Study of Renal Tubular Cell Damage by Oxidative Stress in Potassium Deficient Rats

การศึกษาการทำลายเซลล์เยื่อบุผนังท่อไตเนื่องจากกระบวนการออกซิเดชั่นในภาวะขาดโพแทสเซียมในหนูแรต

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ABSTRACT

Our aim was to study the effect of potassium (K^+) depletion (KD) on oxidative damage of renal tubular cells. Male Sprague - Dawley rats were fed three periods with normal diet (control group) and K^+ deficient diet for 7, 14 and 21 days (KD group). After blood and 24-hour urine samples were collected at the end of each period, rats were scarified to obtain kidneys and soleus muscle. Plasma and muscle were analyzed for K^+ content and kidney for reactive oxygen species (ROS) and superoxide dismutase (SOD; both CuZnSOD and MnSOD) activity and urine for N-acetyl-β-glucosaminidase (NAG). Histological pattern changes of the kidneys were also examined. The results showed that K^+ contents of KD group were significantly decreased in both plasma and muscle. While the ROS formation and MnSOD activity were significantly increased ($p<0.05$), the Cu Zn SOD activity was unchanged in the KD group. The NAG activity in urine of the KD group was significantly increased ($p<0.05$). These changes were clearly observed since after the 7 day of feeding. Changes in histological appearance were less interstitial lesion, diffuse epithelial cell swelling and vacuolar degeneration. Our results indicate potassium depletion can cause renal tubular epithelial oxidative damage especially after 14 and 21 days of feeding .

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อดูผลของภาวะพร่องโพแทสเซียมที่มีต่อการทำลายเซลล์เยื่อบุผนังท่อไต เนื่องจากกระบวนการออกซิเดชัน ทำการเลี้ยงหนูแรตพันธุ์ Sprague-Dawley เพศผู้ด้วยอาหารธรรมดา (กลุ่มควบคุม) และอาหารขาดโพแทสเซียม (กลุ่ม KD) เป็นเวลา 7, 14 และ 21 วัน หลังจากเก็บตัวอย่างเลือดและปัสสาวะ 24 ชั่วโมง ในแต่ละช่วงของการให้อาหารทำการฆ่าหนูเพื่อเก็บตัวอย่างกล้ามเนื้อลายชนิด soleus กับไต ทำการวิเคราะห์หาปริมาณโพแทสเซียมในพลาสมาและกล้ามเนื้อลาย ปริมาณสารอนุมูลอิสระของออกซิเจน (ROS) และการทำงานของเอนไซม์ superoxide dismutase (SOD) ทั้งชนิด CuZnSOD และ MnSOD) ในไตและวัดอัตราการทำงานของเอนไซม์ N-acetyl-β-glucosaminidase (NAG) ในตัวอย่างปัสสาวะรวมทั้งดูลักษณะการเปลี่ยนแปลงของเนื้อเยื่อไต ผลการศึกษาพบว่าระดับโพแทสเซียมในทั้งของพลาสมาและกล้ามเนื้อของหนูกลุ่ม KD ลดลงอย่างมีนัยสำคัญ ($p<0.05$) ในขณะที่ปริมาณของ ROS และอัตราการทำงานของ MnSOD ในกลุ่ม KD เพิ่มขึ้นอย่างมีนัยสำคัญ ($p<0.05$) อัตราการทำงานของ CuZnSOD กลับไม่เปลี่ยนแปลงมากนัก สำหรับอัตราการทำงานของเอนไซม์ NAG ในตัวอย่างปัสสาวะของกลุ่ม KD ก็พบว่าเพิ่มขึ้นอย่างมีนัยสำคัญ ($p<0.05$) การเปลี่ยนแปลงของค่าต่างๆ เหล่านี้สังเกตเห็นได้ชัดเจนตั้งแต่วันที่ 7 ของการให้อาหาร การเปลี่ยนแปลงของเนื้อเยื่อไตทางกายวิภาค พบว่าเซลล์เยื่อบุผนังท่อไตเกิดการบวม และมีการเสื่อมแบบมีถุงน้ำในเซลล์ การศึกษาของเราในครั้งนี้ชี้ให้เห็นว่าภาวะพร่องโพแทสเซียมสามารถทำให้เซลล์บุท่อไตเกิดการเสื่อมและทำให้เกิดการบาดเจ็บจากภาวะออกซิเดชัน โดยเฉพาะในระยะเวลา 14 และ 21 วัน หลังให้อาหารขาดโพแทสเซียม

