Catechin combined with vitamins C and E ameliorates insulin resistance (IR) and atherosclerotic changes in aged rats with chronic renal failure (CRF)

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Abstract

Aging is an inevitable biological process associated with increased oxidative stress and accumulation of asymmetric dimethylarginine (ADMA) a known endogenous inhibitor of nitric oxide synthase. Atherosclerosis and IR constitute major risk factors for cardiovascular mortality in elderly with chronic kidney disease (CKD). We investigated the impact of catechin, vitamins E and C supplementation on insulin sensitivity, redox state, ADMA, nitrate and nitrite (NO\textsubscript{2}/NO\textsubscript{3}) levels and histological picture of heart and large blood vessels of aged rats with CRF. Findings of the present study revealed that aging in rats is associated with hyperinsulinemia, hyperlipidemia, IR indicated by higher homoeostasis model assessment (HOMA)-index, increased lipid peroxidation product malondialdehyde (MDA), ADMA, and blood pressure (BP), but decreased antioxidant capacity and NO\textsubscript{2}/NO\textsubscript{3} levels. CRF exaggerated all these findings and caused thickened intima of carotid arteries and myocardial hypertrophy. Treatment with catechin, vitamins E and C increases the antioxidant capacity and NO\textsubscript{2}/NO\textsubscript{3} production but, decreases MDA, ADMA and BP levels. Also it keeps insulin sensitivity and normal intima/media thickness of carotid arteries. We conclude that decreased nitric oxide (NO) availability due to ADMA accumulation may be responsible for IR and associated atherosclerotic changes in aged rats with CRF. Catechin, vitamins E and C supplementation may moderate oxidative stress of renal
failure, prevent ADMA accumulation, and counteract IR and atherosclerotic changes in the elderly.

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Keywords: Chronic renal failure in aging; Insulin resistance of old rats; Atherosclerosis in rats; Catechin supplementation; Vitamin E; Vitamin C; Asymmetric dimethylarginine (ADMA)

1. Introduction

Aging is an inevitable biological process that affects most living organisms and is an important risk factor for the development of renal failure and cardiovascular diseases (Lakatta, 2001). IR is a characteristic feature of uremia, irrespective of the type of renal disease (DeFronzo et al., 1983) and together with arterial stiffness they are independent risk factors for cardiovascular mortality in this population (Kobayashi et al., 2004; Zoccali et al., 2005).

ADMA is an endogenous inhibitor of nitric oxide synthase (NOS) that was first reported to be elevated in the patients with terminal renal failure by Vallance et al. (1992). Further on more evidence accumulated on the contribution of dysfunction in NO pathway secondary to accumulation of ADMA, leading to high incidence of atherosclerosis in patients with end stage renal disease (Vallance et al., 1992; MacAllister et al., 1996). Significant positive correlation between age and ADMA was reported in a random population sample (Miyazaki et al., 1999) and markedly increased plasma concentration of ADMA is present even in nonsmoking healthy normotensive elderly subjects (Kielstein et al., 2003).

Increased ADMA level reduced NO availability by NOS inhibition and was found to correlate strongly with severity of the atherosclerotic disease, and is a strong predictor of cardiovascular mortality among several risk factors either in patients with renal disease or even in non-renal patients (Kielstein et al., 1999). Independent of the other risk factors, a close relationship has been observed between ADMA levels and IR (Stuhlinger et al., 2002) and intima/media thickening in individuals with varying risk of atherosclerosis (Miyazaki et al., 1999).

It is well known that lipid peroxidation increases with aging resulting in an increase in plasma MDA levels and a decrease in plasma anti-oxidant activity (Rodriguez-Martinez et al., 1999) associated with parallel increase in serum ADMA levels (Xiong et al., 2001). Furthermore, CKD, being a condition of increased oxidant stress, characterized by high production of reactive oxygen species (ROS) (Saran et al., 2003), creates an environment in which NO can oxidatively be inactivated to peroxynitrite and other derivatives (Moreira et al., 2005).

