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Research Article

The Organ Handling of Soluble Klotho in Humans

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Keywords
Klotho · Phosphate · Chronic kidney disease · Hypoxia

Abstract
Background: Chronic kidney disease (CKD) reduces both Klotho expression and its shedding into circulation, an effect that accelerates progression and cardiovascular complications. However, the mechanisms that regulate Klotho release by the human kidney are still unknown. ***Methods:*** We measured plasma Klotho across the kidney, splanchnic organs and lung in 22 patients (71 ± 2 years, estimated glomerular filtration rate [eGFR] 60 ± 5.4 mL/min 1.73 m^2) during elective diagnostic cardiac catheterizations. ***Results:*** Although the Klotho average renal vein concentrations were remarkably higher (by ~9%) than arterial values, the kidney removed Klotho (or was at zero balance) in 7 subjects, indicating that the kidney contribution to systemic Klotho is not constant. Klotho fractional enrichment across the kidney was inversely related to plasma sodium (r = 0.43, p = 0.045) and acid uric acid levels (r = 0.38, p = 0.084) and directly, to renal oxygen extraction (r = 0.56, p = 0.006). In multivariate analysis, renal oxygen extraction was the only predictor of the enrichment of Klotho across the kidney, suggesting the dependence of renal Klotho release on tubular hypoxia or oxidative metabolism. Klotho balance was neutral across the lung. In patients with eGFR <60 mL/min, Klotho was also removed by splanchnic organs (single pass fractional extraction ~11%). ***Conclusions:*** The present study identifies kidney oxygen uptake as a predictor of Klotho release, and

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splanchnic organs as a site for Klotho removal. This study provides new understanding of kidney Klotho release and suggests that modulating kidney oxygen metabolism could increase Klotho delivery, as an option to slow disease progression and blunt organ damage.

Introduction

Klotho, a transmembrane 130 KDa protein, which was initially identified as an aging suppressor [1], is expressed in multiple tissues but is particularly high in the kidney [2–4]. In the human kidney, Klotho is strongly expressed both in the distal and in the proximal convoluted tubule, where it acts as a co-receptor for fibroblast growth factor-23 (FGF23) receptor [2–4]. In addition to membrane Klotho, a secreted form of Klotho protein (soluble Klotho) is generated from the *Klotho* gene through alternative splicing or proteolytic cleavage. Soluble Klotho (sKlotho) is directly released into the extracellular compartment to reach distant tissues, where it is implicated in a variety of processes, including protection of endothelial function, inhibition of phosphate-driven vascular calcification, inflammation, cell senescence and fibrosis [5–10].

Klotho is principally synthesized in the kidney and brain, but it is expressed in multiple organs. The tissue localization of Klotho suggests that the kidney is a major source for the supply of sKlotho to systemic circulation, leading to its systemic effects [11]. However, little information on kidney sKlotho handling is available in humans. sKlotho levels are greater in blood collected from suprarenal inferior vena cava as compared to lower vena cava, suggesting that sKlotho is released into the circulation from the human kidney [12]. Of note, circulating levels of sKlotho decline in various conditions associated with renal dysfunction, such as during ageing [13], hypertension [14] and in patients with chronic kidney disease (CKD) [15]. However, the kidney may also remove sKlotho from the circulation; Hu et al. [12] observed that an intravenous injection of ^{125}I-labeled Klotho protein into normal rats was followed by fast kidney uptake and tubular secretion, indicating that the kidney actively contributes even to the sKlotho clearance.

To study sKlotho handling by the human kidney and other tissues, initially we measured plasma sKlotho across the kidney, splanchnic organs and lung in subjects undergoing heart catheterizations. As a second step, we studied the clinical and biochemical determinants of organ sKlotho handling in the same cohort. Our data shows that the enrichment of sKlotho across the human kidney after a single pass is highly variable and, better than by changes in glomerular filtration rate (GFR), it is predicted by changes in kidney oxygen uptake.

