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Effect of Dietary Salt on Regulation of TGF- β in the Kidney

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Abstract

Dietary sodium chloride (salt) has long been considered injurious to the kidney by promoting the development of glomerular and tubulointerstitial fibrosis. Endothelial cells throughout the vasculature and glomeruli respond to increased dietary salt intake with increased production of transforming growth factor beta (TGF- β) and nitric oxide (NO). High-salt intake activates large conductance, voltage- and calcium-activated potassium channels (BK_{Ca}) channels in endothelial cells. Activation of BK_{Ca} channels promotes signaling through proline-rich tyrosine kinase-2 (Pyk2), c-Src, Akt, and mitogen-activated protein kinase (MAPK) pathways that lead to endothelial production of TGF- β and NO. TGF- β signaling is broadly accepted as a strong stimulator of renal fibrosis. The classic description of TGF- β signaling pathology in renal disease involves signaling through Smad proteins resulting in extracellular matrix (ECM) deposition and fibrosis. Active TGF- β 1 also causes fibrosis by inducing epithelial-mesenchymal transition (EMT) and apoptosis. By enhancing TGF- β signaling, increased dietary salt intake leads to progressive renal failure from nephron loss and glomerular and tubulointerstitial fibrosis.

Keywords

sodium chloride; salt; transforming growth factor-beta; kidney; fibrosis

Background

The deleterious effects of sodium chloride (which will be referred to as “salt” in this paper) have been suspected for at least one hundred years. French physicians were the first to treat hypertension with fluid and salt restriction in the early 1900’s.¹ The French position was met with skepticism and opposition. German researchers were unable to substantiate the benefits of salt restriction. American physicians seemed to ignore the idea of salt restriction altogether until the 1920’s when Allen began treating hypertensive patients with salt and fluid restriction.¹ Allen observed, “In American Literature, salt and fluid restriction in hypertension is mentioned casually and incompletely if at all, and the protein intoxication theory is undoubtedly the dominant one. Treatment consists chiefly in low protein diets, the elimination of supposed toxins, or the artificial reduction of pressure by drugs, bleeding, electricity, and the like. Mental and bodily rest is advised to an extent which largely

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terminates usefulness in life, and many conservative practitioners refrain from any serious attempt to reduce the pressure, and devote themselves to keeping the patient as comfortable as possible, with resignation as to the results.”¹ Dietary salt restriction for treatment of kidney disease and hypertension received a boost in popularity in the 1940’s with Dr. Kempner’s rice and fruit diet that recommended 0.25–0.4 g of sodium per day.²

Detailed laboratory experiments on the effects of salt on the kidneys were undertaken by Meneely *et al.* in the 1950’s.^{3–6} The lab published a series of papers on “Chronic Sodium Chloride Toxicity in the Albino Rat.” In these experiments high-salt diets promoted marked hypertension, higher rates of renal failure, and a dose-dependent reduction in life span.^{3–6} Histologic examination of the rats revealed prominent renal fibrosis and arteriolonephrosclerosis. Based in part upon these early findings, our laboratory became interested in mechanisms of renal injury associated with increased salt intake and focused on Transforming Growth Factor- β (TGF- β) and nitric oxide (NO). Early experiments demonstrated that rats fed a high-salt diet developed significantly increased levels of TGF- β 1 in their glomeruli and tubules.⁷ This increase was significant after just one day on a high-salt diet and occurred *prior* to the development of hypertension, suggesting that the effect of salt on the vasculature is more complex than just increasing blood pressure. Yu *et al.* demonstrated that both normotensive and hypertensive rats fed a high-salt diet for eight weeks had higher levels of TGF- β 1 expression and collagen deposition in the renal interstitium and glomeruli and throughout the cardiovascular system.⁸ These studies highlight the consequence of high levels of dietary salt intake stimulating increased production of TGF- β leading to progressive renal and cardiac fibrosis.

