

The stability of urines containing high concentrations of CR was also clocked over a period of three months to see if there would be a conversion of CR to CRN. It appeared that urines are quiet stable when kept at 4°C as well as at -20°C.

Introduction

Creatine in muscle metabolism :

Muscle contraction and relaxation is fuelled exclusively by free energy liberated during the dephosphorylation of ATP. The storage of skeletal ATP is very small and rapidly used during contraction, and for normal tissue function to continue, ATP must be rapidly resynthesised.

Creatine (CR) is a naturally occurring compound, which, in its phosphorylated forms (phosphocreatine PCR), functions in the maintenance of cellular ATP homeostasis. Approximately 95% of the total CR pool (free CR and PCR) in humans is found in skeletal muscle. In skeletal muscle, PCR accounts for approximately two-thirds of total creatine pool. The presence in cellular compartments of the enzyme creatine kinase allows on the one hand the formation of CR and ATP from PCR and ADP and on the other formation of PCR and ADP from CR and ATP. With rapid increases in energy demands in muscle during exercise, CRP is degraded and the phosphate produced is donated to regenerate ATP. This leads to CR accumulation in muscle which will be rephosphorylated during the recovery process after exercise. PCR, thus, is postulated to act as an energy carrier, transporting energy from the mitochondria to different high energy demanding sites in the cytosol (mainly ATPases, Walliman 1994)

A fraction of CR and CRP pool is daily converted in creatinine (CRN) for excretion. In a 70kg man, about 2g/day of CR must be replaced by exogenous dietary CR (1g/d) and by endogenous CR (1g/d, Crim et al, 1976). Constancy of endogenous CRN production and its release into the body fluids at a constant rate, and constancy for plasma levels of CRN over the 24h of a day make CRN a useful endogenous substance whose clearance may be measured as an indicator of glomerular filtration rate (CRN urinary concentrations are thus used in clinical chemistry to test renal function, and to normalize the concentrations of other substances measured in urine, Saunders 1987). The link of CRN to CR metabolism may suggest that intake of exogenous CR would influence CRN urinary concentrations. The medical importance of CRN makes it important to study the impact of CR supplementation on CRN concentrations.

Creatine intake and exercise performance

Lots of scientific studies have reported the effects of dietary CR supplementation on muscle function and exercise performance. These studies have showed that ingestion of 20g/day of

CR during 5 days leads to an augmentation of 20% of total CR in muscle (Greenhaff et al., 1994 and Harris et al., 1992). Such a supplementation also leads to accelerate CRP resynthesis during the recovery process following exercise (Greenhaff et al., 1994) and to an increase in body mass (~1kg after a week of supplementation, Balsom et al., 1993 and 1994, Harris et al., 1992).

Concerning exercise performance, some studies have reported an improvement in high intensity exercise task following CR intake (Greenhaff et al, 1993 ; Harris et al, 1993 ; Balsom et al, 1993 ; Earnest et al, 1994 ; Birch et al, 1994) and other studies, on the contrary have showed no effect of CR intake on exercise performance (Cooke et al, 1995 ; Balsom et al, 1993 ; Odland et al, 1994). All these studies suggest that high intensity, short exercise, repeated bouts of exercise and specific resting periods are necessary to allow the positive effect of CR on performance.

Relevance of this study

CR doesn't belong to the IOC list of substances whose use are forbidden during competition. Lots of athletes use or abuse creatine supplementation. Thus far, no serious ill-effects have been demonstrated, but some may be revealed in the future.

The interest of this study was to measure the impact of CR supplementation on CRN excretion, to make sure that CRN urinary concentrations can be used as an indicator of renal function and urine dilution, even when high doses of exogenous CR are taken.

Material and Methods

To measure concentrations of CR and CRN in urine and serum, we used an adapted methodology previously described (Jaffé, 1886).

To measure CRN urinary and seric concentrations (**a**), a 250µl aliquot of the biological sample was analysed without pretreatment, with an automated Jaffé reaction with Cobas Mira (Roche ®).