Key Words : Potassium depletion, Oxidative stress, Renal tubular injury
คำสำคัญ : ภาวะพร่องโพแทสเซียม ภาวะออกซิเดชั่น การบาดเจ็บของท่อไต

Introduction

Chronic K^+ deficiency in rats results in prominent tubular and interstitial lesions (Tolins et al, 1987). Though It has been shown that low- K^+ diet is a risk factor in the development of hypertension (Coruzzi et al, 2002, Suga et al, 2001). In addition, low- K^+ concentration result in a high rate of free radical formation in endothelial and MDCK cell lines (Zhou et al, 2002). Moreover, ROS were found marked increased in superoxide anion formation by carotid areterial segment in the rabbit fed with low- K^+ diet (Yang et al., 1998). McCabe et al.,(1994) also found that the physiological K^+ concentration can inhibit the rate of superoxide anion production in endothelial and monocyte cell lines. Therefore the aim of this study is to evaluate the effect of low- K^+ diet on oxidative damage to rat kidneys. The superoxide anion ROS formation, the first line antioxidant defense SOD, and urinary cellular damage enzyme NAG were measured. The kidney tissues morphology were evaluated.

Materials and Methods

1. Chemicals and rat diets

All chemicals were analyzed grade purchased from and Fluka chemika Co. Ltd., Buchs, Switzerland, and Sigma Chemical Co. Ltd., St. Louis, USA.

2. Animals

Male Sprague-Dawley rats (n=30, 250-300g) were used in the present study. After 5 to 7 day of stabilization on a standard chew diet, they were randomly divided into two main groups as control (received normal diet) and low K^+ (KD) groups . These two main groups were further subdivided into three small groups (n=5) each and fed for a period of 7, 14 and 21 days, respectively. During feeding, deionized water was allowed to access *ad libitum*. A 24-h urine specimens was collected with thymol as preservative from each rat at days 7, 14 and 21. All rats were then anesthetized by peritoneal pentobarbital sodium injection (100 mg/kg BW) and venous blood was collected in lithium heparin for plasma preparation. The anesthetized rats were then sacrified and the kidneys were perfused through abdominal aorta with cold phosphate buffer saline plus lithium heparin (0.16 mg/ml) at pH 7.4. Both kidneys were excised and preserved in 10% buffer formalin.

3. Rat diets

The control diet consisting of each ingredient as in g/kg diet as follow 180 casein, 200 corn starch, 500 sucrose, 35 corn oil, 35 peanut oil, 10 $CaHPO_4$, 6 $MgSO_4$, 6 NaCl, 6.8 Na_2HPO_4, 7.1 KCl (Levi et al, 1991), and modified the feed additive mixture. K^+ deficient diet was prepared using the same ingredient as control diet, but NaCl was substituted for KCl.

4. Determination of reactive oxygen species (ROS) and superoxide dismutase (SOD) activity

Rat kidneys were quickly excised and place in cold Krebs- HEPES buffer (composition in mmol / L): NaCl 119; KCl 4.6 ; $CaCl_2$1.2; $MgSO_4$ 1.0; KH_2PO_4 0.4; $NaHCO_3$ 5.0; Glucose 5.5; Na-HEPES 20.0) and then homogenized by glass homogenizer followed with the centrifugation at 12000 rpm for 10 minute (Pagona et al. 1995). The supernatant was used for the determination of ROS. The rate of superoxide anion generation was determined by chemiluminescence of lucigenin (100uM) in a 500 ul solution at 37°C for 5 minute incubation with NADPH (100uM). For each sample, the light signals were counted for 2.5 minute in a luminometer ($20/20^n$ luminometer, Tuner Biosystems). Microtube containing buffer and lucigenin alone or sample blank was counted and the buffer blank values were subtracted from the signals obtained from the kidney tissue. The counts were expressed as relative light unit (RLU) per 10 second per mg protein. For validate the method, before and during the counts, some experiments were performed holding the kidney homogenate for 5 minutes in a solution of SOD (150 U/ml), a superoxide scavenger and NaN_3 for peroxidase inhibition.

