Salt Intake, Endothelial Cell Signaling, and Progression of Kidney Disease

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Abstract—It has been known for decades that increased dietary intake of salt (NaCl) shortens the life span of rats in a dose-dependent fashion. This review focuses specifically on the recently described biological effect and consequences of increased salt ingestion on the endothelium through a mechanism that is independent of blood pressure. Changes in salt intake are recognized by endothelial cells in the vascular tree and glomeruli through a physical process that promotes a series of signaling events involved in transcriptional regulation of genes that include transforming growth factor-β1 (TGF-β1) and the endothelial isoform of nitric oxide synthase (NOS3). A balance is struck between TGF-β1 and NOS3 as salt intake varies and creates a negative feedback loop, because TGF-β1 increased expression of NOS3 and NO inhibited production of TGF-β1 in healthy rats. Changes in this feedback system have been observed in salt-sensitive hypertension and appear to impact end-organ damage, particularly the kidney. The data support an important benefit to reduction of salt intake in the setting of chronic kidney disease. (Hypertension. 2004;43:142-146.)

Key Words: endothelium ■ vascular disease ■ nitric oxide ■ gene expression ■ hypertension

The Guyton hypothesis1,2 regarding the relationship between salt balance and blood pressure continues to be a focus of intense research activity, in part related to the discovery of the association between genetic defects in renal sodium handling and hypertension. There are several excellent reviews of this subject.3–5 Recent studies demonstrating that the endothelium responds to changes in salt intake through a mechanism that is independent of blood pressure, however, have challenged the sole emphasis of the effect of salt intake on blood pressure regulation. This brief review focuses on the important role of the endothelium as a biomechanical sensor that detects changes in dietary salt intake in physiological and pathophysiological states.

Transforming Growth Factor-β and Salt Intake

For physicians and scientists interested in progressive kidney disease, perhaps the most important growth factor yet identified is transforming growth factor-β (TGF-β). After the discovery of TGF-β in 1980,6 numerous studies have shown that this fibrogenic or prosclerotic growth factor participates integrally in renal fibrosis in a variety of disease states.7–30 Several years ago, this laboratory became interested in the role of TGF-β in generation of hypertensive nephrosclerosis, but early experiments demonstrated that dietary salt produced an increase in steady-state mRNA levels of TGF-β1 in the kidney cortex of Sprague-Dawley rats; the effect occurred without a change in blood pressure and developed within 1 day of the increase in salt intake and persisted over the course of the experiment.31 Increasing salt intake also promoted an increase in production of TGF-β1 from aortic ring preparations; the effect was lost after removal of the endothelium.32 These studies provided direct evidence that the endothelium responded to changes in salt intake.

A question that arises is: if TGF-β1 were involved in matrix protein production and deposition, then why should TGF-β1 production increase during high salt intake, because sclerosis and organ dysfunction might be the ultimate result? The answer perhaps lies in the pleiotropic nature of TGF-β, which can also modulate nitric oxide (NO) production. Experiments performed years earlier showed that NO production increased in normotensive rats when dietary salt content was increased,33,34 but not in Dahl/Rapp salt-sensitive (SS) rats.33,35–37 The blood pressure response to an increase in salt intake is dependent on NO production in rats.38 Healthy human subjects demonstrate weight gain and an increase in renal blood flow with an associated decrease in renal vascular resistance when sodium intake increased from 77 mEq/d to 250 mEq/d. These changes in renal hemodynamic parameters were abolished with the addition of Nω mono-methyl-L-arginine (L-NMMA), indicating a dependence on NO production and confirming an important role for NO in the hemodynamic response to salt ingestion.39

