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Endothelial nitric oxide synthase gene polymorphisms and the renal hemodynamic response to L-arginine

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Nitric oxide is generated from L-arginine by nitric oxide synthase (NOS), an enzyme that exists in several isoforms. Some studies found that a polymorphism (G894T) in the endothelial NOS gene was associated with decreased nitric oxide bioactivity and vascular complications. However, it is not known whether the enzyme had a reduced activity. Here we measured the effect of an infusion of L-arginine on renal hemodynamic function in subjects segregated by the presence or absence of the T allele. If this polymorphism represented a functional variant, subjects with the GT/TT form should exhibit a blunted renal hemodynamic response to L-arginine compared to those with a GG allele. All subjects were given a diet controlled for sodium and protein intake. GG subjects had lower mean arterial pressure and an augmented glomerular filtration rate at baseline. In response to a graded L-arginine infusion, this group had significant changes in effective renal plasma flow, glomerular filtration rate, filtration fraction, renal vascular resistance, and renal blood flow. The renal response to L-arginine in GT/TT subjects was blunted. Circulating cGMP levels and endothelial NOS mRNA expression, measured in skin biopsies by real-time PCR, did not differ between the groups. Our study shows that the G894T allele of endothelial NOS is associated with a blunted response to L-arginine, suggesting this polymorphism may be a functional variant in humans.

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Several polymorphisms of the endothelial nitric oxide synthase (eNOS) gene have been identified, including the G894T polymorphism.[1–3] Previous studies have suggested that this missense mutation, which leads to a substitution of aspartate to glutamate in the eNOS protein at position 894, renders this molecular variant more susceptible to proteolytic cleavage, reduced enzymatic activity, and lower basal endothelial nitric oxide (NO) production.[2] Impaired eNOS activity may diminish arteriolar vasodilatation, thereby blunting protective vascular effects that have been attributed to NO.[4–6] From a clinical perspective, decreased NO bioactivity may predispose patients to the development of hypertension, cardiovascular end-organ damage and nephropathy related to diabetes mellitus.[1,2,7–10]

The G894T eNOS gene polymorphism has been associated with impaired endothelial dependent vasodilatation, which may either be on the basis of augmented vasoconstrictor activity or a reduction in the generation of vasodilators, such as NO.[4–6] For example, we have shown in a previous study that the presence of the T allele is associated with an exaggerated renal vasoconstrictive response to a graded infusion of angiotensin II (Ang II).[11] In spite of these studies, the functional status of this polymorphism remains controversial, because other studies have failed to detect functional differences between those with and without the T allele.[5] Whether the G894T polymorphism influences renal hemodynamic functional responses to eNOS enzymatic activation in normal humans is not known.

Accordingly, we examined the effect of a graded L-arginine infusion on renal hemodynamic function in two groups: the first group included subjects who were either homozygous or heterozygous for the T allele (GT/TT subjects), and the second group consisted of those without the T allele (GG subjects). Our rationale reflected the possibility that the pattern of renal hemodynamic responsiveness to L-arginine, the substrate for eNOS, might clarify whether the polymorphism is functional. We also measured baseline eNOS mRNA expression in skin biopsy specimens, hypothesizing that the presence of the T allele would predict a blunted renal

hemodynamic response to L-arginine, in spite of similar eNOS expression.

RESULTS

Baseline characteristics

Baseline characteristics were similar in the GG ($n=16$) and GT/TT ($n=14$) groups (Table 1), except that the GT/TT group was older than the GG group. At baseline, the SBP was lower and the glomerular filtration rate (GFR) was higher in GG vs GT/TT subjects ($P<0.05$; Table 2). The GG group contained 6 men and 10 women and the GT/TT group 10 men and 5 women. These differences were not statistically significant.

Group differences in renal hemodynamic function

Although the systemic impact of L-arginine was similar in the two groups (Table 2), the increased SBP in GT/TT subjects was sustained at the 100 and 250 mg/kg doses of L-arginine. In GG genotype subjects, the L-arginine infusion was associated with increases in GFR, estimated renal plasma flow (ERPF), and renal blood flow (RBF), and a decline in renal vascular resistance (RVR; Table 2; Figures 1 and 2). GFR values were also significantly higher in GG subjects at the 100, 250, and 500 mg/kg doses of L-arginine (Table 2). In contrast, in the GT/TT group, the renal hemodynamic changes were limited to a rise in ERPF at the highest infusion rate, and a decline in RVR. The between-group rise in ERPF in response to L-arginine was augmented in GG subjects at the 100, 250, and 500 mg/kg doses ($P<0.05$ for each of the two infusion rates). There was no significant interaction between renal hemodynamic responsiveness and gender ($P>0.05$ for gender).

Table 1 | Baseline characteristics (mean ± s.e.m.)

