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Age-dependent alterations in Ca\(^{2+}\) homeostasis: role of TRPV5 and TRPV6

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van Abel, Monique, Sylvie Huybers, Joost G. J. Hoenderop, Annemie W. C. M. van der Kemp, Johannes P. T. M. van Leeuwen, and René J. M. Bindels. Age-dependent alterations in Ca\(^{2+}\) homeostasis: role of TRPV5 and TRPV6. Am J Physiol Renal Physiol 291: F1177–F1183, 2006. First published May 16, 2006; doi:10.1152/ajprenal.00338.2006.—Aging is associated with alterations in Ca\(^{2+}\) homeostasis, which predisposes older people to hyperparathyroidism and osteoporosis. Intestinal Ca\(^{2+}\) absorption decreases with aging and, in particular, active transport of Ca\(^{2+}\) by the duodenum. In addition, there are age-related changes in renal Ca\(^{2+}\) handling. To examine age-related changes in expression of the renal and intestinal epithelial Ca\(^{2+}\) channels, control (TRPV5\(^{+/+}\)) and TRPV5 knockout (TRPV5\(^{-/-}\)) mice aged 10, 30, and 52 wk were studied. Aging of TRPV5\(^{+/+}\) mice resulted in a tendency toward increased renal Ca\(^{2+}\) excretion and significantly decreased intestinal Ca\(^{2+}\) absorption, which was accompanied by reduced expression of TRPV5 and TRPV6, respectively, despite increased serum 1,25(OH)\(_2\)D\(_3\) levels. Similarly, in TRPV5\(^{-/-}\) mice the existing renal Ca\(^{2+}\) loss was more pronounced in elder animals, whereas the compensatory intestinal Ca\(^{2+}\) absorption and TRPV6 expression declined with aging. In both mice strains, aging resulted in a resistance to 1,25(OH)\(_2\)D\(_3\) and diminished renal vitamin D receptor mRNA levels, whereas serum Ca\(^{2+}\) levels remained constant. Furthermore, 52-wk-old TRPV5\(^{-/-}\) mice showed severe hyperparathyroidism, whereas PTH levels in elderly TRPV5\(^{+/+}\) mice remained normal. In 52-wk-old TRPV5\(^{-/-}\) mice, serum osteocalcin levels were increased in accordance with the elevated PTH levels, suggesting an increased bone turnover in these mice. In conclusion, downregulation of TRPV5 and TRPV6 is likely involved in the impaired Ca\(^{2+}\) (re)absorption during aging. Moreover, TRPV5\(^{-/-}\) mice likely develop age-related hyperparathyroidism and osteoporotic characteristics before TRPV5\(^{+/+}\), mice, demonstrating the importance of the epithelial Ca\(^{2+}\) channels in Ca\(^{2+}\) homeostasis.