In determination of the SOD activity, both CuZnSOD and MnSOD was determined according to the method of Sun et al., (1988). The principle of the method is based on the inhibition of NBT reduction by xanthine/xanthine oxidase system as a peroxide genaerator. One unit of SOD was defined as the amount of protein that inhibits the rate of NBT reduction by 50%. Bovine SOD was used as standard.

5. N-acetyl-β-glucosaminidase(NAG) activity assay

The 24-hour urine was centrifuged at 900xg

for 10 min and the supernatant was used to assay for NAG activity by the method of Horak et al., (1981). The 0.9% NaCl used as blank and urine blank was count for subtract the urine NAG activity. *p*-nitrophenol working standard solution 10mM used. Measure the absorbance of cuvette at 410 nm (Spectronic 1201, Milton Roy Company). The molar extinction coefficient of *p*-nitrophenol absorption at 410 nm. is 8800 to correct the enzyme level.

6. Potassium content in plasma and muscle

The soleus muscle specimen was measured for K^+ content by the method of Dorup et al., (1988). The soleus muscles were excised and removed all fat and connective tissues, weighted and kept at -70°C until analyses. The sample weighted around 20-30 mg was homogenized by glass homogenizer in 2 ml of 5% tricholoacetic acid (TCA). After centrifugation, the supernatant was determined for K^+ by spectrophotometer (PU 9100X Atomic Absorption Spectrophotometer, Phillips, USA) with lithium as internal standard. The concentration of K^+ was presented as μmol/g wet weight. Plasma concentration of potassium was determined and expressed as mEq/L.

7. Plasma creatinine and protein determination

Plasma creatinine was determined by Jeffe's modified method and protein by standard method.

8. Kidney histological examination

Both cortex and medullary region of the right kidneys were excised and cut into 0.3-0.5 mm., fixed in 10 % buffer formalin and embedded paraffin. The sections will be cut at 5 μm and stained with hemotoxilin & eosin(H&E) for morphological evaluation under the light microscope ((ECLIPSE-TS 100 Nikon, Japan). The tissue damage will be graded into 5 fields/slide. The present of tubular cellularity, basement membrane thickening, dilation, atrophy, sloughing, or interstitial widening were determined.

9. Statistical analysis

Data are expressed as mean ± SE from five rats in each group. Significant differences between mean values were determined by one-way analysis of variance (ANOVA). P values less than 0.05 were considered significantly altered.

Results

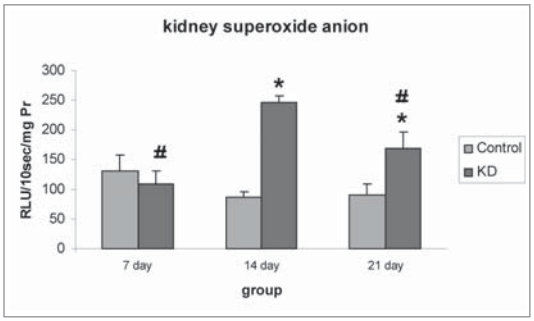
As can be seen from Table 1, hypokalemia was developed within one week after receive the low-K^+ diet, and plasma K^+ remained low at the same level until day 14 and day 21. The K^+ content in soleus muscle was also decreased in the same fashion and even tends to be further decreased when the hypokalemic period prolonged (day 21 compare with day 7). Although, the kidney of hypokalemic rats was larger and had a higher of kidney weight to body weight ratio than that of control group ($p<0.05$), the plasma creatinine levels was not significantly differences.

