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Genetic Suppression of GH-IGF-1 Activity, Combined with Lifelong Caloric Restriction, Prevents Age-Related Renal Damage and Prolongs the Life Span in Rats

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Key Words
GH/IGF-1 activity · Longevity · Kidney pathology · Age-associated nephropathy · Caloric restriction

Abstract
Aim: The aim of this study was to determine the effects of kidney pathology on overall survival and longevity and the combined effects of chronic suppression of growth hormone (GH)/insulin-like growth factor-1 (IGF-1) activity and lifelong caloric restriction on age-associated nephropathy. ***Methods:*** We analyzed the kidneys of rats with suppressed GH activity through genetic manipulation with an antisense GH transgene. Rats were fed normally or with a 30% calorie-restricted diet for 24–26 months. The kidneys of male wild-type young (6 months) and old (24–26 months) rats were compared with male hemizygote transgenic young (6 months) and old (24–26 months) rats fed with either regular diet or 30% calorie-restricted diet for their entire life span. ***Results:*** The transgenic rats had relatively less pituitary GH-secreting cells, and the plasma levels of IGF-1 were decreased by 53% in homozygote rats (tg/tg) and by 28% in hemizygote rats (tg/wt) compared to wild-type rats (wt/wt) of the same age (6 months). Wild-type rats fed the regular diet developed age-associated nephropathy as they aged, showing severe inflammatory cell infiltration, glomerulosclerosis, and tubulointerstitial fibrosis. In addition, about 83% of the wild-type rats allowed to survive naturally showed signs of nephropathy. In contrast, only 26% of the naturally surviving hemizygote rats showed features of nephropathy, despite the fact that these rats lived 8% longer (maximum survival 171 weeks) than the wild-type rats (maximum survival 158 weeks). When chronic suppression of GH/IGF-1 activity was combined with lifelong caloric restriction, however, age-associated nephropathy was nonexistent in hemizygote transgenic rats, and they showed about 30% increase in survival (maximum survival 204 weeks). There was no significant difference in the rate of neoplastic or nonneoplastic lesions (other than in the kidney) in the regularly fed wild-type rats or in the calorie-restricted hemizygote transgenic rats that survived longer. ***Conclusion:*** We concluded that kidney pathology is an important determinant of overall survival, and that prevention of kidney pathology by dietary restriction, combined with chronic suppression of GH/IGF-1 activity, significantly extends overall survival and longevity.

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Introduction

The importance of kidney pathology to survival is generally underestimated. A recent National Health and Nutrition Examination Survey (NHANES III) found that 11% of people over the age of 65 without obvious renal disease had 60% less renal function compared to younger adults [1]. The effects of aging on progressive kidney damage are well known, but the effects of renal damage on overall survival and longevity remain uncharacterized. In this study, we examined the effects of renal damage on longevity in growth hormone (GH)/insulin-like growth factor-1 (IGF-1)-suppressed mutant rats. We selected this model because of the prolonged survival of hemizygote transgenic rats compared with wild-type rats [2].

The beneficial effects of suppressing the GH/IGF-1 activity on aging and survival are well established [2–6]. Recent studies, however, showed that such positive effects could mostly be achieved by controlled suppression of GH/IGF-1 activities [7]. A severely reduced GH/IGF-1 activity could affect overall growth and development, but could also influence survival by facilitating tumorigenesis and by compromising immune function [2]. Hemizygote transgenic rats with reduced GH/IGF-1 activity showed a longer life span than both their homozygote littermates and wild-type control rats of similar genetic background [2]. Importantly, life span was decreased in the homozygote rats as compared with the wild-type controls [2], suggesting that a severely limited GH/IGF-1 activity does not have the same effect on life span [2, 8]. These results support earlier observations of IGF-1 receptor knockout heterozygous mutant mice living approximately 26% longer than wild-type cohorts, while homozygous mice died at birth [3]. Similarly, Sonntag et al. [7] showed that a limited reduction of GH and IGF-1 in a rodent model, initiated in adulthood and continued throughout life, increased life span [7], although the lifelong suppression of GH and IGF-1 in rats of the same genetic background failed to measurably prolong life span [7]. It is, therefore, apparent that only a controlled reduction of the GH/IGF-1 system has long-term positive effects on life span, and such beneficial effects on survival are achieved at least in part by the amelioration of age-associated organ pathology.

Since homozygote antisense GH-transgenic rats have lower survival rates than wild-type rats, we sought to determine the effects of moderately reduced GH/IGF-1 activity on the kidneys of hemizygote transgenic rats, and whether kidney pathology affects overall survival and longevity. This study also investigated the combined effects of lifelong caloric restriction and chronic suppression of GH/IGF-1 activity on spontaneous, age-associated nephropathy in hemizygote transgenic rats.

Materials and Methods

Animals

The transgenic male rats [mini, Jcl:Wistar-TgN(ARGHGEN) 1Nts] used in the present study were obtained from the Nippon Institute for Biological Science (Tokyo, Japan). The generation of these transgenic strains expressing an antisense GH transgene to suppress GH expression has been described previously [8]. Wistar rats with a similar genetic background to that of the transgenic rats were used as wild-type controls. Rats were maintained in accordance with the guidelines for the care and use of laboratory animals, and all protocols were approved by the Ethics Review Committee for Animal Experimentation at Nagasaki University Graduate School of Biomedical Sciences.

