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# Vehicle influence on potassium replacement effectiveness in hypokalemic rats

## *Influência do veículo na eficácia da reposição de potássio em ratos hipocalêmicos*

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*Abstract*

***Introduction:*** **Patients who undergo cardiac surgery are commonly treated with diuretic therapy for the management of volume overload. The concern of hypokalemia in adult before, during and after surgery has already been described since there is risk of cardiac arrhythmia. Clinically, intravenous potassium (K+) replacement dilution is only recommended with sodium chloride 0.9% solution (SF0.9%), likely due to the putative effects of glucose solution 5% (SG5%) on insulin secretion, which influence K+ replacement quality. However, it is not yet experimentally proved the influence of SF0.9% and SG5% on K+ replacement quality in patients with hypokalemia.**

***Objectives:*** **To assess the effects of different vehicles of K+ replacement on blood K+ levels in furosemide hypokalemic rats.**

***Methods:*** **Male Wistar rats were divided into four groups: K++SF, K++SG, SF and SG. Jugular vein was cannulated for K+ replacement and femoral vein was cannulated for blood analysis were performed. Furosemide (50mg/kg) was injected S.C. to induce hypokalemia. It was analyzed potassium plasmatic levels 24 hours before furosemide injection, 24 hours after furosemide injection and after 30 minutes post-replacement.**

***Results:*** **There was reduction in post-injection of K+ levels when compared to the basal values (pre-furosemide) in all groups. However, the levels [K+] returned to baseline in both groups receiving K++SF or K++SG, which was not observed in groups receiving only SF and SG. Only K+SF presented increased after K+ replacement ($P < 0.05$).**

***Conclusion:*** **K+ replacement diluted both in SF and SG did not affect blood K+ levels in rats.**

***Descriptors:*** **Hypokalemia. Potassium. Vehicles.**

*Resumo*

***Introdução:*** **Pacientes após cirurgia cardíaca são comumente tratados com diuréticos para controle de volume plasmático. A preocupação de distúrbios hipocalêmicos em adultos antes, durante ou após a cirurgia já foi ressaltada anteriormente, visto o risco de arritmias cardíacas. Clinicamente, a diluição da solução de potássio (K+) para administração por via intravenosa, em situações que requerem a sua reposição é realizada utilizando-se soro fisiológico (SF) ao invés de soro glicosado 5% (SG5%), possivelmente em vista de poder ocorrer estimulação da secreção de insulina, que interferiria sobre a qualidade da reposição de K+. Porém, não está comprovado experimentalmente se o SF e SG5% poderiam realmente interferir na qualidade da reposição de potássio em pacientes com hipocalemia.**

***Objetivo:*** **Analisar a influência da reposição de K+ diluído em diferentes veículos sobre as concentrações plasmáticas**

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**de K+([K+]p) em ratos submetidos a hipocalemia induzida por furosemida.**

***Métodos:*** **Ratos Wistar adultos foram divididos em quatro grupos: K++SF, K++SG, SF e SG. Foi realizada a canulação da veia jugular para reposição e da veia femoral para coleta de sangue. O diurético furosemida na dose de 50mg/kg foi usado para induzir hipocalemia, foi analisado nível plasmático de potássio 24 h antes da injeção de furosemida, 24 h pós-indução e 30 minutos pós-reposição.**

***Resultados:*** **Os níveis da [K+] pós-injeção de furosemida sofreram redução, comparado aos valores basais (pré-furosemida) em todos os grupos. Entretanto, os níveis [K+] retornaram aos valores basais tanto nos grupos que receberam K++SF ou K++SG, o que não foi observado nos grupos que receberam apenas SF e SG. Quanto ao Na+ plasmático, somente o grupo K+SF apresentou aumento após reposição.**

***Conclusão:*** **A reposição de K+ diluído tanto em SF quanto SG parece não afetar a qualidade da reposição de K+ plasmático em ratos.**

***Descritores:*** **Hipopotassemia. Potássio. Veículos.**

## INTRODUCTION

The fulfillment of preoperative hydration in patients who will undergo surgical trauma and therefore will be at risk of increased release of vasopressin and aldosterone, has aimed to maintain a euvolemic state, as well as ideal plasma concentrations of electrolytes such as sodium (Na+) and potassium (K+). In respect to hydration with sodium overload, it aims to inhibit the release of vasopressin and aldosterone, which can affect the body's response to surgery [1].

