Soy protein diet improves endothelial dysfunction in renal transplant patients

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Abstract

Background. Since it has been demonstrated that soy diet can improve endothelial function, in the present study we evaluated the effect of dietary substitution of 25 g of animal proteins with soy proteins on endothelial dysfunction in renal transplant patients.

Methods. In 20 renal transplant patients (55 ± 11 years, serum creatinine 1.7 ± 0.6 mg/dl), brachial artery flow mediated dilation (FMD) and endothelium-independent vasodilation (sublingual nitroglycerine, 25 μg) were measured at baseline, after 5 weeks of a soy diet and finally after 5 weeks of soy wash-out. Changes in plasma lipids, markers of oxidative stress (lipid peroxides, LOOH) and inflammation (C-reactive protein), isoflavones (genistein and daidzein), asymmetric dimethyl arginine (ADMA) and L-arginine were also evaluated.

Results. At baseline, patients showed a significantly lower FMD as compared with age-matched healthy subjects (3.2 ± 1.8 vs 6.3 ± 1.9, respectively; P < 0.001), while response to nitroglycerine was similar. After soy diet, actual protein intake was not changed, cholesterol and lipid peroxides were significantly reduced, and isoflavones were detectable in plasma. Soy diet was associated with a significant improvement in FMD (4.4 ± 2.0; P = 0.003 vs baseline), while response to nitroglycerine was unchanged. Improvement in FMD was related to L-arginine/ADMA ratio changes, but no significant relation was found to changes in cholesterol, lipid peroxides or genistein and daidzein plasma concentrations. After 5 weeks of soy diet discontinuation, FMD (3.3 ± 1.7%) returned to baseline values and isoflavones were no longer detectable in plasma.

Conclusions. A soy protein diet for 5 weeks improves endothelial function in renal transplant patients. This effect seems to be strictly dependent on soy intake as it disappears after soy withdrawal and is mediated by an increase in the L-arginine/ADMA ratio, independently of change in lipid profile, oxidative stress or isoflavones.

Keywords: ADMA; chronic kidney disease; diet; endothelial dysfunction; L-arginine; renal transplant; soy

Introduction

Renal transplant patients are at increased risk of cardiovascular morbidity and mortality [1–3]. Endothelial dysfunction is a well-known mechanism promoting atherosclerosis and thrombosis [4], contributing to the pathogenesis of cardiovascular events. Thus, endothelial dysfunction has been associated to atherosclerotic damage in coronary [5] and carotid arteries [6]. Moreover, endothelial dysfunction is an independent predictor of cardiovascular events in patients at high cardiovascular risk [7].

Since impaired endothelium-dependent vasodilation has been documented in renal transplanted patients [8–10], improvement of endothelial dysfunction could represent a rational target for therapy aimed to reduce and/or to prevent cardiovascular damage and events [11].

Protein restriction and quality of protein intake are important aspects in nutrition management of patients with chronic renal disease [12,13]. In particular, soy has a special nutritional importance since soy protein foods have high biological value, and contain phytoestrogens (isoflavones) [14] with weak estrogenic [15] and antioxidant activities [16]. The latter might explain the favourable effects which can be exerted by soy diet...
on endothelial function in primate [17] and human studies [18].

In the present study, we evaluated the effect of dietary substitution of 25 g of animal proteins with soy proteins on endothelium-dependent vasodilation in the brachial artery of renal transplant patients. The effect of the dietary periods on plasma isoflavones, markers of oxidative stress and inflammation, asymmetric dimethyl arginine (ADMA) and L-arginine was also assessed.

Subjects and methods

The study population included 20 (55 ± 11 years, 12 males and eight females, six post-menopausal women) renal transplant recipients with stable renal function.

Underline renal diseases were: chronic glomerulonephritis (n = 7), hypertensive nephropathy (n = 4), polycystic kidney disease (n = 2), interstitial nephritis (n = 2), unknown (n = 5). Smoking, obesity, diabetes, nephrotic syndrome or serum creatinine ≥ 2.0 mg/dl and prior cardiovascular events were considered as exclusion criteria. None of the patients received lipid-lowering agents during the study period. Immunosuppressive therapy consisted of prednisone plus ciclosporin plus mycophenolate mofetil or azathioprine in 10 patients, prednisone plus ciclosporin in five patients and ciclosporin alone in five patients. Sixteen patients were on antihypertensive treatment (a mean of two drugs) with calcium channel blockers (n = 12), ACE-inhibitors or sartans (n = 10), beta-blockers (n = 2), alpha-blockers (n = 1), sympathetic modulators (n = 6) and furosemide (n = 3). Treatment was unchanged during the dietary periods. Twenty age- and sex-matched healthy subjects, with normal renal function and lipid profile, were recruited as controls for endothelial response. The protocol was approved by the Ethical Committee of the University of Pisa and all patients gave written consent for the study.