Feeding of Animals

Male rats were kept in a barrier facility (temperature 24 ± 1°C; 12-hour light/dark cycle), which was housed separately and maintained under specific pathogen-free conditions during the study period, as described in detail previously [8]. Briefly, the rats were provided with CR-LPF diet (Oriental Yeast, Tokyo), which is based on the formula of Charles River (CRF-1), although the protein fraction was reduced to 18% for the long-term study. The diet composition was as follows (per 100 g): 18.2 g protein, 4.8 g fat, 6.6 g mineral mixture, 5.0 g fiber, 57.9 g nitrogen-free water-soluble substance, and 7.5 g water. The caloric value of the diet was 348 kcal/100 g. All rats were fed this diet and water ad libitum (AL) after the weaning stage. At 6 week of age, the rats were divided into two groups: AL rats continued to receive food ad libitum and calorie-restricted (CR) rats received approximately 30% less food than the AL controls. The feeding protocol was described in detail previously [8].

Longevity Study in Various Genotypes

Thirty rats in each group were monitored for survival. Wild-type and hemizygote AL and CR rats were monitored until spontaneous death occurred. Dead rats from these experiments were autopsied, and internal organs were collected for histopathological examination to determine renal and extrarenal lesions. Chronic nephropathy was classified according to severity, i.e., mild, moderate, and severe, as proposed by Maeda et al. [9].

Plasma Levels of IGF-1

Plasma samples were collected from young and old wild-type and mutant rats. The concentration of circulating IGF-1 was measured by an enzyme immunoassay (Diagnostic Systems Laboratories, Inc., Webster, Tex., USA), according to the instructions provided by the manufacturer.

Renal Tissue Collection

At least 5 wild-type and hemizygote transgenic rats at each age point were sacrificed at 6 and 24–26 months of age. Both kidneys

were removed via a midline abdominal incision, weighed, and then immediately fixed overnight in 10% formalin for morphometric and immunohistochemical studies.

Histological and Morphometric Analyses

Renal tissues were processed for paraffin embedding, and 4-μm sections were stained with hematoxylin and eosin, periodic acid-Schiff, periodic acid-methenamine silver, and Masson's trichome, as described previously [10, 11]. Histological changes were determined by light microscopy and graded as proposed by Maeda et al. [9].

Immunohistochemical Studies

Immunohistochemical staining was performed as described previously [11, 12]. Briefly, paraffin-embedded tissue sections were deparaffinized with xylene, rinsed thoroughly with 95% ethanol, and then soaked in 0.3% hydrogen peroxide in methanol for 30 min at room temperature to inactivate endogenous peroxidase activity. After a 5-min treatment with 0.05% trypsin (T4799; Sigma, St. Louis, Mo., USA), the tissue sections were incubated with either 10% goat serum or 10% rabbit serum for 30 min, and then with one of the following primary antibodies: anti-proliferating cell nuclear antigen (PCNA, 1:100; Dako, Glostrup, Denmark), anti-ED-1 (1:100; Serotec, Oxford, UK), and anti-α-smooth muscle actin (α-SMA, 1:100; Dako). The slides were washed with phosphate-buffered saline and processed further using a Histofine SAB-PO kit (Nichirei, Tokyo), as recommended by the manufacturer. Antibody binding was visualized by reaction with 3,3′-diaminobenzindine and H_2O_2.

Stained tissue sections were quantitated by counting the numbers of interstitium-infiltrating macrophages (ED-1 stained), proliferating cells (PCNA stained), and phenotypically altered cells (α-SMA stained) in five randomly selected fields of the renal cortex (×40 magnification). The average number of each cell type was then calculated separately in the glomerular and tubulointerstitial compartments as detailed earlier [13].

Statistical Analysis

Statistically significant differences between groups were evaluated using Student's t test or Fisher's test for comparison between two groups, or by one-way analysis of variance followed by Tukey's test for multiple comparisons. Survival was analyzed using Kaplan-Meier estimates and was compared using the log-rank test. All values were expressed as mean ± SEM. $p < 0.05$ was considered statistically significant. All analyses were performed using Microsoft Office Excel (Microsoft Corp., Redmond., Wash., USA) or StatView 5.0 (SAS Institute, Inc., Cary, N.C., USA) software.