Concern about pre- and postoperative hypokalemic disorders in adults undergoing heart surgery has been shown in previous studies [2]. After heart surgery, the patient is usually treated with diuretics to modulation of plasma volume and pulmonary and peripheral edema [3]. Moreover, due to the association of cardiac arrhythmias with disorders of K+, it is necessary to perform an early diagnosis of this condition, as well as physiopathological mechanisms involved with this condition before any surgical procedure, so that therapeutic measures can be taken aiming at maintaining homeostasis [4].

The treatment of hypokalemia can be performed through the administration of $K^+$ intravenously and its dilution is clinically recommended using only saline solution (SS) and not 5% glucose solution (GS 5%). The justification for this behavior is due to the possible effect that could be caused by the 5% GS, or that is, stimulation of insulin secretion that could thus interfere negatively on the quality of the replacement of $K^{+2}$. However, it is not proved that the 5% SS or GS vehicle that would dilute the $K^+$ could actually interfere on the quality of this electrolyte replacement. Therefore, the data from literature seem conflicting in respect to the use of glucose and $K^+$ in coronary artery bypass grafting [4,5].

Therefore, the aim of our study was to analyze the effects of replacement of $K^+$ diluted in 5% SS or GS on plasma concentrations of $K^+$ in rats undergone furosemide-induced hypokalemia.

## METHODS

### Animals

We used 24 adult Wistar rats weighing between 350 and 460 g provided by the bioterium of the ABC Medical School (FMABC). The animals used in the experiment were divided into four groups: potassium and saline solution group (K+SS; N=6); potassium and glucose solution group (K+GS; N=6); saline solution control group (SS; N=6) and 5% glucose solution control group (GS; N=6). The temperature and humidity of the bioterium were monitored and maintained around 22ºC and relative humidity of 60%, respectively. The light/dark cycle was also monitored and established as 12 hours each and before accomplishment of the experiments the animals had free access to water and food (Nuvilab). All procedures in this study were approved by the Animal Experimentation Ethics Committee of FMABC (protocol No. 003/2007).

The induction of hypokalemia was performed five days after surgery of cannulation of the femoral artery and jugular vein, then the animals were maintained for 24 hours without food and water (Nuvilab).

### Cannulation of the jugular and femoral vein

During surgical procedures, the animals were anesthetized with halothane (2% mixed with 100% oxygen) administered via inhalation. The cannula implanted in the external jugular vein consisted of polyethylene tubing PE-50 (Clay Adams, NJ, USA), which is prepared so that to leave an area about 30 mm inside the blood vessel and another spare area with 70 mm at its proximal end. It was performed a melting point between these two tubes to control entry into the vascular bed. The femoral vein was cannulated using polyethylene tube PE-50 (Clay Adams, NJ, USA), 17 cm long connected to a silicone tube PE-10 (VWR International West Chester Pennsylvania, USA) of 10 cm in length . The two tubes were welded and shaped so that to present an angle of about 60° between the silicon tip and the polyethylene

tip (Figure 1) to allow a better anatomical fit during implantation. In addition to that, we performed a silicone button within at ± 5mm from the melting point of the two tubes to narrow its entry into the femoral vein.

During and after the implantation of cannulae, they were maintained patent by infusing of a solution of 1UI/mL of heparin in saline solution. We used low concentration of anticoagulant to avoid the appearance of cases of pseudo-hyperkalemia, which could alter the results of plasma $K^+$ concentrations. The permeability of the cannulae was maintained by infusion of this solution every two days. As a prophylactic measure after the surgical procedure, the animals received intramuscular injections of veterinary pentabiotic (2000 U/mL, Fort Dodge) and were maintained under observation during recovery from anesthesia.

