Klotho is a protein expressed and residing in the cell membrane of the kidney, parathyroid glands, and choroid plexus (12, 14). The extracellular domain of the protein may be cleaved and released into the cerebrospinal fluid and blood (3). The expression of Klotho is a powerful determinant of life span (7). Klotho hypomorphic (klotho<sup>hm</sup>) mice suffer from severe growth retardation and rapid aging (7), leading to premature death within <5 mo (7). Conversely, overexpression of Klotho increases the life span substantially (8).

Klotho increases the cell membrane protein abundance and activity of the renal epithelial Ca<sup>2+</sup> channel TRPV5, thus favoring renal tubular Ca<sup>2+</sup> reabsorption (13). Klotho enhances the Na<sup>+</sup>-K<sup>+</sup>-ATPase activity at a decreased extracellular Ca<sup>2+</sup> concentration in renal epithelial cells and parathyroid glands (4). Moreover, Klotho contributes to the downregulation of the 1α-hydroxylase and thus limits the production of 1,25-dihydroxyvitamin D3 [1,25(OH)<sub>2</sub>D<sub>3</sub>] (10, 14, 17). Dietary vitamin D restriction reverses the growth deficit and increases the life span of klotho<sup>hm</sup> mice (14).

The present study explored the influence of Klotho and Klotho-dependent 1,25(OH)<sub>2</sub>D<sub>3</sub> formation on mineral and electrolyte metabolism. To this end, klotho<sup>hm</sup> mice and wild-type mice (klotho<sup>+/+</sup>) were subjected to a normal (D<sup>+</sup>) or vitamin D-deficient (D<sup>-</sup>) diet or to a vitamin D-deficient diet for 4 wk and then to a normal diet (D<sup>-/+</sup>). At the age of 8 wk, body weight was significantly lower in klotho<sup>hm</sup>/D<sup>-</sup> mice than in klotho<sup>+/+</sup>/D<sup>-</sup> mice, klotho<sup>hm</sup>/D<sup>-</sup> mice, and klotho<sup>+/+</sup>/D<sup>-</sup> mice. Plasma concentrations of 1,25(OH)<sub>2</sub>D<sub>3</sub>, adrenocorticotropic hormone (ACTH), antidiuretic hormone (ADH), and aldosterone were significantly higher in klotho<sup>hm</sup>/D<sup>-</sup> mice than in klotho<sup>+/+</sup>/D<sup>-</sup> mice. Plasma volume was significantly smaller in klotho<sup>hm</sup>/D<sup>-</sup> mice, and plasma urea, Ca<sup>2+</sup>, phosphate and Na<sup>+</sup>, but not K<sup>+</sup> concentrations were significantly higher in klotho<sup>hm</sup>/D<sup>-</sup> mice than in klotho<sup>+/+</sup>/D<sup>-</sup> mice. The differences were partially abrogated by a vitamin D-deficient diet. Moreover, the hyperaldosteronism was partially reversed by Ca<sup>2+</sup>-deficient diet. Ussing chamber experiments revealed a marked increase in amiloride-sensitive current across the colonic epithelium, pointing to enhanced epithelial sodium channel (ENaC) activity. A salt-deficient diet tended to decrease and a salt-rich diet significantly increased the life span of klotho<sup>hm</sup>/D<sup>+</sup> mice. In conclusion, the present observation disclose that the excessive formation of 1,25(OH)<sub>2</sub>D<sub>3</sub> in Klotho-deficient mice results in extracellular volume depletion, which significantly contributes to the shortening of life span.