Dietary counselling

Individual assessment of dietary habits was carried out by the same dietician in all of the patients studied. Nutrient intake assessment was obtained by a 3-day dietary recall by interview and subsequent analysis using the tables of food composition. After the baseline dietary interview, dietary counselling was given individually with the aim of substituting 25 g of animal proteins with 25 g of soy proteins, as suggested by FDA [19], for a 5-week period. This was achieved using common commercially available soy foods, according to each patient’s own preference. All patients were instructed to record the actual daily intake of soy food during the whole study period.

Experimental procedure

Vascular ultrasound scans were performed in the morning, with subjects supine, in a quiet air conditioned room (22–24 °C). A B-mode scan of the right brachial artery was obtained in longitudinal section between 5 cm and 10 cm above the elbow using a 7.0 MHz linear array transducer (AU5 Armonic, ESAOTE Biomedica, Florence, Italy). The transducer was held at the same point throughout the scan by a stereotactic clamp. End-diastolic frames (ECG-triggered) were acquired every second on a personal computer using a commercial software program (miroVIDEO DC30/plus, Pinnacle Systems GmbH, Braunschweig, Germany). A cuff was placed around the forearm just below the elbow.

Experimental protocol

Endothelium-dependent response was assessed as dilation of the brachial artery induced by increased flow (flow mediated dilation, FMD), by inducing forearm reactive hyperaemia (RH) (cuff inflated for 5 min at 250 mmHg) [20]. Endothelium-independent dilation was obtained by administration of a low dose (25 μg) of sublingual glyceril trinitrate (GTN).

Plasma lipid profiles including total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, and apolipoprotein A1 and B were measured after an overnight fast, using conventional enzymatic methods. Oxidative stress was evaluated by measuring plasma lipoperoxides LOOH with a colorimetric method [21]. The isoflavones, genistein and daidzein and their metabolites were measured in serum by HPLC/mass spectrometry [22]. Limit of quantification and detection were respectively 0.005 and 0.001 μM for genistein and 0.05 and 0.005 μM for daidzein. C-reactive protein (CRP) was assessed by a high sensitivity modified laser nephelometry technique [23]. ADMA measured by ELISA [24] and L-arginine by HPLC/mass spectrometry [25].

Vascular responses, lipid profile, LOOH, isoflavones, CRP, ADMA and L-arginine were measured in patients at baseline, after 5 weeks of soy diet and again after 5 weeks of soy wash-out.

Data analysis

Measurements were performed on acquired frames by the computerized edge detection system [26]. FMD and response to GTN were calculated as the maximal percent increase in diameter above baseline. Blood flow volume was calculated by multiplying Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area (πr²). Flow velocity was measured at baseline and within 15 s after cuff release. RH was calculated as percent increase in flow after cuff release as compared with baseline flow.

Data were analysed using an NCSS statistical package. Descriptive data are expressed as mean ± SD. Statistical analysis was performed using the Student’s t-test, ANOVA for repeated measures and Pearson’s correlation test, as appropriate. A value of P < 0.05 was considered statistically significant. The present study was designed to have the 80% power at the 5% level to detect a 1.0% improvement in FMD after intervention.

Results

Renal transplant patients showed significantly reduced FMD as compared with healthy controls (3.2 ± 1.8 vs 6.3 ± 1.9%, respectively; P < 0.001) (Figure 1). No difference was found in RH (renal transplant...
patients 487 ± 248%; control subjects 540 ± 257%; P = NS). Response to GTN was similar in healthy controls and renal transplant patients (7.3 ± 2.9 vs 7.0 ± 3.1%, respectively; P = NS) (Figure 1). At baseline, serum genistein and daidzein were not detectable in renal transplant patients.