Methods

Patients and Procedures

Twenty-two subjects scheduled for elective cardiac catheterization were eligible for enrolment in this protocol at the Department of Internal Medicine, University of Genoa. In 7 (4 males, 3 females, estimated GFR [eGFR] = 58 mL/min 1.73 m^2) of these subjects, data on FGF23 metabolism has been previously reported [14]. The clinical characteristics of subjects studied here are reported in Table 1. Their mean age was 71 years; 10 participants (48%) were male. There were 12 participants with arterial hypertension (45%), 4 (18%) with diabetes; 8 of them (36%) were on renin-angiotensin-aldosterone system (RAAS) blockers, and 6 (27%) on statin treatment. No subject received vitamin D supplements. Their median

Table 1. Clinical characteristics of patients

Number of subjects	22
Age, years	70.7±1.8
Gender, male/female	10/11
Weight, kg	68±3
BMI, kg/m^2	23.5 (18.8–39)
eGFR, mL/min/1.73 m^2	60.1±5.4
Creatinine, mg/dL	1.3±0.1
Haemoglobin, g/dL	12.8 (7.5–16.9)
Phosphate, mg/dL	3.4±0.1
Calcium, mg/dL	8.9 (8.6–10.5)
Glucose, mg/dL	116 (68–256)
Albumin, mg/dL	3.9±0.1
Cholesterol, mg/dL	155.6±11.6
Triglycerides, mg/dL	115.3 (46–274)
Uric acid, mg/dL	6.8±0.5
Na^+, mEq/L	140±0.9
K^+, mEq/L	4.3 (3.4–6.1)
CKD, eGFR ≤55 mL/min/1.73 m^2, yes/no	10/12
Hypertension, yes/no	12/10
Type II diabetes, yes/no	4/18
RAAS blockers, yes/no	8/14
Statins, yes/no	6/16

Data represents mean ± SEM or median (range). BMI, body mass index; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; RAAS, renin-angiotensin-aldosterone system.

eGFR was 60 mL/min per 1.73 m^2 and their mean uric acid levels were moderately increased. The patients were studied in the post-absorptive state, that is, after a 12–14 h fast. Three sets of blood samples were obtained at ~10 min intervals from the femoral artery as well as from the renal veins, the hepatic veins and the pulmonary artery. The right kidney was more often used for sampling. In 2 subjects, both sides were sampled and results averaged. Plasma was quickly separated from blood cells and stored at –80 °C until assayed. Plasma sKlotho levels were determined using an ELISA assay (α-Klotho Assay Kit, IBL, Japan) according to the manufacturer's protocol. Samples of arterial and venous blood were all run in triplicate. All samples from one individual were always run in the same batch (intra-assay CV = 3.5%). Blood pH, pO_2, pCO_2, glucose, lactate and creatinine were measured with an ABL800 Flex apparatus (Radiometer, Copenhagen, Denmark). Blood oxygen content was calculated as previously described [15] from haemoglobin levels, pO2 levels and oxygen saturation.

Calculations and Statistical Methods

The arterio-venous (A-V) difference of sKlotho and other substrates across the kidney, splanchnic organs and lung was calculated as follows: (A) – (V), where (A) and (V) are the concentrations of metabolites in arterial and venous plasma. Fractional extractions and fractional enrichments (FEs) were calculated as 100× ([A] – [V])/[A]). In 7 subjects in whom FGF23 data was available, results on kidney (A-V) differences of both FGF23 and sKlotho were calculated on a molar basis, considering a molecular weight of 32 KDa for FGF23 and of 70 kDa for sKlotho. Statistical analysis was performed using Wilcoxon matched-pairs signed-ranks test to compare arterial data with venous data (Statview Statistical Package, Abacus, Berkeley, CA, USA). The reported *p* values are based on a two-tailed calculation. Linear regression and correlation or Spearman analysis were employed to evaluate the relation