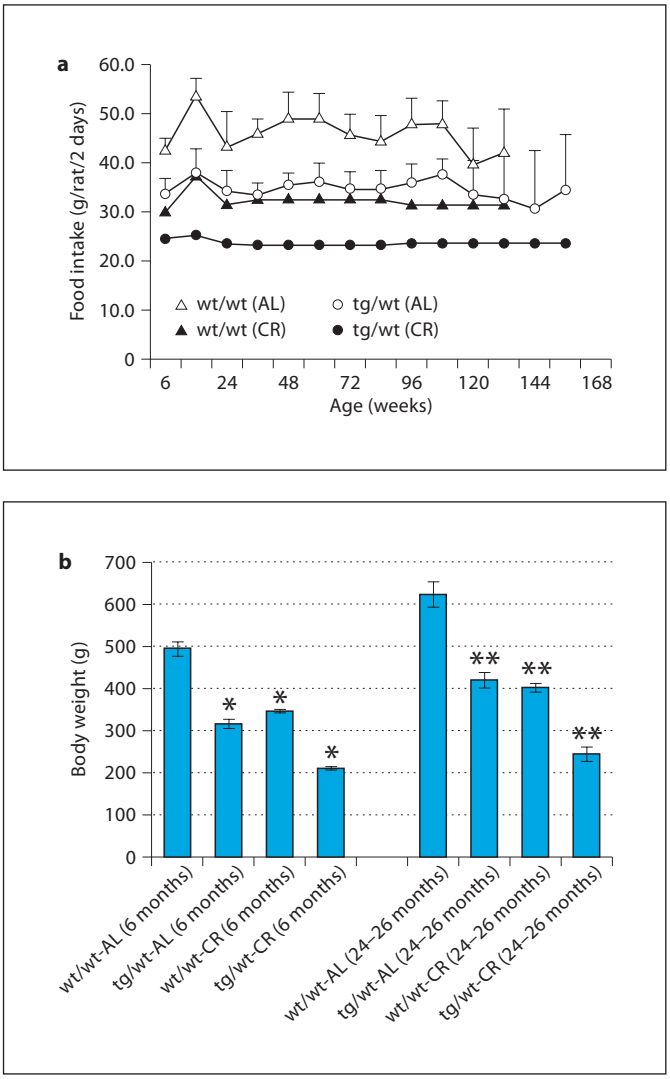

Fig. 1. a Food intake in rat groups in the longevity study (mean ± SD). Data at 12-week intervals are shown but are not given when the number of rats is <5 [from ref. 8, with permission]. **b** Body weight of the corresponding genotypes at 6 and 24–26 months of age (mean ± SD). wt = Wild-type rats; tg = transgenic (hemizygote) animals. * $p < 0.01$, compared to 6 months of wild-type AL rats; ** $p < 0.01$, compared to 24–26 months of wild-type AL rats.

Results

Food Intake and Body Weights of Various Genotypes

Food intake for the wild-type regularly fed (AL) rats was gradually increased from weaning at 4 weeks until 12 weeks of age, and then decreased slightly from 12 to 24 weeks of age (fig. 1). After that, no substantial changes in the food intake were noted with increasing age. Hemizygote AL rats consumed 70–80% of the mean intake of wild-type AL rats, although the food intake patterns were similar between these two groups. The average food intake of hemizygote AL rats was similar to that of the CR wild-type rats. These feeding patterns are consistent with our previous study [8].

All rats gained weight until 96 weeks of age, after which time the body weight either remained constant or gradu-

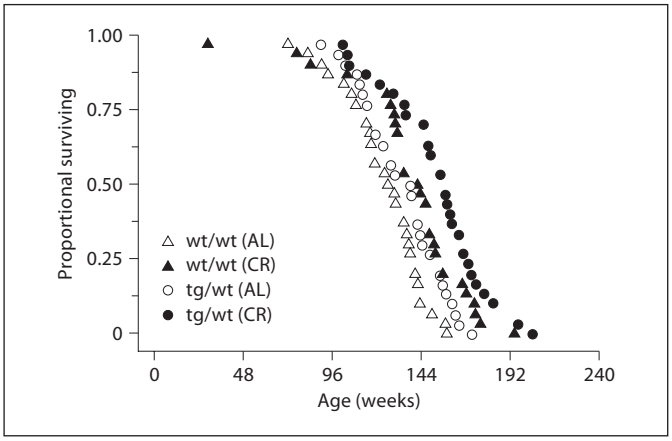

Fig. 2. Survival curves. The number of rats at the start of the study was 30 in each group. $p < 0.05$: tg/wt (AL) vs. wt/wt (AL); $p < 0.05$: wt/wt (CR) vs. wt/wt (AL); $p < 0.05$: tg/wt (CR) vs. tg/wt (AL); not significant: tg/wt (AL) vs. wt/wt (CR) by the log-rank test. wt = Wild-type rats; tg = transgenic (hemizygote) animals [from ref. 8, with permission].

ally decreased. The hemizygote AL rats weighed approximately one third less than the wild-type AL rats. In contrast, the wild-type CR rats were similar in weight to the hemizygote AL rats until 52 weeks of age, after which the hemizygote AL rats continued to gain weight, while weights of wild-type CR rats stabilized. Table 1 details the body weight data for young (6-month-old) and old (24- to 26-month-old) rats of all four groups studied.

Survival and Plasma IGF-1 Levels

The survival rate of the homozygote AL rats was significantly less ($p < 0.01$) than that of the wild-type AL rats. This was predominantly due to the appearance of several tumors in the homozygote AL rats with severely reduced GH/IGF-1 activity. The homozygote life span was decreased by approximately 7 weeks (5%, 50th percentile) and 14 weeks (10%, 25th percentile) compared to wild-type rats. The homozygote rats also showed a higher incidence of leukemia, which was not observed in wild-type rats. In contrast, the hemizygote AL rats showed significantly higher survival rates ($p < 0.03$) than wild-type AL rats; it was approximately 12 weeks (7%, 50th percentile) and 14 weeks (10%, 25th percentile) longer in hemizygote AL rats than in wild-type AL controls (fig. 2). The life span of wild-type CR rats was 10.7% greater at the 25th percentile point and 17.8% greater at the 10th percentile point than that of wild-type AL rats. Similarly, the life span of hemizygote CR rats was extended by about 20 and 30% at the 25th and 10th percentile points, respectively, compared with wild-type AL rats. This extended life span of the hemizygote CR rats prompted us to study the effects of renal injury on overall survival, using these long-lived rats.