(+) -Catechin, a flavonoid from the group of catechins, such as (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechingallate, and (+)-gallocatechin, is known to be present in green tea, black tea and other plant foods (Cooke, 1996). Several epidemiological and in vitro studies suggest that catechins have beneficial effects on human health, serving to protect against congestive heart failure (Ishikawa et al., 1997), cancer (Yamanaka et al., 1997), myoglobinuric acute renal failure (Chander et al., 2003), reduce the incidence of myocardial ischemia and the risk of ischemic heart disease mortality, due to their
antioxidant activities (Arts et al., 2001). Vitamin C is a water-soluble, strongly reducing agent that can react directly with free radicals (Frei et al., 1989). Its features as an antioxidant make vitamin C a potentially attractive substance to prevent atherosclerosis, in which free radicals are involved both at the early stage of endothelial dysfunction and later, during oxidation of the low-density lipoproteins (Van der Loo et al., 2003). In addition, vitamin E is a chain-breaking antioxidant with the particular function of scavenging peroxyl radicals, in order to prevent lipid peroxidation in membrane systems (Sumien et al., 2003).

In view of the above information, old age CRF patients are suggested to be at a higher risk of developing IR and atherosclerosis due to the presence of several risk factors, e.g., age per se, oxidative stress, high ADMA levels, hypertension, dyslipidemia, etc., which predispose them to higher mortality rates than the younger patients. Therefore, studies on agents that can reduce IR and atherosclerosis in these populations are very much deserved. In this regard, the present study aimed at investigating the effect of long term dietary supplementation with (+)-catechin, together with the well known antioxidants, vitamins E and C on IR and atherosclerotic complications associated with CRF in aged rats.

2. Materials and methods

2.1. Materials

2.1.1. Experimental animals

This study was done on male Wistar rats. Thirty old animals (of 1.5–2.0 years of age) weighing 540–560 g, and 10 young rats (of 8–12 weeks of age) weighing 290–310 g, raised in the animal house of Faculty of Medicine and King Khalid University Hospital were investigated. Animals received tap water and food ad libitum, they were housed 4 rats/cage under controlled temperature and a 12-h light–dark cycle. Experimental protocol and housing facilities were conducted in accordance with the standard established guidelines of laboratory animals of College of Medicine Research Council (CMRC), King Saud University. CRF was induced by subtotal nephrectomy (SNx), including a nephrectomy on the right side, and removal of upper and lower thirds of the left kidney (5/6 nephrectomy) through a flank incision (Olson, 1984) under ether anesthesia. Sham operated rats were subjected only to the flank incision. All surgical procedures were conducted under aseptic conditions. Rats were divided into the following experimental groups (\( n = 10 \) rats in each group):

- Group I: Control normal healthy young rats, sham operated, receiving regular diet with no treatment.
- Group II: Control normal healthy old rats, sham operated, receiving regular diet.
- Group III: Old SNx rats receiving no treatment.
- Group IV: Old SNx rats receiving oral catechin (100 mg/kg/day; Anjaneyulu et al., 2003), vitamin E (400 mg/kg food; Yargicoglu et al., 2003), and vitamin C (500 mg/kg food; Mahlfouz and Kummerow, 2004). The treatment started 1 week after the SNx operation and continued for 12 weeks. All drugs were of the highest purity available.
2.2. Methods

2.2.1. Systolic BP measurement
BP was measured by using a non-invasive BP system (NIBP) of the Panlab Technology for research (Spain), 1 week after the surgery (week 0) and at week 12. The average of three measurements was used as a single value for each rat at each time point (Bezerra et al., 2005).

2.2.2. Sample preparation
During the twelfth week of the treatment, rats were placed in metabolic cages for collection of 24-h urine samples. Urine was collected into plastic vials containing isopropanol (0.1 ml), urine was centrifuged, and the supernatant was stored at −70 °C until assays of urinary proteins, creatinine, and NO$_2$−/NO$_3$− levels were performed. Blood samples from the retro-orbital plexus were collected in chilled plain test tubes and centrifuged at 3000 rpm for 10 min at 4 °C. Serum was liquated and kept at −70 °C until the time of biochemical assay. Then animals were sacrificed by i.p. injection of high dose phenobarbital (50 mg/kg; Otero et al., 2005). The remnant kidney of SNx rats and left kidneys of the control rats were removed, washed with ice-cold saline, weighted and kept immediately in liquid nitrogen until being homogenized for measurement of reduced glutathione (GSH), ADMA, MDA and protein concentrations. The heart and carotids were removed, washed with iced saline and fixed in a 10% neutral buffered formalin solution, dehydrated at room temperature through ethanol series and embedded in paraffin. Sections (3 μm thick) were cut and stained with hematoxylin-eosin for routine histology.

