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Original Articles

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Vascular endothelial growth factor C levels are modulated by dietary salt intake in proteinuric chronic kidney disease patients and in healthy subjects

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Abstract

Background. Recent experimental findings demonstrate vascular endothelial growth factor C (VEGF-C)-mediated water-free storage of salt in the interstitium, which prevents a salt-sensitive blood pressure state. It is unknown whether this mechanism plays a role in salt homeostasis and regulation of blood pressure in humans as well. Therefore, we investigated circulating VEGF-C levels and blood pressure during different well-controlled salt intake in chronic kidney disease (CKD) patients and in healthy subjects.

Methods. In two crossover studies, non-diabetic proteinuric CKD patients ($n = 32$) and healthy subjects ($n = 31$) were treated with consecutively a low-sodium diet (LS, aim 50 mmol Na^+/day) and a high-sodium diet (HS, aim 200 mmol Na^+/day) in random order, during two 6-week (CKD patients) and two 1-week periods (healthy subjects).

Results. We found that VEGF-C levels are higher during HS than during LS in CKD patients ($P = 0.034$) with a trend towards higher VEGF-C in healthy subjects as well ($P = 0.070$). In CKD patients, HS was associated with higher NT-proBNP levels ($P = 0.005$) and body weight ($P = 0.013$), consistent with extracellular volume (ECV) expansion and with higher blood pressure ($P < 0.001$), indicating salt sensitivity. In healthy subjects, blood pressure was not affected by dietary salt ($P = 0.14$), despite a rise in ECV ($P = 0.023$).

Discussion. Our findings support a role for VEGF-C-mediated salt homeostasis in humans. Considering the salt sensitivity of blood pressure, this buffering mechanism appears to be insufficient in proteinuric CKD patients. Future studies are needed to provide causality and to substantiate the clinical and therapeutic relevance of this VEGF-C regulatory mechanism in humans.

Keywords: blood pressure; ECV; salt homeostasis; salt sensitivity; VEGF-C

Introduction

Classically, total body salt and extracellular volume (ECV) are thought to be closely linked and controlled by renal salt excretion and dietary salt intake only. Based on the assumption that extracellular body fluids are in equilibrium, excess interstitial salt is considered to be readily mobilized into the bloodstream for renal salt clearance. Blunted renal salt excretion in this concept results in ECV expansion, which can induce a rise in blood pressure, denoted as the salt sensitivity of blood pressure [1, 2]. In support of this concept, we found that salt-sensitive healthy men have a higher ECV than salt-resistant men during high salt intake but not during low salt intake [3].

However, recent experimental findings demonstrating water-free storage of salt question our current understanding on internal environment composition and warrant novel insights into regulatory mechanisms for salt homeostasis [4–9]. Salt can be stored in a newly discovered subcutaneous interstitial compartment by binding to polyanionic proteoglycans and glycosaminoglycans without commensurate water retention [10, 11]. In response to salt-mediated interstitial osmotic stress, mononuclear phagocyte system (MPS) cells secrete vascular endothelial growth factor C (VEGF-C), which stimulates lymphatic growth and endothelial nitric oxide synthase (eNOS) expression [12, 13]. When this system is inhibited, high salt intake induces excess interstitial salt retention and hypertension [4, 5].

In patients with refractory hypertension, a condition which is eminently salt sensitive [14, 15], circulating VEGF-C levels were elevated compared to normotensive subjects [4], suggesting that this extrarenal regulatory mechanism might play a role in salt homeostasis and regulation of blood pressure in humans as well. If so, it can be hypothesized that circulating levels of VEGF-C respond to changes in salt intake, with higher VEGF-C levels during high salt

intake. To test this hypothesis, we investigated circulating VEGF-C levels and blood pressure during steady state on different well-controlled salt intake in two independent studies, in proteinuric chronic kidney disease (CKD) patients and in healthy subjects, respectively.

Materials and methods

This is a *post hoc* analysis of two previous studies described in detail elsewhere [3, 16].

CKD patients

For the current study, we used data and samples collected during placebo treatment on a high-sodium diet (HS, target intake 200 mmol Na^+/day) and low-sodium diet (LS, target intake 50 mmol Na^+/day) from 32 non-diabetic proteinuric CKD patients [age 50 ± 2 years, 73% men, all Caucasian, body mass index (BMI) 27 ± 1 kg/m^2]. Mean achieved sodium intake was above target (90 ± 10 mmol/day) and according to protocol (200 ± 10 mmol/day) during LS and HS diet, respectively. Duration of the dietary interventions was two times 6 weeks, and the order was random. For two subjects from the original study, good quality samples were no longer available.