cell volume; calcium; phosphate; sodium; calcitriol

**MATERIALS AND METHODS**

*Mice.* All animal experiments were conducted according to the guidelines of the American Physiological Society as well as the German law for the welfare of animals and were approved by local authorities. Klotho hypomorphic mice (klotho<sup>hm</sup>) were compared with wild-type mice (klotho<sup>+/+</sup>). The mice were fed either a normal (D<sup>+</sup>) or a vitamin D-deficient (D<sup>-</sup>) diet, or the first 4 wk a vitamin D-deficient diet followed by a normal diet (D<sup>-/+</sup>). As expected, a vitamin D-deficient diet was followed by almost normal growth of klotho<sup>hm</sup> mice. Surprisingly, the excessive formation of 1,25(OH)<sub>2</sub>D<sub>3</sub> in klotho<sup>hm</sup> mice was paralleled by hyperaldosteronism, which was similarly reversed by a vitamin D-deficient diet.
vitamin D₃ (IDS, Boldon, UK). The plasma ADH concentrations were determined utilizing a commercial EIA-Kit (AVP EIA Kit, Phoenix Europe, Karlsruhe, Germany), and the plasma ACTH concentrations were determined utilizing a commercial ELISA-Kit (ACTH_ELISA, MD Bioproducts, Zurich, Switzerland). As the extremely fragile klothohm⁻/⁻ mice cannot be maintained in metabolic cages, spontaneously voided urine was collected for the determination of Na⁺ and creatinine concentrations. The urinary Na⁺ concentrations were determined by flame photometry and the urinary creatinine concentrations by utilizing the Jaffé reaction.

**Determination of plasma volume.** The plasma volume was assessed by dye dilution using Evans Blue (Sigma, Taufkirchen, Germany). Mice were anesthetized with diethyl ether, and 30–50 μl of an Evans Blue stock solution (3 mg/ml in 0.9% NaCl) were injected intravenously into the left retroorbital plexus using a 30-gauge microfine insulin syringe (BD, Heidelberg, Germany). The exact applied volume was determined by weighing the syringe before and after injection. Repeated blood samples (20–25 μl) were drawn from the right retroorbital plexus during superficial diethyl ether anesthesia after 30, 60, and 100 min, which yielded a volume of 10 μl plasma after centrifugation. Absorbance was measured at 620 nm against blank urine stock solution (3 mg/ml in 0.9% NaCl) were injected intravenously into the left retroorbital plexus using a 30-gauge microfine insulin syringe (BD, Heidelberg, Germany). The plasma concentrations of Evans Blue were determined by weighing the syringe before and after injection. Repeated blood samples (20–25 μl) were drawn from the right retroorbital plexus during superficial diethyl ether anesthesia after 30, 60, and 100 min, which yielded a volume of 10 μl plasma after centrifugation. Absorbance was measured at 620 nm against blank urine stock solution (3 mg/ml in 0.9% NaCl) were injected intravenously into the left retroorbital plexus using a 30-gauge microfine insulin syringe (BD, Heidelberg, Germany). The plasma concentrations of Evans Blue were determined by weighing the syringe before and after injection. Repeated blood samples (20–25 μl) were drawn from the right retroorbital plexus during superficial diethyl ether anesthesia after 30, 60, and 100 min, which yielded a volume of 10 μl plasma after centrifugation. Absorbance was measured at 620 nm against blank urine stock solution (3 mg/ml in 0.9% NaCl) were injected intravenously into the left retroorbital plexus using a 30-gauge microfine insulin syringe (BD, Heidelberg, Germany). The plasma concentrations of Evans Blue were calculated using the stock solution dissolved in mouse serum as a standard. To correct for the clearance of Evans Blue during distribution time, linear regression of the log-transformed concentrations was applied to calculate the y-intercept, which represents the imaginary concentration of Evans Blue in its final distribution volume (2). Division of the applied dose of Evans Blue (in mg) by the y-intercept (in mg/ml) resulted in the distribution volume of Evans Blue, which was normalized for body weight. The procedure cannot be tolerated by the fragile klothohm⁻/⁻ mice, and thus plasma volume cannot be determined in those mice. Instead, plasma volume was determined in the more robust klothohm⁻/+ mice, which had a similar hyperaldosteronism as the klothohm⁻/⁻ mice.