The plasma levels of IGF-1 in 6-month-old hemizygote AL rats (1,172.2 ± 73.3 ng/ml) were significantly less

Table 1. Body weight of young and old wild-type and mutant rats fed either the regular diet (AL) or the 30% calorie-restricted diet (CR)

Genotype	Body weight, g	
	6 months	24–26 months
Wild-type AL	494 ± 17	624 ± 29
Hemizygote AL	316 ± 12	421 ± 18
Wild-type CR	345 ± 4	403 ± 9
Hemizygote CR	212 ± 1	244 ± 17

Wild-type AL (6 months) vs. wild-type AL (24–26 months): $p < 0.001$; wild-type AL (6 months) vs. hemizygote CR (24–26 months): $p < 0.001$; wild-type CR (6 months) vs. wild-type AL (24–26 months): $p < 0.001$; hemizygote AL (6 months) vs. wild-type AL (24–26 months): $p < 0.001$; hemizygote CR (6 months) vs. wild-type AL (24–26 months): $p < 0.001$; hemizygote CR (6 months) vs. wild-type CR (24–26 months): $p < 0.001$; hemizygote CR (6 months) vs. hemizygote AL (24–26 months): $p < 0.001$; wild-type AL (24–26 months) vs. wild-type CR (24–26 months): $p < 0.001$; wild-type AL (24–26 months) vs. hemizygote CR (24–26 months): $p < 0.001$; wild-type CR (24–26 months) vs. hemizygote CR (24–26 months): $p < 0.001$; hemizygote AL (24–26 months) vs. hemizygote CR (24–26 months): $p < 0.001$.

Table 2. Plasma IGF-1 concentrations in young and old wild-type and mutant rats fed with the regular diet (AL) or the 30% calorie-restricted diet (CR)

Genotype	IGF-1, ng/ml	
	6 months	24–26 months
Wild-type AL	1,613.6 ± 190.5	1,230.2 ± 270.4
Hemizygote AL	1,172.2 ± 231.9	1,362.3 ± 234.1
Wild-type CR	1,415.8 ± 320	1,152.4 ± 262.1
Hemizygote CR	808.2 ± 103.6	613.4 ± 205.4

Wild-type AL (6 months) vs. hemizygote CR (6 months): $p < 0.001$; wild-type AL (6 months) vs. hemizygote CR (24–26 months): $p < 0.001$; wild-type CR (6 months) vs. hemizygote CR (6 months): $p < 0.001$; wild-type CR (6 months) vs. hemizygote CR (24–26 months): $p < 0.001$; hemizygote AL (24–26 months) vs. hemizygote CR (24–26 months): $p < 0.001$.

