Oxidative stress and nitric oxide deficiency in inflammation of chronic renal failure

Possible preventive role of L-arginine and multiple antioxidants

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ABSTRACT

الأهداف: تقييم تأثير عقار ال–أرجنين ومضادات التأكسد على سيتوكينات الالتهاب ووظائف الكلى وضغط الدم واختلال الدهون في الفشل الكلوي المزمن بالفئران (CRF).

الطريقة: تم إجراء هذه الدراسة خلال الفترة من ديسمبر 2007 حتى نوفمبر 2008 – قسم وظائف الأعضاء – كلية الطب جامعة الملك سعود على 40 فأر ذكر من فصيلة الويستر مصابة بالفشل الكلوي المزمن بطريقة إنقاص كتلة الكلى(RMR) و10 فئران أخرى أجريت لهم جراحة خادعة واستخدمت كمجموعة ضابطة. قسمت الفئران المصابة بالفشل الكلوي إلى 4 مجموعات متساوية تركت أحداها بلا علاج بينما تم علاج المجموعات الأخرى لمدة 12 أسبوع أما بعقار ال–أرجنيين أو مضادات التأكسد (فيتامين ج، هـ، ال–كارنيتين، كاتشيين)أو بكلا من ال–أرجنيين ومضادات التأكسد. تم قياس مستوى السيتوكينات بالبلازما وضغط الدم ووظائف الكلى ومستوى النيتريت والنيترات وكذلك مستوى الدهون بالسيرم.

النتائج: أدى الفشل الكلوي المزمن إلى ارتفاع مستويات السيتوكينات المساعدة على الالتهاب منها انترلوكين–1الفا،–1بيتا، انترلوكين6– و عامل النخر الورمي–الفا، كما ارتفع مستوى جزيء الالتصاق بين الخلوي الذائب–1 بينما انخفض مستوى السيتوكينات المقاومة للالتهاب انترلوكين 4 و 10 في حين ارتفع مستوى الدهون و ضغط الدم. وقد أدى استخدام عقار ال– أرجنيين إلى انخفاض ملحوظ في مستوى السيتوكينات المساعدة على الالتهاب و جزيء الالتصاق بين الخلوي الذائب1– مع تحسن وظائف الكلى وضغط الدم الانقباضي. كما أدى استخدام مضادات التأكسد المتعددة إضافة إلى ذلك إلى انخفاض مستويات السيتوكينات المساعدة على الالتهاب ومستوى جزيء الالتصاق بين الخلوى الذائب–1 وزيادة مستوى انترلوكين–4 المضاد للالتهاب. في حين أظهرت المجموعة التي أعطيت كلا العلاجين معا تحسن أفضل في وظائف الكلى و ضغط الدم ومستوى السيتوكينات بالبلازما وكذلك مستوى الدهون بالدم والنيتريت والنيترايت والكلويسترول وتركيز ثلاثي الجلبسريد.

خاتمة: استعادة التوازن بين مستوى العوامل المؤكسدة ومضاداتها مع التوافر الفعال لأكسيد النتريك يقاوم الالتهاب والاعتلال الكلوي واختلال الدهون في حالات الفشل الكلوي المزمن. هذا يشير إلى الحاجة لمزيد من الاهتمام بدور مضادات التأكسد ومحفزات أكسيد النتريك في علاج الفشل الكلوي ومضاعفاته.

Objectives: To evaluate the effect of L-arginine and multiple antioxidants on the inflammatory cytokines level, renal functions, blood pressure and dyslipidemia in chronic renal failure (CRF) rats.

Methods: This study was carried out between December 2007 and November 2008 in the Department of Physiology, Faculty of Medicine, King Saud University, Kingdom of Saudi Arabia. Chronic renal failure was induced in 40 rats by renal mass reduction (RMR) and 10 rats were sham operated. Renal mass reduction rats were treated for 12 weeks by L-arginine and/or a mixture of antioxidants (L-carnitine, Catechin, Vitamins E and C) and the effect of the treatments on plasma cytokines, soluble intercellular adhesion molecule-1 (sICAM-1), nitrate (NO2) and nitrites (NO3), lipid profile, blood pressure, and renal function was examined.