Biology of TGF- β

TGF was first described in 1980 in the cancer literature due to the ability to potentiate cellular proliferation and transformation.⁹ A large number of structurally related proteins belong to the TGF superfamily. There are three different isoforms of TGF- β that have been identified in mammals, known as TGF- β 1, TGF- β 2, and TGF- β 3.^{10–11} TGF- β 1 is the prototypical member of the TGF family and has multiple roles in development and maintenance of homeostasis. Alterations in TGF- β 1 signaling have been linked to fibrosis, cancer, developmental, and cardiovascular diseases.^{12–17}

TGF- β 1 is secreted from cells in a latent form. The precursor form consists of a large N-terminal latency-associated peptide (LAP) domain and a C-terminal mature TGF- β 1 domain.^{18–19} The pro-TGF- β 1 peptide exists as a dimeric peptide with the LAP responsible for binding another pro-TGF- β 1 peptide. The LAP domain also associates with a binding partner called the latency-associated binding protein (LABP). The dimeric pro-TGF- β 1 complex bound to LABP forms the large latent complex (LLC).^{20–21} The LAP shields the mature TGF- β 1 domain from binding to TGF- β 1 receptors. The complex must undergo proteolytic cleavage to release the mature TGF- β 1 to bind to a receptor and propagate signal.

Two classes of serine/threonine kinase transmembrane receptors, termed TGF- β receptor type I (TBRI) and II (TBR II), form a heteromeric receptor complex responsible for transducing the TGF- β 1 signal (Figure 1).^{22–24} There are at least seven type I receptors and five type II receptors. The TBRI is also known as activin receptor-like kinase (ALK). ALK-5 is responsible for most TGF- β 1 signal propagation. Extracellular active TGF- β 1 binds to TBR II, which recruits TBRI and forms a receptor complex. The receptor complex is responsible for canonical signaling by phosphorylation of Smad 2 or Smad 3.²⁵ Phosphorylated Smad 2/3 forms an oligomeric complex with Smad4 and translocates to the

nucleus. In the nucleus the complex binds to DNA and interacts with other nuclear factors to regulate target genes to modify transcriptional activity.

TGF- β and the Kidney

TGF- β 1-induced fibrosis plays a prominent role in progressive renal failure.^{26–27} The final common pathway in end-stage renal disease (ESRD) is tubulointerstitial fibrosis and glomerulosclerosis with loss of nephrons. TGF- β 1 signaling is broadly accepted as a strong stimulator of renal fibrosis, which is regarded as a vital pathologic event in chronic kidney disease and progression to ESRD.²⁶ The classic description of TGF- β 1 signaling pathology in renal disease involves signaling through Smad proteins resulting in extracellular matrix (ECM) deposition and fibrosis. This pathogenetic process is correct but perhaps too simplistic of an explanation. The pathologic consequences of increased TGF- β 1 signaling in the kidney are also dependent on the physiological microenvironment and the type of cells involved. Renal fibrosis progresses in some regions more quickly than others. TGF- β 1 stimulates some cells, such as mesangial cells and fibroblasts, to proliferate and increase deposition of ECM proteins. Other cells such as tubular epithelial cells, endothelial cells, and podocytes may undergo apoptosis, while other epithelial cells may dedifferentiate and produce ECM proteins.

In addition to primary stimulation of cells to produce ECM, TGF- β is hypothesized to induce fibrosis through induction of epithelial-mesenchymal transition (EMT). Renal epithelial cells are derived developmentally from metanephritic mesenchyme.²⁸ The cells maintain the ability to switch back to mesenchymal myofibroblasts under appropriate signals. Physiologically, this ability to dedifferentiate into myofibroblasts is preserved for growth and development and for tissue injury and repair. Epithelial-mesenchymal transition (EMT) was first implicated in the progression of renal disease in 1996 by Strutz *et al.*²⁹ EMT has been demonstrated in tubular epithelial cells *in vitro* in response to TGF- β .³⁰ *In vivo* histologic studies have examined tissues at an isolated point in time and found evidence supporting a role for EMT in renal fibrosis by identifying cells with biomarkers for both tubular epithelial cells and myofibroblasts.³¹ Fate-mapping studies have traced cells with epithelial origin that have become myofibroblasts.³² Additional evidence has come from studies using inhibitors of EMT to mitigate fibrosis and antifibrotic therapy to suppress EMT.^{33–36} High-salt intake induced TGF- β 1 signaling has been shown to cause EMT in the peritoneal membrane in rats causing peritoneal fibrosis.³⁷ Similar studies linking dietary salt to EMT and fibrosis have not been done in renal tissues. Gene array studies have shown that the Mitogen-Activated Protein Kinase (MAPK), p42/44, is involved in EMT and functions in remodeling and cell motility associated with EMT.³⁸ This is particularly interesting in view of a study that demonstrated p42/44 activation by a high-salt diet was required for TGF- β 1 overproduction.^{39–40} In all, hundreds of papers have been published supporting a role for EMT in renal fibrosis.⁴¹ Recent studies, however, have generated some controversy as fate-mapping studies failed to demonstrate an epithelial origin for renal myofibroblasts.^{42–43} A review article and a debate article highlight the differing views and points of contention in this field.^{28,44}