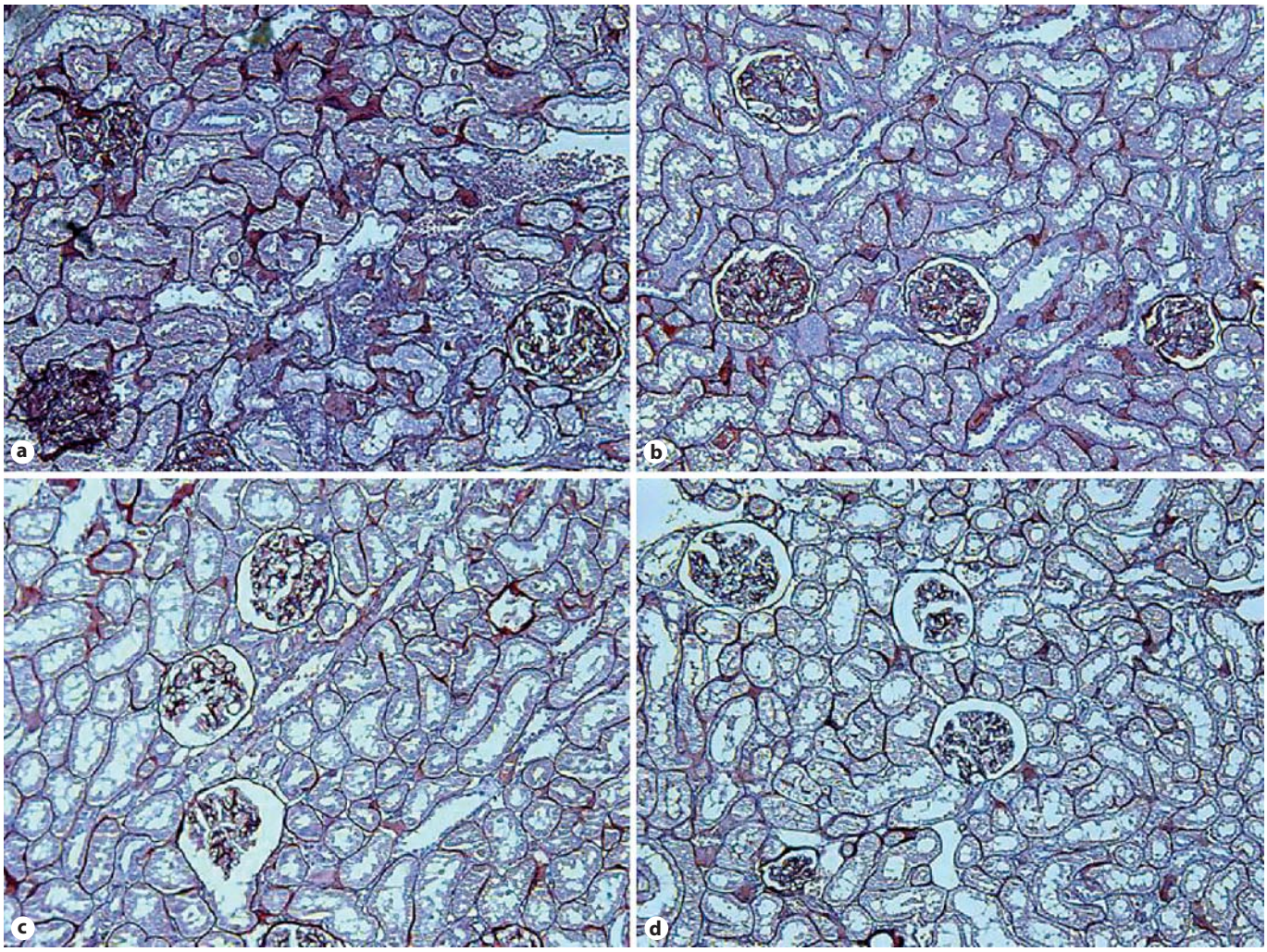

Fig. 3. Histological features of kidneys obtained from a 24- to 26-month-old wild-type AL rat (**a**) and a wild-type CR rat (**b**). **a** Note severe glomerular and tubulointerstitial damage, with massive infiltration of inflammatory cells and interstitial fibrosis, in wild-type rats fed the AL diet. **b** Lifelong dietary restriction significantly reduced such renal lesions in wild-type CR rats of similar age. A similar improvement in renal damage was also noted in 24- to 26-month-old rats with suppressed GH/IGF-1 activity fed either the AL diet (**c**) or the CR diet (**d**). Periodic acid-methenamine silver. Original magnification ×20.

than the levels in wild-type AL rats (1,613.6 ± 57.3 ng/ml) (table 2). Similar reductions were identified in the 24- to 26-month-old hemizygote AL rats (613 ± 205 ng/ml) and wild-type CR rats (1,152 ± 262 ng/ml). In general, both wild-type and hemizygote CR rats had relatively lower levels of plasma IGF-1 compared to their AL counterparts (table 2).

Histological Analysis

The wild-type AL rat kidneys showed no abnormal pathology at 6 months of age, but glomerulosclerosis, severe interstitial inflammatory cell infiltration, and tubulointerstitial fibrosis were present in the kidneys of wild-type AL rats at 24–26 months of age (fig. 3a). Restriction of caloric intake significantly reduced renal damage, as shown in the kidneys of 24- to 26-month-old CR rats (fig. 3b). The hemizygote AL transgenic rats showed no obvious histopathological changes at 6 months of age, but mild features of nephropathy had appeared at 24–26 months of age (fig. 3c); such renal lesions, however, were absent in the 24- to 26-month-old hemizygote CR rats (fig. 3d). These results indicate that prolonged caloric restriction and suppression of GH/IGF-1 confer a variable degree of protection against renal damage.