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Table 2. Klotho and oxygen blood levels, and their FEs or extractions by the kidney, splanchnic organs and lung (n = 22)

eGFR, mL/min	Artery, pg/mL	Renal vein, pg/mL	Kidney A-V, pg/mL	Kidney FE, %	Liver vein, pg/mL	Splanchnic A-V, pg/mL	Splanchnic fractional extraction, %	Lung artery, pg/mL	Lung V-A, pg/mL	Arterial oxygen content, ml/dL	Kidney oxygen fractional extraction, %
94	199.0	213.7	−14.7	−7.4	158.4	40.4	20.3	314.8	−116.0	20.3	8.3
64	300.0	349.6	−49.6	−16.6	247.4	52.5	17.5	412.4	112.5	18.4	11.6
68	233.0	211.0	12.0	5.1	189.0	44.1	18.7	269.4	−36.4	19.3	10.2
98	295.0	253.0	42.0	14.6	295.0	0.0	0.0	340.6	45.6	21.1	7.6
73	240.0	246.0	−6.0	−2.8	314.0	−74.0	−30.9	254.4	15.4	20.0	12.5
81	215.0	226.0	−11.0	−5.5	185.1	29.9	13.8	243.4	28.4	12.3	17.2
97	221.1	376.1	−155.0	−70.8	123.0	98.0	44.3	246.5	25.5	13.5	42.0
86	237.2	170.0	29.1	28.2	237.0	0.00	0.0	272.6	−35.4	17.5	15.4
81	221.0	271.0	−50.2	−23.9	317.9	−96.8	−48.8	229.5	8.5	13.8	24.7
89	683.1	756.1	−73.0	−10.9	592.7	89.4	13.7	748.2	65.2	17.0	19.3
73	491.9	512.0	−20.0	−4.1	537.3	−46.3	−9.6	531.2	39.2	10.9	4.8
79	334.9	352.1	−18.1	−5.1	442.4	−107.5	−32.2	390.0	56.0	19.0	14.1
29	197.2	342.2	−145	−74	166.4	30.8	15.2	253	56.0	17.4	13.9
49	236.0	287.0	−51.0	−21.9	184.01	52.0	22.3	252.7	−16.7	14.4	21.3
30	200.3	219.0	−18.7	−9.1	172.1	28.2	14.1	196.8	−3.4	20.8	12.1
45	232.1	225.3	6.7	2.7	162.5	69.3	29.1	265.1	−33.1	12.0	11.6
29	425.9	343.0	82.9	19.6	210.9	215.0	50.9	507.0	−81.1	7.8	8.0
38	161.1	166.0	−4.9	−3.0	145.1	15.9	9.8	177.1	−16.1	15.1	14.0
47	220.0	253.0	−33.0	−14.8	111.3	108.7	48.4	224.5	4.5	16.8	14.2
35	383.7	299.8	83.9	22.0	271.3	112.4	29.1	409.5	−25.8	16.1	12.4
23	221.0	400.0	−179.0	−79.9	–	–	–	242,4	22.4	10.8	22.3
51	228.1	244.3	−16.2	−7.6	–	–	–	275.0	47.0	16.2	18.6
60±5.4	280±25	307±26	−27±14	−12±6	253±30	32±18	11±6	320±33	35.7±25.1	16±1	14±2.0[a]

Data are expressed as mean ± SEM.FE, fractional enrichment; eGFR, estimated glomerular filtration rate; A-V, arterio-venous; V-A, venous-arterial. Artery versus vein concentration. [a] $p < 0.02$ or less.

Table 3. Univariate and multivariate analyses of the correlation between sKlotho FE in the renal vein and clinical characteristics (n = 22)

Clinical characteristics	Univariate		Multivariate (model r^2 = 0.453, p = 0.011)	
	r	p value	t	p value
Age, years	0.262	0.238		
Body weight, kg	0.331	0.133		
Log BMI, kg/m^2	0.119	0.599		
eGFR, mL/min/1.73 m^2	0.172	0.445		
Creatinine, mg/dL	0.255	0.252		
Log O_2 extraction	0.563	0.006	2.314	0.033
Log Hb, g/dL	0.127	0.583		
Phosphate, mg/dL	0.057	0.808		
Log calcium, mg/dL	0.294	0.197		
Log glucose, mg/dL	0.365	0.113		
S. Albumin, mg/dL	0.147	0.548		
Cholesterol, mg/dL	0.308	0.264		
Log triglycerides, mg/dL	0.347	0.204		
Uric acid, mg/dL	0.376	0.084	–1.390	0.1814 ns
Log Na^+, mEq/L	0.431	0.045	–1.484	0.155 ns
Log K^+, mEq/L	0.155	0.490		