EMT requires multiple changes in cellular structure and function.²⁸ Cells lose epithelial cellular adhesion molecules such as E-cadherins. The epithelial tubular basement membrane is disrupted as cells lose adhesion molecules and undergo cytoskeletal remodeling. Cells gain α -smooth muscle actin and vimentin, cellular markers of myofibroblasts. Transformed myofibroblasts have increased motility and are able to migrate away from the tubular basement membrane into the interstitium. The hypothesis of EMT in renal fibrosis is that cells are able to proliferate and the underlying pathologic consequence of enhanced EMT is

overproduction and deposition of ECM proteins contributing to fibrosis and loss of tubular epithelial cells.

TGF- β 1 also appears to promote fibrosis through apoptotic pathways. It is not clear that TGF- β 1 induces apoptosis on its own but rather participates and contributes to apoptotic signaling cascades. TGF- β 1-induced apoptosis has been observed in a number of nonrenal cell lines including prostate cancer cells, gastric cancer cells, hepatocytes, and uterine epithelial cells.^{45–48} Mice treated with an anti-TGF- β 1 neutralizing antibody had reduced tubule apoptosis and nephron loss in a model of renal injury created by unilateral ureteral obstruction indicating a role for TGF- β 1 in apoptosis.⁴⁹ More evidence for TGF- β 1 involvement was demonstrated in angiotensin II induced apoptosis of cultured proximal tubular epithelial cells.⁵⁰ Inhibition of TGF- β 1 inhibited apoptosis revealing that TGF- β 1 was required. In cultured human epithelial kidney cells, TGF- β 1 potentiated apoptosis induced by staurosporine.⁵¹ In these experiments blockade of the p38 MAPK abolished the ability of TGF- β 1 to potentiate apoptosis. Activation of p38 MAPK is also required for TGF- β 1 overproduction in response to a high-salt diet.⁵²

In summary, it has become increasingly clear that TGF- β , a fibrogenic growth factor, is a critical mediator of progressive kidney failure induced by a wide variety of renal insults. Excess dietary salt intake, which increases vascular and intrarenal TGF- β production, might therefore facilitate renal disease progression independently of the original renal insult. We propose a model by which excess salt intake catalyzes renal disease progression (figure 2). Finally, excess salt intake *per se* may promote chronic kidney disease through the same pathways.

Mechanisms of TGF- β production in the kidney in response to a high-salt diet

Pioneering work by Border *et al.* in the early 1990's implicated TGF- β in the pathogenesis of renal disease.⁵³ Increasing expression of TGF- β was observed in an experimental rat model of immunologic glomerulosclerosis where rats were injected with anti-thymocyte serum.⁵⁴ It was observed that TGF- β was profibrotic and led to increased deposition of extracellular matrix (ECM) proteins. MAPK pathways regulate a number of cellular processes including cell growth, differentiation, proliferation, and stress responses. There are four major families of MAPK including p38, p42/44 (ERK 1/2), ERK 5, and JNK α - δ . TGF- β pathways and MAPK pathways intersect in a number of different ways. Both TGF- β 1 and p38 and p42/44 MAPK signal pathways target nuclear receptors ATF-2 and Elk-1.^{52,55–59} MAPK can be activated by TGF- β 1 signaling. Rats fed a high-salt diet had increased levels of phosphorylation and activity of p38 and p42/44 MAPK along with increased TGF- β 1 expression, but not JNK.^{52,56} Pharmacologic inhibitors of either p38 or p42/44 abolished increased TGF- β 1 levels indicating that TGF- β 1 production in response to dietary salt is dependent on p38 and p42/44.⁵² Levels of ATF-2 and Elk-1 phosphorylation were also increased in response to dietary salt. Thus, renal increases in TGF- β 1 expression in response to a high-salt diet require and enhance both p38 and p42/44 MAPK pathways and effect downstream transcription through ATF-2 and Elk-1.