Healthy subjects

From the original 34 study subjects, samples of sufficient quality were available for 31 subjects (age 23 ± 1 years, 100% men, all Caucasian, BMI 24 ± 1 kg/m^2). Data and samples were obtained after 1 week on a LS diet (target intake 50 mmol Na^+/day) and after one week on a HS diet (target intake 200 mmol Na^+/day), respectively, in random order. Mean achieved dietary sodium intake was below and above target values (34 ± 11 mmol/day and 257 ± 16 mmol/day, respectively) during LS and HS diet, respectively.

Measurements and calculations

At the end of each study period, all participants collected 24 h urine and, after an overnight fast, blood pressure was measured and blood was sampled. Proteinuria was measured by the pyrogallol red-molybdate method. Dietary sodium intake was assessed from 24 h urinary sodium excretion. Blood pressure was measured at 1-min intervals by an automatic device (Dinamap®; GE Medical Systems, Milwaukee, WI), with the patient in semi-supine position. After 15 min of measurements, the mean of the last four readings was used for further analysis. Plasma VEGF-C levels were measured by ELISA (R&D Systems, Wiesbaden-Nordernstadt, Germany). Intra- and interassay variation of the ELISA is 6.6 and 8.5%, respectively. The minimal detection level is 48.4 pg/mL. In the healthy subjects, ECV was measured by the distribution volume of ^{125}-I-iothalamate as described previously [17].

Data analysis

Data are given as mean with SEM or geometric mean with interquartile range (IQR) when skewed. Before statistical testing, skewed variables were subjected to natural log transformed to obtain normality. Comparisons between HS and LS were performed using paired *T*-tests. $P < 0.05$ was considered statistically significant. SPSS 18.0 for Windows (SPSS Inc., Chicago, IL) was used for analyses.

Results

VEGF-C and general parameters in CKD patients

CKD patients had overt proteinuria, a slightly elevated blood pressure and a rather preserved renal function (Table 1). Urinary sodium excretion, a measure of dietary sodium intake, was lower during LS than during HS. VEGF-C levels were significantly higher during HS than during LS [median (IQR) 1228 (1024–1471) versus 1004 (857–1177) pg/mL, respectively, P = 0.034; Figure 1]. NT-proBNP levels and body weight were also higher during HS than during LS, consistent with ECV expansion during HS. Blood pressure and proteinuria were higher during HS as well, indicating salt sensitivity of blood pressure and proteinuria in CKD patients.

VEGF-C and general parameters in healthy subjects

As expected, the healthy subjects had normal blood pressure, normal renal function and no proteinuria (Table 2). Urinary sodium excretion was considerably lower during LS than during HS, indicating excellent dietary compliance. VEGF-C levels tended to be higher during HS than during LS, but the difference was not statistically significant [median (IQR) 881 (758–1023) versus 773 (748–921) pg/mL, respectively, P = 0.070; Figure 1]. Assuming that VEGF-C distributes over the ECV, we calculated the total amount of VEGF-C as product of plasma VEGF-C levels X ECV. Total VEGF-C was higher during HS than during LS [median (IQR) 18176 (14320–26405) versus 14539 (1002–22751) pg, respectively, P = 0.016). In line with the higher ECV during HS, NT-proBNP levels, body weight and creatinine clearance were also significantly higher during HS than during LS. Blood pressure in the healthy young men was not affected by dietary salt intake.

Individual values for blood pressure and VEGF-C during LS and HS in the CKD patients and the healthy subjects are given in Figure 2. No significant correlation could be detected in the healthy subjects nor in the CKD patients. For the pooled data on either sodium intake, a borderline significant correlation was present ($R^2 = 0.217$, P = 0.095 and $R^2 = 216$, P = 0.096 on LS and HS, respectively). However, the correlation disappeared after adjustment for population. The individual change in VEGF-C elicited by HS intake was not correlated with the change in mean arterial pressure in either study population, separately or pooled (Figure 3). Furthermore, no significant associations were found between change in VEGF-C levels/total amount of VEGF-C and change in ECV, NT-proBNP or body weight. VEGF-C levels, however, were significantly higher in CKD patients than in healthy subjects on either sodium intake (P = 0.027 and P = 0.006 on LS and HS, respectively).