FE, fractional enrichment; BMI, body mass index; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; ns, not significant.

between 2 variables. A p value of <0.05 was considered statistically significant. All data are expressed as mean ± SEM.

Results

Handling of sKlotho by the Kidney, Splanchnic Organs and Lung

Individual arterial and venous plasma levels of sKlotho, as well as its A-V differences and enrichments across the kidney, splanchnic organs and lung are reported in Table 2. In accordance with the clinical setting of patients studied here, their arterial sKlotho levels were in the lower normal range (280 ± 25 pg/mL) [16, 17]. Although average renal vein sKlotho concentrations were remarkably higher (by ~9%), than the corresponding arterial values, the kidney removed sKlotho (or was at zero balance) in 7 subjects, indicating that its release by the kidney is not a constant finding (sKlotho arterial vs. venous concentration p = 0.12). In 4 subjects with diabetes mellitus arterial sKlotho level was not different from nondiabetic subjects (208 ± 79 vs. 291 ± 34 pg/mL in diabetic and nondiabetic subjects, respectively, p = ns). The sKlotho enrichment in the renal vein was also not different in diabetic vs. nondiabetic subjects (30 ± 22 vs. 25 ± 14% respectively; p = ns). In all subjects, sKlotho FE across the kidney was, only as a trend, directly related to eGFR (r = 0.205, p = ns), suggesting that renal function per se is not a major determinant of sKlotho enrichment in the renal vein. If only subjects with eGFR >60 mL were taken into consideration, the association between renal Klotho enrichment and eGFR was also very weak (r = 0.194. p = ns).

Although not statistically significant, sKlotho levels in the liver vein were ~10% lower than the arterial ones (Table 2).

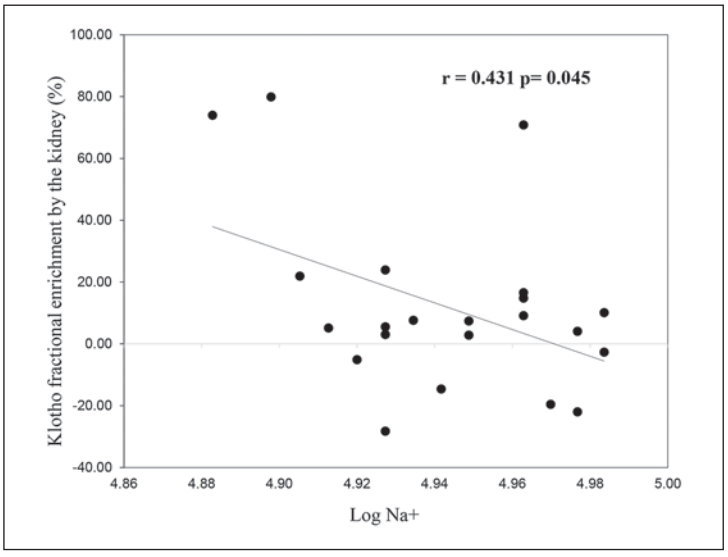

Fig. 1. Relationship between kidney fractional enrichment of Klotho and serum sodium levels.