**Blood pressure.** Systolic arterial blood pressure was determined by the tail-cuff method (model 179, ITC). Application of this method requires certain precautions to reduce the stress of the animals, including appropriate training of the mice over multiple days and adequate prewarming to dilate the tail artery. The animals were placed in a heated chamber at an ambient temperature of 30–32°C, and from each animal blood pressure traces were recorded in one session until the deviation of a minimum of five traces was <5 mmHg. The readings from 3 days were then averaged to obtain a mean blood pressure under the respective treatment. All recordings and data analysis were done using a computerized data-acquisition system and software (PowerLab 400 and Chart 4, ADInstruments, Colorado Springs, CO). All measurements were performed by the same person during a defined time (between 2 and 4 PM).

**Ussing chamber experiments.** ENaC activity was estimated from the amiloride-sensitive potential difference across the colonic epithelium. After removal of the outer serosal and the muscular layer of lateral distal colon under a microscope, tissues were mounted onto a custom-made mini-Ussing chamber with an opening diameter of 0.99 mm and an opening area of 0.00769 cm². The serosal and luminal perfusate contained (in mM) 145 NaCl, 1 MgCl₂, 2.6 Ca-glucanate, 0.4 KH₂PO₄, 1.6 K₂HPO₄, and 5 glucose. To assess ENaC-mediated transport, 50 μM amiloride (in ethanol; Sigma, Schnelldorf, Germany) were added to the luminal perfusate. In all Ussing chamber experiments, the transepithelial potential difference (V₀) determined continuously and the transepithelial resistance (Rₑ) were estimated from the voltage deflections (ΔV₀) elicited by imposing test currents (Iₜ). The resulting Rₑ was calculated according to Ohm’s law.

**Statistics.** Data are provided as means ± SE; n represents the number of independent experiments. All data were tested for significance using ANOVA or a paired or unpaired Student t-test. Where applicable, GraphPad InStat version 3.00 for Windows 95 (GraphPad Software, San Diego, CA) or SAS Imp version 8.0.1 (SAS Institute, Cary, NC) was used. Only results with P < 0.05 were considered statistically significant.

**RESULTS**

At the age of 8 wk, the body size was smaller for klothohm⁻/⁻ mice than for klotho⁻/+D mice (Fig. 1A). Both transient and sustained vitamin D deficiency fully reversed the growth retardation of Klotho-hypomorphic mice; i.e., the body weight of klothohm⁻/⁻ and klothohm⁻/+ mice was not significantly different from the body weight of klotho⁻/+D mice (Fig. 1B).

The plasma volume per gram body weight was smaller in klothohm⁻/⁻ than in klotho⁻/+D mice, pointing to extracellular volume depletion of Klotho-deficient mice (Fig. 2A). The volume depletion occurred despite a significantly larger fluid intake of klotho⁻/+D mice than of klothohm⁻/+ mice (Fig. 2B). The plasma volume could not be determined in klothohm⁻/+ mice.

Extracellular volume depletion is expected to decrease renal urea clearance and thus to increase plasma urea concentration. As illustrated in Fig. 3, the plasma urea concentration was indeed markedly higher in klothohm⁻/⁻ than in klotho⁻/+D mice. Sustained vitamin D deficiency led to a significant decrease in plasma urea concentration (Fig. 3).

The plasma Na⁺, Ca⁺, phosphate, but not K⁺ concentrations were significantly higher in klothohm⁻/⁻D+ mice than in klotho⁻/+D+ mice (Fig. 4) and were not significantly different between klotho⁻/⁻D⁻ mice and klotho⁻/+D⁻ mice or between klotho⁻/⁻D⁻ mice and klotho⁻/+D⁻ mice (Fig. 4). Thus lack of Klotho increased the plasma Na⁺, Ca⁺, and phosphate concentrations, effects partially or fully reversed by transient or sustained vitamin D restriction. The plasma K⁺ concentration was not significantly different between the genotypes and was not significantly influenced by dietary vitamin D in Klotho hypomorphic mice (Fig. 4).