Results: Chronic renal failure increased plasma Interleukin (IL)-1alpha, IL-1beta, IL-6, tumor necrosis factor-alpha, soluble intercellular adhesion molecule-1 (sICAM-1) levels and decreased anti-inflammatory cytokines IL-4 and 10 levels. In addition, hypertension, and dyslipidemia were found. L-arginine treatment improved kidney functions, decreased systolic blood pressure and decreased inflammatory cytokines levels. Antioxidants administration decreased inflammatory cytokines and sICAM-1 levels and increased IL-4 levels. Combined use of L-arginine and the antioxidants mixture were very effective in their tendency to recover normal values of kidney functions, plasma cytokines, sICAM-1, blood pressure, NO2/NO3, cholesterol and triglycerides concentrations.

Conclusion: Restoration of the pro-oxidant/ antioxidants balance with increased NO bio-availability counteracts inflammation, renal impairment and dyslipidemia in CRF. This may open new perspectives for the role of antioxidants and NO precursors in the treatment of uremia and its complications.

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Limited availability of the substrate L-arginine due to decreased production after renal mass reduction (RMR) may reduce nitric oxide (NO) synthesis, and there is some evidence that superoxide production may be increased.[1] Although renal NO production is deficient in RMR; increased inducible NO-synthase (iNOS) activity can be induced in several cell types including inflammatory and non-inflammatory cells by a big list of cytokines.[2] This was reported to be associated with decrease L-arginine level in plasma and with enhanced inflammatory response that potentate tissue damage. On the other hand, L-arginine supplementation was reported to modulate the immune status in several pathological processes.[3] Other than the abnormalities in NO synthetic pathway, the pro-oxidant and antioxidant capacity is disturbed in CRF resulting in increased oxidative stress. This potentially mediates cardiovascular, neurological and several other complications of CRF. Furthermore, components of the anti-oxidant defense mechanisms including vitamins C and E, selenium and glutathione (GSH), and scavenging system are deficient in patients with CRF and a protein oxidation patterns consistent with leukocyte myeloperoxidase-mediated events has been demonstrated in these patients.[4,5] L-carnitine is another important antioxidant that gained specific interest in CRF and that was found to be deficient in these patients.[6] Uremic patients, as well as patients with CRF, appear to have abnormal renal handling of carnitine leading to dyslipidemia, lethargy, muscular weakness, hypotension, cardiac dysfunction and arrhythmias, and recurrent cramps.[6,7] Most of these symptoms were found to be improved by L-carnitine administration.[8] Catechins are a group of flavonoids, present in vegetables and plant-derived beverages and food, such as tea and chocolate, that attract particular attention due to their relative high antioxidant capacity and free radical scavenging activities in biological systems.[9] Owing to the multiple complications caused by oxidative stress and inflammatory response associated with disordered L-arginine-NO pathway and deficient anti-oxidant defense mechanisms in CRF. This study aimed to evaluate the effects of L-arginine either alone or in combination with L-carnitine, Catechin, Vitamins E and C on the pro-inflammatory cytokines production (interlukin [IL]-1 α, IL-1 β, IL-6, tumor necrosis factor-alpha [TNF-α]) and anti-inflammatory cytokines (IL-4 and IL-10), soluble intercellular adhesion molecule-1 (sICAM-1), NO level, blood pressure, lipid profile, and renal function in CRF induced in rats by RMR.

Methods. This study was carried out between December 2007 and November 2008 in the Physiology Department, Faculty of Medicine, King Saud University (KSU), Saudi Arabia. Fifty male Wistar rats (2-3 months old), weighing (200-230 g), raised in the animal house of the Faculty of Medicine, KSU were used in the study. Animals received tap water and food ad libitum. They were housed at 4/cage in 12-hour light-dark cycle. The study design was approved by the Research Ethics Committee of the Faculty of Medicine, KSU, Saudi Arabia. The experimental protocol and housing facilities were conducted according to the standard established guidelines of laboratory animals of Collage of Medicine Research Center (CMRC), KSU. Rats were divided into 5 groups (n=10 rats each): Group A: healthy control rats receiving no treatment, Groups A1, A2, A3, and A4 are RMR rats. Group A1 received no treatment, group A2 received L-arginine (1.25 g/L) in the drinking water (approximately 0.2 g/kg/day), and group A3 received antioxidants mixture of Vitamin E (400 mg/kg food), Vitamin C (500 mg/kg food), L-carnitine 500 mg/kg intraperitoneal (i.p), Catechin 100 mg/kg/day mixed with food and group A4 received both L-arginine and antioxidant treatment in the same doses used in groups A2 and A3. Renal mass reduction was conducted under pentobarbitol anesthesia (40 mg/kg, i.p), by removing approximately 5/6 of the kidney tissue, involving the right nephrectomy and removal of the upper and lower poles of the left kidney.[10] The RMR procedure was accomplished via dorsal incision and was carried out under strict hemostasis and aseptic technique. Control rats were sham operated by flank incision under anesthesia. After the operation, rats were allowed to recover and have free access to the standard laboratory diet and tap water. Treatment was started one week after RMR (week 0) and continued for 12 weeks. All drugs were purchased from Sigma Chemical Co. (Sigma, Ltd, USA). Blood samples were collected at weeks 0 and 12 from the retro-orbital plexus in iced plain and EDTA containing tubes. Serum and plasma were separated and stored at -70°C until assay. Urine was collected in sterile containers containing isopropranolol (0.1 mL) by putting the animals individually in sterile metabolic cages for 24 hours. Urine volume was measured in (ml/24 hours), then the urine was centrifuged and part of the supernatant was used to measure creatinine and protein content, and the rest was stored at -70°C until assay of NO2/NO3. Plasma IL-1 α, IL-1β, IL-4, IL-6, and IL-10 were assessed by a specific sandwich enzyme linked immunosorbent assay (ELISA) kits (R & D Systems, UK) according to the manufacturer's instructions. The optical density was read at 450nm for IL-4, IL-6 and IL-1α and at 490 nm for IL-10 in automated microplate reader-model ELX800, serial number 191720, (Bio-tek Instruments Inc, Vermont, USA). Soluble intercellular adhesion molecule-1 was measured using a specific ELISA kit (R&D Systems, UK). The optical density