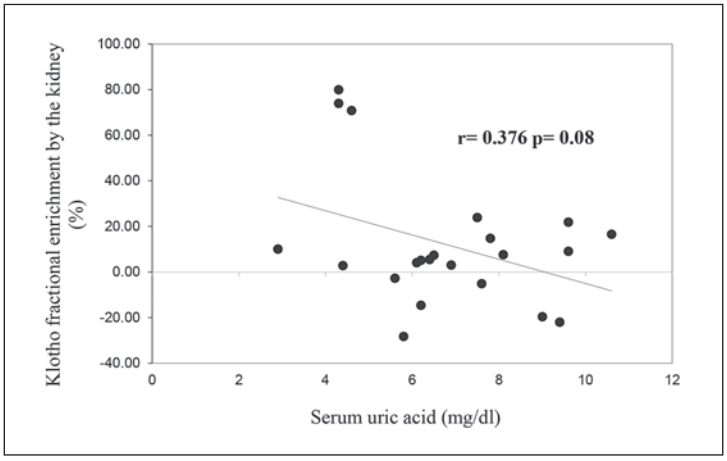

Fig. 2. Relationship between kidney Klotho fractional enrichment and serum uric acid levels.

Arterial sKlotho levels were almost identical to systemic venous (pulmonary artery) whole body levels (Table 2), documenting maintained zero balance of sKlotho across these organs systems.

Mainly due to moderate degree anaemia, arterial oxygen content was slightly reduced in patients studied here. Kidney fractional oxygen extraction was on the average 14 ± 2% of arterial content, varying from 8 to 42%.

Determinants of sKlotho Enrichment in the Renal Vein

As a next step, we studied whether the enrichment of sKlotho by the kidney can be predicted by clinical findings. Table 3 summarizes the associations between individual clinical data and sKlotho enrichments across the kidney. At univariate analysis, sKlotho FE was inversely related to plasma sodium ($r = 0.431$, $p < 0.045$; Fig. 1) and, as a trend, to uric acid plasma levels ($r = 0.376$, $p = 0.084$; Fig. 2), but neither to plasma phosphate nor to eGFR.

In addition, sKlotho FE was strongly associated with renal oxygen fractional extraction both in subjects with normal renal function ($n = 12$; $r = 0.79$, $p < 0.002$) and in all the study subjects ($n = 22$; $r = 0.563$, $p = 0.006$; Fig. 3).

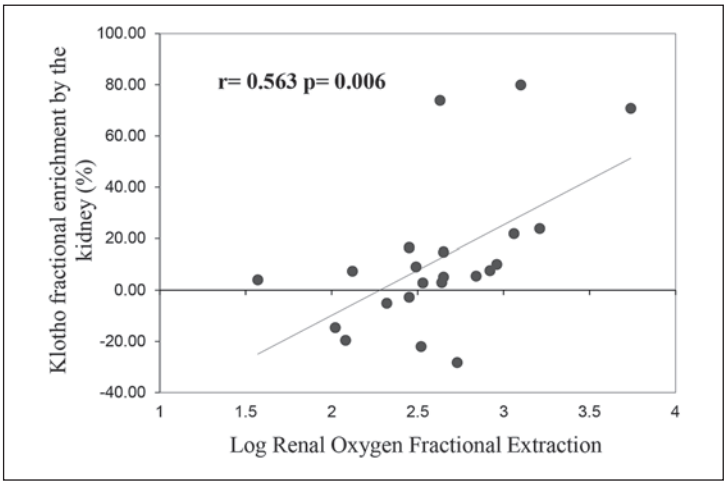

Fig. 3. Relationship between fractional enrichment of Klotho and oxygen extraction by the kidney.

Table 4. Klotho FE ("minus" sign) or extraction ("plus" sign) across the kidney and splanchnic organs according to eGFR

	Klotho kidney FE, %	Klotho splanchnic fractional extraction, %
eGFR >60 mL/min (*n* = 12)	–8.3±7.0	+0.14±11.6
eGFR <60 mL/min (*n* = 10)	–12±10	+11.3[a]±5.5

Data is presented as mean ± SEM. eGFR, estimated glomerular filtration rate; FE, fractional enrichment. Artery versus vein. [a] $p < 0.05$.