The hypercalcemia and hyperphosphatemia could have resulted from enhanced formation of 1,25(OH)₂D₃, with subsequent stimulation of intestinal Ca⁺ and phosphate absorption. The plasma concentration of 1,25(OH)₂D₃ was indeed significantly higher in klotho⁻/+D+ mice than in klotho⁻/+D⁻ mice (Fig. 5A). A vitamin D-restricted diet significantly decreased 1,25(OH)₂D₃ formation. Accordingly, the plasma 1,25(OH)₂D₃ concentration was significantly lower in klothohm⁻/⁻D⁻ mice than in klothohm⁻/+D+ mice (Fig. 5A).

The plasma PTH concentration tended to be lower in klothohm⁻/+D+ mice than in klotho⁻/+D+ mice, a difference, however, not reaching statistical significance (Fig. 5B).

The extracellular volume depletion is expected to stimulate ADH release. The plasma ADH concentration was significantly higher in klothohm⁻/⁻D+ mice than in klotho⁻/+D+ mice (Fig. 5C). Sustained, but not transient, dietary vitamin D restriction decreased the plasma ADH concentration to levels similar to control animals. The plasma ADH concentration tended to be lower in klotho⁻/⁻D⁻ mice than in klotho⁻/+D⁻ mice, but again tended to be higher in klothohm⁻/+D⁻ mice than in klotho⁻/+D⁻ mice (Fig. 5C).

The plasma aldosterone concentration was significantly higher in klotho⁻/+D+ mice than in klotho⁻/+D+ mice (Fig. 5D). Again, sustained, but not transient, dietary vitamin D restriction reversed the hyperaldosteronism. Accordingly, the plasma aldosterone concentration was significantly lower in klothohm⁻/⁻D⁻ mice than in klotho⁻/+D+ mice, but was again
significantly higher in klothohmD−/+ mice than in klothohm+/-D+ mice (Fig. 5D).

The plasma ACTH concentration was significantly higher in klothohmD−/+ mice (149 ± 17 nM, n = 4) than in klothohm+/-D+ mice (75 ± 12 nM, n = 5) but was not significantly affected by transient or sustained vitamin D depletion; i.e., it was similarly high in klothohmD−/- mice (157 ± 27 nM, n = 4).

The extracellular volume depletion and subsequent stimulation of aldosterone release could have resulted from hypercalcinemia. To explore this possibility, additional experiments were performed with a Ca2+-deficient diet. As illustrated in Fig. 6A, the plasma aldosterone concentration in klothohmD−/− mice significantly declined following treatment of the mice with a Ca2+-deficient diet. Nevertheless, the plasma aldosterone concentration remained significantly higher in klothohmD−/+ mice than in klothohm+/-D+ mice even under a Ca2+-deficient diet (Fig. 6A). Plasma aldosterone levels in klothohm+/-D+ mice were unaffected by a Ca2+-deficient diet.

Further experiments were performed to study whether urine salt wasting contributes to the hyperaldosteronism of klothohm mice. To this end, the urinary Na+ and creatinine concentra-

Fig. 1. Body weight of Klotho wild-type (klothoh+/+) and Klotho hypomorphic mice (klothohm) with and without a vitamin D-deficient diet. A: photograph of 8-wk-old klothohm and klothoh+/+ mice on a vitamin D-containing control diet (klothohm+/-D+ and klothoh+/+D+) as well as klothohm mice maintained on a continued vitamin D-deficient diet (klothohmD−/−) or on an initial vitamin D-deficient diet for 4 wk followed by a control diet for a further 4 wk (klothohm+/-D−/-). B: arithmetic means ± SE (n = 7–12) of the body weight of klothohm+/-D+, klothoh+/-/D−, klothohmD−/−, and klothohmD−/− mice. *,#Significant difference from klothoh+/+D+ mice and from klothohmD−/− mice (ANOVA, P < 0.001).

