

Original Article

Five-sixth Nephrectomy in Female Common Marmosets (*Callithrix jacchus*) as a Chronic Renal Failure Model

—A Longitudinal Course of Serum Biochemical, Hematological and Histopathological Changes—

Itaru Yamaguchi^{1*}, Kensuke Myojo¹, Hiroko Sanada¹, Atsuko Takami¹, Yui Suzuki^{1, 2}, Minami Imaizumi¹, Chie Takada¹, Naoya Kimoto¹, Koji Saeki¹, Jyoji Yamate², and Katsumi Takaba¹

¹ Fuji Research Park, Kyowa Hakkō Kirin Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411–8731, Japan

² Veterinary Pathology, Osaka Prefecture University, 1–58 Rinkuu Ourai Kita, Izumisano, Osaka 598–8531, Japan

Abstract: To assess the relevance and availability of subtotal nephrectomized common marmoset monkeys as a chronic renal failure (CRF) model, we observed for 26 weeks the pathophysiological condition of female marmosets subjected to five-sixth surgical nephrectomy (5/6Nx) by a two-step surgical method. The 5/6Nx marmosets showed a significant increase in serum levels of urea nitrogen, creatinine and cystatin-C immediately after 5/6Nx surgery. These renal disorder parameters subsequently tended to decrease with the passage of time but remained higher than the control levels by the end of the study. Hyperplastic parathyroid glands, a high turnover state of osteodystrophy in the femoral bone with higher serum ALP activity and anemia with hypocellularity of bone marrow were evident. The 5/6Nx marmosets showed a stable CRF condition for a long time and some characteristic disorders similar to those observed in CRF patients. These diagnostic aspects might be a species-specific anatomical and physiological signature, reflecting the nutritional condition. The CRF model using 5/6Nx marmosets might become a useful method of evaluating the unique mechanism of CRF development. (DOI: 10.1293/tox.2013–0055; J Toxicol Pathol 2014; 27: 183–196)

Key words: common marmoset, 5/6 nephrectomy, chronic renal failure, bone, anemia

Introduction

The remnant kidney model has been a mainstay of experimental studies of chronic renal failure (CRF). This model has been produced mostly by unilateral nephrectomy and either partial infarction or amputation of the poles of the remaining kidney^{1–3}. Rodent models remain essential tools that are widely employed by nephrological investigators. Rat models are usually able to faithfully recapture the major pathological manifestations of human diseases, whereas mice are notorious for resistance to development of clinical lesions after injury^{4, 5}. Several critical factors, such as animal species, strain, gender, age and body weight, may play a decisive role in determining the outcome of a particular

experiment^{5–9}.

Nonhuman primates are used when they represent a well-established model of a compound class or when they are the relevant species (especially for biotechnology-derived products) for detecting known side effects. On the other hand, with the increase of biotechnology-derived pharmaceuticals, the use of nonhuman primates has also increased, as much as to raise public concern.

The common marmoset, a new world primate, is the shortest-lived of the primates, with an average lifespan of nine to 13 years and a maximum life span of 22 years¹⁰. Due to its small size and its relatively easy adaptation to laboratory conditions, the marmoset is increasingly used in many fields of biomedical research, including fundamental biology, pharmacology and toxicology studies^{11, 12}. In addition, regulatory authorities worldwide recognize the use of marmosets in nonclinical development, and as they are one of the more primitive, nonhuman primate species and the most phylogenetically distant from humans, their use requires less ethical justification than the larger “old world monkey”¹³. The marmoset has been used successfully by a number of pharmaceutical and biotechnology companies to support clinical trials and for product registration with the

Received: 30 September 2013, Accepted: 4 June 2014

Published online in J-STAGE: 3 July 2014

*Corresponding author: I Yamaguchi (e-mail: itaru.yamaguchi@kyowa-kirin.co.jp)

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Table 1. Individual Data of the Marmosets

Group	Animal No.	Individual No.	Age (months)	Starting weight (g)	Sacrifice
Non-treated control	001	I3294F	40	349	5W
	002	I3306F	42	348	5W
	003	I3698F	42	323	5W
	004	I3714F	41	316	5W
	005	I3166F	49	319	13W
	006	I3297F	43	373	13W
	007	I3283F	57	385	26W
	008	I3661F	43	317	26W
	009	I3697F	41	296	26W
	010	I3701F	41	362	26W
5/6Nx	101	I3286F	42	392	Day 10 ^{a,#}
	102	I3298F	42	390	5W
	103	I3304F	56	376	5W
	104	I3710F	41	298	5W
	105	I3720F	41	322	5W
	106	I3301F	42	306	13W
	107	I3302F	42	305	13W
	108	I3303F	42	399	13W
	109	I3293F	42	337	13W
	110	I3169F	63	347	26W
	111	I3665F	42	423	26W
	112	I3671F	42	361	Day 16 ^{b,#}
	113	I3674F	42	348	26W
	114	I3693F	41	324	Day 96 ^{a,§}

^a Moribund, ^b Dead [#] These animals (No. 101 and 112) survived only a short time (10 or 16 days) after 5/6Nx; therefore, the histopathological findings were excluded from the results focused on chronic renal failure related changes. [§] Due to failure of blood collection, serum biochemical and hematological analyses could not be performed.

United States (US) Food and Drug Administration (FDA) and the European Medicines Agency (EMA)^{14, 15}. In addition, the common marmoset shows a higher incidence of renal lesions including glomerulo- and tubulointerstitial nephritis or progressive nephropathy^{16–21} compared with that in the cynomolgus monkey^{22, 23}.

The aim of this study was to investigate the relevance and availability of the subtotal nephrectomized common marmoset as a CRF model. In a previous study, we carried out five-sixth surgical nephrectomy (5/6Nx) on female marmosets and acquired data regarding the parameters of the renal disorder and the pathological changes of the remnant kidneys up to 13 weeks after the operation²⁴. The 5/6Nx marmosets showed an increase in urine volume and elimination of urinary protein immediately after nephrectomy, but there was no progressive increase in the elimination of urinary protein (proteinuria). In the remnant kidney in 5/6Nx marmosets, slight degenerative glomerular abnormalities and interstitial fibrosis with inflammatory mononuclear cells were observed. There was no further progression of the glomerular changes over time²⁴. In this study, we focused on the longitudinal course of serum biochemical, hematological and histopathological changes in 5/6Nx female marmosets for up to 26 weeks after the surgical operation.

Materials and Methods

Animals and surgical procedure

Common marmoset monkeys were obtained at one or two years old from CLEA Japan Inc. (Kawasaki, Japan). One or two animals were housed in a steel cage (width 390 × depth 550 × height 700 mm; Shin Toyo Seisaku-sho Ltd., Saitama, Japan) with environmental enrichments, generally a roost, an aerial bar and a table tennis ball. The animal room was kept under controlled conditions of temperature (24–30°C), relative humidity (30–70%) and ventilation (air exchange rate of 15 to 17 times/hr) with a 12-hour light/12-hour dark cycle. They were allowed free access to a commercial diet (CSM-1M, CLEA Japan Inc.) and tap water throughout both the acclimation and experimental periods. Additional supplements (dry fruits, boiled quail eggs or milk powder) were provided as considered appropriate. During the acclimation period, animals were checked daily by cage-side observation. Detailed clinical observation and body weight measurement were conducted weekly. At the end of the acclimation period, 24 female marmosets aged 40 to 63 months old were selected. Ten animals were allocated into the non-treated group, and 14 animals were allocated into the 5/6Nx group. The individual data for the animals are presented in Table 1. The experiment and care of the animals were conducted in accordance with the guiding principles for “The Care and Use of Laboratory Animals of Kyowa Hakko Kirin Co, Ltd”.