Inoue et al used bovine aortic endothelial cells in culture to show that TGF-β1 increased the transcriptional rate of the
endothelial isoform of nitric oxide synthase (NOS3). These findings prompted an investigation into the effect that up-regulation of TGF-β1 production had on NOS3 production while on a high-salt diet. Both steady-state mRNA and protein levels of NOS3 increased in the aorta and glomeruli when salt intake increased. Incubation of these tissues with a neutralizing antibody directed against TGF-β1 decreased NOS3 levels toward baseline, indicating a direct role for TGF-β1 in control of NOS3 levels and, subsequently, NO production. Expression of TGF-β1 and NOS3 in the aorta and glomeruli were tightly coordinated in Sprague-Dawley rats and Dahl/Rapp salt-resistant (SR) rats. Experiments using SR rats further demonstrated an inhibitory effect of NO on TGF-β1 production. Levels of TGF-β1 and NO correlated directly in the Dahl/Rapp SS rat, but baseline production of TGF-β1 was greater, NO production was less, and the inhibitory effect of NO on TGF-β1 production was diminished, compared with tissue samples from SR rats. Thus, dietary salt intake regulates TGF-β1, which in turn regulates expression of NOS3, in potential targets of hypertension-related renal damage. 30 When provided sufficient substrate for NOS3 production, increased production of NO produces feedback inhibition of TGF-β1 production and further serves a vasodilatory function, which decreases shear forces. The system appears to become dysfunctional when NO production is impaired, such as in the SS rat. Progressive kidney failure related in part to abnormal expansion of the mesangium and accumulation of extracellular matrix proteins in the preglomerular arterioles develops in SS rats. Expression of TGF-β1 is increased in the kidneys of these animals and administration of a neutralizing antibody to TGF-β1 attenuates the renal damage. When provided sufficient substrate for NO production, renal injury does not occur; these findings further support a counter-regulatory effect of NO on TGF-β1.

**Signaling Events in the Endothelium Stimulated by Salt Intake**

The signaling events that modulate endothelial cell function during changes in salt intake have been clarified further. Because salt intake suppresses the renin–angiotensin system and intrarenal production of angiotensin II of healthy animals, the mechanism is therefore independent of angiotensin II (Figure 1). Endothelial cells in culture produce TGF-β after activation of an inwardly rectifying potassium channel by shear force. Because an increase in salt intake expands blood volume and increases blood flow, experiments were designed to determine if the mechanism by which salt intake modified production of TGF-β1 was mediated through this mechanism. Dahl SS and SR rats also demonstrate an increase in cardiac output and plasma volume with high salt intake, although SS rats do not require increased plasma volume for hypertension to develop. In this situation in which plasma volume is controlled experimentally, the in vivo effects of plasma volume on activities of the p38 mitogen-activated protein kinase (MAPK) and p42/44 MAPK. Activation of both pathways is required to generate TGF-β1, which is required to generate NO through NOS3. NO can directly inhibit production of TGF-β1 and promote vasodilation, which reduces shear forces, completing the negative feedback system. The inhibitors used to delineate this pathway are shown.

**Figure 1.** Diagram representing the interrelationship among dietary salt intake, the renin–angiotensin system, and renal TGF-β1 production. While the relationship between salt intake and angiotensin II is well appreciated, perhaps the ability of angiotensin II and increased salt intake to stimulate independently and independently of angiotensin II is less well appreciated. In an attempt to control intrarenal production of TGF-β1, both pathways should be considered.

**Figure 2.** Diagram representing the proposed mechanism by which salt intake controls production of TGF-β1 and NO in endothelium. An increase in blood flow results in the opening of an inwardly rectifying potassium channel, which activates both the p38 mitogen-activated protein kinase (MAPK) and p42/44 MAPK. Activation of both pathways is required to generate TGF-β1, which is required to generate NO through NOS3. NO can directly inhibit production of TGF-β1 and promote vasodilation, which reduces shear forces, completing the negative feedback system. The inhibitors used to delineate this pathway are shown.
type II receptor, which in turn phosphorylates Smad2/3. Phosphorylated Smad2/3 then binds to Smad4 in the cytoplasm and the heteromeric Smad complex migrates into the nucleus, where it binds to specific cis elements in gene promoter regions. This Smad complex, however, binds DNA with low affinity and requires other binding partners for efficient and specific induction of gene transcription. For example, Smad4 directly binds ATF-2, whereas Smad3 interacts with activator protein (AP)-1. Cross-talk between the Smad signaling pathway and p42/44 MAPK and p38 MAPK pathways has been demonstrated to regulate TGF-β–induced gene transcription in chondrocytes. In addition to the Smad pathway, the AP-1 binding site has been shown to be essential for transcription of TGF-β. The p42/44 MAPK can also directly phosphorylate and activate Smad2 to permit nuclear translocation and initiation of gene transcription without activation of the TGF-β receptor. Activation of the Smad signaling pathway is important in TGF-β1–induced upregulation of NOS3 transcriptional rates in endothelial cells. The combined data suggest that transcription factors that are activated by the MAPK pathways and the Smad heteromeric complex synergize to initiate TGF-β1, as well as NOS3, gene transcription (Figure 3). This interaction perhaps explains experiments in which short-term administration of TGF-β1 to glomeruli isolated from rats on a high-salt diet, which activates the MAPK pathways, further augmented NOS3 expression but had no effect on production of NOS3 in glomeruli isolated from rats on a low-salt diet.