Discussion

We found that VEGF-C levels are modulated by salt intake in two different independent studies, with higher VEGF-C levels during high salt intake. Firstly, in proteinuric CKD patients after two 6-week periods of dietary intervention and secondly, in healthy subjects, after two 1-week periods of dietary intervention, albeit the latter of borderline statistical significance. In the CKD patients, higher salt intake was associated with higher blood pressure, whereas in the healthy subjects, the measured blood pressure was not affected by dietary salt, despite a rise in ECV.

Animal studies have found that during high salt diet, the content and polyanionic character of glycosaminoglycans increase, accompanied by hypertonic salt storage in the ensuing reservoir tissue [7, 18]. VEGF-C, which is secreted by MPS cells in response to interstitial hypertonicity, induces eNOS expression by binding to VEGFR-2 [12] and stimulates lymphangiogenesis by binding to VEGFR-3

Table 1. General parameters in CKD patients during LS and HS diet

General parameters in CKD patients ($n = 32$)			
	LS	HS	P-value
Proteinuria (g/day)	3.0 ± 0.4	3.8 ± 0.4	<0.001
Systolic blood pressure (mmHg)	137 ± 3	143 ± 3	<0.001
Diastolic blood pressure (mmHg)	83 ± 1	86 ± 2	0.004
Mean arterial pressure (mmHg)	101 ± 11	105 ± 15	0.001
Creatinine clearance (mL/min)	82 ± 6	89 ± 5	0.217
NT-proBNP (pg/mL)	62 (41 – 93)	91 (60 – 137)	0.005
Body weight (kg)	89.1 ± 2.9	91.2 ± 3.0	0.013
Plasma VEGF-C (pg/mL)	1004 (857 – 1777)	1228 (1024 – 1471)	0.034
Plasma Na^+ (mmol/L)	139.0 ± 0.4	139.1 ±0.4	0.666
Urinary Na^+ (mmol/day)	90 ± 10	200 ± 10	<0.001

LS = low-sodium diet, HS = high-sodium diet, Reference value NT-proBNP < 125 ng/L.

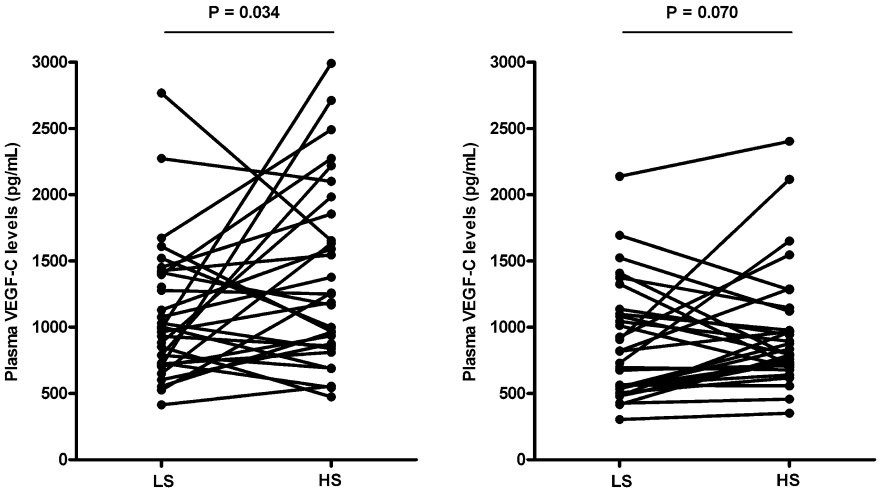

Fig. 1. VEGF-C levels in CKD patients (left panel) and healthy subjects (right panel) during LS and HS diet.