ECCaC; CaT1; VDR; PTH; 1,25(OH)\(_2\)D\(_3\); aging; osteoporosis

REGULATION OF THE CA\(^{2+}\) ABSORPTIVE ACTIVITY OF THE INTESTINE AND KIDNEY IS CRUCIAL FOR THE MAINTENANCE OF NORMAL EXTRACELLULAR CA\(^{2+}\) LEVELS. Intestinal Ca\(^{2+}\) absorption and renal tubular (re)absorption of filtered Ca\(^{2+}\) are modulated on the basis of the body’s overall need for Ca\(^{2+}\) gain or loss. Part of the Ca\(^{2+}\) (re)absorption occurs through the active transcellular pathway, which can be envisaged as a three-step process. In the distal convoluted tubule (DCT) and connecting tubule (CNT) cells of the kidney, Ca\(^{2+}\) from the pro-urine enters the epithelial cells via the luminal Ca\(^{2+}\) channel TRPV5. Subsequently, Ca\(^{2+}\) is transported across the cell in association with the Ca\(^{2+}\)-binding protein calbindin-D\(_{9K}\) and is finally extruded into the bloodstream via the Na\(^{+/Ca^{2+}}\)-exchanger (NCX1) and the plasma membrane Ca\(^{2+}\)-ATPase (PMCA1b) in the basolateral membrane. In enterocytes, the main proteins involved in active Ca\(^{2+}\) absorption are the homologous TRPV6 channel at the luminal membrane, calbindin-D\(_{9K}\) as the Ca\(^{2+}\)-binding protein, and only PMCA1b at the basolateral side. In general, TRPV5 seems to be the major isoform in kidney, whereas TRPV6 is ubiquitously expressed with the highest concentrations in the prostate, stomach, brain, lung, and small intestine (21, 22, 32, 38). Moreover, active Ca\(^{2+}\) (re)absorption is the primary target for regulation by the calcitropic hormones 1,25-dihydroxyvitamin D\(_3\) [1,25(OH)\(_2\)D\(_3\)], the active metabolite of vitamin D\(_3\), and parathyroid hormone (PTH). Also, estrogens can stimulate active Ca\(^{2+}\) (re)absorption in both kidney and duodenum (46).

It is well known that aging is accompanied by alterations in Ca\(^{2+}\) homeostasis, which predisposes older patients to certain Ca\(^{2+}\)-related disorders, including hyperparathyroidism and osteoporosis. For instance, intestinal Ca\(^{2+}\) absorption decreases with aging and, in particular, active transport of Ca\(^{2+}\) by the duodenum (6, 14). Furthermore, there are age-related changes in renal Ca\(^{2+}\) handling, like reduced renal tubular function and a decline in response of the kidney to PTH during aging (7, 17, 35, 36). Renal and intestinal calbindins play an important role in active Ca\(^{2+}\) transport, and their expression decreases with age in parallel with the age-related decline in Ca\(^{2+}\) (re)absorption (2, 6, 26). In addition, the capacity of 1,25-dihydroxyvitamin D\(_3\) to stimulate Ca\(^{2+}\) absorption also reduces with age, whereas circulating levels of PTH rise in rats and humans (5, 12). Moreover, age-related increases in PTH levels may play an important role in bone remodeling. Bone loss occurs with aging, leading to reduced bone strength and, therefore, of osteoporotic fracture risk in the elderly (31, 36, 41).

Interestingly, TRPV5 knockout (TRPV5\(^{-/-}\)) mice display several alterations in Ca\(^{2+}\) homeostasis. For instance, they are impaired in active Ca\(^{2+}\) (re)absorption, despite enhanced vitamin D levels, causing severe hypercalciuria (23). In contrast to the excessive renal Ca\(^{2+}\) wasting, intestinal TRPV6 and calbindin-D\(_{9K}\) expression and Ca\(^{2+}\) absorption are increased, which could function as a compensatory mechanism to maintain a normal serum Ca\(^{2+}\) concentration (23). These findings suggest an essential role for both TRPV5 and TRPV6 in Ca\(^{2+}\) homeostasis. Moreover, dysfunction of these channels may contribute to disturbances in Ca\(^{2+}\) homeostasis and have implications for age-related changes in Ca\(^{2+}\) metabolism.

The present study investigated the physiological role of TRPV5 and TRPV6 in the aging kidney and intestine. Our
results suggest that downregulation of the renal and duodenal proteins involved in transcellular Ca\(^{2+}\) transport, including TRPV5 and TRPV6, are responsible for the impaired renal Ca\(^{2+}\) reabsorption and duodenal Ca\(^{2+}\) absorption in aging. Moreover, TRPV5\(^{-/-}\) mice develop age-related hyperparathyroidism and osteoporotic characteristics earlier compared with control mice, demonstrating the importance of these epithelial Ca\(^{2+}\) channels in Ca\(^{2+}\) homeostasis.