**Connector for blood collection and infusion solutions**

In order to not perform the infusion of replacement solutions by the same route of blood collection, it was prepared a connector with marlex mesh of 10x10mm and two stainless steel tubes (23 Gauge) with 2 mm long, twisted at an angle of 90°, which were settled on this mesh with epoxy glue sustained on the skin of the back of the animal (Figure 1). In order to not allow blood reflux when using this connector, its outlet was protected with polyethylene PE-50 (Clay Adams, NJ, USA) of 10 mm with one of its end fused.

**Analysis of plasma**

After 24 hours without solid food, it was performed collection of 1 mL of blood through the cannula in the jugular vein and transferred to an Eppendorf tube. The blood was centrifuged (Costar microcentrifuge) for 10 min at a speed of 1000 rpm to separate serum and plasma. After this procedure, 500 µL of plasma was pipetted and transferred to a new Eppendorf tube that was immediately sent to the Laboratory of Clinical Analysis of FMABC. The levels of plasma sodium and potassium were determined using ion-selective electrode, through the equipment RAPID LAB 348-BAYER. Blood samples for analysis of $[K^+]_p$ were obtained in three separate stages: 1) before hypokalemic induction (control), 2) 24 h after furosemide injection and

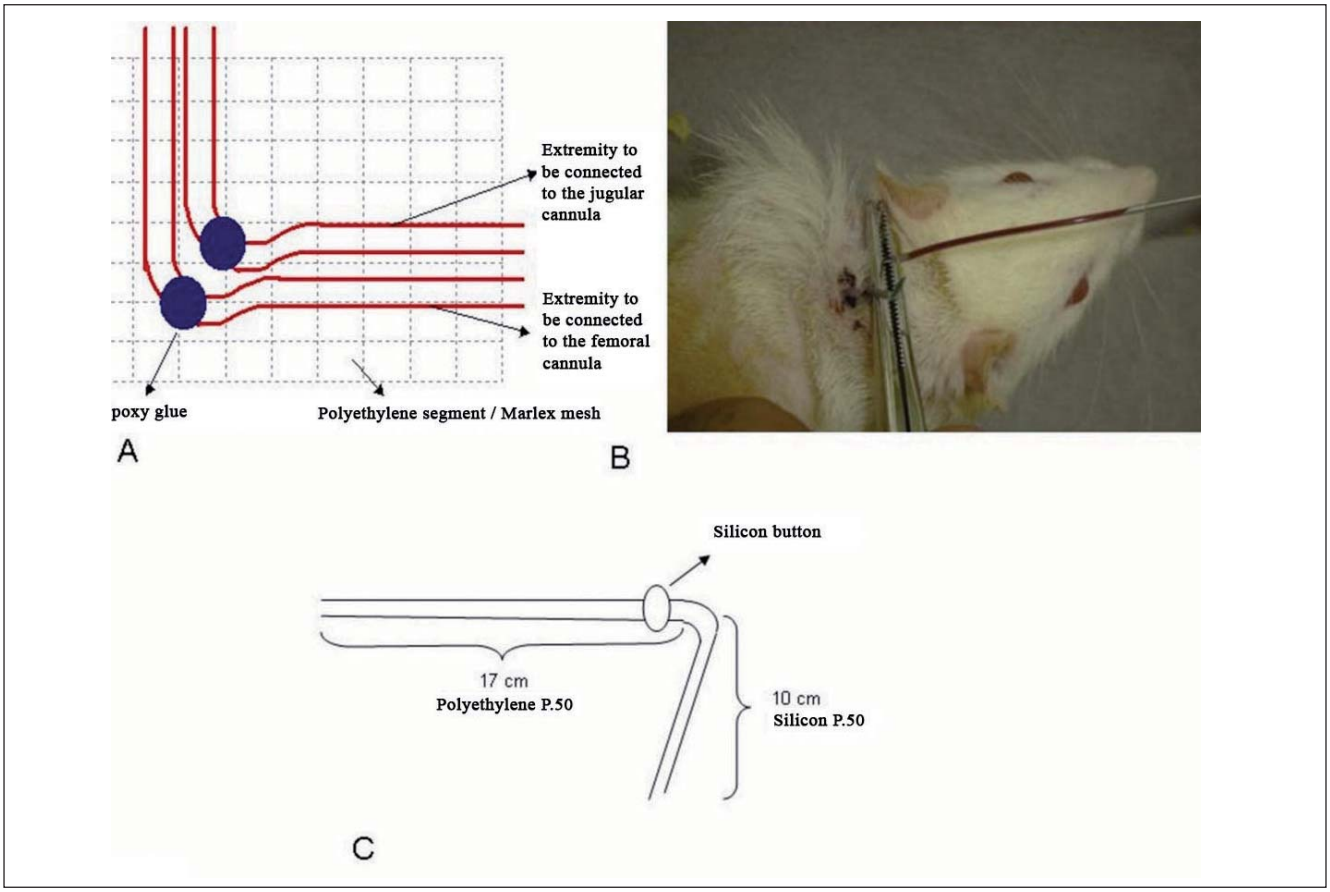


***Fig. 1** – Scheme of venous and arterial cannulas and connectors for blood replacement and collection*

detection of hypokalemia and 3) 30 minutes after infusion of solutions replacement.

**Hypokalemic induction**

Five days after surgery, induction of hypokalemia was performed, using furosemide (50 mg/kg, SC). The animals were maintained in individual boxes and supplied with 200 mL of water from water fountain for 24 hours.