**Importance of Salt Intake in the Progression of Chronic Kidney Disease**

Clues to the pathological significance of the effect of salt intake independent of blood pressure come from studies that used animals and human subjects. Using the uninephrectomized spontaneously hypertensive rat (SHR), which served as a model of progressive renal failure, Benstein et al showed that a low-salt (0.09% sodium) diet alone resulted in less proteinuria and glomerular sclerosis than did administration of a diuretic while continuing a standard 0.45% sodium diet; systolic blood pressures determined by tail-cuff methodology did not differ among the groups over the 36 weeks of the study. More recently, Yu et al showed that administration of 8.0% NaCl diet to normotensive Wistar-Kyoto rats and SHR increased TGF-β1 production and produced fibrosis in the kidney and left ventricle. The authors concluded that excessive salt intake might play a direct role in cardiovascular disease. In another study, administration of 8.0% NaCl diet to Fisher-Lewis rats after orthotopic renal transplantation accelerated the development of chronic allograft nephropathy. An increase in tubulointerstitial fibrosis and glomerular sclerosis was demonstrated; an associated increase in urinary excretion rate and renal cortical levels of TGF-β1 was also observed (unpublished observations from this laboratory, 2001).

In a retrospective analysis of progression of chronic kidney disease, 57 subjects with baseline creatinine clearances between 10 and 40 mL/min were divided into 2 groups based on consistent urine sodium excretion rates of either less than 100 mEq/d or greater than 200 mEq/d. Mean blood pressures of the groups did not differ and both glomerular and tubulointerstitial diseases were represented in both groups. The rate of decline in creatinine clearance was greater in the high-salt group, compared with the low-salt group (0.51±0.09 versus 0.25±0.07 mL/min per month; P<0.05). Proteinuria increased in the high-salt group and decreased in the low-salt group.

Several points are worth emphasizing to clinicians who care for patients who have chronic kidney disease. A mainstay of therapy continues to be ACE inhibitors or angiotensin receptor antagonists, both of which appear to slow progression of kidney failure, in part related to inhibiting the stimulation of TGF-β production by angiotensin II. The present data suggest that an additional approach to the management of intrarenal TGF-β production might be salt reduction, which works through a mechanism that is independent of angiotensin II (Figure 1). Institution of a diuretic may be important in the management of hypertension, but administration of a diuretic to animals that continued the high salt intake did not reduce intrarenal production of TGF-β. Reduction of salt intake also enhances the anti-proteinuric...
effect of ACE inhibitors. Thus, efforts to monitor and reduce salt intake through dietary restriction therefore may produce beneficial effects that are independent of blood pressure. Finally, another interesting observation in rats was that serum TGF-β1 levels were not affected by salt intake, whereas intrarenal production and urinary excretion of TGF-β1 increased as salt intake increased; these findings suggest that urinary TGF-β1 levels may reflect intrarenal production of this growth factor and may be a parameter that will prove useful to follow in chronic kidney disease.

**Summary**

In summary, endothelial cells lining the arteries and glomeruli of the rat serve as sensors of changes in dietary salt intake, which generates signal transduction events that lead to production of TGF-β1 and NOS3. Changes in expression of these effector molecules contribute to the vascular and renal responses to salt intake. Extrapolating the findings to humans, dietary salt intake probably poses no substantial risk to healthy individuals. However, in patients predisposed to progressive renal failure, such as those with chronic kidney disease, individuals with reduced renal mass, such as the renal transplant recipient, or patients with endothelial dysfunction that produces an imbalance between TGF-β1 and NOS3, an excess of salt intake may accelerate loss of renal function by permitting the fibrogenic effect of TGF-β1 to be the predominant feature of this tightly integrated system.

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**References**


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