was read at 490 nm. Total body nitric oxide production was evaluated by measuring the stable end products of nitric oxide metabolism NO2/NO3 in serum and 24 hours urine by special kit (Cayman Chemical, USA) utilizing nitrate reductase and the Griess reagents and using ELISA reader as previously reported.[11] Kidney function tests and serum lipid analysis were measured by colorimetric methods using specific kits for serum urea and creatinine, urine creatinine, protein excretion, total serum cholesterol and triglycerides levels from Spinreact- Spain according to the instructions provided with the kits. All rats were subjected to systolic blood pressure measurement by Non Invasive Blood Pressure system (NIBP) using the tail-cuff plethysmography (Letica LE 5100, Panlab, Barcelone, Spain) one week after the surgery and every 4 weeks: the average of 3 measurements was used as a single value for each rat at each time point.

Statistical analysis of the results was carried out using the SPSS package for statistical analysis version 12.0. Comparison between all the groups at each time point was carried out using one-way analysis of variance. A post Hoc LSD test was used when ANOVA shows significant differences. Comparison between the 0 and 12 weeks data of the same group was carried out by paired sample t-test. All data are presented as mean ± S.E.M. Results were considered significant ($p<0.05$).

Results. One week after RMR (0 time) kidney function tests of groups A1, A2, A3 and A4 showed a picture of CRF characterized by significant reduction in creatinine clearance ($p=0.000$) and significant marked increase in serum creatinine ($p=0.000$), urea and urinary protein loss in comparison to sham operated controls ($p=0.000$). After 12 weeks, group A1 showed further significant increase in serum urea, creatinine, urinary protein loss and decreased creatinine clearance in comparison to week 0 ($p=0.000$ for all variables). L-arginine supplementation caused serum urea and creatinine levels and the proteinuria to remain significantly lower in group A2 rats compared to group A1 ($p=0.000$) and also keep their creatinine clearance significantly higher ($p=0.000$). L-carnitine, Catechin, Vitamins E and C treatment to group A3 also caused a significant reduction in serum urea and creatinine levels and urinary protein loss and increased creatinine clearance in comparison to the untreated rats ($p=0.000$ for each parameter). However, the effect of the antioxidant mixture on serum urea, and creatinine was significantly less than that caused by L-arginine treatment ($p=0.000$). Combined treatment by L-arginine and the multiple antioxidants caused further improvement in kidney function in group A4 rats showing no significant difference from the sham operated control regarding serum creatinine, creatinine clearance, and proteinuria ($p=0.000$ for all) (**Table 1**).

Serum and urinary NO2/NO3 decreased significantly in all rats subjected to RMR at week 0 compared with the sham-operated controls ($p=0.000$) (**Table 1**). Group A1 rats showed further decrease in their serum and urinary NO2/NO3 at week 12 ($p=0.000$). Treatment with L-arginine significantly increased serum and urinary NO2/NO3 in group A2 rats in comparison to the untreated group A1 ($p=0.000$) an effect that was greater than that caused by L-carnitine, Catechin, Vitamin E and Vitamin C treatment in group A3 rats ($p=0.000$). Rats receiving combined L-arginine and antioxidants showed further significant increase in serum and urinary NO2/NO3 production in comparison to rats receiving L-arginine alone ($p=0.007$, $p=0.000$).