Table 2. General parameters in healthy subjects during LS and HS diet

General parameters in healthy subjects ($n = 31$)			
	LS	HS	P-value
Proteinuria (g/day)	<0.2	<0.2	-
Systolic blood pressure (mmHg)	123 ± 2	124 ± 1	0.138
Diastolic blood pressure (mmHg)	68 ± 1	69 ± 1	0.453
Mean arterial pressure (mmHg)	86 ± 8	87 ± 7	0.251
Creatinine clearance (mL/min)	103 ± 5	123 ± 5	0.003
NT-proBNP (pg/mL)	14 (11 – 19)	26 (20 – 35)	0.002
Body weight (kg)	79.9 ± 2.1	81.6 ± 2.1	<0.001
ECV (L)	19.8 ± 0.5	20.8 ± 0.5	0.023
Plasma VEGF-C (pg/mL)	773 (748 – 921)	881 (758 – 1023)	0.070
Toatl amount of VEGF-C (pg)	14539 (1002 – 22751)	18176 (14320 – 26405)	0.016
Plasma Na^+ (mmol/L)	138.5 ± 0.4	139.8 ± 0.4	0.001
Urinary Na^+ (mmol/day)	46 ± 11	257 ± 16	<0.001

LS = low-sodium diet, HS = high-sodium diet, Total amount of VEGF-C is calculated as plasma VEGF-C× ECV. Reference value NT-proBNP < 125 ng/L.

[13]. The resulting vasodilatory response and electrolyte removal from the interstitium prevents a salt-sensitive blood pressure state [4, 5, 19–21]. This non-osmotic VEGF-C–macrophage–lymphangiogenesis pathway may act alongside the osmotic storage of salt that translates into ECV excess. As currently no methods are established for investigation of salt storage in patient-oriented research, we can only speculate that dietary salt induces salt storage in specific reservoirs as well. However, the close association between changes in dietary salt intake followed by parallel

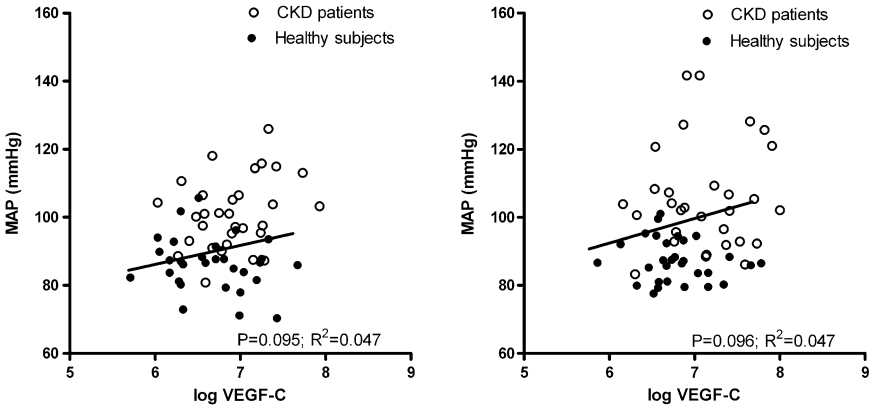

Fig. 2. Association between VEGF-C levels and mean arterial pressure (MAP) in CKD patients and healthy subjects during LS (left panel) and HS diet (right panel).

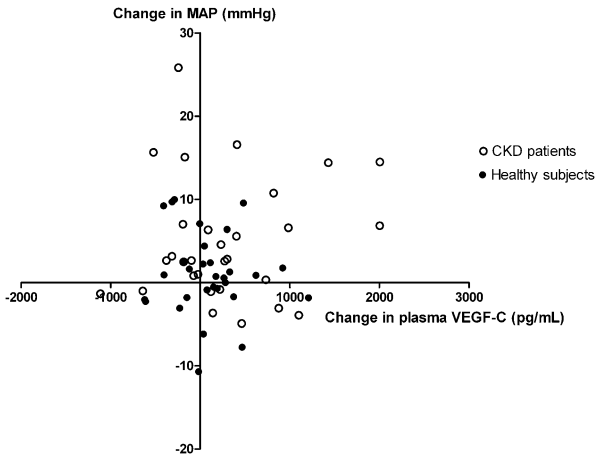

Fig. 3. Association between change in VEGF-C levels and change in mean arterial pressure (MAP) in CKD patients and healthy subjects.

changes in plasma VEGF-C levels supports the notion that changes in MPS-derived VEGF-C levels might serve as a clinical indicator for salt overload and salt storage in humans. We believe that this new research area warrants further investigation in patient-oriented research.