**MATERIALS AND METHODS**

**Animals.** TRPV5\(^{-/-}\) mice were generated and genotyped as described previously (23). TRPV5\(^{-/-}\) and wild-type mice (TRPV5\(^{+/+}\)) were fed standard chow and given water ad libitum. Seven to nine littermates of both genotypes of different ages were used. At the age of 10, 30, and 52 wk, mice were placed in metabolic cages (Techniplast, Buguggiate, Italy) for 48 h. Mice were allowed to adapt to these cages for 1 day, and then 24-h urine samples were collected. Mice were killed, and blood, kidney, and duodenum samples were collected. The animal ethics board of the Radboud University Nijmegen approved all animal experimental procedures.

**Analytic procedures.** Serum and urine Ca\(^{2+}\) concentrations were analyzed using a colorimetric assay kit as described previously (10). Serum phosphorus levels were measured on a Hitachi analyzer (Hitachi, Tokyo, Japan). Serum PTH levels were measured using a mouse intact PTH ELISA kit (Immunotopics, San Clemente, CA). Serum and urine Ca\(^{2+}\) were analyzed using a colorimetric assay kit as described previously (10). Serum PTH levels were measured using a radioimmunoassay (RIA) (50) and immunoextraction followed by a radioimmunassay (RIA) (50) and immunoextraction followed by quantitation by \(^{125}\) I-RIA (IDS, Boldon, UK) (13), respectively.

In vivo \(^{45}\) Ca\(^{2+}\) absorption assay. Ca\(^{2+}\) absorption was assessed by measuring serum \(^{45}\) Ca\(^{2+}\) at early time points after oral gavage. Mice were fasted overnight (12 h) before the test. Animals were hemodynamically stable under anesthesia (urethane, 1.4 mg/g body wt) during the experiment. The solution used to measure \(^{45}\) Ca\(^{2+}\) absorption contained 0.1 mM CaCl\(_2\), 125 mM NaCl, 17 mM Tris, and 1.8 g/l fructose and was enriched with 20 μCi \(^{45}\) CaCl\(_2\)/ml (18 Ci/g; New England Nuclear, Newton, MA). For the oral test, 15 μl/g body wt of this solution was administrated by gavage as described previously (49). Blood samples were obtained at 2, 4, 8, and 12 min after oral gavage, and serum (10 μl) was analyzed by liquid scintillation counting. The change in the serum Ca\(^{2+}\) concentration was calculated from the \(^{45}\) Ca\(^{2+}\) content of the serum samples and the specific activity of the administered \(^{45}\) Ca\(^{2+}\).

**RNA isolation and quantitative PCR.** Total RNA from kidney and duodenal mucosa was isolated using TRIzol reagent (GIBCO BRL, Life Technologies, Breda, The Netherlands) according to the manufacturer's protocol. Total DNase-treated RNA (2 μg) was reverse-transcribed using Moloney murine leukemia virus reverse transcriptase (GIBCO BRL) as described previously (19). Expression of TRPV5, TRPV6, calbindin-D\(_{28k}\), calbindin-D\(_{9k}\), NCX1, PMCA1b, and the vitamin D receptor (VDR) as well as mRNA levels of the housekeeping gene hypoxanthine-guanine phosphoribosyl transferase (HPRT), as an endogenous control, were determined by quantitative real-time PCR on an ABI Prism 7700 Sequence Detection System (PE Biosystems, Rotkreuz, Switzerland). The following sequences for mouse VDR primers and probe were used: forward, 5'-AATGGAGATTGGCGGCATAC-3'; reverse, 5'-TGTCACCGACGGTTGACA-3'; probe, 5'-AGGACAAACCCGGCAGACT GCCA-3'. The sequences of other target genes used are as described previously (45, 46).