As in **Table 1** serum cholesterol showed no significant difference between all the studied groups at week 0 ($p=0.774$), but serum triglycerides increased significantly ($p=0.000$) in RMR rats in comparison to the sham operated group. However, at week 12 serum cholesterol and triglycerides levels increased significantly in untreated group A1 rats in comparison to sham operated controls ($p=0.000$ for each). Antioxidant treatment caused greater reduction in serum triglyceride levels in comparison to L-arginine treatment ($p=0.000$). Combined use of both L-arginine and the antioxidant mixture nearly normalized serum triglyceride levels to the control value ($p=0.4$). All the used treatment regimens caused significant reduction in serum cholesterol levels in the treated groups in comparison to untreated group A1 ($p=0.000$). But no significant difference between the serum cholesterol level between the 3 treated groups was found ($p=0.5$). Soluble intercellular adhesion molecule-1 increased significantly in group A1 CRF rats in comparison to sham operated controls ($p=0.000$). Use of the antioxidant treatment caused significant reduction in the level of sICAM-1 in group A3 in comparison to group A1 rats ($p=0.035$). The level of sICAM-1 in the antioxidant treated rats showed no significance difference from the sham operated control group ($p=0.51$). Furthermore, L-arginine treatment either alone or in combination with the antioxidant mixture also decreased sICAM-1 levels in treated rats in comparison to group A1 rats, but this reduction was not significant ($p=0.20$, $p=0.78$) (**Table 2**).

Plasma inflammatory cytokines analysis revealed significant increase in IL-1α and 1β levels in untreated group A1 rats in comparison to the sham operated group ($p=0.000$) (**Table 2**). Treatment by L-arginine and the antioxidant mixture either separately or in combination significantly decreased IL-1α and IL-1β in treated rats in comparison to the untreated group ($p=0.000$). However, the antioxidant mixture caused greater reduction in

IL-1β in comparison to L-arginine (p=0.000). On the other hand, L-arginine treatment was more powerful in decreasing IL-1α level than the antioxidant mixture (p=0.001). Other inflammatory cytokines as IL-6 was also found to be significantly higher in group A1 rats in comparison to the sham operated control (p=0.000). Treatment by L-arginine and the antrioxidants mixture either individually or in combination significantly decreased IL-6 levels in comparison to the untreated group. But the reduction of IL-6 levels achieved by the combined use of L-arginine and the antioxidants was greater than that caused by either of them alone (**Table 2**). At the same time, TNF- α level showed significant increase in untreated CRF rats in comparison to the sham operated controls. Treatment by either L-arginine or antioxidants significantly decreased TNF- α levels in groups A2 and A3 rats in comparison to A1 group (p=0.000) the effect of the 2 treatments was comparable (p=0.23). But the combined use of L-arginine and the antioxidant treatment in group A4 rats caused greater reduction in plasma TNF- α level in comparison to the use of either of the 2 treatments (p=0.000) (**Table 2**).

Regarding the anti-inflammatory cytokines; **Table 2** shows that IL-4 and IL-10 levels decreased significantly

Table 1 - Kidney function, nitrate/nitrite (NO2/NO3) production and lipid profile of chronic renal failure rats treated with L-arginine, antioxidant mixture of catechin, L-carnitine, Vitamins E and C, or both L-arginine and antioxidant mixture for 12 weeks and their sham operated control.