**Replacement solutions**

After detection of hypokalemia by plasma analysis, the rats were connected to the syringe that was filled with one of the following solutions: 0.4 mL of 19.1% KCl (1 mEq) + 1.6 mL of saline solution in the K+SS group; 0.4 mL of 19.1% KCl (1 mEq) + 1.6 mL of glucose solution in the K+GS group; 2 mL of saline solution in the SS group (Control) and 2 mL of 5% glucose solution in the GS group (Control). These solutions were infused into the animal with the aid of a volumetric infusion pump of SAMTRONIC ST 690 syringe scheduled in 30 minutes (0.15 mL/min).

**Statistical analysis**

The results were expressed as mean ± standard error of the mean and subjected to two-way Analysis of Variance (ANOVA), followed by Tukey post-test. The level of significance was accepted as $P<0.05$.

RESULTS

**Hypokalemic Induction**

In the first pilot experiments (data not shown) performed to induce hypokalemia, it was used the dose of furosemide of 10mg/Kg. This was used in previous studies to induce sodium appetite, however, it was not found a decrease in plasma concentration of $K^+$ in our pilot studies. Thus, the doses of 20 mg/kg, 30 mg/kg, 40 mg/kg and 50 mg/kg were incremented in pilot studies to assess the dose-dependent responses, being observed hypokalemia only at a dose of 50 mg/kg, which determined the dose of 50 mg/kg adopted in this study.

**Analysis of plasma $K^+$**

Prior to subcutaneous injection of furosemide was not observed significant difference ($p>0.05$) of values of plasma $K^+$ between the groups K+SS (4±0mEq/L), (4±0mEq/L), SS (4m±0Eq/L) e GS (4±0mEq/L) (Figure 2). All animals showed significant decreases ($P<0.05$) of values of plasma K+ after 24 hours of administration of furosemide (K+SS =3±0mEq/L, K+GS = 3±0mEq/L, SS = 3±0mEq/L and GS = 3±0mEq/L), and there was no significant difference ($P>0.05$) between groups. Shortly after 30 minutes of the K+ replacement (Figure 2), there was no significant difference between group SSK and GSK groups (P> 0.05). However, there was a significant difference when comparing the values between the control SS and GS groups ($P<0.05$) K+SS = 4±0mEq/L, K+GS = 4±0mEq/L, SS = 3±0mEq/L and GS= 3±0mEq/L).