2.2.3. Preparation of tissue homogenate
Small fragments of kidney tissue (~250 mg) were homogenized in ice bath for 30 s in 0.3 M perchloric acid (HClO$_4$) using an Ultra Turax Homogenizer (Janken Kunkel IKa-Werk, Staufen, Germany). The homogenate was centrifuged at 2300 × g for 15 min at 4 °C. After neutralization with trioctylamine (0.2 vol.) and trichlorofluoro-methane (0.8 vol.) (Frank et al., 2003). The supernatant was kept at −70 °C for determination of MDA levels, GSH and protein concentrations. For ADMA assay homogenates (25%, w/v) of 0.25 g kidney tissue of each animal were prepared in chilled potassium chloride (1.17%) and protease inhibitor cocktail (SC-29130) (Santa Cruz Biotechnology Inc., California), 1 tablet/50 ml using a Polytron. The homogenate was centrifuged at 1500 × g for 5 min at 4 °C to separate the nuclear debris. The supernatant so obtained was kept at −70 °C until the time of the assay.

2.2.4. Analytical procedures
The following parameters were measured in all studied groups:

(i) Serum glucose was measured by oxidase method (enzymatic glycemic kit, Spinreact, Spain) (Varley et al., 1988).
(ii) Serum insulin was measured by rat insulin RIA kit produced by Linco Research, St. Charles, MO, USA (Andersen et al., 1993).

(iii) Serum IR was assessed using the HOMA-IR index originally described by Matthews et al. (1985), applying the following formula: fasting glucose (mmol/l) \times fasting insulin (\mu U/ml)/22.5. As a cut-off point to define IR, a HOMA-IR value of 2.6 was considered, as reported previously (Ascaso et al., 2003).

(iv) The serum MDA reflecting the level of lipid peroxidation, was measured using a spectrophuorometer, as described by Yagi (1976).

(v) GSH: The non-protein sulphydryl (NPSH) as a marker for reduced GSH, was measured in serum and kidney tissue using the method of Ellman (1959).

(vi) Catalase (CAT) activity was measured in serum by a commercial kit produced by Cayman Chemical Company, Ann Arbor, MI. The method based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H2O2. The formaldehyde produced is measured spectrophotometrically at 540 nm (Johansson and Borg, 1988).

(vii) Superoxide dismutase (SOD) activity was measured by SOD-assay kit purchased also from Cayman Chemical Company, Ann Arbor, MI. The nitro-blue tetrazolium (NBT) reduction by superoxide anion to blue formazan was followed at 450 nm (Wheeler et al., 1990).

(viii) ADMA was measured in serum and kidney homogenates by ELISA kit produced by DLD Diagnostica, Germany, as reported previously (Schulze et al., 2004). Controls and samples were pipetted into ADMA-coated microtiter strips, and 50 \mu l rabbit-anti-N-acyl-ADMA antiserum was added. The plate was covered and incubated for 15–20 h at 2–8 °C. Then goat anti-rabbit-IgG-peroxidase enzyme conjugate was added to all wells and incubated for 60 min at room temperature. The 3,3', 5,5'-tetramethylbenzidine (TMB) substrate solution was added followed by 100 \mu l of 0.3 M sulphuric acid (stop solution). Optical density has been read at 450 nm.

(ix) Total body nitric oxide production was evaluated by measuring the stable end products of NO metabolism, NO2− and NO3− in plasma and 24-h urine by special kit (Cayman Chemical Company, USA) utilizing nitrate reductase and the Griess reagent (Green et al., 1982).