**Immunohistochemistry.** Kidney tissue was cut into pieces, placed in 1% (wt/vol) periodate-lysine-parafomaldehyde fixative for 2 h at room temperature, and incubated overnight at 4°C in PBS containing 15% (wt/vol) sucrose. Subsequently, kidney tissue was frozen in liquid nitrogen and 7-μm sections were cut for the staining procedure. For detection of TRPV5 abundance, kidney sections were stained with guinea pig anti-TRPV5 antiserum (1:50) (19). To visualize TRPV5, sections were stained with goat anti-guinea pig Alexa 488-conjugated anti-IgG (1:300, Sigma). To quantify TRPV5 protein expression, digital images of the kidney sections were taken with a Zeiss Axioskop microscope (Thornwood, NY), and the integrated optical density was measured by computer analysis with Image-Pro Plus version 3.0 software (Media Cybernetics, Silver Spring, MD).

**Immunoblotting.** For protein analysis, frozen kidney tissue was homogenized in ice-cold solubilization buffer as previously described (48). Total protein fractions (10 μg) were separated on 12% (wt/vol) SDS-PAGE gels and blotted to polyvinylidene difluoride-nitrocellulose membranes (Immobilon-P, Millipore, Bedford, MA). Blots were incubated with rabbit anti-calbindin-D\(_{28k}\) (1:10,000) (9) or mouse anti-β-actin (1:25,000, Sigma) and thereafter with peroxidase-conjugated goat anti-rabbit/mouse antibody (1:2,000, Sigma). Immunoreactive protein was detected using the enhanced chemiluminescence method as described by the manufacturer (Amersham, Buckinghamshire, UK). Protein expression was quantified by computer-assisted densitometry with the use of Image-Pro Plus version 3.0 software (Media Cybernetics).

**Statistical analysis.** Values are expressed as means ± SE. Statistical significance of differences between groups was determined by ANOVA followed by pairwise comparisons using the method of least significant difference. Differences in means with \(P < 0.05\) were considered statistically significant.

**RESULTS**

**Serum parameters.** No significant differences were observed in body weight between TRPV5\(^{+/+}\) and TRPV5\(^{+/+}\) mice, except at the age of 52 wk TRPV5\(^{+/+}\) mice have an increased body weight compared with TRPV5\(^{-/-}\) mice. With advancing age, body weight increased significantly (Table 1). Serum Ca\(^{2+}\) concentrations were slightly elevated in TRPV5\(^{-/-}\) mice compared with TRPV5\(^{+/+}\) mice but did not significantly change with increasing age in either strain. In addition, serum

**Table 1. Effect of age on serum parameters in TRPV5\(^{+/+}\) and TRPV5\(^{+/+}\) mice**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>10 wk old</th>
<th>30 wk old</th>
<th>52 wk old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>25.0 ± 1.3</td>
<td>23.2 ± 1.1</td>
<td>27.9 ± 1.5</td>
</tr>
<tr>
<td>Ca(^{2+}), mM</td>
<td>2.76 ± 0.02</td>
<td>2.84 ± 0.02(^a)</td>
<td>2.70 ± 0.03(^a)</td>
</tr>
<tr>
<td>Phosphorus, mM</td>
<td>2.10 ± 0.25</td>
<td>2.25 ± 0.28</td>
<td>2.06 ± 0.27</td>
</tr>
<tr>
<td>PTH, pg/ml</td>
<td>7.9 ± 1.4</td>
<td>23.3 ± 3.5</td>
<td>24.1 ± 8.0</td>
</tr>
<tr>
<td>1.25(OH)(_{2})D(_3), pM</td>
<td>121 ± 13(^a)</td>
<td>539 ± 81</td>
<td>295 ± 65(^a)</td>
</tr>
<tr>
<td>Osteocalcin, μg/l</td>
<td>1172 ± 4(^a)</td>
<td>164 ± 10(^a)</td>
<td>53 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. \(n\), No. of mice: TRPV5\(^{+/+}\), wild-type mice; TRPV5\(^{-/-}\), homozygous knockout mice. \(^aP < 0.05\) vs. all. \(^bP < 0.05\) vs. 10-wk-old TRPV5\(^{+/+}\) and 10-wk-old TRPV5\(^{+/+}\); \(^cP < 0.05\) vs. TRPV5\(^{-/-}\), all ages. \(^dP < 0.05\) vs. 30- and 52-wk-old TRPV5\(^{+/+}\); \(^eP < 0.05\) vs. TRPV5\(^{+/+}\), all ages. \(^fP < 0.05\) vs. 30-wk-old TRPV5\(^{+/+}\).
PTH levels were not different between TRPV5−/− and TRPV5+/+ mice, except for a substantial increase in 52-wk-old TRPV5−/− mice. Levels of serum phosphorus were higher in 30-wk-old TRPV5−/− mice compared with all other groups (Table 1). Circulating levels of 1,25(OH)2D3 were significantly elevated in TRPV5−/− mice compared with TRPV5+/+ mice at 10 wk of age. Interestingly, in TRPV5+/+ mice, serum 1,25(OH)2D3 concentrations increased with aging, whereas in TRPV5−/− mice, levels remained constantly elevated (Table 1). Serum concentrations of the bone turnover marker osteocalcin were significantly higher in TRPV5−/− mice compared with TRPV5+/+ mice and decreased in both mice strains with age. Moreover, osteocalcin levels rose again in 52-wk-old TRPV5−/− mice (Table 1).