**Analysis of plasma $Na^+$**

The concentration of plasma $Na^+$ before the effects of furosemide was higher in group K+SS ($P>0.05$), while the GS group showed the lowest values ($P>0.05$) (Figure 3) (K+SS = 143,17±3mEq/L, K+GS = 142±3mEq/L, SS = 142±1mEq/L and GS = 141±1mEq/L). After 24 hours of administration of the diuretic, the K+SS showed again the highest values of plasma $Na^+$ ($P>0.05$), while the two control groups showed the lowest values ($P>0.05$). It was observed signficant drop of plasma levels of $Na^+$ in the K+SS, K+GS, SS and GS groups ($P<0.05$) (K+SS = 141±3mEq/L, K+GS = 140±2mEq/L, SS = 139±2mEq/L and GS = 139±2mEq/L). After 30 minutes of the $K^+$ replacement (Figure 3) we found significant differences ($P<0.05$) of plasma concentrations of $Na^+$ between K+SS and K+GS ($P<0.05$). On the other hand, no significant difference was found when comparing the values between the SS and GS control groups ($P>0.05$) (K+SS = 143±3mEq/L, K+GS = 140±3mEq/L, SS = 139±2mEq/L and GS = 139±2mEq/L).

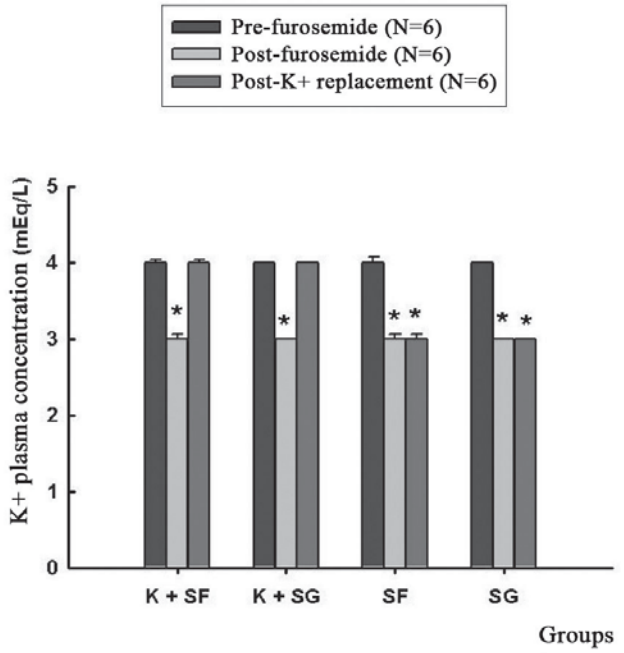


***Fig. 2** – $K^+$ Plasma concentration (mEq/L) in rats pretreated with furosemide, in the groups receiving potassium replacement with saline solution (0.9% K+SS) or glucose solution (5% K+GS) and the control groups that received only saline solution (0.9% SS) or glucose solution (5% GS) and did not receive $K^+$ replacement. *P<0.05; Unlike from before hypokalemic induction*

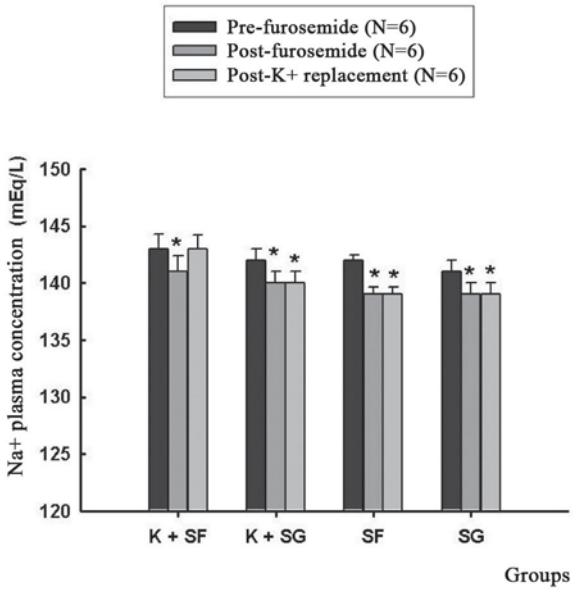


***Fig. 3*** *– $Na^+$ plasma concentration (mEq/L) in rats pretreated with furosemide, in the groups receiving potassium replacement with saline solution (0.9% K+SS) or glucose solution (5% K+GS) and in the control groups that received only the saline solution vehicle (0.9% SS) or glucose solution (5% SS) and did not receive $K^+$ replacement. *P <0.05; Unlike before hypokalemic induction*