(x) Serum total cholesterol, triglycerides, urea, creatinine levels, as well as urinary protein and creatinine were measured calorimetrically using commercial kits (Spinreact, Spain) according to the manufacturer’s instructions and reported techniques (Buccolo and David, 1973; Meiattini et al., 1978; Bruits and Ashwood, 2001). The results were measured by spectrophotometry. Creatinine clearance over 24-h was calculated as \( \frac{U_{\text{creatinine}} \times V}{P_{\text{creatinine}}} \), where \( U_{\text{creatinine}} \) is the urinary creatinine concentration, \( P_{\text{creatinine}} \) the plasma creatinine, and \( V \) is the flow rate (ml/day), i.e., 24-h volume of urine.

2.2.5. Histopathological examination of large vessels and cardiac tissues

For evaluation of effect of the treatment regimen on heart muscle and arterial wall pathology, paraffin embedded 3- \( \mu \)m sections were cut and hematoxylin-eosin staining was performed. Tissues were examined at 200 \( \times \) with an Olympus microscope, the images were taken by an Olympus digital camera. The area between the internal and external elastic
laminae was taken as arterial media, and the area between internal elastic lamina and the luminal boundary taken as neointima, as previously described (Tulis et al., 2000).

2.2.6. Statistical analysis

Results were statistically analyzed using a statistical software program (SPSS for Windows). Results were expressed as mean ± S.D. of 10 values in each group. Differences between groups were tested for statistical significance by ANOVA followed by Tukey’s post hoc test, and \( p < 0.05 \) was considered significant.

3. Results

3.1. Serum insulin and glucose levels and HOMA-IR index

As shown in Table 1, old control rats had significant increase (\( p < 0.05 \)) in body weight, blood glucose, serum insulin and HOMA-IR index in comparison to young rats. CRF in old rats caused significant increase in serum insulin level and HOMA-IR index (\( p < 0.05 \)), while blood glucose level showed no significant change in comparison to control old rats (\( p > 0.05 \)). Treatment of CRF rats (Group IV) with catechin, vitamins E and C for 12 weeks prevented significant changes in insulin level, and HOMA-IR index from that of the control old group (\( p > 0.05 \)).

3.2. Systolic BP

CRF rats exhibited significant elevation in arterial pressure during the observation period in comparison to the control rats (\( p < 0.05 \), Table 1). Catechin, vitamins E and C therapy results in correction of CRF-induced hypertension in comparison to the untreated CRF rats (Group III) (\( p < 0.05 \)).

3.3. MDA, CAT, SOD and GSH levels

Aging enhanced lipid peroxidation manifested by significant increase in serum and kidney MDA levels in aged rats (Group II) in comparison to young control (Group I)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Young controls</th>
<th>Old controls</th>
<th>Old CRF untreated</th>
<th>Old CRF treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>304 ± 12</td>
<td>542 ± 14(^*)</td>
<td>482 ± 9(^*)</td>
<td>534 ± 7(^*)</td>
</tr>
<tr>
<td>Insulin ((\mu)U/ml)</td>
<td>5.48 ± 0.8</td>
<td>7.52 ± 1.1(^*)</td>
<td>19.2 ± 2.1(^*)</td>
<td>8.58 ± 1.8(^*)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>8.76 ± 0.49</td>
<td>11.8 ± 1.1(^*)</td>
<td>11.27 ± 1.2(^*)</td>
<td>11.64 ± 1.2(^*)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.1 ± 0.28</td>
<td>3.8 ± 0.3(^*)</td>
<td>9.2 ± 1.4(^*)</td>
<td>4.86 ± 2.8(^*)</td>
</tr>
<tr>
<td>BP at week 0</td>
<td>111.8 ± 2.0</td>
<td>120.3 ± 2.5(^*)</td>
<td>155.9 ± 4.0(^*)</td>
<td>154.1 ± 4.09(^*)</td>
</tr>
<tr>
<td>BP at week 12</td>
<td>113.0 ± 3.0</td>
<td>124.3 ± 2.9(^*)</td>
<td>211.1 ± 11.6(^*)</td>
<td>168.4 ± 3.864(^*)</td>
</tr>
</tbody>
</table>

Notes: \(^*p < 0.05\) vs. young control, \(^p < 0.05\) vs. old control, \(^\dagger p < 0.05\) vs. untreated CRF rats.