In our proteinuric CKD patients, blood pressure increased during the high-salt diet, in line with the well-established salt sensitivity of blood pressure in CKD [22, 23] and along with a rise in body weight and NT-proBNP, suggesting ECV expansion. Concomitantly, VEGF-C levels were increased, suggesting that high salt intake induces an extrarenal homeostatic pathway in these patients as well. This increase in VEGF-C was present despite the fact that during LS dietary sodium intake was substantially higher than the target of 50 mmol/day, thus limiting the difference with the HS period.

Animal data support a role for the VEGF-C–macrophage–lymphangiogenesis pathway in the protection against developing hypertension in response to a HS intake [5]. Furthermore, subjects with refractory hypertension show higher plasma VEGF-C levels than controls, suggesting that this pathway is relevant in the pathogenesis of human hypertension as well [4]. In our study populations we did not find a between-individual correlation between levels of VEGF-C and blood pressure, or between the responses of VEGF-C and blood pressure to HS when analysing for individual responses, neither in the separate populations, nor for pooled data. This could implicate either absence of an association, or complete protection against a sodium-induced rise in blood pressure by the adaptive response of the VEGF-C–macrophage–lymphangiogenesis pathway. Whereas we want to emphasize that a head-to-head comparison between the two populations should be interpreted with caution, due to differences in the experimental design and patient characteristics, nevertheless, it is noteworthy that VEGF-C levels were higher in the CKD patients, i.e. in the population where blood pressure was sodium sensitive.

The mechanism for the higher VEGF-C levels in CKD patients is of interest, but cannot be derived with certainty from our data. The data are consistent with the assumption that in CKD patients VEGF-C is stimulated more than in healthy controls on a similar sodium intake, which can be hypothesized to reflect a less effective response to sodium intake and hence a persisting stimulus. Whether this is due to differences in osmotic storage, non-osmotic storage, or to blunted sodium excretion in CKD leading to difference in overall sodium balance cannot be ascertained from our data. However, the higher NT-proBNP levels in CKD on each sodium intake are consistent with a higher ECV and hence differences in overall balance and osmotically stored sodium in CKD patients.

The rise in blood pressure during HS in CKD patients suggests that the presumed extrarenal, MPS-driven regulatory mechanism is not sufficient to preclude a rise in blood pressure in response to HS. Of note, as VEGF-C reduces the permeability of the glomerular filtration barrier and promotes podocyte survival [24, 25], an increase in VEGF-C is theoretically expected to reduce proteinuria, independently of blood pressure. At variance with this consideration, in our patients proteinuria increased during high salt intake, probably secondary to the rise in blood pressure.

In an independent study in healthy subjects, VEGF-C levels were also increased by a 1-week period on high-salt diet, with a concomitant rise in the ECV and creatinine clearance, whereas blood pressure was salt resistant. These data suggest that the MPS-driven VEGF-C–macrophage–lymphangiogenesis regulatory pathway, which is specific for local tissue salt

storage, is stimulated by high salt intake, alongside the conventional renal osmotic pathways of salt homeostasis. The rise in creatinine clearance can be considered part of the integrative homeostatic response to HS and is considered instrumental in facilitating excretion of the excess sodium and sodium resistance of blood pressure. This is consistent with our current observation of a rise in creatinine clearance in our sodium-resistant healthy subjects and a smaller, non-significant rise in creatinine clearance in our sodium-sensitive CKD patients. Of note, we previously demonstrated that the rise in GFR on HS closely corresponds to the rise in ECV, i.e. the osmotic storage pathway, in healthy subjects [17].

Our data are the first to document an effect of a salt intake on VEGF-C, a crucial step in the newly identified VEGF-C–macrophage–lymphangiogenesis pathway as an extrarenal homeostatic mechanism in the response to an increase in salt intake in humans, in a salt-sensitive as well as a salt-resistant condition. Unfortunately, we have no data on total body composition and salt content. Furthermore, it would be of great interest to directly monitor local interstitial changes in humans during dietary salt intervention in future research.

To conclude, VEGF-C levels are increased by a high-salt diet in proteinuric CKD patients and in healthy subjects, supporting a role for VEGF-C-mediated interstitial regulatory mechanisms in salt homeostasis in humans. Considering the rise in blood pressure during a high-salt diet, this buffering mechanism for salt-sensitive hypertension appears to be insufficient in proteinuric CKD patients. Future studies should investigate the clinical relevance, the reasons for failure in CKD and potential targets for intervention, of VEGF-C mediated interstitial electrolyte and volume homeostasis in humans.

Conflict of interest statement. None declared.

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