## DISCUSSION

Our study examined the effect of $K^+$ replacement diluted in saline solution or 5% glucose solution on plasma concentratins of $Na^+$ and $K^+$ in rats undergone furosemide-induced hypokalemia. It was observed that the administration of furosemide at a dose 50 mg/kg was sufficient to cause hypokalemia. Regarding the $K^+$ replacement, the results obtained during this study were not different from the findings on glucose to affect the $K^+$ replacement in cases of hypokalemia observed in humans. However, interestingly, the values of plasma $Na^+$ after the replacement were higher in the group treated with saline solution when compared to other groups.

In order to induce hypokalemia to perform this study, it was taken as basis a model of $Na^+$ depletion induced by treatment with furosemide (Stellar and Epstein [6], Denton [7], Grossman [8] and Fitzsimons [9]), which has been used in studies on the mechanisms of thirst and sodium appetite. The animals in our study were fed only with water supply. Studies by Pereira et al. [10] reported that the dose of 10 mg/kg (SC route) of furosemide induces a maximum and rapid loss of water and electrolytes - including $Na^+$ and $K^+$. Our results, however, showed no hypokalemia using this dose. Thus, this study has began in accordance with the model described, but without success. In order to induce hypokalemia in experimental study with animals, we used the principle of dose-dependence, increasing the dose of furosemide with subsequent assessment of the levels of plasma $K^+$. The doses of 20, 30, 40 and 50 mg/kg (SC route) were used, and hypokalemia was observed only when we used the dose of 50 mg/kg of this drug.

We observed during the development of this experimental protocol, that after three days of treatment with subsequent doses of furosemide, the animals did not present hypokalemia, a fact that may be related to the declining of furosemide action in rats under $K^+$ depletion, as suggested in studies of Hropot et al. [11]. In this study, it was reported that deprivation of $K^+$ can reduce the binding of furosemide in its areas of action. Moreover, this phenomenon may be related to the findings of Stummer et al. [12], which suggest that the increased influx of $K^+$ to the intracellular environment in acute hypokalemia is compensated by the stimulation of K+ efflux via $Na^+$-$K^+$-ATPase to restore the balance of $K^+$ in cerebrospinal fluid. However, this was not observed in our study. Thus, adaptation to the doses of furosemide in our experimental conditions remains to be determined.

Since the negative consequences of potassium replacement orally in patients after CABG procedure were observed previously [3], we used the intravenous administration in our study as a proposal treatment. The results of our study showed that replacement of $K^+$ diluted in saline solution and separately in glucose solution, did not change the quality of the $K^+$ replacement in animals undergone furosemide-induced hypokalemia.

A study by Sweeney [13] showed that the association of insulin with glucose could therefore promote the appearance of hypokalemia. This could difficult the correction of $K^+$ levels, due to the redistribution of this ion to the intracellular environment. Study by Muntzei et al. [14] showed that hyperinsulinemia increases sympathetic activity and reduces $K^+$ levels in plasma, because it activates the renin-angiotensin-aldosterone system. Their study showed that hyperinsulinemia caused decreased levels of $K^+$ plasma, but in our study there was no injection of insulin, which seems to be one of the hypotheses for the absence of changes in the replacement of the groups assessed.

It is noteworthy to emphasize that in humans it is not recommended to use the 5% GS vehicle during the $K^+$ replacement, since glucose stimulates an increase in insulin and some authors [15-17] reported that this fact stimulates the influx of $K^+$ to the intracellular invironment, suggesting a difficulty in reaching a state of normokalemia. However, this effect was not observed in experimental animals in our study. One hypothesis that could explain the non-interference of this vehicle in replacement of $K^+$ is the fact that it is iso-osmotic in relation to plasma. The differences

between the SS is mainly due to the osmolar gap when compared with the 5% GS. The first has its osmolarity around 308 mOsm/L while the second 252 mOsm/L, which makes it clear that, despite the difference of 46 mOsm/L, the two solutions are iso-osmotic in relation to plasma and such solutions are considered important in volume replacement in clinical practice because they do not affect the osmotic balance. Thus, further studies are needed to clarify whether there would be changes in insulin concentration and $K^+$ plasma during the infusion of hyperosmolar solutions of glucose in experimental animals.