Aging results in increased renal Ca2+ excretion and decreased expression of Ca2+ transport proteins. To investigate the effect of aging on renal Ca2+ handling, mice were placed in metabolic cages and 24-h urine samples were collected to determine the amount of Ca2+ excretion. Urinary Ca2+ loss in TRPV5−/− mice was significantly higher compared with TRPV5+/+ mice. Moreover, Ca2+ excretion increased during aging. Similarly, in TRPV5+/+ mice there was a tendency toward elevated Ca2+ excretion with increasing age (Fig. 1A).

Furthermore, the expression of genes encoding the Ca2+ transport proteins involved in transcellular Ca2+ reabsorption was examined using quantitative real-time PCR. In TRPV5+/+ mice, a decline in TRPV5 mRNA expression was observed with increasing age (Fig. 1B). In addition, a similar reduction in calbindin-D28K expression was detected in TRPV5+/+ mice during aging, whereas the expression was significantly decreased in TRPV5−/− mice independent of age (Fig. 1D). Expression of the extrusion protein NCX1 was also lower in TRPV5−/− mice, but no changes were measured in either TRPV5−/− or TRPV5+/+ mice with increasing age (Fig. 1E).

Subsequently, examination of renal Ca2+ transport protein expression by immunohistochemistry revealed a marked decrease in TRPV5 protein abundance with increasing age, as indicated by the reduced immunopositive staining (Fig. 2A). The corresponding integrated optical density analysis confirmed a significant decline in TRPV5 protein abundance (Fig. 2B). Furthermore, Western blot analysis of calbindin-D28K demonstrated decreased protein abundance in TRPV5+/+ mice compared with the TRPV5−/− mice. In addition, a downregulation of calbindin-D28K abundance in 52-wk-old TRPV5+/+ mice was observed compared with younger wild-type animals (Fig. 2C). Densitometrical analysis of the intensity of the immunocomplexes confirmed this decrease in calbindin-D28K protein expression with age in TRPV5+/+ mice (Fig. 2D). The corresponding β-actin bands did not vary significantly in density, which precludes unequal loading as an explanation for the differences.

Aging results in reduced Ca2+ absorption and duodenal expression of Ca2+ transport proteins. Following renal analysis, the effect of aging on intestinal Ca2+ absorption was examined. Although the 45Ca2+ absorption rate in TRPV5−/− mice was significantly higher compared with TRPV5+/+ littersmates (Fig. 3A), aging reduced the amount of 45Ca2+ ab-