Fig. 2. Plasma volume and fluid intake of klothoh+/+ mice and klothohm mice. A: arithmetic means ± SE of the plasma volume (n = 9–14) of klothohm mice on an initial vitamin D-deficient diet for 4 wk followed by a control diet for a further 4 wk (klothohm+/-D−/-) and klothoh+/+ mice on a vitamin D-containing control diet (klothoh+/+D+). *Significant difference from klothoh+/+D−/− mice (Student’s t-test, P < 0.01). B: arithmetic means ± SE of the fluid intake (n = 4–9) of klothohm and klothoh+/+ mice on a vitamin D-containing control diet (klothohm+/-D+ and klothoh+/+D+) or on an initial vitamin D-deficient diet for 4 wk followed by a control diet for a further 4 wk (klothohm+/-D−/−). *Significant difference from klothoh+/+D+ mice (ANOVA, P < 0.05).

Fig. 3. Plasma urea concentration of klothoh+/+ mice and klothohm mice with and without a vitamin D-deficient diet. Shown are arithmetic means ± SE (n = 3–8) of the plasma urea concentrations of klothohm and klothoh+/+ mice on a vitamin D-containing control diet (klothohm+/-D+ and klothoh+/+D+) as well as klothohm mice maintained on a continued vitamin D-deficient diet (klothohm+/-D−/-) or on an initial vitamin D-deficient diet for 4 wk followed by a control diet for a further 4 wk (klothohmD−/−). *Significant difference from klothoh+/+D+ mice. #Significant difference from klothohmD−/− mice (ANOVA, P < 0.05).
tions of klotho\textsuperscript{−/−}D\textsuperscript{+} and klothohm\textsuperscript{−/−}D\textsuperscript{+} mice were determined under a normal diet and a low-salt diet for 3 days each. As shown in Fig. 6B, salt depletion dramatically reduced the urinary Na\textsuperscript{+}/creatinine ratio in klotho\textsuperscript{−/−}D\textsuperscript{+} mice but not in klothohm\textsuperscript{−/−}D\textsuperscript{+} mice.

The volume depletion was expected to decrease blood pressure. Due to the small size of klothohm\textsuperscript{−/−}D\textsuperscript{+} mice, it was technically impossible to determine their blood pressure. Blood pressure was, however, significantly lower in klothohm\textsuperscript{−/−}D\textsuperscript{+} mice than in klotho\textsuperscript{−/−}D\textsuperscript{+} mice (Fig. 7). Blood pressure in klothohm\textsuperscript{−/−}D\textsuperscript{+} mice was between levels in klothohm\textsuperscript{−/−}D\textsuperscript{+} mice and klotho\textsuperscript{−/−}D\textsuperscript{+} mice (Fig. 7).

The volume depletion and lowered blood pressure in view of the hyperaldosteronism may have resulted from decreased aldosterone sensitivity of mineralocorticoid target tissues. To explore this possibility, Ussing chamber experiments were performed in the terminal colon of klothohm\textsuperscript{−/−}D\textsuperscript{+} mice and klotho\textsuperscript{−/−}D\textsuperscript{+} mice (Fig. 8). As a result, the

Fig. 4. Plasma Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, and phosphate concentrations of klotho\textsuperscript{−/−}D\textsuperscript{+} mice and klothohm\textsuperscript{−/−} mice with and without vitamin D-deficient diet. Shown are arithmetic means ± SE (n = 6-16) of the plasma Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, and phosphate concentrations of klothohm\textsuperscript{−/−} and klotho\textsuperscript{−/−} mice on a vitamin D-containing control diet (klothohm\textsuperscript{−/−}D\textsuperscript{−} and klotho\textsuperscript{−/−}D\textsuperscript{−}) as well as klothohm\textsuperscript{−/−} mice maintained on a continued vitamin D-deficient diet (klothohm\textsuperscript{−/−}D\textsuperscript{−}) or on an initial vitamin D-deficient diet for 4 wk followed by a control diet for further 4 wk (klothohm\textsuperscript{−/−}D\textsuperscript{−/+}). *Significant difference from klotho\textsuperscript{−/−}D\textsuperscript{+} mice (ANOVA, P < 0.05).