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Table 2
ADMA, NO\textsubscript{2}/NO\textsubscript{3}, MDA, GSH levels and antioxidant enzyme activities in the studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Young controls</th>
<th>Group I</th>
<th>Old controls Group II</th>
<th>Old CRF untreated Group III</th>
<th>Old CRF treated Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Serum ADMA (μmol/l)</td>
<td>1.12 ± 0.06</td>
<td>1.61 ± 0.12\textsuperscript{A}</td>
<td>5.11 ± 0.45\textsuperscript{A}</td>
<td>2.13 ± 0.29\textsuperscript{t}</td>
<td></td>
</tr>
<tr>
<td>Kidney ADMA (μmol/l)</td>
<td>6.22 ± 0.49</td>
<td>6.86 ± 0.86</td>
<td>8.77 ± 1.06\textsuperscript{A}</td>
<td>6.9 ± 1.33\textsuperscript{i}</td>
<td></td>
</tr>
<tr>
<td>Serum NO\textsubscript{2}/NO\textsubscript{3} (μmol/l)</td>
<td>47.16 ± 2.8</td>
<td>26.96 ± 1.86\textsuperscript{A}</td>
<td>12.79 ± 2.83\textsuperscript{A}</td>
<td>18.83 ± 1.60\textsuperscript{1A}</td>
<td></td>
</tr>
<tr>
<td>Urine NO\textsubscript{2}/NO\textsubscript{3} (μmol/24 h)</td>
<td>23.60 ± 2.36</td>
<td>18.63 ± 1.64\textsuperscript{A}</td>
<td>7.17 ± 0.80\textsuperscript{A}</td>
<td>10.00 ± 1.04\textsuperscript{1A}</td>
<td></td>
</tr>
<tr>
<td>Serum MDA (nmol/ml)</td>
<td>3.05 ± 0.186</td>
<td>4.58 ± 0.33\textsuperscript{A}</td>
<td>10.47 ± 0.62\textsuperscript{A}</td>
<td>5.44 ± 0.48\textsuperscript{1A}</td>
<td></td>
</tr>
<tr>
<td>Kidney MDA (nmol/ml)</td>
<td>244.9 ± 4.04</td>
<td>649.6 ± 56.18\textsuperscript{A}</td>
<td>725.5 ± 26.7\textsuperscript{A}</td>
<td>628.75 ± 25.8\textsuperscript{1A}</td>
<td></td>
</tr>
<tr>
<td>Serum SOD (IU/ml)</td>
<td>1.73 ± 0.10</td>
<td>1.59 ± 0.06\textsuperscript{A}</td>
<td>1.19 ± 0.085\textsuperscript{A}</td>
<td>1.54 ± 0.023\textsuperscript{1}</td>
<td></td>
</tr>
<tr>
<td>Serum CAT (μmol of H\textsubscript{2}O\textsubscript{2} consumption/(min mg protein))</td>
<td>71.8 ± 4.95</td>
<td>56.6 ± 3.63\textsuperscript{A}</td>
<td>31.5 ± 3.08\textsuperscript{A}</td>
<td>46.2 ± 2.86\textsuperscript{1A}</td>
<td></td>
</tr>
<tr>
<td>Serum GSH (μg/mg protein)</td>
<td>236.80 ± 11.28</td>
<td>214.77 ± 11.52\textsuperscript{A}</td>
<td>181.22 ± 5.57\textsuperscript{A}</td>
<td>208.9 ± 9.78\textsuperscript{1A}</td>
<td></td>
</tr>
<tr>
<td>Kidney GSH (μg/mg protein)</td>
<td>11.09 ± 0.6</td>
<td>8.17 ± 0.64\textsuperscript{A}</td>
<td>64 ± 0.51\textsuperscript{A}</td>
<td>4.39 ± 0.49\textsuperscript{i}</td>
<td></td>
</tr>
</tbody>
</table>

Notes: \textsuperscript{A}p < 0.05 vs. young control, \textsuperscript{t}p < 0.05 vs. old control, \textsuperscript{i}p < 0.05 vs. untreated CRF rats.
(p < 0.05, Table 2). On the other hand, antioxidant enzymes CAT and SOD activities and the serum and kidney levels of the non-enzymatic antioxidant GSH were significantly lower in aged rats in comparison to young rats (p < 0.05). CRF caused further significant (p < 0.05) increase in MDA levels and decreased activity of CAT and SOD and decreased serum and kidney GSH in older rats (Group III) in comparison to their controls (Group II) (p < 0.05, Table 2). Catechin, vitamins E and C treatment (Group IV) significantly decreased serum MDA level and increased serum levels of the antioxidant enzymes CAT and SOD, and both serum and kidney GSH levels as compared to the untreated Group III rats (p < 0.05).