No correlation was found between arterial oxygen content and sKlotho per cent enrichment in the renal vein ($r = 0.19$, $p = 0.38$, ns). There was also no relationship between kidney sKlotho enrichment and age, weight, haemoglobin, serum albumin, BUN, glucose, bicarbonate, triglycerides, fibrinogen and iron levels. Using the studied variables for inclusion into multivariate analysis, models revealed that the kidney oxygen fractional extraction was the only predictor of enrichment of sKlotho in the renal vein, suggesting the dependence of renal sKlotho systemic release on kidney oxidative metabolism.

sKlotho Enrichment across the Kidney according to eGFR

As a next step, to evaluate how eGFR may influence sKlotho kidney enrichment, we studied patients separately, according to eGFR values >60 mL/min ($n = 12$, mean eGFR 81 ± 3 ml/min 1.73 m^2) or <60 mL/min ($n = 10$, mean eGFR 38 ± 2 mL/min 1.73 m^2; Table 4). Renal vein sKlotho enrichment was similarly variable in subjects with different eGFR. Fractional oxygen extraction was similar in patients with normal and decreased eGFR (14.8 ± 1.43 vs. 15.6 ± 2.76%, respectively, p = ns).

In patients with lower eGFR, a removal of sKlotho by splanchnic organs was detectable (artery 257 ± 34 vs. liver vein 177 ± 17 pg/mL, $p < 0.025$, fractional extraction ~11%).

sKlotho and FGF23 Kidney Handling

In 7 subjects in whom the measure of FGF23 was already available [14], it was possible to correlate the kidney A-V differences of both FGF23 and sKlotho. In these subjects FGF23

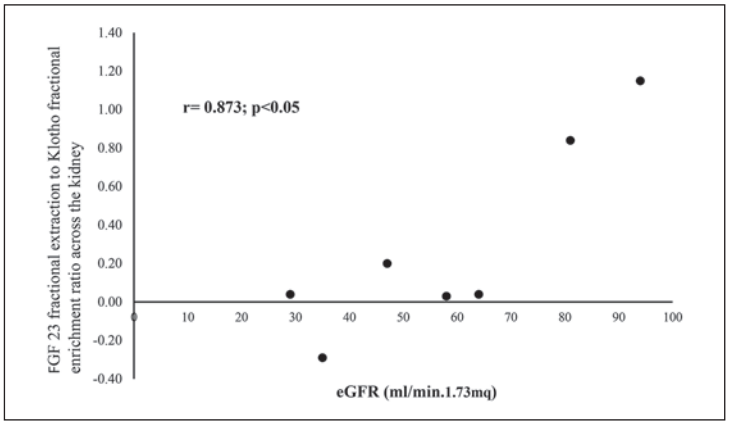

Fig. 4. Relationship between FGF23 to Klotho A-V difference ratio across the kidney and eGFR. FGF23, fibroblast growth factor-23; eGFR, estimated glomerular filtration rate.

was removed by the kidney with a single pass extraction of 15 ± 2.2%. On a molar basis, the ratio of FGF23 to sKlotho A-V difference across the kidney (the ratio between the A-V differences cancels out the role of blood flow, and expresses the ratio of absolute exchange rates) was directly related to eGFR (r = 0.873, p < 0.05; Fig. 4). This ratio was close to 1 (i.e., FGF23 kidney uptake was 1:1 with respect sKlotho kidney release) at eGFR values of ~90 mL/min, but declined to 0 when eGFR declined to 30 mL/min. This finding is in keeping with the unique characteristics of FGF23-Klotho signalling in the kidney and suggests a greater dependency on a high eGFR of FGF23 kidney removal than sKlotho kidney release.

Discussion

Several new observations have been made in this study. First, in contrast with the current assumption that the kidney constantly releases sKlotho to distant organs, we observed that the FE of sKlotho across the human kidney is highly variable. Another new observation is that a decrease in renal function per se does not to modify the kidney ability to add sKlotho to the renal vein, as shown by similar sKlotho enrichments in subjects with different eGFR. Third, this study shows that, better than by changes in eGFR, kidney oxygen extraction from blood predicts the enrichment of sKlotho in the renal vein, suggesting that oxygen uptake is the major determinant of kidney sKlotho handling and release into the circulation. As a further point, we observe for the first time that splanchnic organs can remove sKlotho from the circulation.