Groups	Weeks	Control	RMR	RMR+L-arg.	RMR+antiox	RMR+L-arg.+antiox
S. urea (mg/dl)	0	34.96 ± 0.37	81.18 ± 0.37*	82. 12 ± 1.3*	81.58 ± 1.43*	82.42 ± 1.25*
	12	34.7 ± 0.7	128.27 ± 0.6*,a	72.18 ± 1.8*,†, a	102.76 ± 1.4*,†,‡,a	42.1 ± 1.4*,†,‡,$,a
S.cr. (mg/dl)	0	0.43 ± 0.005	1.62 ± 0. 07*	1.57 ± 0.08*	1.59 ± 0.08*	1.58 ± 0.07*
	12	0.43 ± 0.005	2.34 ± 0.1*,a	1.25 ± 0.1*,†	1.70 ± 0.06*,†,‡	0.54 ± 0.02†,‡,$,a
Cr.Cl (ml/min)	0	2.22 ± 0.09	1.12 ± .05*	1.05 ± 0.04*	1.01 ± 0.04*	1.08 ± 0.04*
	12	2.15 ± 0.06	0.77 ± 0.03*,a	1.84 ± 0.11†,a	1.83 ± 0.14*,†,a	2.15 ± 0.07†,‡,$,a
S.NO2/NO3 (Mmol/l)	0	47.2 ± 0.85	39.87 ± 0.68*	39.73 ± 0.55*	39.84 ± 0.46*	39.60 ± 0.50*
	12	47.36 ± 0.80	20.84 ± 0.72*,a	42.8 ± 1.78*,†	29.6 ± 1.2*,†,‡,a	46.17 ± 1.51†,‡,$,a
Ur.Vol. (ml/24h)	0	13.53 ± 0.43	14.8 ± 0.39*	14.63 ± 0.45*	14.59 ± 0.45*	14.54.4 ± 0.41
	12	15.09 ± 0.46	30.98 ± 0.4*,a	27.7 ± 2.3*,a	28.27 ± 0.72*,a	13.38 ± 0.18†,‡,$,a
Ur.protein (mg/24 hours)	0	8.19 ± 0.21	36.17 ± 1.4*	36.12 ± 1.3*	35.8 ± 1.32*	37.78 ± 1.67*
	12	8.56 ± 0.24	104.79 ± 3.24* a	49.22 ± 2.15*,†,a	61.15 ± 3.2*† a	16.63 ± 3.93†,‡,$,a
Ur.NO2/NO3 (Mmol/24 h)	0	24.08 ± 0.59	18.12 ± 0.58*	17.87 ± 0.58*	17.65 ± 0.75*	18.10 ± 0.46*
	12	23.70 ± 0.52	8.98 ± 0.36*,a	18.17 ± 0.38*,†,a	15.06 ± 0.27*,†,‡,a	20.46 ± 0.85*,†,$,a
S.cholesterol (mg/dl)	0	101.67 ± 3.22	103.94 ± 3.16	95.73 ± 9.83	94. 37 ± 10. 8	105.76 ± 2.9
	12	101.67 ± 3.22	155.87 ± 2.0*,a	130.17 ± 3.04*,†,a	133.31 ± 5.05*,†,a	130.19 ± 5.31*,†,a
S.Triglyceride (mg/dl)	0	64.93 ± 2.00	78.10 ± 2.40*	77.26 ± 1.59*	77.43 ± 2.06*	77.26 ± 1.70*
	12	65.71 ± 1.62	112.98 ± 3.6*,a	89.71 ± 0.88*,†,a	74.31 ± 1.2*,†,‡	68.65 ± 1.62†,‡,$,a

Data are presented as mean ± SEM. S - serum, Ur - urine, Cr. - creatinine, Cl. - clearance, Vol - volume, Control - sham operated healthy rats receiving no treatment, RMR - renal mass reduction rats without treatment, RMR+arg - renal mass reduction rats receiving L-arginine treatment, RMR+antiox - renal mass reduction rats receiving antioxidants treatment, RMR+ antiox+arg - renal mass reduction rats receiving L-arginine and antioxidant treatment. *p<0.05 significant versus control animals. †p<0.05 significant versus untreated renal mass reduction animals. ‡p<0.05 significant versus L-arginine treated group. $p<0.05 significant versus the antioxidants treated group, ap< 0.05 significant versus the 0 time of this parameter in the same group.

Table 2 - Plasma cytokines and soluble intercellular adhesion molecule-1 (sICAM-1) levels at 12 weeks in all studied groups.

Groups	Control	RMR	RMR+L-arg.	RMR+antiox	RMR+L-arg.+antiox
IL-1 alpha (pg/ml)	7.56 ± 0.25	69.40 ± 3.47*	47.91 ± 1.23*,†	59.10 ± 2.7*,†,‡	53.61 ± 1.90*,†
IL-1beta (pg/ml)	16.95 ± 0.49	86.68 ± 1.26*	51.52 ± 1.61*,†	39.75 ± 1.55*,†,‡	31.17 ± 0.79*,†,‡,a
Il-4 (pg/ml)	28.34 ± 0.44	27.14 ± 0.34*	26.73 ± 0.2*	27.43 ± 0.35	27.12 ± 0.34*
IL-6 (pg/ml)	32.64 ± 0.98	89.30 ± 1.89*	45.81 ± 1.31*,†	44.39 ± 1.01*,†	34.42 ± 1.03*,†,‡,a
Il-10 (pg/ml)	116.00 ± 1.74	96.49 ± 0.89*	98.66 ± 1.39*	97.23 ± 3.8*	97.88 ± 1.8*
TNF-alpha (pg/ml)	6.06 ± 0.34	26.44 ± 1.01*	17.97 ± 0.65*,†	12.85 ± 0.78*,†	19.12 ± 0.57*,†,‡,a
sICAM-1 (pg/ml)	672.38 ± 63.49	1081.11 ± 65.56*	956.11 ± 71.0*	871.38 ± 76.22†	1064.44 ± 70.39*