3.4. ADMA and NO\textsubscript{2}/NO\textsubscript{3} levels

Table 2 shows that serum and urinary concentrations of NO\textsubscript{2}/NO\textsubscript{3} were significantly lower in older rats (Group II) in comparison to young rats (Group I) (p < 0.05). CRF caused serum and urinary NO\textsubscript{2}/NO\textsubscript{3} level to be significantly lower in Group III rats compared with the sham-operated controls (Group II) (p < 0.05). Treatment with catechin, vitamins E and C increased both serum and urinary NO\textsubscript{2}/NO\textsubscript{3} production in treated CRF rats in comparison to the untreated group (p < 0.05). In addition, Table 2 also shows that control old rats had significantly higher serum ADMA level in comparison to young ones (p < 0.05), while kidney tissue ADMA was comparable in both age-groups (p > 0.05). Untreated CRF rats (Group III) showed marked increase in their serum and kidney tissue ADMA level in comparison to age-matched sham operated rats (p < 0.05). Supplementation with catechin, vitamins E and C significantly decreased both serum and kidney tissue ADMA level in CRF in comparison to the untreated Group III rats (p < 0.05).

3.5. Lipid profiles and kidney functions

As shown in Table 3, serum contents of cholesterol, triglycerides and 24 h urinary protein excretion were significantly higher in old rats (Group II) compared to the young ones (Group I) (p < 0.05). However, the serum urea and creatinine levels and creatinine clearance showed no significant difference between control young and old rats (p > 0.05). After 12 weeks of SNx operation, aged CRF rats (Group III) showed a significant increase in their serum and kidney tissue ADMA level in comparison to age-matched sham operated rats (p < 0.05).
in serum creatinine, urea nitrogen and urinary protein loss and reduction in creatinine clearance, compared to the age-matched controls rats \((p < 0.05, \text{Table } 3)\). Treatment with catechin, vitamins E and C (Group IV) caused a significant reduction \((p < 0.05)\) in serum urea, creatinine and protinurea, and increased creatinine clearance in comparison to the untreated rats (Group III).

### 3.6. Histopathological examination of heart and blood vessels

Microscopic examination of the myocardial tissue of old CRF rats (Group III) showed disarray of myocardial fibers, eosinophilic cytoplasm, hyperchromatic nuclei, foci of inflammation and hydropic degeneration indicating myocardial hypertrophy in comparison to the controls (Group II) (Fig. 1). Treatment with catechin, vitamins E and C (Group IV) produced marked improvement in the picture of the myocardium that shows less degeneration, less eosinophilic cytoplasm than the untreated Group III (Fig. 1). Wall of the carotid arteries of old CRF rats showed thickening of the intima and focal mild infiltration with mononuclear cells (Fig. 2). Treatment of CRF with catechin, vitamins E and C for 12 weeks prevented the intimal thickening and the inflammatory cellular infiltration in the blood vessel walls (Fig. 2).

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![Fig. 1](image1.png)

**Fig. 1.** (A) Section of myocardium of old control rats. Some cells show disarray of myocardial fibers and eosinophilic cytoplasm (the short black arrows), (B) myocardium of old CRF with no treatment showing focal areas of disarray of myocardial fibers with eosinophilic cytoplasm (the thin complete arrow), foci of inflammation around blood vessels (the interrupted arrow) and hydropic degeneration (the empty arrow), and (C) myocardium of old CRF treated with catechin, vitamins E and C shows less degeneration, less eosinophilic cytoplasm than untreated group (200×).