The kidney has the highest level of expression of Klotho, but little is known on how important sKlotho circulating in serum is derived from the kidney. Serum sKlotho levels in patients with CKD are extremely variable [16, 17]. In contrast, renal Klotho levels are uniformly reduced in experimental CKD [18–20]. In their seminal study, Hu et al. [12] observed in 7 subjects with a GFR >50 mL/min that suprarenal inferior vena cava enrichment of sKlotho was >50%, thus demonstrating an outstanding kidney feature of sKlotho metabolism. In patients studied here, the fractional release of sKlotho by the kidney represented on the average ~9% of its arterial plasma concentration, a figure much lower than that previously observed from sampling from the vena cava [12]. In addition, in 7 out of 22 subjects studied here, the kidney A-V difference balance of sKlotho was neutral or even positive, actually indicating either sKlotho removal or zero balance from blood. Accordingly, our data obtained in humans indicate that in the basal, post-absorptive state, the kidney can either produce or remove sKlotho from blood.

In this study, besides expressing a quantitative result of sKlotho enrichment across the kidney, we also describe the association between sKlotho kidney handling and several clinical/biochemical parameters. At univariate analysis, both dehydration and low kidney oxygen first pass extraction predicted sKlotho kidney output. The finding that high sodium and high uric acid plasma levels were associated with low sKlotho enrichment in the renal vein suggests that hydration status may be a determinant of kidney Klotho production. This observation is in accordance with the evidence that a low fluid intake is a risk factor for CKD [21, 22] and with experimental models showing that water can protect against kidney injury [23]. It is interesting that in mice as well as in human embryonic kidney cells, dehydration causes a significant decline of renal Klotho transcript levels and protein abundance [23]. Besides downregulating Klotho, an elevated serum osmolality can also activate various metabolic processes, including vasopressin release and activation of the aldose reductase-fructokinase pathway that associate with renal injury [22] and excess mortality in the elderly [24].

However, at multiple regression analysis, oxygen fractional extraction by the kidney was the only predictor of sKlotho kidney enrichment. To explain this association, several hypotheses can be made. The first is that oxygen fractional extraction and sKlotho enrichment are both dependent on the amount of viable kidney tissue. However, this hypothesis can be likely ruled out on the ground that the association between oxygen extraction and sKlotho enrichment across the kidney was also observed in subjects with preserved eGFR.

Another possibility is that sKlotho release is associated with tubular function. As a matter of fact, the high renal oxygen demand is associated primarily with tubular oxygen consumption, which is necessary for solute reabsorption. In this regard, FGF23/Klotho axis acts on sodium reabsorption in the proximal and distal tubules [4, 25]. Therefore, changes in solute reabsorption in different conditions of fluid homeostasis, oxidative stress, segmental tubular dysfunction and disease could represent conditions underlying changes in kidney sKlotho release.

As a possibility, we also have to consider the role of kidney hypoxia on the development and progression of CKD. A role for renal hypoxia as a major determinant of renal disease progression was first proposed by Fine and Norman [26] and this hypothesis has been recently supported by many animal studies [27, 28] and by studies in humans obtained in vivo by MRI [29]. In subjects studied here, arterial oxygen content was slightly reduced, owing to their reduced haemoglobin concentrations, implying a decreased oxygen delivery to tissues. However, we were not able to detect an association between arterial oxygen content or arterial haemoglobin and fractional sKlotho enrichment in the renal vein. In accordance with the large excess of oxygen supply to the human kidney, we observed that ~14% of arterial oxygen content was removed by the kidney after a single pass, a percentage similar to that observed in the normal condition [30, 31]. However, the measure of cortical oxygenation was not feasible in our study, so we cannot exclude that changes in oxygen supply to different intrarenal sites are associated with changes in Klotho release.