Table 1 Plasma K^+, muscle K^+, plasma creatinine, and kidney weight/BW ratio of the rats. (* $p < 0.05$ Low- K^+ diet vs. control, all n=5 excepted muscle K^+ n=3)

	Control diet	Low- K^+ diet
Plasma K^+(mEq/L)		
7 days	4.8 ± 0.20	3.25 ± 0.20*
14 days	5.58 ± 0.39	3.30 ± 0.16*
21 days	4.70 ± 0.20	3.14 ± 0.08*
Plasma creatinine (mg/dl)		
7 days	0.66 ± 0.062	0.87 ± 0.05
14 days	0.77 ± 0.019	0.78 ± 0.03
21 days	0.83 ± 0.02	0.76 ± 0.02
Muscular K^+ (μM/gww)		
7 days+	85.13 ± 3.64	73.24 ± 1.62*
21 days+	83.52 ± 4.48	65.60 ± 2.52*
Kidney weight/Body weight ratio		
7 days	3.56 ± 0.19	4.22 ± 0.13*
14 days	3.24 ± 0.19	4.06 ± 0.04 *
21 days	3.66 ± 0.08	4.25 ± 0.037*

Superoxide anion formation

Figure1. showed the rate of superoxide anion formation in kidney tissue determined by chemiluminescence of lucigenin at the 100 μM concentration. The rate of O_2^- formation was expressed as relative light unit (RLU)/10sec/mgPr. We found a marked increased in O_2^- generation from kidney of the 14 days (245.99 ± 11.41) and the 21 days (168.19 ± 27.76) of low- K^+ fed rats when compared to the control diet fed rats (7 days;131.52 ± 26.64 , 14 days; 86.85 ± 9.43 and 21 days; 90.65 ±18.04) and the 7 days low- K^+ diet group (108.97 ± 23.23) ($p<0.05$).

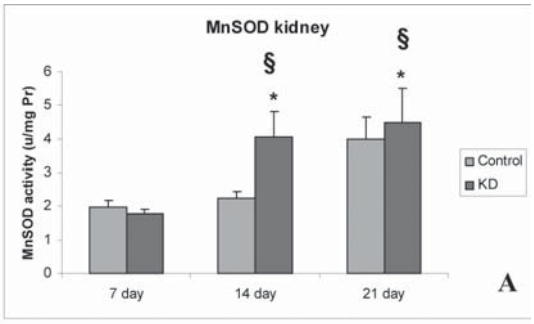


* $p < 0.05$ vs. control , # $p < 0.05$ vs. 14 day KD

Figure 1 Histograme showing superoxide anion level of rat kidneys in each group (n=5). Control = normal diet, KD = low-potassium diet

SOD activity

MnSOD activity of the 14 and 21 days (4.05 ± 0.75, 4.48 ± 1.01 23) low- K^+ fed rats were significantly higher than those of the control (2.57 ± 0.18, 0.97 ± 0.69, $p < 0.05$) and the 7 days low- K^+ diet group. However, the Cu/ZnSOD activity was not significantly changed in all groups.



* $p < 0.05$ vs.control, $^{s}p < 0.05$ vs 7day KD

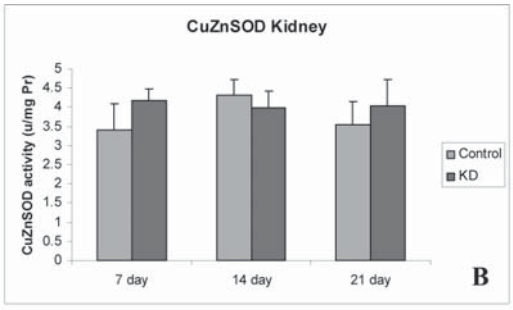
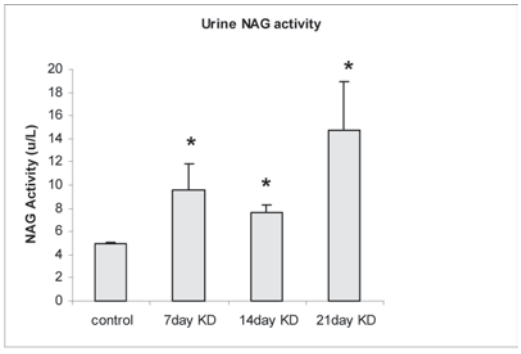


Figure 2 MnSOD(A) and CuZnSOD (B) level of rat kidney in each group (n=5). Control = normal diet, KD = low-K^+ diet

Urinary N-acetyl-β-glucosaminidase activity

The 24-hours urine NAG activity was significantly elevated in all groups of low- K^+ diet when compared to control group ($p < 0.05$).