To measure CR concentrations, the pH of the urine samples was adjusted between 1.3 and 2.5 with HCl concentrated. Then 2ml of the sample were heated at 150°C for 30min. 250µl of the sample was analysed with Cobas Mira. The measured concentration of CRN is including the CR transformed in CRN and the CRN present in the urine and partly degraded by the conditions of transformation (**d**).

Then, calibration curves for CRN (**Crn**), CRN degraded by the transformation conditions (**Crn***), and CR transformed into CRN (**Cr***) are established.

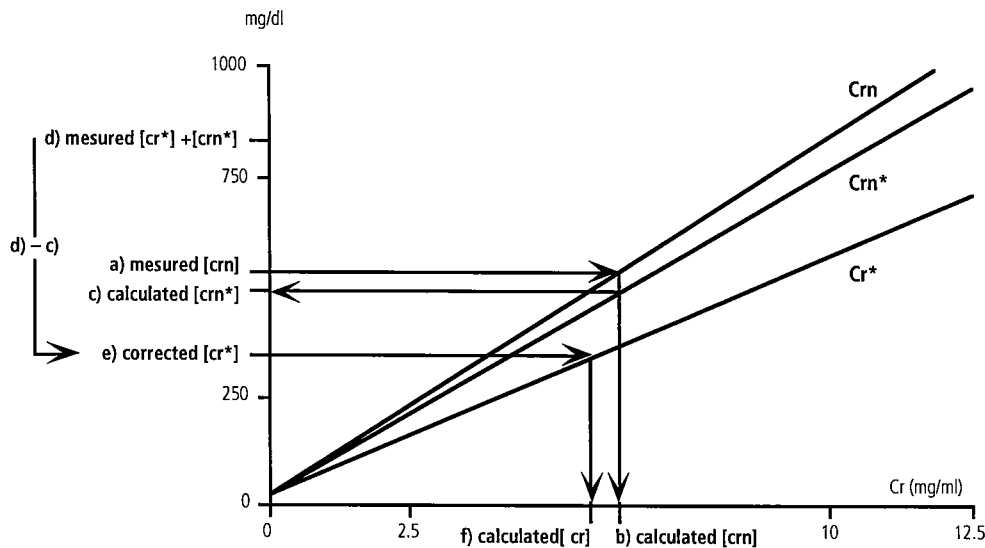


Fig. 1 : Principle of application of the transformation method of CR in CRN. Measure with the Cobas Mira CRN concentrations (**a**) and CR + partly degraded CRN concentrations (**d**). Calculate CRN real concentration with the calibration curve **Crn** (**b**). Then, establish the amount of degraded CRN (**c**) with the calibration curve **Crn***. Calculate the concentrations of CR without CRN (**e**) subtracting **d-c** and finally the real concentration of CRN (**f**) using calibration curve **Cr***. Values **b** and **f** correspond to CRN and CR concentrations present in the urine sample.

Results

Excretion study

Two male volunteers were supplemented with 20g CR per day for 5 days. (Kreatin by Alcofit, Galenica, Bern). Each miction was analysed (volume, CR concentration, CRN concentration, pH, specific gravity, T/E ratio).

Evolution of different biochemical parameters in urine (see Fig 2)

- *CRN concentration per miction.* CR intake doesn't influence CRN excretion in urine. There is no difference in CRN excretion between, during and after CR intake. The fluctuations correspond to daily normal variations. These observations agree with another study

showing that CRN excretion in urine varies little with the quantity and nature of the diet (Narayanan and Appelton, 1980).

- *CR in miction.* Intake of CR influences its excretion in urine. We observe a peak of excretion about 5 hours after the intake of 20g CR. The concentrations are back to physiologic values after about 24 hours.
- *CR and CRN per day.* CRN excreted per day is not modified by the CR supplementation. The values we measured (~30mg/day/kg) tally with the mean excretion of normal non supplemented person (Ciba Geigy, 1981).

CR excreted per day is highly increased by CR supplementation. The values we measured (~143mg/day/kg) are about 350 fold higher than that of mean excretion of normal non supplemented person (~0.42mg/day/kg, Ciba Geigy, 1981). About 10g per day are excreted when 20g CR is taken. The other 10g may be captured in muscles or excreted in perspiration, bile, etc. Another possibility is the excretion in urine into another form.