Soy diet did not change total dietary protein intake, body weight or albumin levels (Table 1). Renal function, urinary protein excretion, serum calcium and phosphorus were not significantly modified, while total and LDL cholesterol and LOOH levels were significantly reduced (Table 2). The estimated amount of isoflavones administered was 28 ± 16 mg/day of genistein and 24 ± 16 mg/day of daidzein. After 5 weeks of dietary soy, serum genistein concentrations were above the limit of detection in 100% and the limit of quantification in 80% of the patients, while serum daidzein concentrations reached the limit of detection in 100% but the quantification in only 20% of the patients. As compared with baseline, CRP and ADMA levels were not significantly modified, while serum creatinine, L-arginine/ADMA ratio, and CRP levels were not signifi-
cantly changed (Table 2). Genistein and daidzein serum concentrations were below limit of quantification or not detectable. Brachial artery diameter and RH were unchanged, while FMD returned to baseline (Figure 3).

Improvement in FMD was significantly related to increase in L-arginine/ADMA ratio (R = 0.49; P < 0.05). FMD changes after soy diet tended to be inversely related to ADMA (R = -0.37; P = 0.10) and FMD values at baseline (R = -0.38; P = 0.09), while no correlation was found between changes in FMD and changes in serum LDL cholesterol, LOOH or CRP levels. Moreover, improvement in FMD was not related to the genistein and daidzein estimated amount in the soy diet or measured serum concentration after the dietary period.

Five weeks of soy diet discontinuation, total and LDL cholesterol and LOOH were still significantly lower as compared with baseline values. ADMA, L-arginine/ADMA and CRP levels were not significantly changed (Table 2). Genistein and daidzein serum concentrations were below limit of quantification or not detectable. Brachial artery diameter and RH were unchanged, while FMD returned to baseline values and response to GTN remained unmodified (Figure 3).

**Discussion**

The present study demonstrates, for the first time, that the substitution of animal proteins with soy proteins improves endothelial function in the brachial artery of renal transplant recipients, suggesting that soy diet might exert a vascular protective effect in these patients.

Reduced endothelium dependent vasodilation is an alteration which represents an independent predictor
of cardiovascular events in patients at high cardiovascular risk [7]. Endothelial dysfunction is a common feature of patients with renal transplantation [8–10], thus it can contribute to the high risk of cardiovascular morbidity and mortality of these patients [1–3].

It can be hypothesized that treatment of endothelial dysfunction might have favourable effects on cardiovascular prognosis in renal transplant patients.

Protein restriction and quality of protein intake are important aspects in nutrition management of renal patients [13]. Vegetable protein induces lower glomerular filtration rate, renal plasma flow and fractional albumin excretion than animal protein [27], and can exert more favourable effects on lipid metabolism, acid-base status and blood pressure control [28–30]. Soy has a special nutritional importance, since soy protein foods have high biological value and contain mono- and poly-unsaturated fatty acids, fibres and phytoestrogens (isoflavones) [14]. Isoflavones have weak estrogenic [15] and antioxidant activity [16], which can favourably affect endothelial function [17,18].

To address the effect of soy diet, we selected a group of stable renal transplant patients who at baseline presented a markedly reduced brachial artery FMD as compared with healthy subjects. Since patients showed values for both RH (the stimulation evoked to activate endothelial relaxation) and response to GTN (administered at a dose causing a degree of vasodilation comparable with FMD) similar to those obtained in healthy controls, our results confirm the presence of impaired endothelium-dependent vasodilation in the conduit arteries of renal transplant patients [8–10].

A 5-week diet with soy protein substitution for animal proteins was able to improve FMD in these patients. Since RH and response to GTN did not change after soy diet, it indicates that soy can selectively improve endothelium-dependent vasodilation in the brachial artery of renal transplant patients. These data are in line with previous results obtained in hypercholesterolaemic males [31] and post-menopausal women [18,32], while it is controversial whether this effect is detectable in healthy normocholesterolaemic post-menopausal women [33,34].

Interestingly, after 5 weeks of soy withdrawal, in the renal transplant patients FMD returned to baseline values. Thus, it is conceivable that the observed improvement in endothelial function strictly depended on soy diet, since it disappeared after the soy wash-out period.

The effect of the dietary intervention is very likely to be specific. In the study population, other factors which could independently affect endothelial function, such as smoking habits and pharmacological treatment were unchanged during the overall experimental period. Moreover, an adequate experimental setup including computerized analysis of brachial artery diameter, assures good reproducibility for FMD measurement [26], thus excluding methodological-related problems.

Several mechanisms could be responsible for the beneficial effect of soy on brachial artery endothelium-dependent vasodilation.