Each marmoset in the 5/6Nx group underwent 5/6Nx by a two-step surgery²⁵. In the first operation, marmosets were anesthetized by inhalation of isoflurane (Mylan Seiyaku, Tokyo, Japan) and held in a lateral position. The surgical site was shaved free of hair and disinfected with povidone-iodine and 70% ethanol. After identification of the left kidney by palpation, the skin, muscle and peritoneum in the region were dissected about 1 to 1.5 cm in length. The kidney was exteriorized by pressing the abdomen with the fingers, and then held by clipping the proximal ureter and renal vessels with a hemoclipper. The upper and lower poles of the left kidney were resected with a pair of surgical scissors, leaving one-third of the renal parenchyma and renal hilum intact. An ablation hemostatic sheet (SURGICEL, Ethicon Inc., NJ, USA) was used for hemostasis by covering the ablation surface. A few minutes later, the hemostat clipping was removed, and the ablation surface was checked for bleeding. The renal remnant was returned to the abdomen cavity. A continuous suturing method was used for suture of the abdominal muscle and peritoneum, and a buried suture method was used for suture of skin. The suture site was disinfected with povidone-iodine. In the second operation, the right kidney was removed after ligation of the proximal ureter and renal vessels with silk thread. After each operation, the animals were gently laid to rest on a hot plate kept at 38°C until awaking from anesthesia. The animals were then kept in an incubator set at 38°C. After checking their health status, animals in good condition were returned to the animal feeding cages in the animal room. In addition, all operated animals received a daily intramuscular dose of about 10 mg/kg lincomycin hydrochloride (Lincocin[®], Pfizer Inc.) and about 0.3 mg/kg of meloxicam (Metacam[®], Boehringer Ingelheim Vetmedica, Inc.) for 3 or 4 days after each operation.

Clinical signs

Clinical signs were recorded by daily cage-side monitoring of behavioral changes (apathy, loss of appetite).

Serum biochemistry, hematology and histopathology

At 5, 13 and 26 weeks after the 5/6Nx operation, 10 non-treated control and 11 5/6Nx animals were sacrificed. Under deep anesthesia with isoflurane, blood samples were collected from the abdominal vein of animals for selected blood chemistry and hematological examinations, and then the animals were euthanized by exsanguination to prevent them from suffering. The animals were then dissected for pathological observation.

The serum biochemical analyses were performed with an automatic analyzer (H7170S, Hitachi Ltd.) and an automated electrolyte analyzer (PVA-EXII, A&T Corporation). Hematological examination was performed using an automatic hematological analyzer (ADVIA 120, Siemens Healthcare Diagnostics). However, since this system could not clearly fractionate neutrophils and eosinophils in marmoset peripheral blood, the total number of both cell types was used as the granulocyte number. Blood coagulation pa-

rameter analyses were performed using an automated blood coagulation analyzer (CA-1500, Sysmex).

All major organs were collected and fixed in 10% neutral buffered formalin, embedded, sectioned at 4 to 6 μm , and stained using hematoxylin and eosin (H-E). The bone tissues were decalcified with 10% EDTA solution. The kidney sections were also stained with periodic acid-Schiff (PAS) reaction and Masson's trichrome. These preparations were observed by light microscopy.

Statistics

Data were expressed as means \pm SD. All parameters were evaluated with the Student's *t*-test and an unpaired *t*-test with Welch's correction. All *p* values resulted from two-sided statistical tests, and $p < 0.05$ was considered to be significant.

Results

All animals in the non-treated control group survived to the end of study, whereas three animals in the 5/6Nx group died or were moribund. One animal (No. 101) was moribund on day 10, and another animal (No. 112) died on day 16 after the 2nd surgery. The moribund animal (No. 101) showed a marked change in renal functional parameters (an increase in serum urea nitrogen (UN), creatinine, cystatin-C (Cys-C)), electrolytes (an increase in inorganic phosphorus and potassium and a decrease in sodium and chloride), lipids (a decrease in total cholesterol, triglycerides and phospholipids), liver function (an increase in aspartate aminotransferase (AST) and choline esterase), and an increase in alkaline phosphatase (ALP) with severe anemia and thrombopenia. These animals were considered to be at the onset of the acute renal failure state. Another animal (No. 114) showed emesis and low appetite on day 94, loss of appetite and defecation, incomplete opening of the eyelids, and a decrease in locomotor activity on day 96. This animal showed hydronephrosis in the remnant kidney, alveolar hemorrhage in the lungs, parathyroid gland hyperplasia, an increase of adipose tissue in the bone marrow, and mineralization at renal tubules, tracheal cartilage and aortic tunica media. Hence this animal was considered to have been in a severe renal failure state due to a shift to hydronephrosis in the remnant kidney. Tremors and bradykinesia were observed in one animal (No. 111) of the 5/6Nx group. Reduction of appetite was also observed in some animals in the 5/6Nx group. These changes diminished within one or two weeks after surgery.

In the serum blood chemistry, the 5/6Nx marmosets showed a marked increase in the serum UN, creatinine and Cys-C levels at week 5. These levels subsequently tended to decrease with the passage of time, but the serum creatinine and Cys-C levels were sustained at higher levels at weeks 13 and 26 in the 5/6Nx marmosets. The serum calcium and inorganic phosphorus levels were significantly higher than the control level at week 5. The calcium and inorganic phosphorus levels were restored to normal levels at weeks 13

and 26. In one 5/6Nx animal (No. 108) at week 13, a higher level of inorganic phosphorus was observed. This animal revealed not only higher values of renal disorder parameters (serum UN, creatinine and Cys-C) but also a higher value of potassium and lower values of sodium, chloride and lipids (total cholesterol, triglyceride and phospholipids). The serum ALP levels were also increased at week 5, and the higher levels continued at weeks 13 and 26 in most of the 5/6Nx animals (except one case). The serum phospholipids concentration of the 5/6Nx marmosets was significantly higher than the control value at weeks 5 and 26. The mean serum total cholesterol, triglyceride and total bilirubin levels at week 26 also tended to be higher in the 5/6Nx group than in the control group (Table 2).

Mean red blood cell counts, and hemoglobin, hematocrit and MCH levels decreased in the 5/6Nx marmosets. The grade of these changes was marked at week 13 after surgery. Regarding the reticulocyte counts, one 5/6Nx animal (No. 104) showed a lower level at week 5, whereas another animal (No. 109) showed a higher level at week 13. Mean granulocyte and platelet counts increased significantly at week 5 in the 5/6Nx marmosets. These parameters were similar to the control values at the end of the study. The mean serum concentration of fibrinogen in the 5/6Nx marmosets increased and continued to remain higher than the control level until the end of the study (Table 3).