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**Fig. 1.** Effect of aging on renal Ca2+ excretion and mRNA expression levels of genes encoding renal Ca2+ transport proteins. A: total excretion of Ca2+ in 24-h urine samples from 10-, 30-, and 52-wk-old TRPV5+/+ (filled bars) and TRPV5−/− (open bars) mice. Using real-time quantitative PCR, renal mRNA expression of TRPV5 (B), TRPV6 (C), calbindin-D28K (D), Na+/Ca2+ exchanger (NCX1; E), and vitamin D receptor (VDR; F) of the different experimental groups was measured and presented as a ratio to hypoxanthine-guanine phosphoribosyl transferase (HPRT) expression (n = 7–9). Values are means ± SE; n = 9 mice, 3 mice/cage. *P < 0.05 vs. all. #P < 0.05 vs. TRPV5+/+. †P < 0.05 vs. TRPV5−/−. ‡P < 0.05 vs. 10-wk-old TRPV5+/+. In addition, a similar reduction in calbindin-D28K expression was detected in TRPV5+/+ mice during aging, whereas the expression was significantly decreased in TRPV5−/− mice independent of age (Fig. 1D). Expression of the extrusion protein NCX1 was also lower in TRPV5−/− mice, but no changes were measured in either TRPV5−/− or TRPV5+/+ mice with increasing age (Fig. 1E). No differences in TRPV6 mRNA expression were observed between TRPV5−/− and TRPV5+/+ mice with aging, except for a remarkable upregulation in 52-wk-old TRPV5−/− mice (Fig. 1C). Furthermore, VDR mRNA levels were significantly lower in TRPV5−/− mice compared with TRPV5+/+. During aging, expression of VDR was decreased in both groups of mice (Fig. 1F).
The present study demonstrated that an age-related decrease in the expression of the key players responsible for active $\text{Ca}^{2+}$ transport, including TRPV5 and TRPV6, contributes to the decline in intestinal and renal $\text{Ca}^{2+}$ absorption with aging. Furthermore, our results indicated that mice lacking TRPV5 develop severe hyperparathyroidism with aging, suggesting that these mice are more susceptible to age-related osteoporosis.

A significant increase in the rate of $\text{Ca}^{2+}$ absorption was observed in TRPV5$^{-/-}$ mice compared with their wild-type littermates, which was accompanied by an upregulation of duodenal TRPV6 and calbindin-D$_{9K}$ expression. Importantly, despite the renal $\text{Ca}^{2+}$ wasting in TRPV5$^{-/-}$ mice, serum $\text{Ca}^{2+}$ levels remained normal and were even slightly increased compared with TRPV5$^{+/+}$ mice. As suggested previously, the increased duodenal expression of TRPV6 and calbindin-D$_{9K}$, thereby increasing $\text{Ca}^{2+}$ absorption, could act as a compensatory mechanism triggered by the elevated $1,25(\text{OH})_2\text{D}_3$ levels in TRPV5$^{-/-}$ mice (23). This was confirmed recently in a study by Renkema et al. (40) demonstrating that the compensatory upregulation of intestinal $\text{Ca}^{2+}$ transporters and $\text{Ca}^{2+}$ hyperabsorption were abolished in TRPV5/25-hydroxyvitamin-D$_3$-1α-hydroxylase double knockout mice, which have undetectable serum $1,25(\text{OH})_2\text{D}_3$ levels. The slightly elevated serum $\text{Ca}^{2+}$ levels in these knockout mice could be due to an overcompensation by $1,25(\text{OH})_2\text{D}_3$ and intestinal $\text{Ca}^{2+}$ absorption to correct renal $\text{Ca}^{2+}$ loss (8, 27). Moreover, aging was associated with a decline in duodenal $\text{Ca}^{2+}$ absorption in both mice strains. In addition, the expression of duodenal VDR was observed during aging (Fig. 3B). To address the molecular mechanism responsible for the decreased absorption, the expression of genes encoding proteins involved in intestinal $\text{Ca}^{2+}$ absorption was examined. The expression of TRPV6 and calbindin-D$_{9K}$ mRNA was significantly upregulated in TRPV5$^{-/-}$ mice (Fig. 3, C and D). Moreover, aging resulted in a decline in TRPV6 levels in both mice strains (Fig. 3C), whereas no changes were observed in the expression of calbindin-D$_{9K}$ (Fig. 3D). PMCA1b mRNA levels decreased with age in TRPV5$^{+/+}$ mice, whereas no significant changes were present in the expression of PMCA1b in TRPV5$^{-/-}$ mice with increasing age (Fig. 3E). In addition, no significant changes in the expression of duodenal VDR were observed during aging in TRPV5$^{+/+}$ or TRPV5$^{-/-}$ mice (Fig. 3F). Finally, aging did not affect the expression of the housekeeping gene HPRT in both kidney and duodenum (data not shown).