Parameter	GG group n=16	GG/TT group n=14
Age (years)	24 ± 1	28 ± 1*
Body mass index (kg/m^2)	23 ± 1	24 ± 1
Estrogen (pmol/l in women)	60 ± 16	80 ± 14
Sodium excretion (mmol/24 h)	230 ± 31	270 ± 31
Protein intake (gram/kg/day)	1.2 ± 0.3	1.1 ± 0.3

*$P<0.05$ vs GG subjects.

Table 2 | The hemodynamic response to L-arginine in men and women by eNOS genotype (mean ± s.e.m.)

	Baseline	100 mg/kg	250 mg/kg	500 mg/kg
GG subjects				
SBP	108 ± 2	110 ± 3	105 ± 3	109 ± 3
DBP	62 ± 2	63 ± 2	61 ± 2	60 ± 2
MAP	78 ± 2	79 ± 2	76 ± 3	75 ± 3
GFR	137 ± 8	143 ± 4	147 ± 7	149 ± 6*
ERPF	693 ± 26	723 ± 35*	787 ± 40*	847 ± 48*
FF	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.18 ± 0.01*
RBF	1103 ± 45	1151 ± 59*	1234 ± 65*	1300 ± 73*
RVR	73 ± 4	71 ± 4	64 ± 5*	60 ± 4*
GT/TT subjects				
SBP	116 ± 3†	117 ± 3†	114 ± 4†	114 ± 3
DBP	64 ± 2	64 ± 2	63 ± 2	62 ± 2
MAP	80 ± 3	80 ± 2	77 ± 3	79 ± 3
GFR	126 ± 6†	120 ± 5†	125 ± 6†	131 ± 7†
ERPF	686 ± 49	668 ± 43§	721 ± 49§	754 ± 48*,§
FF	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
RBF	1164 ± 104	1109 ± 80	1181 ± 89	1220 ± 87
RVR	75 ± 8	78 ± 6	64 ± 7*	67 ± 7*

ERPF, effective renal plasma flow in ml/min per 1.73 m^2; FF, filtration fraction; GFR, glomerular filtration rate in ml/min per 1.73 m^2; MAP, mean arterial pressure (mm Hg); RBF, renal blood flow in ml/min per 1.73 m^2; RVR, renal vascular resistance in mm Hg/l/min.
*$P<0.05$ vs within-group baseline value.
$^\dagger P<0.05$ for between-group differences.
$^\S P<0.05$ for the response vs GG subjects.

Circulating RAS components, cGMP, and skin eNOS expression in GG vs GT/TT polymorphism subjects

The impact of the L-arginine infusions on circulating cyclic 3′,5′-guanosine monophosphate (cGMP) and RAS mediators was similar (Table 3). Skin eNOS expression was similar in the GG compared to the GT/TT group (Figure 3). There was no significant interaction between gender and circulating RAS components, cGMP, or eNOS expression ($P>0.05$).

DISCUSSION

Several previous studies have suggested that the G to T substitution at position 894 of the eNOS gene is associated with dose-dependent reductions in enzymatic activity and lower basal endothelial NO production,[7–9] however this

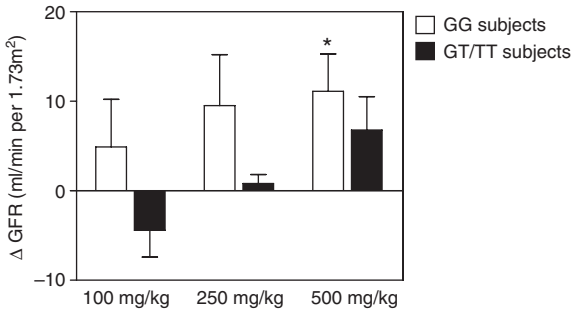

Figure 1 | The effect of L-arginine on GFR in GG vs GT/TT subjects (mean ± s.e.m.). GFR = glomerular filtration rate ml/min per 1.73 m^2. *$P<0.05$ vs baseline GFR.

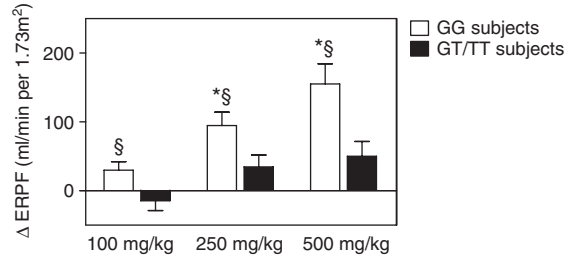

Figure 2 | The effect of L-arginine on ERPF in GG vs GT/TT subjects (mean ± s.e.m.). ERPF = effective renal plasma flow in ml/min per 1.73 m^2, *$P<0.05$ vs baseline value, $^\S P<0.05$ for the response vs GT/TT group.