- *pH.* CR intake seems to have a slight regulating effect on pH. The pH fluctuations seems to follow the appearance of CR in miction every day of supplementation.
- *Specific gravity.* Important variations of specific gravity are visible during and after the supplementation. The mean value of the specific gravity during supplementation is 1.029, when after supplementation it's 1.024. To test whether the high specific gravity values are correlated with high CR concentrations, the ratios CR/specific gravity and CRN/specific gravity were calculated.

Excretion study

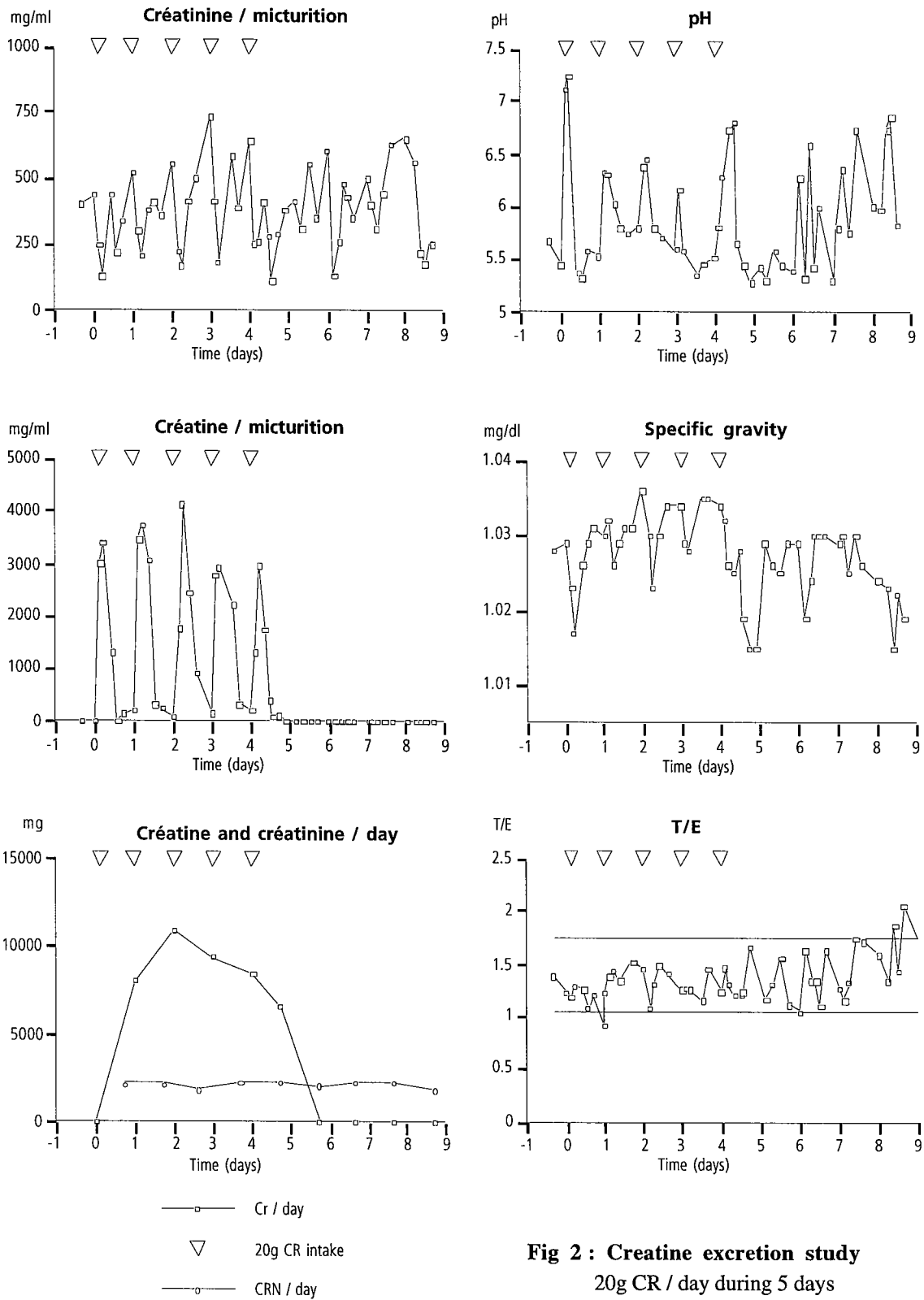


Fig 2 : Creatine excretion study
20g CR / day during 5 days

- *CR/specific gravity and CRN/specific gravity ratio* (fig. 3). These ratios represent the proportion of CR (CRN) among all the molecules composing urine (and so their contribution to the urine specific gravity). We can see that the days of CR supplementation, there are falls of the ratio CRN/specific gravity corresponding to peak of the ratio CR/specific gravity. The presence of high concentration of CR in urine seems to increase slightly its specific gravity.

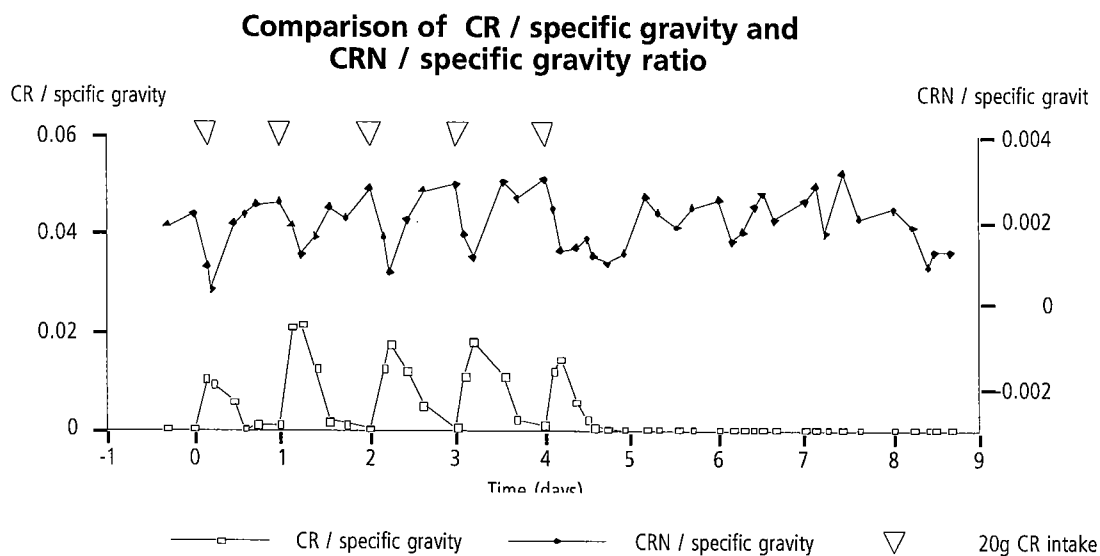


Fig 3 : Comparison of CR/specific gravity and CRN/specific gravity ratio, during CR supplementation (20g CR / day during 5 days).

- *Testosterone/Epitestosterone ratio (T/E)*. This ratio was tested to make sure that CR intake has no effect on the production (excretion) of these hormones. A hypothesis was that the ergogenic effect of CR was due to a modification in the production (excretion) of these hormones. No difference is observable before, during and after CR supplementation. The fluctuations do not exceed 25% of the mean, which is accepted as normal intra-individual variations. (Data not shown)

Evolution of CR and CRN concentrations in serum

As shown on fig 4, CRN concentrations are not modified by CR intake. The CR concentrations show an important peak about 3 hours after the intake of 20g CR (0.4mg/ml) the concentrations fall to physiological values (0.01mg/ml) 9 hours after the intake. It is important to remember that our method of quantification of CR and CRN leads to a slight over evaluation of the concentrations, due to the presence in the serum of non-specific chromagens. The kinetic of apparition of CR in the serum shown here is similar to the one previously described by another study (Harris et al, 1993).