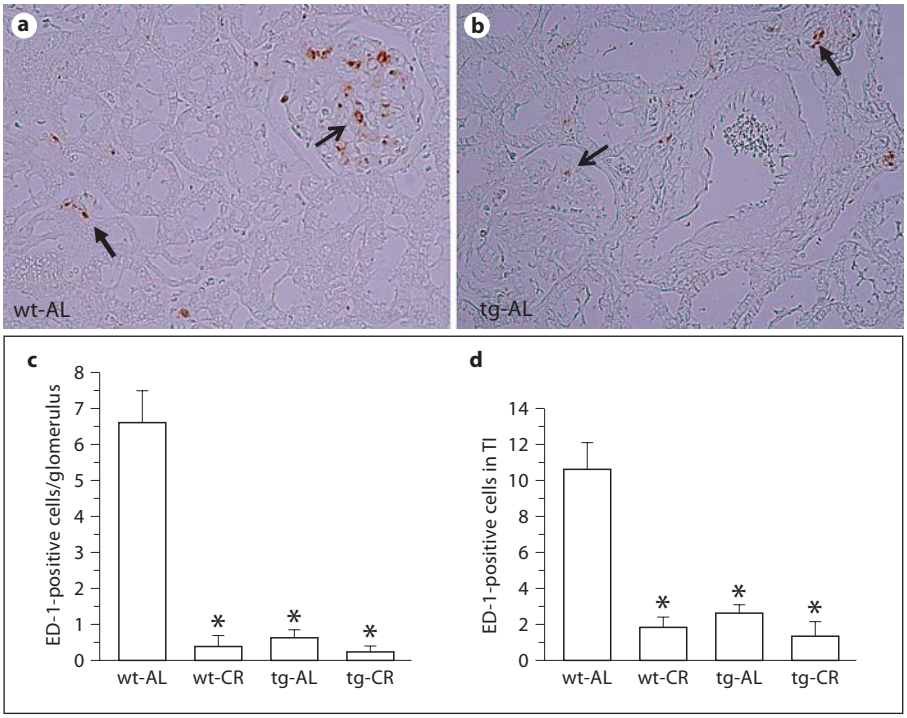

Fig. 4. Immunohistochemical staining of ED-1 in kidney sections prepared from a 24- to 26-month-old wild-type (wt) AL rat (**a**) and a hemizygote transgenic (tg) AL rat of similar age (**b**). Lifelong suppression of GH/IGF-1 activity significantly reduced ED-1-positive macrophage infiltration in hemizygote transgenic AL rats (**b**). Original magnification ×20. The arrows indicate macrophages. The average numbers of ED-1-positive macrophages in glomeruli (**c**) and tubulointerstitium (TI) (**d**) in the various groups of rats indicate that dietary restriction and chronic suppression of GH/IGF-1 activity could reduce renal inflammation and possibly affect longevity in these rats (see also table 1). * $p < 0.05$ compared to wild-type AL rats.

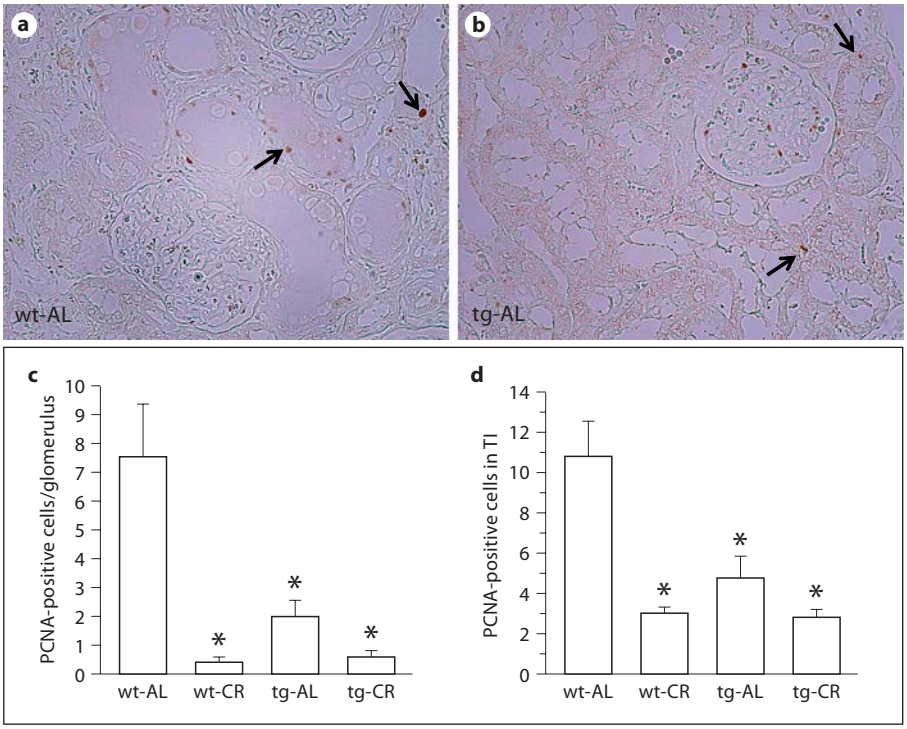

Fig. 5. Immunohistochemical staining of PCNA in kidney sections prepared from a 24- to 26-month-old wild-type (wt) AL rat (**a**) and a hemizygote transgenic (tg) AL rat of similar age (**b**). Lifelong suppression of GH/IGF-1 activity significantly reduced the proliferation of resident renal cells, as demonstrated by fewer PCNA-stained cells in hemizygote transgenic AL rat kidneys (**b**). Original magnification ×20. The arrows indicate PCNA-positive cells. The average numbers of PCNA-positive macrophages in the glomeruli (**c**) and tubulointerstitium (TI) (**d**) in the various groups of rats indicate that dietary restriction and chronic suppression of GH/IGF-1 activity could reduce cell proliferation in renal tissue. * $p < 0.05$ compared to wild-type AL rats.

Renal Expression of ED-1-Positive Macrophages

The 24- to 26-month-old wild-type AL rats showed increased renal infiltration of ED-1-positive macrophages (fig. 4a) compared to kidneys of age-matched hemizygote AL rats (fig. 4b). However, hemizygote AL and CR rats of the same age contained similar numbers of ED-1-positive macrophages in both the glomeruli (fig. 4c) and the tubulointerstitium (fig. 4d). These results suggested that