The histopathological changes in the 5/6Nx marmosets are presented in Table 4. The remnant kidneys from 5/6Nx marmosets at week 5 revealed hypertrophic glomeruli with an increase in size of the Bowman's capsule (Fig. 1B) and degenerative and/or regenerative renal tubules with interstitial fibrosis and inflammatory or mononuclear cell infiltration (Fig. 2A, D). In addition, atrophic or regenerative renal tubules and dilated lumens of distal and/or collecting tubules were also observed. At week 13, hypertrophic glomeruli showed increased dilatation of glomerular capillaries, but there was slight if any mesangial change (Fig. 1C). There were only a few degenerative glomeruli containing swollen glomerular epithelium and necrotic mesangial cells. Atrophic renal tubules surrounded by thickened basement membrane in the fibrosis area with prominent infiltration of mononuclear cells were apparent (Fig. 2B, E). At week 26, the glomerular changes disappeared except in one 5/6Nx animal (Fig. 1D). The interstitial mononuclear cell infiltration remained at week 26 (Fig. 2C). In one 5/6Nx marmoset, hypertrophic renal tubules were evident at week 26 (Fig. 2F). On the other hand, most of the kidneys in non-treated control marmosets revealed slight to moderate interstitial inflammatory and/or mononuclear cell infiltration. Normal or degenerative renal tubules were surrounded by infiltrated mononuclear cells, but interstitial fibrosis was not apparent (Fig. 2G, H).

Hyperplasia of the chief cells in the parathyroid glands was observed in the 5/6Nx marmosets at weeks 5, 13 (Fig. 3) and 26. One animal with parathyroid hyperplasia (No. 113) showed mineralization at bronchial and tracheal cartilage (Fig. 4A) and aortic tunica media (Fig. 4B). Multiple site

of mineralization were also observed in a moribund animal at week 14 (No. 114). The 5/6Nx marmosets showed bone resorptive changes in the lumens of Haversian canals (Fig. 5B, C) with microfibrils (Fig. 5D) and in the subperiosteal cortical bone (Fig. 5F) in the femur. In one 5/6Nx animal (No. 107), trabecular bone decreased and was surrounded by fibrous tissue (Fig. 5H). This animal revealed moderate parathyroid hyperplasia (Fig. 3B). In addition, an increase in adipose tissue with a decrease in marrow cells in femoral bone marrow appeared in the 5/6Nx marmosets at weeks 13 (Fig. 6B) and 26 (Fig. 6D) as compared with the levels in non-treated control animals at weeks 13 (Fig. 6A) and 26 (Fig. 6C), respectively.

Discussion

The most widely studied model for the pathophysiology of CRF is the 5/6Nx or the remnant kidney model. Five-sixth experimental nephrectomy has been shown to cause systemic hypertension, proteinuria, a decrease in renal function and progressive glomerulosclerosis²⁶⁻²⁸.

In the 5/6Nx marmosets, the urine volume and elimination of urinary protein increased immediately after nephrectomy, and recovery was slower than that in 5/6Nx rats²⁴. The serum UN, creatinine and Cys-C levels remained higher than those of the control until the end of the study, suggesting that the uremic condition persisted for a long time in the 5/6Nx marmosets.

The remnant kidney of 5/6Nx marmosets showed hypertrophic glomeruli at weeks 5 and 13. The change almost diminished at week 26. The dilatation of glomerular capillaries and increase in size of the Bowman's capsule might be caused by glomerular hyperperfusion and hyperfiltration depending on a reduction in renal mass²⁹. There were no progressive degenerative or sclerotic glomerular changes in the remnant kidney from the 5/6Nx marmosets until week 26. We previously reported that the thick glomerular basement membrane of marmosets might resist glomerular hyperfiltration and prevent glomerular injury²⁴. In addition, elevated systemic pressure is accompanied by elevated glomerular capillary pressure in several experimental models of hypertensive renal disease, including renal ablation^{30, 31}. In a two-kidney one-clip renal hypertension model, common marmosets showed high blood pressure and plasma renin activity during the first 3 to 5 weeks after renal arterial clipping and return to the control level within 10 weeks³², while rats showed renal hypertension at 4 months after clipping³³. Hence, the recovery of systemic blood pressure in marmosets after renal nephrectomy may be correlated with the disappearance of the glomerular hypertrophic changes, which consequently prevent further progressive glomerular injury or prominent proteinuria in the remnant kidney of 5/6Nx marmosets.

Loss of renal function is also associated with development of interstitial fibrosis, which is characterized by loss of the renal parenchyma, tubular atrophy and accumulation of extracellular matrix proteins^{34, 35}. In the 5/6Nx marmosets,