![Fig. 2](image2.png)

**Fig. 2.** (A) Section of blood vessel of old control rats showing focal thickening of the intima (the empty arrow), (B) blood vessels of old CRF with no treatment has focal thickening of the intima (the double head arrow), infiltration with occasional mononuclear cells (the black triangle), and (C) blood vessels of old CRF treated with catechin, vitamins E and C showing normal intimal-medial thickening with absent inflammatory cellular infiltration (the long black arrow) (200×).
4. Discussion

IR plays an important role in clustering risk factors of atherosclerosis such as hypertension, dyslipidemia, and abnormal glucose metabolism (Shinohara et al., 2002) and was reported to be present early in the course of renal disease (Chen et al., 2003). Aging and age-related diseases, especially CRF are associated with oxidative stress from the over-production of ROS (Augustyniak et al., 2005). ROS can cause IR by decreasing insulin mediated glucose uptake (Hansen et al., 1999).

Food components may increase and decrease cellular oxidative ability. (+)-Catechin, one of the natural components that are present in green and black tea, fresh vegetables and fruits (Ruidavets et al., 2000), is proved to have a free radical scavenging and antioxidant effect (Rice-Evans et al., 1996). Catechin is reported to be diminished in old age (Augustyniak et al., 2005). In the present study a combination of oral (+)-catechin, vitamins E and C were investigated to test their effect on IR and atherosclerosis in chronic renal disease. Serum and kidney MDA levels were found to be increased while, CAT and SOD enzyme activities and GSH levels were found to be decreased in old rats in this study.

Our data agree with previous works of Rodriguez-Martinez et al. (1998) and Anisimov et al. (1999), who reported that serum total antioxidant activity decreased and lipid peroxidation levels increased with age, as indicated by high plasma MDA, mainly in processes associated with oxidative stress and vascular injury (Janero, 1990). These disturbances in the prooxidant/antioxidant balance (redox status) were so much exaggerated when CRF was induced in the old rats in this study. This raised the potential for tissue damage due to oxidative stress (Sies, 1991).

The activity of methylating enzymes such as the $S$-adenosylmethionine-dependent arginine methyltransferase (Type I) responsible for the ADMA synthesis and the activity of ADMA-hydrolyzing enzymes such as dihydro-dimethylaminohydrolase (DDAH) is redox-sensitive (Boger et al., 2000). This could explain that increased lipid peroxidation product MDA level a marker of oxidative stress induced in CRF rats was found to be associated with increased blood and kidney tissue ADMA levels.

The inhibition of endothelial nitric oxide synthase (eNOS) by high ADMA levels could be the leading cause of decreased NO production measured by its stable metabolites, NO$_2^−$/NO$_3^−$ in serum and urine of CRF in this study. Decreased NO bioavailability could be the cause of increased HOMA-IR index observed in untreated CRF rats, as it was reported that a normal insulin secretion and function requires an intact endothelium (Steinberg et al., 1996). Current thinking about the link between IR, atherosclerosis and NO focuses on endothelial function (Chan and Chan, 2002), thus the intact functioning of eNOS seems to be of central importance in insulin sensitivity (Steinberg et al., 1996).

Several mechanisms could contribute to the hypertension in old control and CRF rats in this study. First, increased ADMA level through inhibition of NO synthesis by competitive inhibition of NOS could be the leading cause of hypertension. In addition, the observation that ADMA levels increases early in the development of athero-sclerosis suggests that ADMA has the potential to be not only a marker, but a mediator of vascular lesions (Boger, 2004). The role of increased ADMA level in stimulating the atherosclerotic changes in the carotid arteries marked by increased intima-media thickness in CRF rats recruited in the current study could be explained by the proposed hypothesis of Zsuga et al. (2005) that...
ADMA by inhibiting both the neuronal and the endothelial forms of NOS, results in IR as well as in atherosclerosis, therefore ADMA is a molecule responsible for the coexistence of these two conditions.