Data are presented as mean ± SEM. Control; sham operated healthy rats receiving no treatment, RMR - renal mass reduction rats without treatment, RMR+ arg - renal mass reduction rats receiving L- arginine treatment, RMR+antiox - renal mass reduction rats receiving antioxidants treatment, RMR+ antiox+arg - renal mass reduction rats receiving L-arginine and antioxidant treatment, IL - interlukin, TNF - tumor necrosis factor. *p<0.05 significant versus control animals. †p<0.05 significant versus untreated renal mass reduction animals. ‡p<0.05 significant versus L-arginine treated group. ap<0.05 significant versus the antioxidants treated group

Table 3 - Systolic blood pressure (BP) of chronic renal failure rats at 0, 4, 8, 12 weeks of treatment by L-arginine, antioxidant mixture of catechin, L-carnitine, Vitamins E and C, or both L-arginine and antioxidant mixture.

Groups	Systolic BP (mm Hg) week 0	Systolic BP (mm Hg) week 4	Systolic BP (mm Hg) week 8	Systolic BP (mm Hg) week 12
Control	112.3±0.44	112.5±0.60	113.0±1.00	113.5±0.47
RMR	149.2±0.84*	174.5±1.84*	197.00±1.61*	207.00±1.85*
RMR+ arg	150.5±0.61*	147.9±1.19*,†	161.6±1.55*,†	169.1±1.61*,†
RMR + atiox	150.0±0.66*	150.1±1.15*,†	154.5±2.1†,‡	160.90±1.9*,†,‡
RMR + arg+antiox	150.4±0.58*	124.9±0.64^{*,†,‡,a}	125.3±0.81^{*,†,‡,a}	121.6±1.09^{*,†,‡,a}

Data are presented as mean ± SEM. Control - sham operated healthy rats receiving no treatment, RMR - renal mass reduction rats without treatment, RMR+ arg - renal mass reduction rats receiving L-arginine treatment, RMR+antiox - renal mass reduction rats receiving antioxidants treatment, RMR+ antiox+arg - renal mass reduction rats receiving L-arginine and antioxidant treatment. $^{*}p<0.05$ significant versus control animals. $^{\dagger}p<0.05$ significant versus untreated renal mass reduction animals. $^{\ddagger}p<0.05$ significant versus L-arginine treated group.
$^{a}p<0.05$ significant versus the antioxidants treated group

in CRF rats in comparison to the sham operated group (p=0.019, p=0.000). Antioxidant mixture treatment increased IL-4 in group A3 rats showing no significant difference from the control group (p=0.07), but it showed no statistically significant difference from the level in A1 group (p=0.5). However, L-arginine treatment either alone or in combination with the antioxidants fail to cause significant change in IL-4 levels in comparison to the untreated A1 (p=0.4, p=0.98) and it remained lower than the control value (p=0.002, p=0.015). Interlukin-10 does not change significantly in group A2, A3, and A4 in comparison to the untreated A1 group (p=0.4, p=0.8, p=0.6). Blood pressure measurement showed marked elevation in systolic arterial blood pressure in CRF rats starting from one week after RMR operation (week 0) (p=0.000) and continuously during the observation period (**Table 3**). Use of either L-arginine or the antioxidant mixture caused significant partial attenuation of the CRF-induced hypertension (p=0.000 for each at weeks 4, 8, and 12). The effect of both treatments was comparable at week 4 (p=0.17), but that of the antioxidants was more powerful at weeks 8 and 12 (p=002, p=000). Combined use of L-arginine and antioxidant therapy results in greater correction of CRF- induced hypertension in comparison to treatment by either of them (p=0.000 at all time points).