* $p < 0.05$ vs control

Figure 3 Urinary NAG activity in each groups of the rats (n=5). Control = normal diet, KD = low-potassium diet

Kidney histology

As aforementioned, the kidney of KD rats was much larger than that of the control group (Table.1). Histological sections present in Figure4. reveal the changes in kidney of the low- K^+ fed rats compared to control. There was no obviously changed in the 7 day low- K^+ diet fed group when compared to control excepted for the swelling of medullar with mild hyperplasia. However, the 14 day and 21 day low-potassium diet fed group showed various degree of histological changes, including, medullary swelling and hyperplasia, intratubular deposition of amorphous materials, tubular atrophy, interstitial mononuclear cell infiltration and interstitial expansion. The most prominent injury was seen in the medulla region especially at inner strip. The degree of injury was augmented as the hypokalemic period prolonged.

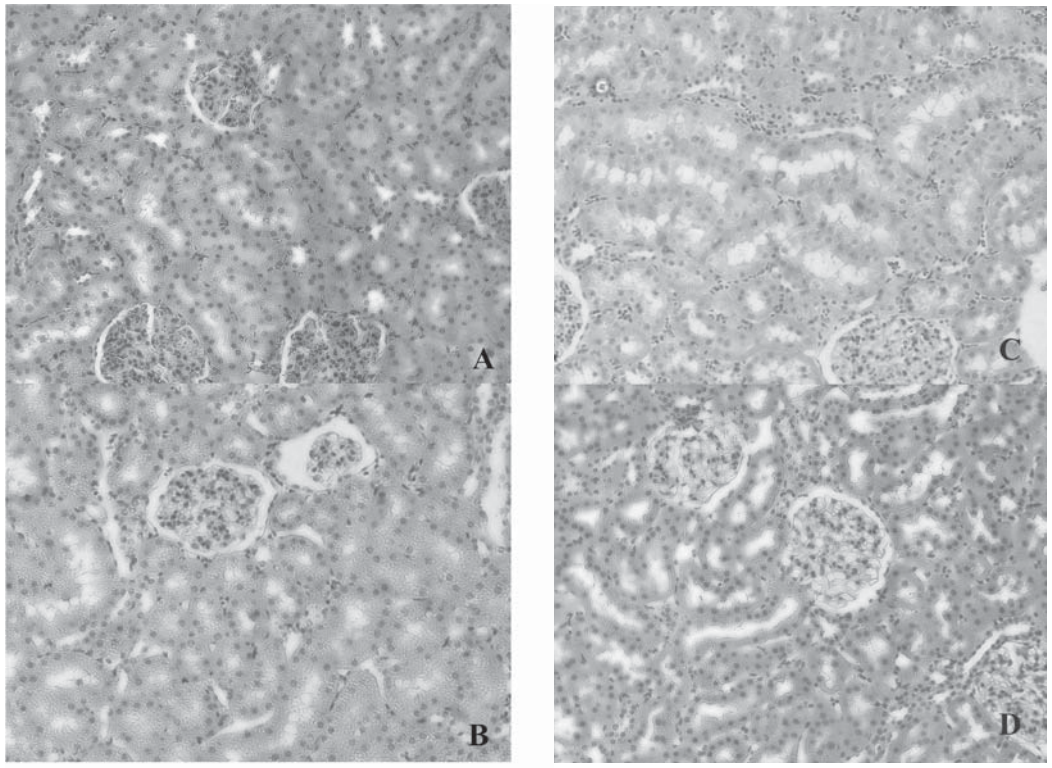


Figure 4 Histological observation of kidney tissue(A) control (B) 7KD(C) 14 KD (D) 21 KD show tubular epithelial swelling, vacuolar degeneration, tubular dilatation, tubulointerstitial cell infiltration and tubular atrophy (200x).