Table 2. Individual Serum Chemistry Data

Group	Weeks after	Animal No	TP (g/dL)	ALP (U/L)	AST (IU/L)	ALT (IU/L)	Glu (mg/dL)	UN (mg/dL)	CRE (mg/dL)	T-Chol (mg/dL)	PL (mg/dL)	TG (mg/dL)	T-Bil (mg/dL)	ChE (U/L)	Cys-C (mg/L)	IP (mg/dL)	Ca (mEq/L)	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	
Non-treated contro	5	001	4.3	206	114	8	137	18.7	0.26	138	162	77	0.07	5992	0.3	2.1	4.5	150	3.0	113	
		002	4.8	311	136	8	141	24.5	0.21	158	177	63	0.08	6643	0.4	2.7	4.8	152	3.0	113	
		003	5.3	287	97	7	114	18.4	0.27	92	161	161	46	0.10	5350	0.3	1.7	4.6	154	3.2	117
		004	6.1	460	58	2	112	18.7	0.30	89	115	115	62	0.24	5397	0.3	3.0	4.7	150	3.4	111
		Mean	5.13	316.0	101.3	6.3	126.0	20.1	0.260	119.3	153.8	153.8	62.0	0.123	5845.5	0.34	2.38	4.65	151.5	3.15	113.5
SD	0.77	106.0	33.0	2.9	15.1	3.0	0.037	34.2	26.8	26.8	12.7	0.079	606.7	0.04	0.59	0.13	1.9	0.19	2.5		
13	5/6Nx	005	7.0	170	165	36	156	19.8	0.20	96	174	132	0.07	6074	0.2	1.6	5.3	156	3.1	111	
		006	3.8	269	117	0	88	20.3	0.21	132	165	165	46	0.08	3448	0.2	2.4	4.2	151	3.3	116
		Mean	5.40	219.5	141.0	18.0	122.0	20.05	0.205	114.0	169.5	169.5	89.0	0.075	4761.0	0.20	2.00	4.75	153.5	3.20	113.5
		SD	1.25	353.0	135.8	3.0	27.1	41.65	0.101	48.1	52.2	52.2	18.2	0.036	2737.1	0.10	3.69	0.61	8.8	1.15	6.9
		Mean	5.45	308.8	99.3	3.8	109.8	18.08	0.243	99.0	144.8	144.8	56.0	0.090	6862.5	0.33	2.48	4.40	152.8	2.98	115.8
SD	0.69	57.6	34.3	1.7	20.2	4.0	0.0	20.2	12.5	12.5	23.4	0.026	3414.9	0.06	0.72	0.08	1.3	0.26	1.0		
5/6 Nx Day 10 [#]	5	101	6.7	1550	367	9	275	372.0	4.71	60	84	14	0.30	18623	2.4	25.5	4.8	125	10.4	96	
		102	5.1	297	181	20	110	65.4	0.68	152	193	193	59	0.07	6322	0.8	3.5	5.2	154	2.9	114
		103	5.5	481	179	14	107	72.6	0.90	132	190	190	65	0.12	7993	0.7	3.9	4.9	150	3.1	110
		104	5.2	1644	178	9	130	151.3	1.68	151	202	202	64	0.10	5523	1.5	7.4	5.2	149	2.8	111
		105	5.0	832	94	2	128	112.7	1.41	121	177	177	90	0.08	5565	1.1	5.3	5.4	154	2.9	119
Mean	5.20	813.5	158.0	11.3	118.8	100.50	1.168	139.0	190.5*	190.5*	69.5	0.093	6350.8	1.02	5.03*	5.18**	151.8	2.93	113.5		
SD	0.22	596.5	42.7	7.6	11.9	39.75	0.459	15.1	10.3	10.3	13.9	0.022	1154.8	0.35	1.76	0.21	2.6	0.13	4.0		
13	5/6Nx	106	5.0	488	91	3	114	33.4	0.53	161	174	43	0.07	5511	0.6	1.9	4.8	153	3.2	117	
		107	4.4	1149	83	1	152	67.1	0.65	116	155	155	67	0.08	9480	0.7	2.7	3.7	153	3.3	116
		108	3.0	435	347	7	105	125.0	0.73	49	71	71	28	0.14	9562	0.8	9.8	3.9	135	5.5	102
		109	6.0	414	57	7	88	40.3	0.52	137	187	187	63	0.06	4226	0.6	2.8	4.9	152	3.1	111
		Mean	4.60	621.5	144.5	4.5	114.8	66.45	0.608	115.8	146.8	146.8	50.3	0.088	7194.8	0.68	4.30	4.33	148.3	3.78	111.5
SD	1.25	353.0	135.8	3.0	27.1	41.65	0.101	48.1	52.2	52.2	18.2	0.036	2737.1	0.10	3.69	0.61	8.8	1.15	6.9		
26	5/6Nx	110	5.5	552	99	2	125	44.8	0.72	112	187	134	0.12	9844	0.6	3.4	4.5	155	3.2	122	
		111	6.0	574	219	12	176	18.7	0.62	168	211	211	61	0.18	4837	0.8	2.3	4.7	152	2.9	112
		113	6.6	234	155	10	89	34.6	0.70	157	218	218	110	0.15	6442	0.6	1.7	5.1	158	2.9	114
		Mean	6.03	453.3	157.7	8.0	130.0	32.70	0.680	145.7	205.3*	205.3*	101.7	0.150**	7041.0	0.67	2.47	4.77	155.0	3.00	116.0
		SD	0.55	190.3	60.0	5.3	43.7	13.15	0.053	29.7	16.3	16.3	37.2	0.030	2556.7	0.12	0.86	0.31	3.0	0.17	5.3

Significant different from the non-treated control group by Student's *t*-test and an unpaired *t*-test with Welch's correction (**p*<0.05; ***p*<0.01). # The animal (No. 101) was moribund and was euthanized to prevent it from suffering on day 10.

Table 3. Individual Hematology Data

Group	Weeks after 5/6Nx	Animal No	RBC ($\times 10^4/\text{mL}$)	Hb (g/dL)	Ht (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT ($\times 10^4/\text{mL}$)	RET ($\times 10^4/\text{mL}$)	WBC ($\times 10^2/\text{mL}$)	Gra ($\times 10^2/\text{mL}$)	Lymph ($\times 10^2/\text{mL}$)	Mono ($\times 10^2/\text{mL}$)	Baso ($\times 10^2/\text{mL}$)	Others ($\times 10^2/\text{mL}$)	PT (sec)	APTT (sec)	FGB (mg/dL)	
Non-treated control	5	001	657	13.6	44.5	67.7	20.7	30.6	76.4	26.3	39.9	18.9	17.0	2.0	0.1	1.8	NE	NE	NE	
		002	638	14.1	45.8	71.7	22.1	30.8	79.7	25.5	37.3	14.1	19.2	2.5	0.1	1.4	NE	NE	NE	
		003	548	10.4	36.2	66.1	19.0	28.8	81.9	33.9	24.3	24.3	11.4	11.4	1.1	0.2	0.3	5.4	30.1	148.9
		004	429	10.7	33.1	77.1	25.0	32.4	66.3	52.0	72.3	24.8	24.8	39.7	4.8	0.4	2.6	5.3	37.9	272.5
		Mean	568.0	12.20	39.90	70.65	21.70	30.64	76.08	34.43	12.31	20.41	5.88	12.35	1.58	0.14	0.96	5.35	34.00	210.70
SD	104.2	1.92	6.22	4.90	2.54	1.47											NC	NC	NC	
	13	005	674	13.5	42.2	62.6	20.0	32.0	42.8	16.9	36.8	8.6	26.9	0.7	0.1	0.4	5.3	22.3	261.2	
		006	499	11.0	37.2	74.5	22.0	29.6	103.5	37.9	22.3	11.9	11.9	8.9	0.6	0.0	1.0	5.4	27.2	214.4
		Mean	586.5	12.25	39.70	68.55	21.04	30.78	73.15	27.39	29.55	10.25	17.93	0.63	0.07	0.68	5.35	24.75	237.80	
		SD																		
		007	522	13.1	41.4	79.4	25.1	31.6	56.6	28.2	27.8	13.5	13.5	13.3	0.8	0.1	0.1	6.8	38.3	183.9
5/6Nx	Day 10 [#]	008	634	13.8	45.0	71.0	21.8	30.7	62.9	31.7	43.4	33.4	8.5	1.1	0.0	0.2	MV	MV	26.6	215.9
		010	514	11.5	37.9	73.7	22.4	30.3	54.7	22.6	36.2	16.4	16.4	18.9	0.7	0.1	0.1	MV	MV	197.4
		Mean	556.7	12.80	41.43	74.70	23.10	30.87	58.07	27.50	35.80	21.1	13.57	0.87	0.07	0.13	6.80	32.45	199.07	
		SD	67.1	1.18	3.55	4.29	1.76	0.67	4.29	4.59	7.81	10.8	10.8	5.21	0.21	0.06	0.06	NC	NC	16.06
		101	144	3.0	10.8	83.0	23.5	28.4	47.5	21.3	218.2	182.2	182.2	32.1	2.2	0.7	1.1	NE	NE	NE
	5	102	398	7.0	24.5	61.6	17.6	28.6	98.1	34.6	54.1	41.1	11.3	1.4	0.0	0.3	5.3	29.1	306.0	
		103	453	10.6	35.2	77.8	23.4	30.1	101.0	25.4	45.4	24.8	19.5	0.8	0.2	0.6	MV	MV	341.5	
		104	633	12.8	43.3	68.4	20.2	29.6	85.7	20.9	50.6	25.3	23.4	0.6	0.2	1.2	NE	NE	NE	
		105	635	13.1	44.5	70.0	20.6	29.4	95.8	26.7	61.4	30.4	30.4	27.6	0.4	0.2	2.8	NE	NE	NE
		Mean	529.8	10.88	36.88	69.45	20.46	29.42	95.15**	26.89	52.88	30.40*	20.44	0.80	0.13	1.22	5.30	31.85	323.75	
SD	122.5	2.81	9.23	6.65	2.37	0.62	6.65	5.71	6.71	7.57	7.57	6.93	0.43	0.09	1.10	NC	NC	NC		
	13	106	458	10.0	33.8	73.8	21.8	29.6	85.3	23.8	25.2	16.1	8.4	0.3	0.1	0.4	5.3	27.0	306.7	
		107	397	8.6	29.5	74.2	21.7	29.2	79.7	33.7	37.0	21.8	12.9	0.7	0.1	1.4	5.5	26.1	257.7	
		109	375	7.2	25.8	68.9	19.2	27.9	78.3	50.3	40.0	25.7	11.7	0.7	0.1	1.8	5.2	26.5	344.6	
		Mean	410.0	8.60	29.70	72.30	20.90	28.88	81.10	35.94	34.07	21.20	10.99	0.54	0.08	1.22	5.33	26.53	303.00	
		SD	43.0	1.40	4.00	2.95	1.47	0.87	3.70	13.35	7.82	4.83	4.83	2.31	0.23	0.03	0.76	0.15	0.45	43.57
	26	110	501	10.3	34.6	69.0	20.6	29.8	74.1	21.0	55.7	35.8	17.3	1.2	0.1	1.2	6.0	34.0	252.4	
		111	531	10.8	39.0	65.3	20.3	31.1	57.3	20.2	37.1	14.9	19.1	3.0	0.0	0.1	5.4	30.4	297.5	
		113	530	11.1	34.7	73.6	20.9	28.5	65.5	40.8	42.6	25.3	25.3	15.6	1.2	0.0	0.5	5.4	37.4	360.8
		Mean	520.7	10.73*	36.10	69.30	20.60	29.80	65.63	27.33	45.13	25.33	17.33	1.80	0.03	0.60	5.60	33.93	303.57*	
		SD	17.0	0.40	2.51	4.16	0.30	1.30	8.40	11.67	9.56	10.45	10.45	1.75	1.04	0.06	0.56	0.35	3.50	54.45