**DISCUSSION**

The present study demonstrated that an age-related decrease in the expression of the key players responsible for active $\text{Ca}^{2+}$ transport, including TRPV5 and TRPV6, contributes to the decline in intestinal and renal $\text{Ca}^{2+}$ absorption with aging. Furthermore, our results indicated that mice lacking TRPV5 develop severe hyperparathyroidism with aging, suggesting that these mice are more susceptible to age-related osteoporosis.
explained by the fact that 1,25(OH)2D3 in these knockout mice results suggest that TRPV5 is a key component in renal Ca2+

Future investigations should unravel the role of TRPV6 in the expression of genes encoding duodenal Ca2+ transport proteins. A: changes in serum Ca2+ within 12 min after 45Ca2+ administration by oral gavage in TRPV5+/+ (●) and 10-wk-old TRPV5−/− (▲) mice. The change in the serum Ca2+ concentration (ΔμM) is obtained by the equation (cpm 10 μl serum/cpm 10 μl stock solution) × 10^2 μM. B: comparison of ΔμM at 4 min after 45Ca2+ administration by oral gavage between 10-, 30-, and 52-wk-old TRPV5+/+ (filled bars) and TRPV5−/− (open bars) mice. Using real-time quantitative PCR, duodenal mRNA expression of TRPV6 (C), calbindin-D28K (D), PMCA1b (E), and VDR (F) of the different experimental groups was measured and presented as a ratio to HPRT expression. Values are means ± SE; n = 7–9. *P < 0.05 vs. all. #P < 0.05 vs. TRPV5+/+. †P < 0.05 vs. 30-wk-old TRPV5+/+. §P < 0.05 vs. 10- and 30-wk-old TRPV5−/−.

decline in expression of this protein is the main cause of the decrease in duodenal Ca2+ absorption with aging.

In the kidneys of TRPV5+/+ mice, expression of TRPV5 and calbindin-D28K mRNA decreased during aging. Previous studies demonstrated a reduction in renal calbindin-D28K expression with increasing age (2, 26). Decreased expression of TRPV5 and calbindin-D28K could lead to a reduced Ca2+ reabsorption ability, resulting in hypercalciuria, as shown in the TRPV5−/− mice and described previously (23, 47). These results suggest that TRPV5 is a key component in renal Ca2+ reabsorption. In accordance with the decreased expression of TRPV5, a tendency toward increased urinary Ca2+ excretion was observed in the aging TRPV5+/+ mice. Although the hypercalciuria is even more pronounced in older TRPV5−/− mice, no further decline in the expression of the Ca2+ transporting proteins is observed with increasing age. Interestingly, TRPV6 expression was increased in the 1-yr-old TRPV5−/− mice. TRPV6 is present in DCT and CNT as well as other tubular segments (34). However, the presence of TRPV6 does not rescue the renal Ca2+ loss in TRPV5−/− mice (23) and, therefore, its role in active Ca2+ reabsorption is questionable. Future investigations should unravel the role of TRPV6 in the various renal tubules and the possible involvement during aging.