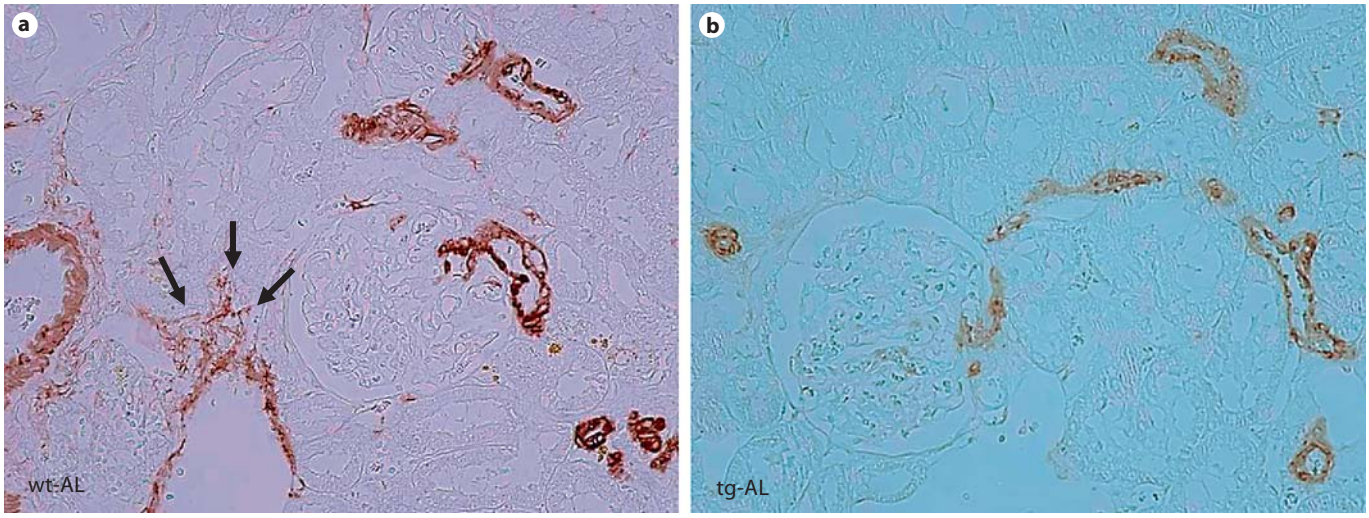

Fig. 6. Immunohistochemical staining of α-SMA in kidney sections prepared from a 24- to 26-month-old wild-type (wt) AL rat (**a**) and a hemizygote transgenic (tg) AL rat of similar age (**b**). Lifelong suppression of GH/IGF-1 activity significantly reduced the α-SMA-positive interstitial cells in hemizygote transgenic AL rat kidneys (**b**). The arrows in **a** depict the α-SMA-positive intestinal cells. Original magnification ×20.

Table 3. Survival analysis and organ pathology in wild-type and mutant rats: note that the severity of chronic nephropathy is associated with percent survival in various genotypes

Genotype	Neoplastic lesions %	Chronic nephropathy, %	Maximum survival weeks	Survival over wild-type AL %
Wild-type AL	50	83	158	–
Hemizygote AL	73	26	171	8.2
Wild-type CR	62	6.8	194	22.7
Hemizygote CR	46	0	204	29.1

lifelong suppression of the GH/IGF-1 system or caloric restriction elicited renoprotective effects by reducing the inflammatory response.

Renal Expression of PCNA and α-SMA

The number of PCNA-positive cells was higher in the kidneys of 24- to 26-month-old wild-type AL rats (fig. 5a) compared with the age-matched hemizygote AL kidneys (fig. 5b). The kidneys of the 24- to 26-month-old hemizygote AL and CR rats contained similar numbers of these cells, in both glomeruli (fig. 5c) and tubulointerstitium (fig. 5d). These results suggest that lifelong suppression of GH/IGF-1 activity or dietary restriction reduces cell proliferation in the kidney. Furthermore, prolonged caloric restriction or suppression of GH/IGF-1 activity, separately or in combination, reduces phenotypic transformation of glomerular and tubulointerstitial cells in 24- to 26-month-old rats; this was evidenced by the α-SMA-positive cells in the glomeruli and tubulointerstitium of wild-type AL rats (fig. 6). It is worth mentioning that proliferation and transdifferentiation of resident renal cells, in response to injury, can contribute significantly to developing glomerulosclerosis and tubulointerstitial fibrosis by producing excessive matrix proteins.

Survival and Kidney Lesions

The effects of renal damage on survival in diet-restricted wild-type and hemizygote transgenic rats are summarized in table 3. We found that 83% of the wild-type AL rats showed renal damage including inflammatory cell infiltration and tubulointerstitial fibrotic changes. In contrast, only 26% of the hemizygote AL rats showed features of renal injury, and such reduced renal injury was associated with an 8.2% increase in survival compared with wild-type AL rats. Interestingly, chronic dietary restriction significantly reduced the incidence of renal injury, and only 6.8% of the wild-type CR rats showed features of nephropathy, which was again associated with a 22.7% increase in survival over wild-type AL rats. Chronic suppression of GH/IGF-1 activity in combination with dietary restriction completely suppressed chronic nephropathy, and the hemizygote transgenic CR

rats survived about 30% longer than the wild-type AL rats. These observations clearly show that reduced renal pathology is associated with increased survival. As shown in table 3, there were no major differences in the rate of neoplastic lesions in wild-type and hemizygote transgenic rats, neither in the AL nor CR group.

Discussion

Maintaining water, electrolyte, and mineral balance and eliminating metabolic waste are the main functions of the kidney [14–18]. The renal transport system is essential for the physiological regulation of organic and inorganic ion balance. Most of the chronic renal diseases, without therapeutic intervention, usually progress to irreversible renal failure to affect survival [19–23].