Increased MDA and ADMA levels in old CRF rats in the present study were found to be associated with hypercholesterolemia and hypertriglyceridemia. This was in accordance with previous reports of an association between ADMA and MDA in hypercholesterolemic rabbits (Xiong et al., 1994). IR associated with hypercholesterolemia, hyperlipidemia, hyperglycemia, uremia, hypertension, obesity and the metabolic syndrome is attained by the elevation of ADMA via variable mechanisms (oxidative stress, impaired renal clearance, etc.) that inhibits neuronal NOS, and consequently impairs post-prandial insulin sensitization (quoted by Zsuga et al., 2005). On the other hand, accelerated atherosclerosis associated with the uremia is a consequence of the inhibition of the endothelial isoforms (Shinohara et al., 2002). It is also worth of notice that metabolic abnormalities induced by IR further elevate the systemic ADMA concentration, thus accelerate the progression of both atherosclerosis and IR (Zsuga et al., 2005).

Treatment of our CRF rats with a mixture of catechin, vitamins E and C resulted in marked reduction in MDA, serum and kidney tissue ADMA levels in contrast to increased GSH levels and antioxidant enzyme activity. This could be attributed to synergistic antioxidative functions of catechin, vitamins E and C that modulate the oxidative stress (Frei et al., 1989; Rice-Evans et al., 1996; Sayed-Ahmed et al., 2001), or may potentially imply a direct effect in modifying the activity of DDAH, an enzyme thought to be pivotally important in the regulation of ADMA levels (Cooke, 2000). Alternatively, it is possible that catechin, vitamins E and C may function via modulation of lipid peroxides and oxidized low-density lipoproteins (ox-LDL) levels which are known to increase ADMA level either through increased production or decreased degradation (Ishikawa et al., 1997; Jiang et al., 2004). This could inhibit oxidative endothelial damage (Arduini et al., 1990) and prevent the oxidative modification of proteins (Sayed-Ahmed et al., 2001). The decreased ADMA level by antioxidant mixture in the present study is in accordance with previous lowering effect of high dose of vitamin E on ADMA level in CRF patients (Saran et al., 2003).

Decreased cholesterol and triglyceride levels in treated rats in this study could be attributed to the lipid lowering effect of vitamin C, which exerts a protective role against the peroxidative damage (Dalla Libera et al., 2001). In addition, catechin is reported to decrease cholesterol absorption and enhance cholesterol excretion (Tokunaga et al., 2002). Correction of the lipid profile could work together with BP lowering effect of increased NO level to protect the blood vessel wall against atherosclerotic manifestations and the myocardium against hypertension-induced changes.

Amelioration of CRF-induced oxidative stress with catechin, vitamins E and C in the current study was accompanied not only by reversal of CRF-induced elevation of ADMA level in the remnant kidney and serum, but also with an expected rise in NO production capacity as demonstrated by increased serum and urinary $\text{NO}_2^-/\text{NO}_3^-$ levels. This observation clearly points to the role of increased ADMA level in the pathogenesis of NO-deficiency in CRF. The combination of increased NO production capacity and increased antioxidant pool could be the contributing factors in lowering BP, amelioration of atherosclerotic changes and IR in our treated CRF animals. This is in accordance with previous reports that catechins present in tea protect against the development of severe
atherosclerosis, as assessed by radiographic films of the abdomen, in population-based follow-up study among men and women (Geleijnse et al., 1999). In addition, catechin intake was reported to be inversely proportionally associated with ischemic heart disease mortality in elderly men (Arts et al., 2001). Other than its role in lowering ADMA level, vitamin E may play a direct role is decreasing IR due to its ability to preserve the intracellular redox balance and prevent the activation of the stress sensitive serine kinase cascades mostly associated with transcriptional and mitogenic effects of insulin (Vinayaga Moorthi et al., 2006).

5. Conclusion

The findings of the present study help us to conclude that NO deficiency may be responsible about the atherosclerotic changes and associated IR in old age people with CRF and even in the absence of renal disease. Catechin, vitamins E and C supplementation resulted in improvement in the antioxidant forces, decreases lipid peroxidation and ADMA levels and increased NO production that may be responsible about keeping the normal insulin sensitivity and preventing atherosclerotic changes. Taken together, these results indicate that the increase in endogenous ADMA levels in aged healthy and CRF rats may be secondary to an elevation of lipid peroxides. And that a mixture of antioxidant intake in old age peoples especially those with CKD may be of specific benefits in holding the disease progression and counteracting IR and atherosclerotic complications in this population.

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