Aging in TRPV5+/+ mice was accompanied by increasing serum 1,25(OH)2D3 levels, whereas no differences were observed in aging TRPV5−/− mice. This latter finding could be explained by the fact that 1,25(OH)2D3 in these knockout mice is already maximally elevated. 1,25(OH)2D3 is a known stimulator of intestinal and renal Ca2+ (re)absorption and upregulates the expression of proteins involved in active Ca2+ transport, including TRPV5 and TRPV6 (20, 24, 45). Therefore, the decline in the expression of the Ca2+ transporting proteins shown in our study suggests that there is a decrease in 1,25(OH)2D3 sensitivity. Indeed, VDR mRNA levels in kidneys of both TRPV5+/+ and TRPV5−/− mice gradually decrease with age, suggesting that the kidney develops a refractoriness to 1,25(OH)2D3 levels. Evidently, Li et al. (28) showed that VDR knockout mice have impaired Ca2+ conservation capability in the kidney, despite increased serum 1,25(OH)2D3 levels. Furthermore, intestinal Ca2+ absorption as well as expression of duodenal TRPV6 are decreased in VDR knockout mice (49). However, no significant differences were found in duodenal VDR mRNA levels during aging in both TRPV5+/+ and TRPV5−/− mice. Although several reports described a decrease in intestinal VDR expression (25, 29), other studies showed that receptor occupancy was reduced in elder rats (43). Our findings indicate an age-related intestinal resistance to the action of 1,25(OH)2D3, which is apparently not due to a reduction in duodenal VDR mRNA levels, thereby resulting in the decline in TRPV6 expression and Ca2+ absorption with aging.

Interestingly, aging of TRPV5+/+ mice was not associated with a change in serum PTH levels, whereas 52-wk-old TRPV5−/− mice showed severe hyperparathyroidism. Also of interest, serum phosphorus levels were elevated in 30-wk-old TRPV5−/−. Hyperphosphatemia leads to parathyroid cell proliferation (33), which could result in the observed hyperpara-
thyroidism in 52-wk-old TRPV5 /−/ mice, thereby reducing the serum phosphorus levels back to normal. Furthermore, PTH, in addition to 1,25(OH)₂D₃, is involved in renal and intestinal Ca²⁺ reabsorption (31, 36, 42). Indeed, the relatively constant levels of serum Ca²⁺ and the decline in renal and intestinal Ca²⁺ (re)absorption in aged animals observed in the present study may suggest that bone resorption increases with aging. Osteocalcin levels in adult animals, serum osteocalcin levels increased in 52-wk-old TRPV5 /−/ mice have also been found in humans and may play an important role in age-related bone loss or senile osteoporosis (31, 36, 42). The relative expression levels of osteocalcin in TRPV5 /−/ mice are significantly elevated compared with TRPV5 /+/+ mice, suggestive of increased bone turnover, which could be the result of the reduced bone thickness in TRPV5 /−/ mice described previously (23). After the decline in osteocalcin levels in both mice strains, which is probably due to the lower need of bone formation and turnover to build the skeleton in adult animals, serum osteocalcin levels increased in 52-wk-old TRPV5 /−/ mice in accordance with the elevation of serum PTH. PTH indirectly activates osteoclasts and modifies the phenotype of the osteoblast from a cell involved in bone formation to a cell directing bone resorption (37, 44). Thus the age-associated hyperparathyroidism in TRPV5 /−/ could lead to increased bone turnover, thereby resulting in net bone loss and aggravating the existing reduction in bone volume.

Previous studies indicated that estrogens can upregulate active Ca²⁺ (re)absorption in both kidney and duodenum (46). This was accompanied by an increased expression level of the Ca²⁺ transport proteins in female compared with male mice in both the kidney and duodenum. However, our data do not allow us to conclude that the age-dependent regulation of Ca²⁺ transporters is gender (in)dependent.

In summary, the present findings demonstrated a decline in renal and intestinal Ca²⁺ (re)absorption through TRPV5 and TRPV6 during aging, which is of physiological significance in understanding the imbalance in mineral metabolism associated with aging. Moreover, TRPV5 /−/ mice likely develop age-related hyperparathyroidism and osteoporotic characteristics earlier compared with TRPV5 /+/+ mice, corroborating the importance of the epithelial Ca²⁺ channels in Ca²⁺ homeostasis.

GRANTS

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