Discussion

Chronic K^+ deficient nephropathy could be occurred if prolonged and progressive irreversible tubulointerstitial damage to end stage kidney diseases (Tolins et al, 1987). Moreover, the recent cellular and animal studies on K^+ deficiency condition found the association of ROS generation and its activity increased in vessels altered function (Yang et al, 1998, Zhou et al, 2002). As has been previously reported by Everts et al. (1993) and Piyachaturawat et al. (1999), we also found that the K^+-depleted rats had a rapidly declined on both plasma K^+ and muscular K^+ content within the first week and remained low at the same level until day 14 and day 21.

The KD rat in our study demonstrated an increased in the levels of O_2^- formation in kidney tissue. This result is in agreement with the studies of Zhou et al. (2002) and Yang et al. (1998) who found the increased in the O_2^- level and it activity *in vitro*. According to the recent studies NADH/NADPH oxidase has been reported as the major oxidase mediated production and action of O2- in thick ascending limb of Henle's loop (TALHs) and kidney homogenate in rat (Li et al, 2002, Zou et al, 2001). The high levels of O_2^- in the TALHs may associate with its function and metabolic activity (Ferialle et al, 2001). In the kidney, NADH oxidase has been reported to mediate the generation of O_2^- in response to

Angiotensin II (Ang II) in mesaingial cell (Jaimes et al, 1998). In addition, Ang II was activated by NADH in hypertensive K^+-depleted rat which accordanced to osteopontin (OPN), a macrophage adhesive protein was up regulated in the medulla (Ray et al, 2001). These may under going hyperplastic the kidney enlargement and tubulointerstitial mononuclear cell recruitment which can caused tubulointerstitial damages (Ray et al, 2001, Franco et al, 2006).

Beside this, the MnSOD, mitochodria located, activity was correlation with O_2^- formation in our study. MnSOD has been shown to be and inducible enzyme and protect against free radical harmful in mitochondria matrix (Taylor et al, 2003). On the other hand, oxidative damage can be extensive activation by cytochrome c release subsequently progressive cell damages and apoptosis (Zager et al, 2004, Jaing and Wang, 2004). This indicated that the increased of MnSOD activity should protected against cellular damages during oxidative stress induction (Maijima et al, 1998). Although, CuZnSOD activity was not affect on K^+ deficiency fed rats, there may affect by extracellular source of CuZnSOD (Adler and Huang, 2004)

Although in a mild degree of architecture changes was seen, the levels of renal tubular epithelial injury enzymes such as NAG activity was immediately changed. ?ktem et al., (2004) demonstrated that the NAG activity increased significantly in the oxidative stress condition involved in diabetic tubulonephropathy. Thus, increase activity of urinary NAG may suggest that the kidney molecular functional changes should association in early tubular damage.

In conclusion, chronic dietary K^+ fed for one week induced the oxidative damages in rat kidney and progressively damage further in the next two to three week of the experiment. The severity of damages are depend on the level of K^+ intake and time course deficit as revealed by less tubulointerstitial lesion, diffuse epithelial swelling and tubulointerstial injury. The ROS increased production and antioxidant defense system are rapidly response and remain progress throughout the experimental period. In addition, urinary NAG activity increased in earlier response than the ROS and antioxidant defense system. These results suggested that chronic potassium depletion could first promote cellular dysfunction and then lead to oxidative damages progression occur. Thus, the balance between ROS and others antioxidant defense system would concerned for further investigation.

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References

Adler, S. and Huang H. 2004. Oxidant stress in kidneys of spontaneously hypertensive rats involves both oxidase overexpression and loss of extracellular superoxide dismutase. Am J Physiol Renal Physiol 287: F907-F913.

Coruzzi, P., Brambilla, L., Gualerzi, M., Rossi, M., Parati, G., Di Rienzo, M., Tadonio, J. and Novarini, A. 2001. Potassium depletion and salt sensitivity in essential hypertension. J Clin Endocrin Metab. 86: 2857-2862.