Additional studies focused on the mechanism of TGF- β 1 and MAPK pathway activation in response to dietary salt. Proline rich tyrosine kinase-2 (Pyk2) was investigated due to its role in transduction of extracellular events and stress signals and ability to participate in activation of MAPK pathways.^{60–63} Pyk2 is activated by a number of stimuli including shear stress, angiotensin II, and elevations in intracellular calcium. Rats fed a high-salt diet had dose-dependent increases phosphorylation of Pyk2 in glomeruli.⁶³ Pyk2 recruited c-Src as a binding partner for complex formation, and c-Src was phosphorylated.^{63–64} Addition of

a Pyk2 inhibitor, tyrphostin 9, or a c-Src inhibitor, PP2, blocked TGF- β 1 production in rats fed a high-salt diet.⁶³ These studies demonstrate that TGF- β 1 production in renal glomeruli in response to dietary salt is dependent on Pyk2 and c-Src signaling with MAPK's.

Dietary salt increases nitric oxide (NO) production in normal rat and human kidneys.^{65–68} In cultured bovine aortic endothelial cells, TGF- β 1 increased endothelial nitric oxide synthase (NOS3) expression.⁶⁹ Rat glomeruli from animals fed high-salt diets had increased NOS3 levels.⁷⁰ The levels are reduced to near baseline with neutralizing antibodies directed against TGF- β 1. Activation of NOS3 can either occur through binding of a calcium/calmodulin complex or through calcium independent posttranslational modifications.^{71–74}

Posttranslational modifications have a longer lasting effect than calcium/calmodulin binding. Protein kinase B (Akt) is one of the kinases responsible for phosphorylation of NOS3 at serine 1176 which has been shown to increase NOS3 activity.⁷⁴ Pyk2 and c-Src are directly involved in activation of Akt and phosphorylation of NOS3 in glomeruli.⁷⁰ This is consistent with data from the cardiovascular literature demonstrating a role for Pyk2 in the activation of NOS3 in ischemia and angiogenesis.⁷⁵ As already mentioned dietary salt increases the activity and complex formation of Pyk2 and c-Src.⁷⁰ Inhibitors of either Pyk2 or c-Src blocked NOS3 production in glomeruli in response to dietary salt.

In Dahl/Rapp salt-sensitive rats compared to salt-resistant rats, NO production in response to dietary salt was not increased, TGF- β 1 levels were higher, and the inhibitory effect of NO on TGF- β 1 was not as great.^{65–66} NO production in response to dietary salt is likely a protective mechanism to shield against the deleterious effects of increased TGF- β 1 production.⁷⁶ However, over time and in the continued presence of a high-salt diet, the effects of TGF- β 1 become the dominant influences on renal pathology. In salt-sensitive rats the process is accelerated and the balance is more easily tilted toward TGF- β 1 overproduction.

Increases in dietary salt lead to increases in blood volume and renal blood flow.^{77–78} Salt-sensitive and salt-resistant rats both develop increased plasma volume and cardiac output in response to a high-salt diet. We have proposed that increased salt intake increases blood volume and promotes increases in shear stress on endothelial cells lining the arteries and glomeruli. Exposure of cultured endothelial cells to shear stress *in vitro* increases production of TGF- β 1 through activation of large-conductance, voltage- and calcium-activated potassium (BK_{Ca}) channels.⁷⁹ In a similar fashion, increased salt intake *in vivo* promoted activation of BK_{Ca}.⁸⁰ These channels are sensitive to tetraethylammonium (TEA) and iberiotoxin (IB), a specific inhibitor of BK_{Ca} channels. TEA and IB blocked production of TGF- β 1 in aortic rings and in glomeruli.^{37,80} Activation of potassium channels activates p38 and p42/44 MAPK as indicated by data demonstrating that rats fed a high-salt diet treated with TEA had decreased p38 and p42/44 phosphorylation.³⁷ Collectively, these data indicate that high-salt intake activates BK_{Ca} channels on endothelium, leading to activation of a signaling cascade that involves Pyk2, c-Src, Akt, and MAPK pathways that promote the vascular production of TGF- β 1 and NO.