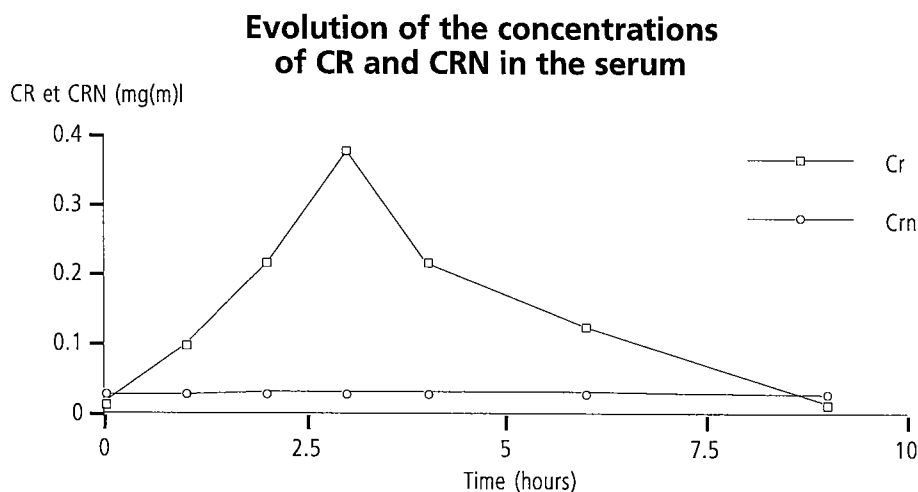


Fig 4 : Evolution of the concentrations of CR and CRN in serum, after 20g CR intake.

Stability study (see Fig 5)

Anti doping routine controls are performed on two urines samples (A and B). The laboratory has to keep sample B as long as three months. It is thus important, as so many athletes take high doses of CR, to make sure that high concentrations of CR in urine do not lead to urine modifications (variations of CRN concentrations due to transformation of CR into CRN for exemple). This hypothesis made us test the stability of urines over a three months period.

Five urine samples were analysed during three months. Each sample was divided into two parts, of which one was spiked with 10mg/ml CR, and the other analyzed without any treatment. Each part was then separated into three parts, conserved at different temperatures : one part at -20°C (2ml aliquots), another at 4°C and the last at room temperature (RT). We analysed over three months the variations of pH, CR and CRN concentrations.

- *pH*. We observe an increase in pH values over three months specially at 4°C and RT. An explanation for this augmentation is the growth of bacteria in the urine sample, which modifies the pH.
- *CR and CRN*. The CRN concentrations are stable over a three months period. A slight augmentation is visible for the sample 4°C. This augmentation could be explained by the apparition in the urine of non specific chromagens reacting with the colorimetric test.

The CR concentration is less stable than the CRN concentrations, and we can observe, especially at 4°C after the third week of conservation, a decrease in the CR concentrations. We explain this by the fact that there could be special conditions in urine leading to CR instability. The CR is not converted into CRN as there is no corresponding augmentation of CRN concentrations, but could be present in another form of degradation. Another explanation could be the precipitation of CR in the sample leading to the analysis of only part of the CR present in the sample.

Conclusions

- Creatine concentrations in both urine and serum are not altered by CR supplementation. This means that CRN concentrations are stable and can be used as an indicator of urine concentration, even when high doses of CR are taken.
- Creatine concentrations in urine are greatly raised by CR supplementation. About half of the dose is found in the urine, the rest is either stocked in muscle, or excreted under another form of excretion.
- The specific gravity of the urine seems to be rised by CR supplementation, then CR could mask a low specific gravity.
- CR seems to have a slight modulating effect on pH.
- CR intake doesn't alter T/E ratio in urine.
- The presence of high concentrations of CR in urine doesn't alterate the conservation of urine over three months. CRN concentrations remain stable.