NE, not examined; NC, not calculated; MV, missing value. Significant different from the non-treated control group by Student's *t*-test and an unpaired *t*-test with Welch's correction (***p*<0.05; ***p*<0.01). # The animal (No. 101) was moribund and was euthanized to prevent it from suffering on day 10.

Table 4 Individual histopathological findings of the kidney, parathyroid gland, lung, trachea, aorta, bone and bone marrow

Organ	Histopathological findings	Non-treated control													5/6Nx							
		Groups					Weeks after 5/6Nx operation								5/6Nx							
		Animal No.					Weeks after 5/6Nx operation								5/6Nx							
		001	002	003	004	005	006	007	008	009	010	102	103	104	105	106	107	108	109	110	111	113
Kidney																						
Glomerulus																						
	Glomerular hypertrophy	0	0	0	0	0	0	0	0	0	0	1	0	2	1	1	1	2	2	2	0	1
	Degeneration/necrosis, glomerular cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	Atrophy, glomeruli	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
	Tubulointerstitium																					
	Degeneration/necrosis, renal tubular epithelium	0	0	1	2	1	0	1	0	0	0	1	1	2	2	1	1	2	2	0	1	1
	Atrophy, renal tubules	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2	2	3	2	0	0	2
	Cellular infiltration, inflammatory/mononuclear cells, interstitial	1	1	3	3	2	1	2	1	2	0	1	1	3	3	2	3	2	2	3	2	1
	Fibrosis, interstitial	1	0	0	0	0	0	0	0	0	0	1	0	2	2	1	2	1	1	1	2	0
	Dilatation, lumen, distal and/or collecting ducts	0	0	2	0	0	0	0	0	0	0	1	3	2	1	1	1	1	1	0	2	0
	Regeneration, renal tubular epithelium	0	0	0	1	1	1	0	0	0	2	1	0	2	1	2	2	2	2	0	1	1
	Hypertrophy, renal tubular epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3
	Hydronephrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mineralization, renal tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
	Parathyroid gland																					
	Hyperplasia, chief cells	0	0	0	0	0	0	0	0	1	0	2	1	2	2	2	3	0	2	2	0	1
Lung																						
	Mineralization, bronchial cartilage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	Hemorrhage, alveolar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Trachea																						
	Mineralization, tracheal cartilage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Aorta																						
	Mineralization, tunica media	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Bone (femur)																						
	Microfibrosis, lumen, Haversian canal and subperiosteal	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	2	0	0	1	0	1
	Decrease, trabecular bone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
	Resorption cavity, subperiosteal and/or trabecular bone	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	1	1	0
Bone marrow (femur)																						
	Congestion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0
	Decrease, marrow cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0
	Fibrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
	Increase, adipose tissue	0	0	0	0	0	1	1	0	1	2	2	0	0	3	3	2	0	1	2	2	3

Histopathological grades: 0, no remarkable change; 1, very slight; 2, slight; 3, moderate; P, present. # The animal (No. 114) was moribund and was euthanized to prevent it from suffering on day 96.