Public health implications of increased TGF- β production in the kidney

According to the latest data from the United States Renal Data Systems 2010 annual report, 547,982 patients in the United States are living with ESRD.⁸¹ An additional 165,639 patients are living with a functional kidney transplant required due to kidney failure or worsening kidney function.⁸¹ Program expenditures for ESRD therapy rose to \$39.6 billion for the year, and Medicare expenditures for ESRD therapy consumed 5.8% of total Medicare expenditures.⁸¹ The monetary cost of kidney disease is at an all time high.

According to the latest National Health and Nutrition Examination Survey (NHANES) data less than 10% of those surveyed met national recommendation for sodium intake in the United States.⁸² Dietary guidelines for higher risk individuals recommended reduced sodium intake of 1.5 g per day or less for hypertensives, middle aged and older adults, and blacks. However, only 5.5% of adults in this high-risk group met the dietary sodium recommendations.⁸² At a time when much is known about the deleterious effects of salt intake, many Americans continue to consume a high-salt diet.

Increased morbidity and mortality in response to a high-salt diet has been clearly demonstrated in carefully controlled animal experiments.³⁻⁶ Such carefully controlled laboratory studies cannot be performed in humans. A recent retrospective analysis of 3681 participants from the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) and European Project on Genes in Hypertension (EPOGH) trials who had 24-hour urinary sodium collections at baseline reported an increase in cardiovascular mortality for the low-salt tertile based on 50 cardiovascular deaths in that group over a 21 year follow-up period (mean follow-up of 7.9 years).⁸³ The outcome, while statistically significant, represents a low number of cardiovascular deaths for such a long period of time and for such a large population. Urine sodium was only measured once at the beginning of the study. The population consisted of relatively young (mean age 40.9), healthy white Europeans.

In contrast, most population-based studies have demonstrated increased morbidity and mortality in humans on a high-salt diet. We will focus on the detrimental effects of salt on the kidney, but, from a public health view, it should be mentioned that dietary salt has additionally been implicated in increased hypertension, stroke, and cardiovascular disease (Table 1). A French study evaluated 839 individuals with 24-hour urine collection.⁸⁴ Those in the top 25% of sodium excretion had significantly increased urinary albumin excretion. A Dutch cohort of 7850 people also showed a positive relationship between salt intake and urinary albumin excretion.⁸⁵ The effect was more pronounced in overweight patients. Intervention studies to reduce salt intake have shown renal benefits. A crossover trial of type II diabetic patients with microalbuminuria showed increased blood pressure and albuminuria on a high-salt diet compared to a low-salt diet.⁸⁶ A randomized, double-blind, crossover trial in blacks, whites, and Asians demonstrated that a modest reduction in dietary salt resulted in a significant reduction in blood pressure and urinary albumin excretion in all three groups.⁸⁷ A randomized, double-blind, placebo controlled trial in black hypertensive patients showed that reduced salt intake resulted in decreased blood pressures and decreased albuminuria.⁸⁸ Weinberger *et al.* demonstrated an increase in all-cause mortality for both normotensive and hypertensive salt-sensitive patients compared with salt-resistant.⁸⁹ The trials of hypertension prevention (TOHP) randomized over 3000 participants to a sodium reduction intervention.⁹⁰ Intention-to-treat analysis of patients randomized to a reduction in dietary salt revealed a 20% reduction in all cause mortality and a 25% reduction in cardiovascular events.

In summary, most clinical studies represent an impressive collection that supports a call to action to restrict salt intake in vulnerable populations. Pre-clinical findings support a role for dietary salt intake in increasing renal TGF- β 1 production, which has a central role in the pathogenesis and progression kidney disease. Cooperation among government, academia, nonprofit organizations, health care professionals, and the food industry is necessary for development and implementation of effective strategies to reduce public salt consumption.⁹¹⁻⁹³ Population-based efforts to reduce salt intake are both feasible and cost-effective.⁹⁴⁻⁹⁵ Many countries have undertaken such initiatives with success.⁹³ Salt reduction should be a major public health priority for those individuals, organizations, and institutions interested in improving the health of the population.

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Figure 1.
Schematic illustrating the signal propagation of TGF- β

Figure 2. Cellular mechanisms of renal pathology associated with excess TGF-β. See section, Mechanisms of TGF-β Production in the Kidney in Response to a High-salt Diet, for details.

Table 1

Public Health Implications of Excess Dietary Salt Intake

Kidney Damage and Progression of CKD ^{84–89}
Increased Cardiovascular Disease and Cardiovascular Events ⁹⁰
Hypertension and Vascular Disease ^{86–88}