Discussion. Chronic inflammation is a highly prevalent pathological process in CRF that predicts poor clinical outcome, enhancing cardiovascular complications and mortality in end stage renal disease.[12] We reported previously that CRF induced in rats by subtotal nephrectomy depleted the antioxidant enzyme pool, manifested by declined catalase and superoxide dismutase enzymes activities and reduced glutathione levels with accumulation of the nitric oxide synthase inhibitor asymmetric dimethyle arginine (ADMA) that inhibits the activity of endothelial nitric oxide synthase (e-NOS).[13] Accordingly, the combination of NO inactivation by reactive oxygen species (ROS) and depressed NO biosynthesis reduce the availability of NO and thus contribute to endothelial dysfunction and arteriosclerosis that may lead to increased renovascular resistance and hypertension.[13,14]

In the present study, RMR was followed by the development of CRF presented by systolic hypertension, increased serum urea and creatinine levels, proteinuria and decreased creatinine clearance. Although the results of the current study may be limited by the inability to measure the activities and expression of the various isoforms of the NOS enzyme, decreased urinary and serum NO2/NO3 content was found in untreated RMR rats suggesting that systemic NO production is impaired. The inappropriate NO level in CRF could be attributed in part to accumulation of ADMA and methylguanidinen.[13,15] In addition, the effects of NO may be limited by its rapid inactivation by oxygen free radicals, which are also increased in the uremia.[4,13] These findings are in accordance of previous results documenting that NO release is impaired in CRF, and may be involved in the pathogenesis of hypertension in this disease.[14,16] L-arginine administration (1.25g/L in drinking water) for 12 weeks to group A2 rats in the current study was found to be associated with blunting the increase in systolic blood pressure, increased NO production measured by its stable metabolites NO2/NO3, decreased proteinuria and increased creatinine clearance. The increased production of NO could explain the lowered blood pressure after L-arginine treatment and is consistent with reports of significant blood pressure reduction by L-arginine whether it is provided through natural foods or as pharmacological preparations in humans.[17] The reported increase in renal blood flow (RBF) and decrease in glomerular capillary pressure and efferent arteriolar resistance in response to L-arginine treatment[18] could provide further explanation

of decreased arterial blood pressure and increased creatinine clearance in CRF rats treated with L-arginine in the current study. Other than its ability to increase NO production, L-arginine is suggested to decrease the levels of the NOS antagonist ADMA, homocystiene and malondialdehyde (MDA) and myeloperoxidase (MPO) activity in hemodialysis patients.[19] Clinical and experimental evidences suggest that the enzymatic activity of NOS is regulated by the ratio between the concentration of L-arginine (the natural substrate) and that of ADMA (the endogenous inhibitor).[20] Because ADMA is a competitive inhibitor of NOS, its inhibitory action can be overcome by increasing the concentration of the enzyme's substrate, L-arginine. Thus, nutritional supplementation with L-arginine may help to restore the physiological status by normalizing the L-arginine: ADMA ratio.[20] In fact, NO inhibits the mitogenic response and the expression of extracellular matrix proteins induced by various agonists in mesangial cells, including endothelin.[21] This is supported by reduced sICAM-1 levels in association with increased NO levels in treated rats in the current study. Furthermore, L-arginine may decrease the oxidative stress.[19,22] Meanwhile, the blood pressure lowering effect of the antioxidant mixture used in the current study could be due to decreased NO breakdown potentially related to lower release of ROS.[13] However, other effects like increased expression or activity of e-NOS are not rejected and are supported by increased serum concentration of NO metabolic end products (NO2/NO3) level in all the CRF treated rats. We observed that antioxidant therapy resulted in partial correction of hypertension as opposed to complete correction with the addition of L-arginine. This point to the role of numerous factors in the pathogenesis of CRF- induced hypertension.