Everts, M.E., L∅mo, T. and Clausen, T. 1993. Changes in K^+, Na^+ and calcium contents during invivo stimulation of rat skeletal muscle. Acta Physiol Scand. 147: 357-368.

Franco, M., Martínez, F., Rodr?guez-Iturbe, B., Johnson, R.J., Santamaría, J., Montoya, A., Nepomuceno, T., Bautista, R., Tapia, E. and Herrera-Acosta, J. 2006. Angiotensin II, interstitial inflammation, and the pathogenesis of salt-sensitive hypertension. Am J Physiol Renal Physiol. 291: F1281-F1287.

Féraille, E. and Doucet, A. 2001. Sodium-potassium-adenosinetriphosphatase-dependent sodium transport in the kidney: hormonal control. Physiol. Rev. 81: 345-418.

Dorup, I., Skajaa, K. and Clausen, T. 1988. A simple and rapid method for the determination of the concentrations of magnesium, sodium, potassium and sodium, potassium pumps in human skeletal muscle. Clin Sci (Lond) 74(3): 241-248.

Hiraishi, H., Terano, A., Razandi, M., Sugimoto, T., Harada, T. and Ivey, K.J. 1992. Role of cellular superoxide dismutase against reactive oxygen metabolite injury in culture bovine aortic endotherial cells. J Biol Chem. 267: 14821-14817.

Hodgson, E.K. and Fridovich, I. 1975. The intervention of bovine erythrocyte superoxide dismutase with hydrogen peroxide:Inactivation of the enzyme. Biochemistry. 14: 5294-5299.

Hoffmann, S., Spitkovsky, D., Radicell, J.P., Epe, B. and Wiesner, R.J. 2004. Reactive oxygen species derived from the mitochondrial respiratory chain are not responsible for the basal levels of oxidative base modifications observed in nuclear DNA of mammalian cells. Free Radic Biol Med. 36(6): 765-773.

Horak, E., Hopfer, S.M. and Sunderman, F.W, Jr. 1981. Spectrophotometric assay for urinary *N*-acetyl-β-D-glucosaminidase activity. Clin Chem. 27(7): 1180-1185.

Jaimes, E.A., Galceran, J.M. and Raij, L. 1998. Angiotensin II induces superoxide production by mesangial cells. Kidney Int. 54: 775-784.

Jaing, X. and Wang, X. 2004. Cytochrme *C*-mediated apoptosis. Annu Rev Biochem. 73: 87-106.

Knochel, J.P. and Schlein, E.M. 1972. On the mechanism of rhabdomyolysis in potassium depletion. J Clin Invest. 51(7): 1750-1758.

Levi, M., McDonald, L.A., Preisig, P.A. and Alphern, R.J. 1991. Chronic K depletion stimulates rat renal brush-border membrane Na-citrate cotransporter. Am J Physiol. 261 (Renal Fluid Electrolyte Physiol. 30): F767-F763.

Li, N., Yi, F.X., Spurrier, J.L., Bobrowitz, C.A. and Zou, A.P. 2002. Production of superoxide through NADH oxidase in thick ascending limb of Henle's loop in rat kidney. Am J Physiol Renal Physiol. 282(6): F1111-1119.

McCabe, R.D., Bakarich, M.A., Srivastava, K. and Young, D.B. 1994. Potassium inhibits free radical formation. Hypertension. 24(1): 77-82.

Majima, H. J.; Oberley, T. D.; Furukawa, K.; Mattson, M. P.;Yen, H.-C.;Szweda, L. I. and St.Clair, D. K. 1998. Prevention of mitochondrial injury by manganese superoxide dismutase reveals a primary mechanism foralkaline-induced cell death. J Biol Chem. 273: 8217-8224.

Okada, Y. and Maeno, E. 2001. Apoptosis, cell volume regulation and volume-regulatory chloride channels. Comp Biochem Physiol A Mol Integr Physiol. 130(3): 377-383.