As previously shown [31,32], soy diet reduced total and LDL-cholesterol, without modifying HDL cholesterol, and these changes in lipid profile can explain the improvement of endothelial function as reported in hypercholesterolaemic male patients [31]. However, the improvement in FMD was not related to changes in total or LDL cholesterol levels, suggesting that the beneficial effect of soy on endothelial function was not related to changes in lipid profile after soy. Moreover, the lipid-lowering effect of the soy was maintained after the wash-out period, while the beneficial effect on FMD disappeared. Thus, the effect of soy on endothelial function seems to be independent of its lipid-lowering effect. However, larger trials evaluating normocholesterolaemic post-menopausal women...
failed to demonstrate a beneficial effect of soy supplementation on endothelial function [33,34].

Soy diet could be particularly effective in renal transplant patients, since they demonstrated a markedly reduced endothelial function [8–10]. This possibility is in agreement with the fact that the improvement of FMD after soy is inversely related to baseline FMD values. Since the most important mechanism determining endothelial dysfunction in renal transplant patients seems to be the production of oxidative stress [10], reduction of oxidative stress could be another mechanism explaining the improvement of endothelial function induced by soy diet.

In the present study, this possibility was indirectly explored by measuring plasma LOOH, which was significantly reduced by soy diet, an effect which can be related to the antioxidant properties of isoflavones [16] as well as to changes in lipid profile. However, the change in LOOH was not related to the improvement in FMD. Moreover, LOOH were still significantly lower after soy withdrawal, although they tended towards baseline values. These results would suggest that the beneficial effect of soy diet on endothelial function, could be independent of an antioxidant effect. However, it cannot be excluded that the observed changes of systemic markers of oxidative stress may not reflect the effect of dietary period on intracellular oxidative mechanisms.

The positive effect of soy diet on endothelial function might be mediated by isoflavones. Genistein, the predominant isoflavone, caused nitric oxide-dependent relaxation in the human forearm microcirculation [36] and administration of the soy phytoestrogen genistein, at the dose of 54 mg/day for 6 months or 1 year, improved FMD in healthy post-menopausal women [37,38]. In the present study, the goal to increase serum concentration of soy isoflavones by the dietary period using commercially available whole soy foods was achieved. The estimated total amount of isoflavone dietary intake was similar to the dosage of genistein which improved endothelial function in post-menopausal women [37,38]. The increase in serum concentration of isoflavones was in parallel with improvement of conduit artery endothelial function in transplant patients. Although no significant relationship was found between plasma concentration of genistein and daidzein and the improvement of FMD, our data suggest that soy isoflavones might mediate, at least in part, the beneficial effect of soy diet in renal transplant patients.

No significant change in CRP was found after soy diet; in addition, no improvements in FMD were related to changes in CRP levels, suggesting that the effect of soy diet on endothelial function is not mediated by the reduction of low-grade inflammation.

Finally, the improvement in FMD could be mediated by an effect on ADMA, the endogenous inhibitor of endothelial NO synthase [39], since the link between ADMA, renal function and endothelial function is well-documented [40] and high ADMA plasma levels have been described in renal transplant patients, despite functioning renal grafts [41]. In our study ADMA levels did not significantly change after soy diet. However, changes in FMD after soy were significantly related to the modification in L-arginine/ADMA ratio. This is in agreement with previous results showing, in healthy volunteers, a parallel neutralization of lipaemia-induced endothelial dysfunction by soy protein and an increase in L-arginine/ADMA ratio [42]. This effect could be related to soy protein, including high L-arginine content, since it has been shown that chronic administration of soy isoflavones were not able to lower ADMA plasma levels in a large cohort of healthy post-menopausal women [43].

The present study has some limitations. Patients were not randomized to soy diet or dietary counselling. Moreover, 5 weeks is a relatively short period of time to evaluate the chronic effect of a soy diet, but it was chosen to ensure patients' compliance to the study protocol. However, this preliminary observation could represent the basis of a study on the effect of long-term administration of soy in renal transplant patients.

In conclusion, this study indicates a specific beneficial effect of substituting 25 g of soy proteins for animal proteins on conduit artery endothelium-dependent vasodilation in renal transplant patients. This effect seems to be mediated, at least in part, by an increase in L-arginine/ADMA ratio.

Since endothelial dysfunction is an independent promoter of cardiovascular events, improvement of endothelial dysfunction by a soy diet may have a beneficial influence in reducing cardiovascular morbidity and mortality in renal transplant patients.

Conflict of interest statement. All authors disclose any significant primary arrangement with commercial companies that produce or sell products that are the subjects of the study reported in the present manuscript.

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