Fig. 5. Plasma 1,25(OH)\textsubscript{2}D\textsubscript{3}, parathyroid hormone (PTH), antidiuretic hormone (ADH), and aldosterone concentrations of klotho\textsuperscript{−/−}D\textsuperscript{+} and klothohm\textsuperscript{−/−} mice with and without a vitamin D-deficient diet. Shown are arithmetic means ± SE of the plasma 1,25(OH)\textsubscript{2}D\textsubscript{3} (n = 12–20), PTH (n = 5–14), ADH (n = 12–17), and aldosterone concentrations (n = 10–18) of klothohm\textsuperscript{−/−} and klotho\textsuperscript{−/−} mice on a vitamin D-containing control diet (klothohm\textsuperscript{−/−}D\textsuperscript{−} and klotho\textsuperscript{−/−}D\textsuperscript{−}) as well as klothohm\textsuperscript{−/−} mice maintained on a continued vitamin D-deficient diet (klothohm\textsuperscript{−/−}D\textsuperscript{−}) or on initial vitamin D-deficient diet for 4 wk followed by a control diet for a further 4 wk (klothohm\textsuperscript{−/−}D\textsuperscript{−/+}). *Significant difference from klotho\textsuperscript{−/−}D\textsuperscript{+} mice. #Significant difference from klothohm\textsuperscript{−/−}D\textsuperscript{+} mice (ANOVA, P < 0.05).
every mouse 3–4 days under a normal diet and 3–4 days under a low-salt diet. The values are the means of the individual values determined for klotho mice and klothoD mice with and without a low-Ca2+ diet (Fig. 8). The observations revealed enhanced ENaC activity in Klotho-deficient mice (Fig. 8).

Amiloride-sensitive current was significantly larger in klothoD mice than in klothoD mice (Fig. 8). The observations revealed enhanced ENaC activity in Klotho-deficient mice (Fig. 8).

Additional experiments were performed to elucidate whether salt intake modified the survival of klothoD mice. Dietary salt diet indeed modified the life span of Klotho-deficient mice. When treated with a low-salt diet, the klothoD mice tended to die earlier (at an age of 62.9 ± 8.9 days, n = 14) than klothoD mice on a normal-salt diet (at an age of 66.8 ± 5.6 days, n = 26). In contrast, the life span of klothoD mice on a salt-rich diet was significantly more extended (age of 214.0 ± 19.1 days, n = 4) than the life span of animals on either a control diet or salt-deficient diet. (Fig. 9).
DISCUSSION

The present observations confirm the marked influence of Klotho deficiency on 1,25(OH)_{2}D₃ formation (10, 14, 17) as well as plasma Ca²⁺ (6) and phosphate (11) concentration. Klotho participates in the inhibition of 1α-hydroxylase and thus decreases 1,25(OH)₂D₃ production (10, 14, 17). As 1,25(OH)₂D₃ stimulates intestinal and renal Ca²⁺ and phosphate transport (9, 11), the unrestrained formation of 1,25(OH)₂D₃ presumably accounts for the hypercalcaemia and hyperphosphatemia in Klotho hypomorphic mice (10, 14, 17). In view of the scatter of the present data, however, other mechanisms contributing to the deranged Ca²⁺ and phosphate metabolism cannot be excluded.