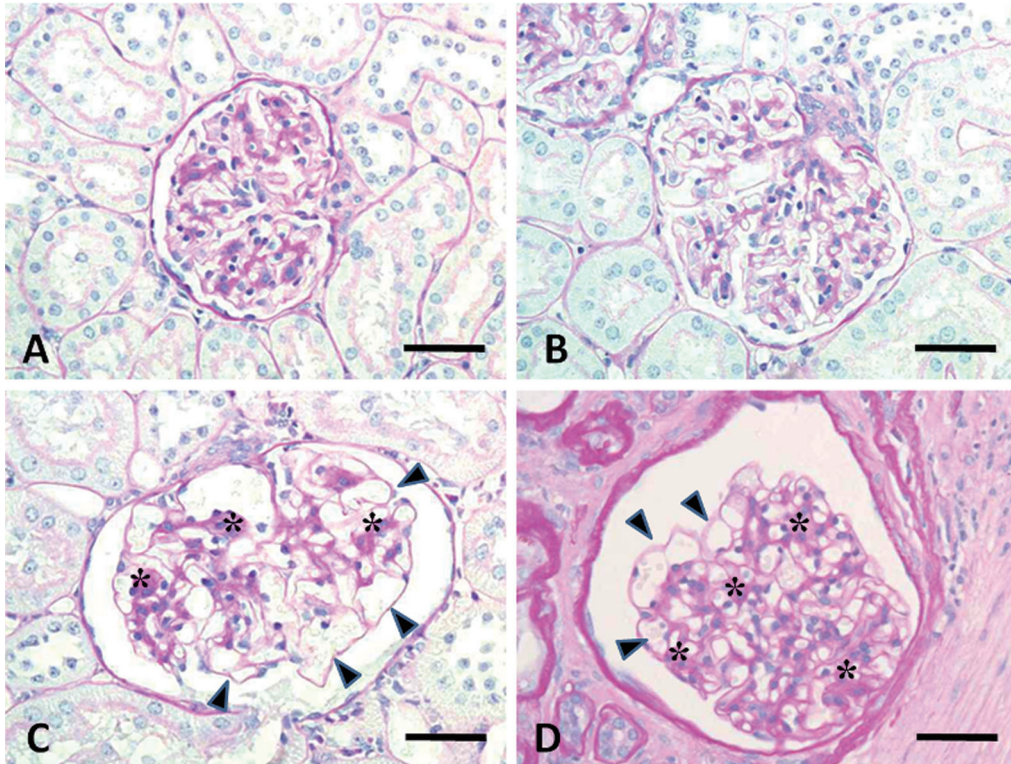


Fig. 1. Photomicrograph of glomerular changes in kidneys from a non-treated control marmoset (A) and 5/6Nx marmosets at weeks 5 (B), 13 (C) and 26 (D). Hypertrophic glomeruli show dilatation of glomerular capillaries (arrowheads) and an increase in the size of the Bowman's capsule. A few glomeruli show a very slight increase in mesangial area (asterisks) . Animal Nos.: A, 001; B, 104; C, 109; D, 111. PAS reaction. Scale bar = 50 μ m

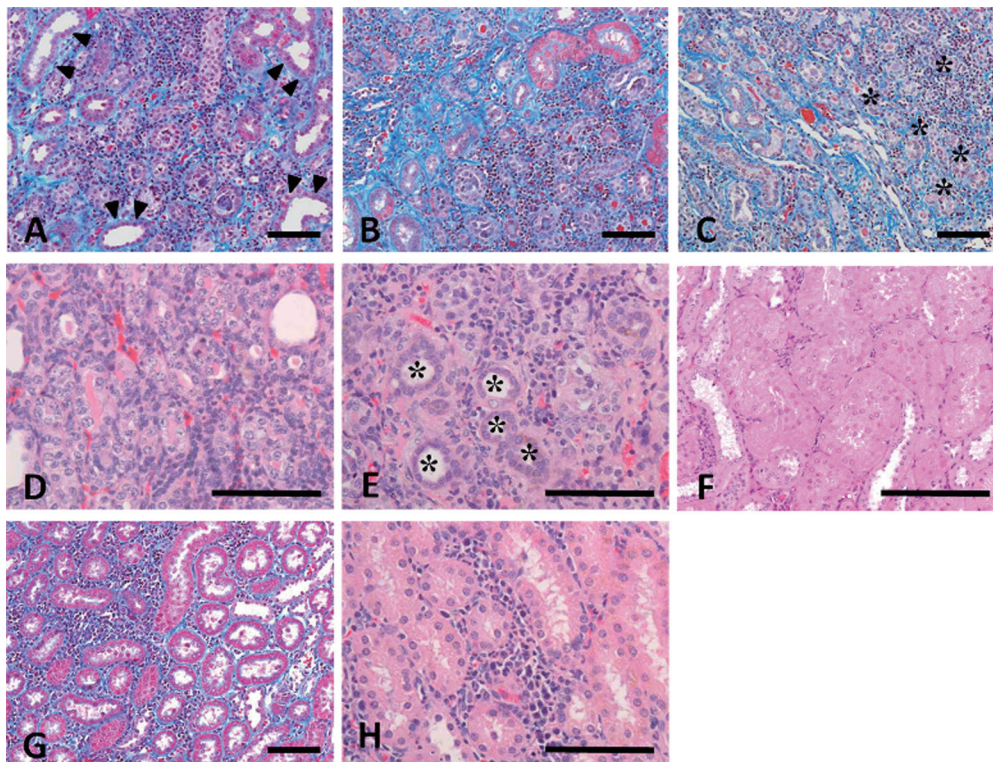


Fig. 2.

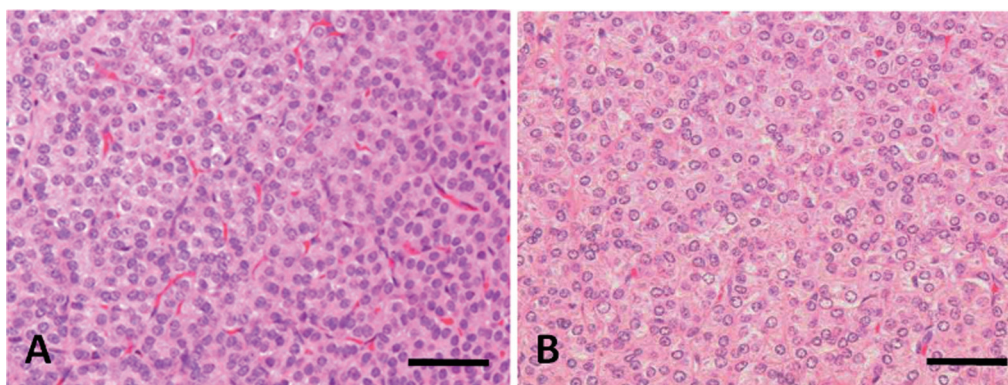


Fig. 3. Photomicrograph of parathyroid glands from a non-treated control marmoset (A) and a 5/6Nx marmoset (B) at week 13. Hyperplasia of the chief cells appeared in the 5/6Nx animal. Animal Nos.: A, 006; B, 107. H-E stain. Scale bar = 100 μ m

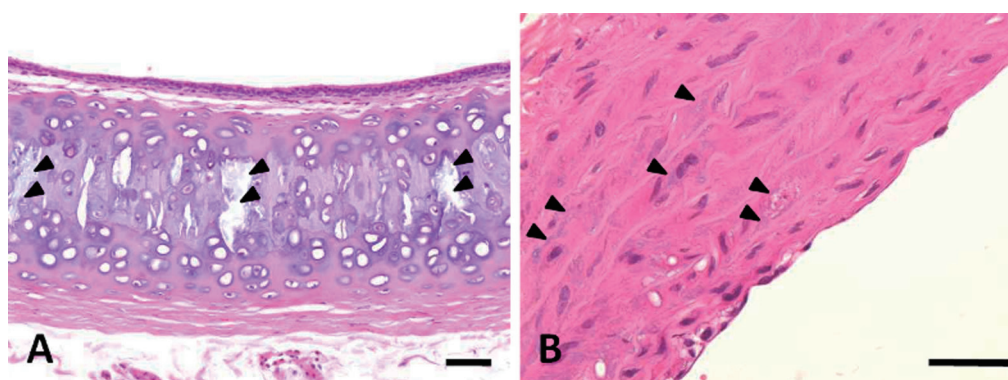


Fig. 4. Photomicrograph of mineralization in the trachea (A) and aorta (B) in a 5/6Nx marmoset at week 26. Arrowheads show mineralization in tracheal cartilage and aortic tunica media. Animal No. 113. H-E stain. Scale bar = 100 μ m

higher serum creatinine and Cys-C levels, which are renal functional disorder parameters, were sustained until week 26, and the damaged renal tubules surrounded by thickened basement membrane or interstitial fibrosis with infiltrated mononuclear cells remained at week 26. Plasma fibrinogen, which is a marker of acute inflammation, also continued to be detected at a high level for a long time in the 5/6Nx marmosets, suggesting that the inflammation should continue in the bodies of these animals. In recent years, many researchers have suggested that inflammation has deleterious effects on the progression of renal disease, including diabetes nephropathy^{36, 37}. Continuous renal non-resolving inflammation might be correlated with the formation of tubulointerstitial lesions in the remnant kidney^{38, 39} and sus-

tain the chronic renal failure condition for a long time in the 5/6Nx marmosets.