Öktem, F., Ozguner, F., Yilmaz, H.R, Uz, E. and Dündar, B. 2006. Melatonin reduces urinary excretion of *N*-acetyl-β-D-glucosaminidase, albumin and renal oxidative markers in diabetic rats. Clin Exp Phar Physiol. 33: 95-101.

Pagano, P.J., Ito, Y., Tornheim, K., Gallop, P.M., Tauber, A.I. and Cohen, R.A. 1995. An NADPH oxidase superoxide-generating system in the rabbit aorta. Am J Physiol. 268 (Heart Circ. Physiol. 37): H2274-H2280.

Piyachaturawat, P., Muchimapura, S., Sophasan, S., Jariyawat, S., Pholpramool, C., Satayavivad, J. and Endou, H. 1999. Effect of chronic K^+ defiency on contractile properties of soleus muscle in rats: evidence of sex differences. Clin Exp Phar Physiol. 26: 323-329.

Ray, P.E., Suga, S., Liu, X.H., Huang, X. and Johnson, R.J. 2001. Chronic potassium depletion induces renal injury, salt sensitivity, and hypertension in young rats. Kidney Int. 59(5): 1850-1858.

Saitoh, D., Shirani, K.Z., Croffi, W.G., Kizaki, T., Ohno, H., Okada, Y., Mason, A.D. Jr. and Pruitt, B.A. Jr. 2001. Changes in the tissue and plasma superoxide dismutase (SOD) levels in a burened rat model.Tohoku J Exp Med. 193: 27-36.

Stetson, D.L., Wade, J.B. and Giebisch, G. 1980. Morphologic alterations in the rat medullary collecting duct following potassium depletion. Kidney Int. 17(1): 45-46.

Suga, S.I., Phillips, M.I., Ray, P.E., Raleigh, J.A., Vio, C.P., Kim, Y.G., Mazzali, M., Gordon, K.L., Hughes, J. and Johnson, R.J. 2001. Hypokalemia induces renal injury and alterations in vasoactive mediators that favor salt sensitivity. Am J Physiol Renal Physiol. 281: F620-F629.

Sun, Y., Oberley, L.W. and Li, Y. 1988. A simple method for clinical assay of superoxide dismutase. Clin Chem. 34(3): 497-500.

Taylor, E.R., Hurrell, F., Shannon, R.J., Lin, T.K., Hirst, J. and Murphy, M.P. 2003. Reversible glutathionylation of complex I increases mitochondrial superoxide formation. J Biol Chem. 278(22): 19603-19610.

Tejada, F., Cremades, A., Aviles, M., Castell, M.T., Penafiel, R. 1998. Hypokalemia alters sex hormone and gonadotropin levels: evidence that FSH may be required for luteinization. Am J Physiol. 275(6 Pt 1): E1037-1045.

Tolins, J.P., Hostetter, M.K. and Hostetter, T.H. 1987. Hypokalemic nephropathy in the rat. Role of ammonia in chronic tubular injury. J Clin Invest. 79(5): 1447-1458.

Yang, B., Li, D., Weng, Y., Lynch, J., Wingo, C. and Meh,T.A. J. 1998. Increased superoxide anion generation and alterd vasoreactivity in rabbits on low potassium diet. Am J Physiol. 274 (Heart Circ. Physiol. 43): 111955-111961.

Yu, B.P. 1994. Cellular defenses against damage from reactive oxygen species. Physiol Rev. 74(1): 139-162.

Yu, S.P. 2003. Regulation and critical role of potassium homeostasis in apoptosis. Prog Neurobiol. 70: 363-386.

Zager, R.A., Johnson, A.C.M. and Hanson, H.Y. 2004. Proximal tubular cytochrome *c* efflux: Determinant, and potential marker, of mitochondrial injury. Kidney Int. 65: 2123-2134.

Zhou, X., Yin, W., Doi, S.Q., Robinson, S.W., Takeyasu, K. and Fan, X. 2003. Stimulation of Na,K-ATPase by low potassium requires reactive oxygen species. Am J Physiol Cell Physiol. 285: 319-326.

Zou, A.P., Li, N., Cowley, A.W.Jr. 2001. Production and actions of superoxide in the renal medulla. Hypertension. 37: 547-553.