Finally, in our study we also show that the kidney exchange of FGF23 and sKlotho is stoichiometrically related. We previously observed that in humans, the kidney removes from blood FGF23 [14], an effect that is seemingly accounted for by glomerular filtration and tubular reabsorption. As a further extension of our previous findings, we now report that kidney FGF23 single-pass extraction is related to sKlotho single pass enrichment (Fig. 4). On a molar basis, the ratio of FGF23 to Klotho to A-V difference across the kidney declines from 1.0 at eGFR values of ~90 mL/min, to values close to 0 when eGFR reaches ≈30 mL/min. This finding is in keeping with the finding about the dependency on GFR of FGF23 kidney removal, while ongoing sKlotho release may expresses tubular adaptation to nephron loss.

It is commonly assumed that the sKlotho production by the kidney declines progressively along with the decrease in GFR. To our surprise, we observed that the sKlotho enrichment in the renal vein (which expresses the kidney tissue metabolic activity) was similar in patients

with different eGFR studied here. On the one hand, this finding may be, at least in part, the expression of a lower plasma flow and Klotho wash-out from the kidney in CKD. However, another possible explanation is that proteolytic cleavage of the extracellular domain of the transmembrane form may be accelerated to compensate the Klotho reduced availability.

Even less studied than its source, the route of elimination of *s*Klotho is completely unknown. In our study, although not statistically significant, sKlotho levels in the liver vein were ~10% lower than the arterial ones. This difference was statistically different in patients with reduced GFR. However, we cannot understand from our study whether this finding expresses an effect of CKD per se or if it simply represents reduced variability in Klotho measurement in the cohort of patients with more reduced GFR. Our results suggest that other organs, besides the kidney, can remove sKlotho from the circulation and are in keeping with the observation (12) that an intravenous injection of ^{125}I-labeled *s*Klotho protein into normal rats is followed by its removal not only by the kidney, but also by the liver and spleen.

The current study has several strengths, as it provides an example of in vivo sKlotho regulation in humans in a clinical setting. As a matter of fact, this study includes a cohort of non-selected patients, in which sampling of central veins was feasible. However, a potential limitation of our work is that the cohort studied might represent an aged and disease-prone population, in which sKlotho metabolism may have been influenced by the combined events occurring in aging and disease. In addition, hypertension and/or diabetes, which can down-regulate sKlotho [32], were present in 12 out of 22 patients. Moreover, our findings may represent some inaccuracies in the methods currently available to detect small A-V differences of Klotho.

Results presented here express qualitative, but not quantitative, data on sKlotho exchange across organs, since the measure of blood flow was not feasible in our study. In addition, the net balance data obtained from a simple A-V measurement can result from a variety of different rates of uptake and release. Therefore, zero net balance does not imply that uptake and release are also zero, but rather that their difference is such [33]. Although unlikely, this means that a given organ still produces and utilizes sKlotho but at comparable rates.

In conclusion, the present study provides the first report of sKlotho handling measured across the human kidney and major organ systems in humans and identifies oxygen uptake as predictor of kidney sKlotho release. Our data suggests that kidney sKlotho release is part of a homeostatic system that links kidney energy metabolism to the release of survival factor(s) to extrarenal tissues. Besides providing a better understanding of physiology of sKlotho metabolism, the data reported in this study suggests that modulating kidney oxygen metabolism could increase kidney sKlotho regulation and export, as an option to blunt organ damage and slow disease progression.

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Statement of Ethics

The study was part of a larger protocol on the study of inter-organ substrate exchange [13] approved by the Ethical Committee of the Department of Internal Medicine of the University of Genoa. All subjects were informed about the nature, purposes, procedures, and possible risks of the study, before their informed consent was obtained. The procedures were in accordance with the Helsinki declaration.

Disclosure Statement

The authors declare that they have no conflicts of interest to disclose.

Author Contributions

G.G., M.B., and F.A. performed primary clinical experiments and wrote the manuscript. D.V. and S.M. performed measurements. M.S. and A.M. collected clinical findings, A.S. provided intellectual input into primary experiments. F.V. and D.P. provided intellectual input and contributed to writing and editing the manuscript and G.M.R. oversaw the project and contributed to writing and editing the manuscript.

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