Enhanced inflammatory condition in CRF rats in the current study was demonstrated by increased plasma pro-inflammatory cytokines IL-1α,IL-1β, IL-6, TNF-α, and decreased anti-inflammatory cytokines IL-4 and IL-10 levels. These findings are in accordance with previous reports in CRF patients and rats.[12,23] The observed inflammatory response in CRF rats in the current study was found to be associated with enhanced hyper adhesive state demonstrated by increased levels of plasma sICAM-1. This increase may be due to inadequate clearance and enhanced synthesis/release and it reflects the presence of CRF complications as malnutrition, inflammation and cardiovascular diseases.[24,25] A significant correlation between sICAM-1 and other inflammatory cytokines as TNF-α and C-reactive protein was reported suggesting that the circulating level of this molecule may be regarded as a marker of inflammatory process.[26] Decreased L-arginine as a part of the malnutrition of uremia could enhance this inflammatory state. This is supported by the reported increase of circulating cytokines such as TNF-α, IL1-β in malnourished uremic patients.[27] This further exacerbates the oxidative and inflammatory milieu in uremia with increased aggregability of platelets taken from these patients.[27] We observed that L-arginine administration to CRF rats significantly decreases the inflammatory cytokines IL-1α and IL-1β, IL-6 and TNF-α production, but it did not influence the anti-inflammatory cytokines IL-4 and IL-10. This may indicate that the beneficial effects of L-arginine on CRF do not involve promoting anti-inflammatory cytokines production. However, in addition to a direct anti-inflammatory action of L-arginine that decreases the production of inflammatory cytokines in CRF; an indirect action could be attributed to its enhancing effect on NO production that was reported to decrease the leukocyte adhesion in post ischemic tissue.[28] L-carnitine, Catechin, Vitamins E and C treatment in the present study decreased the inflammatory cytokines IL-1α and IL-1β, IL-6 and TNF- α levels and increased the anti-inflammatory cytokine IL-4 levels. This was associated with lowered plasma sICAM-1 levels. This inhibition of sICAM-1 up regulation after the antioxidant treatment could be attributed to increased NO levels with its reported ability to modulate leukocyte adhesion.[29] These beneficial effects of the antioxidant therapy in CRF may result from the interruption of several pathophysiological mechanisms. Vitamin E protects glomerular basement membrane integrity, prevents neutrophil chemotaxis and inhibits platelet aggregation. It also inhibits 5-lipoxygenase in peripheral blood monocytes of hemodialysis patients, resulting in it decreases lipid peroxidation and leukotriene B4 content.[30] Vitamin C, functions as a co-factor for biosynthesis of carnitine and it exerts a protective role against the peroxidative damage through its lipid lowering effect.[31] Carnitine itself has antioxidant action and a strong inhibitory effect on free radical production.[32] Furthermore, recent data showed that L-carnitine treatment is able to modify some particular aspects of the inflammatory status characterizing CRF patients.[7] This is supported by the findings of the present study of decreased inflammatory cytokines in the plasma of CRF rats receiving L-carnitine in combination with the other antioxidant. The Catechin part of the antioxidant mixture, being a flavenoid, could help through the direct scavenging of some radical species or may also act as a chain-breaking antioxidant or may recycle other chain antioxidants such as α-tocopherol by donating a hydrogen atom to the tocopheryl radical.[9] Therefore, the beneficial effects of antioxidant treatment in CRF rats in the current study may imply additive effects of Vitamin E, Vitamin C, Catechin and L-carnitine that modulate oxidant stress. Increased levels of these antioxidants could explain the

improvement in kidney function and prevention of the oxidative stress induction of inflammatory cytokines in the treated uremic rats in the current study.

Hyperlipidemia in renal failure rats in the current study confirms previous reports of disordered lipid metabolism in this disease.[33] Elevated serum cholesterol level is known to be associated with impaired endothelial function and may induce superoxide formation.[34] Dietary L-arginine supplementation appears to affect the metabolism of lipoproteins through the formation of polyamines by arginase enzyme in extrahepatic tissues.[35] This may explain the reduction of cholesterol and triglycerides in CRF rats receiving L-arginine treatment either alone or in combination with antioxidants in the present study, similar finding are recently reported in hemodialysis patients receiving oral L-arginine supplementation.[32]Multiple recent studies reported the ability of each of the antioxidants used in the current study to decrease blood lipids in different diseases.[36,37] Catechins and L-carnitine were reported to have lipid lowering effect in addition to their antioxidant effects.[36,37] So, the combined hypo-lipidimic effect of catechin, L-carnitine, Vitamins E and C may underlie the reduction of cholesterol and triglycerides levels in the antioxidant treated group. This correction of dyslipidemia by antioxidants treatment in the current study was augmented by L-arginine addition and this finding was in accordance with other studies that showed improvement in lipid, lipoprotein and apoproteins profile during L-arginine treatment in diabetic rats and in healthy human volunteers.[38]

In conclusion, increased levels of pro-inflammatory cytokines and sICAM-1 in CRF is a marker of inflammation, malnutrition and cardiovascular diseases and predicts mortality. Both L-arginine and antioxidant mixture (L-carnitine, catechin, Vitamins E and C) treatments showed beneficial effects in their tendency to lower pro-inflammatory cytokines and sICAM1 levels, recover normal values of kidney functions , blood pressure, NO2/NO3, cholesterol and triglycerides concentrations. Indeed, the effects of L-arginine and the antioxidants in CRF may open new perspectives in the treatment of uremia and may raise the strong recommendation of immunonutrition in critically ill patients. Diets containing high concentration of L-arginine is now commercially available and findings of the present study support the beneficial effect of combined L-arginine and multiple antioxidants in CRF induced by RMR however further studies in human patients are required.

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