The present study was conducted in long-lived, genetically altered rats with suppressed GH/IGF-1 activity, with and without caloric restriction, to determine the effect of kidney pathology on overall survival. We found that the CR hemizygote transgenic rats survived 30% longer than the regularly fed wild-type rats despite a similar prevalence of neoplastic lesions (50% in wild-type AL vs. 46% in hemizygote CR rats) [8]. Similarly, nonneoplastic lesions not associated with kidney disease were seen in similar numbers in both groups of rats allowed to die naturally (50% in wild-type AL vs. 53% in hemizygote CR rats). One significant finding, however, was that 83% of the naturally surviving wild-type AL rats developed kidney lesions, whereas no kidney lesions were seen in the naturally surviving hemizygote CR rats. This finding suggests that prevention of kidney pathology by dietary restriction, combined with chronic suppression of the GH/IGF-1 system, could significantly extend the life span. Our speculation is further supported by the observation that, in contrast to the naturally surviving wild-type AL rats, the incidence of kidney lesions in the naturally surviving wild-type CR rats diminished from 83 to 6.8%. More importantly, such renoprotection was accompanied by a 22% increase in survival of the wild-type CR rats. Therefore, we conclude that extended longevity in wild-type CR rats is influenced by kidney function.

Controlled reduction of the GH/IGF-1 activity and prolonged caloric restriction may produce a similar milieu to that which promotes survival in mammals [8, 21, 24, 25]; however, we found that combining these experimental interventions had additive effects on life span, reflected by the 30% increased survival in the hemizygote CR rats compared to an 8% increased survival in the hemizygote AL rats. Data on the ages at the 25th and 10th percentile survival points also suggested an additive effect on life span extension when long-lived heterozygote rats were subjected to caloric restriction. Our results are in accordance with earlier observations in long-lived Ames dwarf mice, in which pituitary GH, prolactin, and thyroid-stimulating hormone were nearly absent due to mutation of the *prop-1* gene [26]. These animals survived even longer with caloric restriction [27], although the investigators proposed a difference in the life-prolonging effect between the Ames phenotype and CR animals. The Ames phenotype appeared to delay the onset of aging, while the CR animals showed a decelerated rate of aging [27].

Interestingly, we achieved a 22.7% increase in survival in wild-type rats just by reducing calorie intake, suggesting that several effects of caloric restriction might be masked to some extent in the hemizygote rats with reduced GH/IGF-1 activity. The survival points and hazard ratios for mortality also suggested that caloric restriction had a slightly greater effect on life span than that produced by the reduced GH/IGF-1 axis per se. Again, the 8, 22.7, and 30% increases in survival of hemizygote AL rats, wild-type CR rats, and hemizygote CR rats, respectively, over wild-type AL rats showed that survival is affected by the presence of kidney lesions. Of relevance, 83% of the naturally surviving wild-type AL rats showed kidney lesions.

One important issue that needs further clarification is why the GH/IGF-1-suppressed homozygote rats in our previous studies had poorer survival rates than the wild-type rats despite the absence of kidney lesions [2, 28]. It is possible that, in contrast to 50% of the wild-type AL rats, 96.9% of the naturally surviving homozygote AL rats developed some form of neoplastic lesions, consistent with the notion that a severely reduced GH/IGF-1 axis could promote tumorigenesis. In addition, natural killer cell numbers and activity were reduced in homozygote rats [2], thus further promoting tumorigenesis by reducing immune function, and eventually affecting the overall survival.

One obvious benefit of reduced GH/IGF-1 activity or caloric restriction or the combination of both was renoprotection. These genetic or dietary manipulations delayed or prevented the development of age-associated glomerulosclerosis and interstitial damage to varying degrees by reducing proliferative activity (as determined by PCNA staining) and by decreasing phenotypic cellular transformations (as determined by α-SMA staining) of

resident renal cells. Furthermore, reduced GH/IGF-1 activity and/or caloric restriction exerted renoprotective effects by reducing renal infiltration of inflammatory cells, including macrophages (as determined by ED-1 staining), resulting in delay or prevention of age-associated renal disease. Klotho is an anti-ageing factor and mostly produced in the kidney. Klotho can influence kidney function by influencing renal calcium and phosphate metabolism [16, 29–32]. Further studies will determine whether klotho plays any role in renoprotection achieved through reduced GH/IGF-1 activity, caloric restriction, or the combination of both.

We propose that tumors and kidney lesions might have differential effects on survival. Tumors are usually associated with premature death [33], which would, therefore, be reduced by modulating tumorigenesis. However, such an effect might not extend survival beyond normal life expectancy. On the other hand, once survival goes past that point, additional nonneoplastic factors might determine the extent of longevity. The present study implicated kidney pathology as one of the most important nonneoplastic lesions that affect overall survival, and suggested that the prevention of kidney pathology by dietary restriction, combined with chronic suppression of the GH/IGF-1 activity, could significantly extend overall survival and longevity [21]. Further studies will determine whether the results of our animal studies are of clinical relevance to humans, as to date, there is no concrete evidence that GH deficiency or caloric restriction could increase the human life span.

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