Spontaneous progressive nephropathy dominated by glomerular lesions in common marmosets has been reported^{20, 21}. The historical renal lesion in common marmosets is characterized by glomerular lesions with an increase in mesangial matrix, which progresses with aging, and secondary tubulointerstitial lesions, including tubular hyperplasia²⁰. In our study, two non-treated control animals showed remarkable tubulointerstitial nephritis without any glomerular changes and interstitial fibrosis. Both animals were about 42 months old at necropsy and revealed no changes in renal disorder parameters. Tubulointerstitial inflammation is primarily a visible lesion in young marmosets. The intensity of

Fig. 2. Photomicrograph of tubulointerstitial lesions in kidneys from 5/6Nx marmosets at weeks 5 (A and D), 13 (B and E) and 26 (C and F) and a non-treated control marmoset at week 5 (G and H). A–C: Interstitial fibrosis with inflammatory or mononuclear cells in the remnant kidney of 5/6Nx marmosets at weeks 5 (A) and 13 (B). The interstitial mononuclear cell infiltration remained at week 26 (C). Dilated lumens of distal and/or collecting tubules (arrowheads) and atrophic renal tubules (asterisks) are observed. D: Degenerative, necrotic and regenerative renal tubules at week 5. E: Atrophic renal tubules (asterisks) surrounded by thickened basement membrane in the fibrosis area with mononuclear cell infiltration at week 13. F: Hypertrophic renal tubules in one 5/6Nx animal at week 26. G and H: Interstitial mononuclear cell infiltration without interstitial fibrosis in the kidney of a non-treated animal. Animal Nos.: A, 104; B and E, 109; C and F, 113; D, 105; G and H, 003. A–C and G, Masson's trichrome stain; D–F and H, H–E stain. Scale bar = 100 μ m

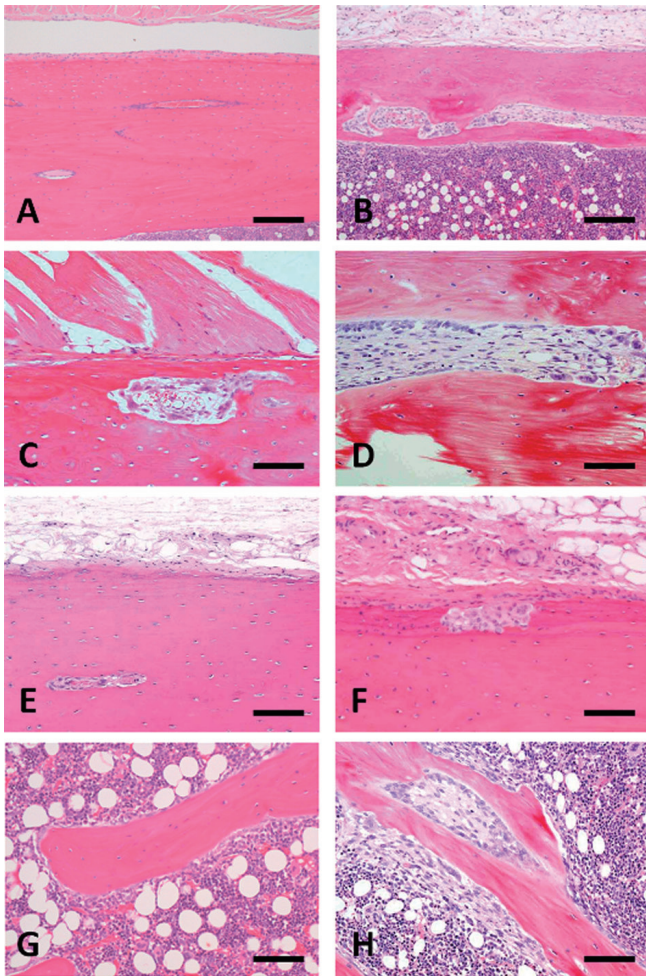


Fig. 5. Photomicrograph of femoral bone changes in non-treated control marmosets at weeks 13 (A and G) and 26 (E) and the 5/6Nx marmosets at weeks 13 (B, C, D and H) and 26 (F). Histological structure of femoral cortical bone at week 13 (A) and 26 (E) and trabecular bone at week 13 (G) in non-treated control animals. Bone resorptive change (B and C) and microfibrosis (D) in the lumens of Haversian canals of cortical bone and a decrease in trabecular bone with surrounding fibrous tissue (H) present in 5/6Nx animals at week 13. Resorptive changes appear in the subperiosteal cortical bone (F) of 5/6Nx animals at week 26. Animal Nos.: A and G, 005; B, C, D and H, 107; E, 007; F, 113. H-E stain. Scale bar = 100 μ m

spontaneous glomerular changes with deposition of immunoglobulin might be higher in marmosets over 7 years old²⁹. The present study used young animals that were about 3.5 to 5 years old. When older animals are used, the renal changes with spontaneous glomerular lesions in the remnant kidney may develop into progressive lesions showing glomerular- and tubulointerstitial nephropathy.

Renal osteodystrophy is an almost universal consequence in CRF patients. Two major subtypes of renal osteodystrophy can be distinguished in humans on the basis of histology: lesions characterized by high bone turnover and low bone turnover⁴⁰. High-turnover renal osteodystrophy is related to a marked and persistent increase in circulat-

ing levels of parathyroid hormone (PTH) with parathyroid cell hyperplasia⁴¹ and shows characteristic bone histology, including an increase in number and size of osteoclasts, increased osteoblastic activity, enhanced resorption and osteoid surfaces. The 5/6Nx marmosets showed higher serum ALP activity and parathyroid cell hyperplasia throughout the study period. In addition, a transient increase of serum calcium and inorganic phosphorus levels was noted. Some of the 5/6Nx animals had bone resorptive changes with microfibrosis in the lumens of Haversian canals and subperiosteum in cortical bone and trabecular bone. An animal with a marked bone lesion also had higher levels of serum ALP activity and a higher grade of hyperplastic lesion of parathyroid. Hence the bone in the 5/6Nx marmosets might be in a high turnover state. On the other hand, 5/6Nx rats previously showed an increase of PTH and bone resorption but suppressed bone formation⁴². During progressive CRF development in 5/6Nx rats, the total plasma calcium and phosphate concentrations were almost unchanged and ALP levels were reduced⁴³.