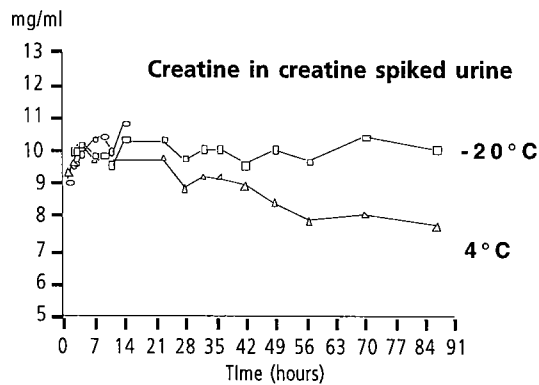
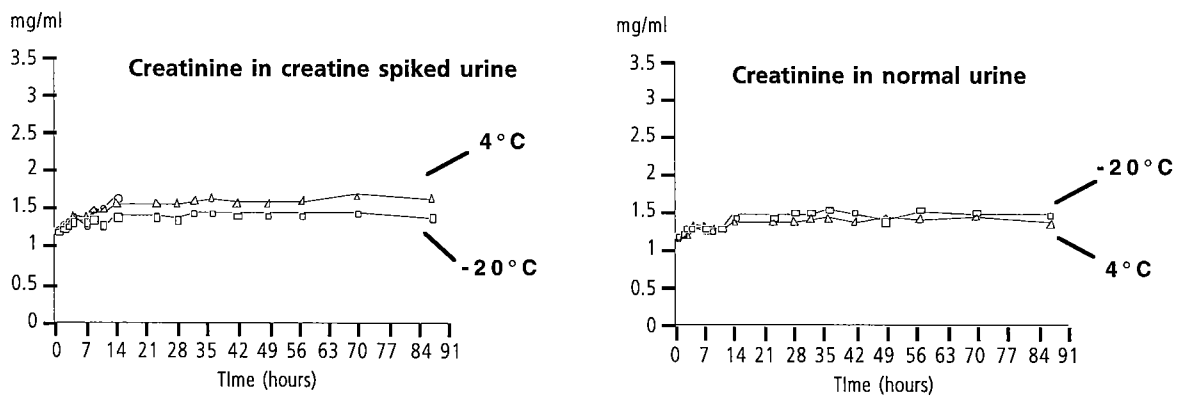
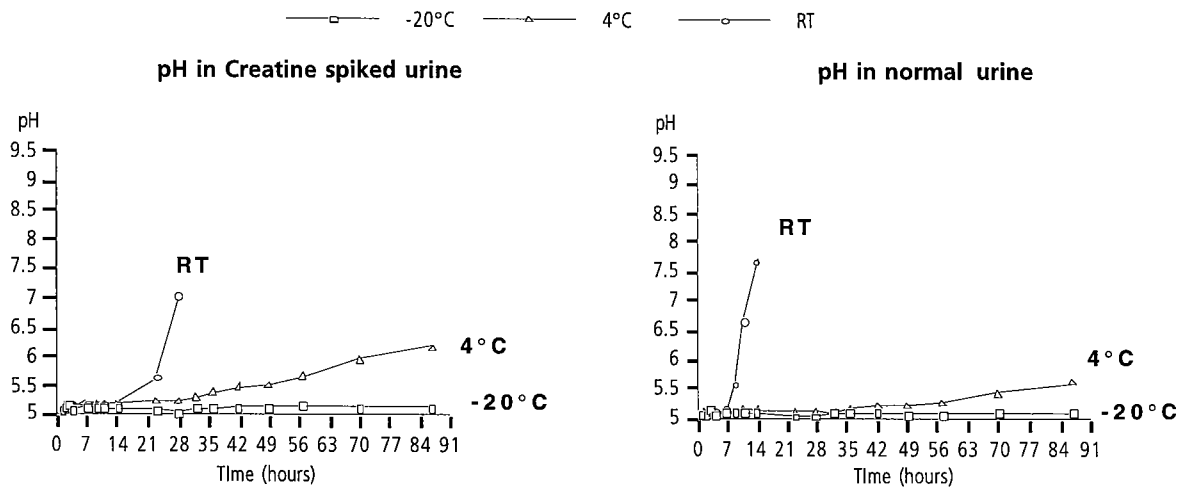


Fig 5 : Stability study over a period of three months
 Spiked urines contain 10mg/ml CR

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