The intriguing fact that the plasma concentration of $Na^+$ after the $K^+$ replacement was higher in the K + SS compared with other groups suggests that the replacement of $K^+$ diluted in SS in the case of hypokalemia, corroborates for maintenance of plasma levels of $Na^+$, in addition to effectively restore $K^+$ plasma levels.

Summarizing, according to our findings, potassium replacement in SS or 5% GS vehicles did not affect the quality of replacement of $[K^+]_p$ in rats with furosemide-induced hypokalemia.

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REFERENCES

1. Moriya T, Martins ACP, Cherri J, Piccinato CE, Okano N, Carneiro JJ, et al. Hidratação e equilíbrio ácido-base em pacientes cirúrgicos. Acta Cir Bras. 2000;15(supl 2):34-8.

2. Hastings LA, Wood JC, Harris B, Von Busse S, Drachenberg A, Dorey F, et al. Cardiac medications are not associated with clinically important preoperative electrolyte disturbances in children presenting for cardiac surgery. Anesth Analg. 2008;107(6):1840-7.

3. Norris W, Kunzelman KS, Bussell S, Rohweder L, Cochran RP. Potassium supplementation, diet vs pills: a randomized trial in postoperative cardiac surgery patients. Chest. 2004;125(2):404-9.

4. Sánchez LT, Fernández FA, Infante ED, Ramos JM. Arrhythmias and cardiac electrophysiology. Rev Esp Cardiol. 2008;61(Suppl 1):27-36.

5. Barcellos CS, Wender OCB, Azambuja PC. Glicose insulina e potássio (GIK) na revascularização do miocárdio de pacientes diabéticos: ensaio clínico randomizado. Rev Bras Cir Cardiovasc. 2007;22(3):275-84.

6. Stellar E, Epstein AN. Neuroendocrine factors in salt appetite. J Physiol Pharmacol. 1991;42(4):345-55.

7. Denton D. The hunger of salt: an anthropological, physiological and medical analysis. 2nd ed. London:Springer-Verlag;1984.

8. Grossman SP. Extracelular thirst and sodium appetite: physiological basis. London:Academic Press;1999. p.43-60.

9. Fitzsimons JT. Angiotensin, thirst, and sodium appetite. Physiol Rev. 1998;78(3):583-686.

10. Pereira DT, David RB, Vendramini RC, Menani JV, De Luca LA Jr. Potassium intake during cell dehydration. Physiol Behav. 2005;85(2):99-106.

11. Hropot M, Klaus E, Unwin R, Giebisch G. Diminished diuretic and natriuretic response to furosemide in potassium-depleted rats. Ren Physiol Biochem. 1994;17(1):10-20.

12. Stummer W, Betz AL, Keep RF. Mechanisms of brain ion homeostasis during acute and chronic variations of plasma potassium. J Cereb Blood Flow Metab. 1995;15(2):336-44.

13. Sweeney RW. Treatment of potassium balance disorders. Vet Clin of North Am Food Anim Pract. 1999;15(3):609-17.

14. Muntzei MS, Joseph T, Onwumere O. Effect of insulin-induced hypokalemia on lumbar sympathetic nerve activity in anesthetized rats. J Hypertens. 2004;22(9):1755-60.

15. Phillips LD. Manual de terapia intravenosa. 2ª ed. Porto Alegre:Art Med;2001. p.86-90.

16. Potter PA, Perry AG. Grande tratado de enfermagem prática: clínica e prática hospitalar. 3ª ed. São Paulo:Santos Livraria Editora;2001. p.805-20.

17. Riella MC. Princípios de nefrologia e distúrbios hidroeletrolíticos. 4ª ed. Rio de Janeiro:Guanabara Koogan;2003. p.215-35.