More importantly, the present observations reveal a novel functional consequence of Klotho deficiency, i.e., extracellular volume depletion with subsequent increase in ADH release and hyperaldosteronism. The volume depletion further leads to decreased blood pressure. At least in theory, the volume depletion of Klotho hypomorphic mice could be due to hypercalcaemia and subsequent activation of the Ca²⁺-sensing receptor CasR. CasR regulates the renal tubular Na⁺ reabsorption; i.e., stimulation of the receptor inhibits renal tubular Na⁺ transport, leading to subsequent renal salt loss (15). Vitamin D-induced hypercalcaemia has previously been shown to down-regulate Na-K-2Cl cotransporter expression in the thick ascending limb of Henle’s loop, which is expected to foster renal salt wasting and extracellular volume contraction (16). Accordingly, treatment of the mice with a Ca²⁺-deficient diet significantly and substantially blunted the hyperaldosteronism. The plasma volume was decreased and plasma aldosterone levels were enhanced in animals receiving a transiently vitamin D-deficient diet. In those mice, the unrestrained 1α-hydroxylase activity is expected to result in excessive 1,25(OH)₂D₃ formation as soon as vitamin D is added to the diet.

During a Ca²⁺-deficient diet, the plasma aldosterone level still remained significantly higher in klotho⁰⁰/D⁺ mice than in klotho⁺⁺/⁺⁺ mice. Thus additional mechanisms may contribute to the volume depletion in klotho⁰⁰/D⁺ mice, and more than a single disorder contributes to the derangement of electrolyte metabolism in Klotho hypomorphic mice. Klotho has previously been shown to upregulate the Na⁺-K⁺-ATPase (4) and, at least in theory, Klotho deficiency could result in decreased renal tubular Na⁺-K⁺-ATPase activity, thus compromising renal tubular salt reabsorption. The defect is particularly apparent under a salt-deficient diet, which leads to a rapid decrease of urinary Na⁺ output in wild-type mice but not in Klotho hypomorphic mice.

Klotho deficiency led to a significant increase in ACTH release. Unlike the aldosterone plasma level, the increased ACTH level could not be reversed by vitamin D deficiency and is presumably caused by mechanisms other than 1,25(OH)₂D₃ excess. It should be kept in mind that Klotho is not only expressed in parathyroid glands and the kidney, but as well in the choroid plexus (12, 14) and is released into cerebrospinal fluid (3). Thus Klotho deficiency could modify cerebral functions and hypothalamic control of hormone release more directly.

Klotho deficiency may not only affect the endocrine system but similarly compromise the function of the autonomous nerve system. In an earlier study, Klotho deficiency has been shown to impair the increase in catecholamine release during stress (12).

The hyperaldosteronism leads to an increase in electrogenic Na⁺ transport in the terminal colon, an epithelium similarly mineralocorticoid sensitive as the renal collecting duct (1). The observation illustrates that aldosterone is effective in Klotho hypomorphic mice and that extracellular volume as well as blood pressure are decreased despite increased aldosterone release and action.

In view of the hyperaldosteronism, the possibility was explored of whether the volume depletion may contribute to the decreased life span of Klotho-deficient mice. The results indeed demonstrate that a salt-rich diet significantly and substantially extended the life span of Klotho-deficient mice. These observations do not contradict the role of other pathophysiological mechanisms leading to the dramatic decrease in the life span of Klotho hypomorphic mice but clearly demonstrate a strong impact of extracellular volume depletion on the survival of those mice.

Similar to what has been shown for the life span (14), growth deficit (14) and erythrocyte survival (5), vitamin D restriction reverses the effect of Klotho deficiency on the plasma mineral and electrolyte concentrations as well as on hyperaldosteronism. The observations reveal that excessive formation of 1,25(OH)₂D₃ substantially contributes to or even accounts for the hyperaldosteronism in Klotho hypomorphic mice.

In conclusion, lack of Klotho leads to profound derangements not only of mineral but as well of electrolyte metabolism, resulting in a decrease in blood pressure and hyperaldosteronism. The effect could be largely reversed by vitamin D deficiency and is thus at least in part secondary to excessive formation of 1,25(OH)₂D₃. Salt repletion significantly enhances the life span of Klotho hypomorphic mice, and salt deficiency thus significantly contributes to the early death of those animals.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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