Calcitriol administration was associated with a significant suppression of bone resorption and a marked increase in all osteoid parameters in the CRF condition^{43, 44}. The common marmoset has extremely high circulating levels of 1,25-(OH)₂D₃ without exhibiting hypercalcemia^{45, 46} and has an abnormal 1,25-(OH)₂D₃ receptor system, suggesting that the marmoset monkey has end-organ resistance to 1,25(OH)₂D₃. Common marmosets at one year of age with low serum 25-hydroxyvitamin D₃ and 24R, 25-dihydroxyvitamin D₃ low calcium levels and high ALP levels may exhibit osteomalacia-like bone changes resembling vitamin D-dependent rickets type II⁴⁷⁻⁴⁹. The incidence of bone abnormalities in marmosets dramatically declines, especially in young, growing animals. This may be due to the large quantities of vitamin D₃ in the commercial diet (2750 IU of vitamin D₃ in 100 g of CSM-1M pellets). The 5/6Nx marmosets in the present study showed a decrease in body weight immediately after 5/6Nx surgery; however, their body weights were stable, and feeding did not cause any marked difference between the 5/6Nx animals and control animals (data not shown). Hence, the osteodystrophy-like changes in the 5/6Nx marmosets were probably secondary to renal failure and not nutritional in origin, although feeding of a high vitamin D₃ diet may have affected the grade of the bone lesions. Control of the vitamin D₃ concentration in the diet may influence bone lesion progression in 5/6Nx marmosets.

In chronic kidney disease (CKD) patients, a relationship between disorders of mineral metabolism (elevated phosphorus levels), abnormal bone (osteodystrophy), and vascular calcification appeared⁵⁰. In the 5/6Nx marmosets, a transient increase of serum calcium and inorganic phosphorus levels was noted, and the levels gradually returned to normal by the end of the study. Hence, most of the 5/6Nx animals at weeks 13 and 26 did not show systemic mineralization including the vascular system (excluding two animals). One 5/6Nx animal at week 26 and the moribund

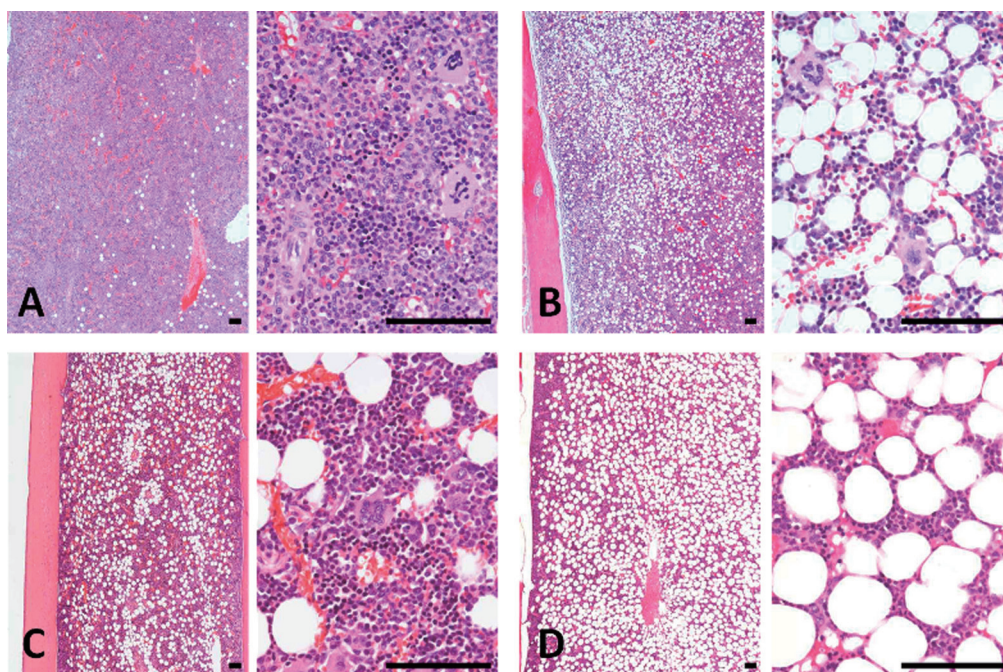


Fig. 6. Photomicrograph of femoral bone marrow changes in non-treated control marmosets at weeks 13 (A) and 26 (C) and 5/6Nx marmosets at weeks 13 (B) and 26 (D). An increase in adipose tissue with a decrease in marrow cells appeared at week 13 (B) and 26 (D) in the 5/6Nx animals compared with each of the histological structures in non-treated control animals, respectively. Animal Nos.: A, 006; B, 107; C, 009; D, 113. H-E stain. Scale bar = 100 μ m

animal at week 14 showed mineralization in the medial artery and tracheal and/or bronchial cartilage with hyperparathyroid-related bone changes. The 5/6Nx animal at week 26 revealed hypercalcemia (5.1 mg/dL vs 4.3 to 4.5 mg/dL in control animals). On the other hand, the moribund animal might have been in a severe renal failure state due to a shift to hydronephrosis.

In the other biochemical parameters, the 5/6Nx marmosets demonstrated slight changes in liver function markers, showing higher values of AST and phospholipids at weeks 5 and 26 and higher values of total cholesterol, triglyceride and total bilirubin at week 26. Liver metabolism dysfunction could also play a role in the lipid profile changes encountered in the CRF rats: increased values of total cholesterol and tryglycerides⁵¹. Lipid abnormalities are observed in CKD patients^{52, 53} and are a risk factor for cardiovascular events in CKD patients⁵⁴. The lipid changes encountered in CKD patients might be reproduced in 5/6Nx marmosets.

Anemia is a common complication of CKD. In the 5/6Nx marmosets, the red blood cell counts and hemoglobin and hematocrit values were lower at week 5. Moreover, the reticulocyte count was also lower, which suggests a failure of the erythropoietin (EPO) response mechanism due to putatively insufficient erythropoietin production associated with the renal mass reduction. At week 13, despite the increase in reticulocyte counts, cellularity in the femoral bone marrow was reduced at weeks 13 and 26. Mechanisms involved in the pathogenesis of renal anemia include deficiency of EPO, chronic inflammation, iron deficiency and a shortened half-

life of erythrocytes. Although the prevalence of anemia increases with diminishing renal function, normochromic and normocytic anemia is observed at a relatively early stage of renal dysfunction. Hypoplasia of the erythroid precursors usually found in the bone marrow with normal leukopoiesis and megakaryocytopoiesis⁵⁵. Renal EPO is mainly produced by the interstitial fibroblast in the deep cortex and outer medulla in the kidney^{56, 57}. Patients with renal failure may develop anemia and refractory EPO because of mechanisms associated with chronic inflammation⁵⁸. In addition, hepcidin that evolves as a potent regulator of the body's iron distribution, is indicated as a potential of dysregulation of iron metabolism in CKD patients^{59, 60}, and excessive hepcidin production contributes to the functional iron deficiency and associated anemia during inflammation states. Hence, the continuous chronic inflammation in the remnant kidney of 5/6Nx marmosets might contribute to the pathogenesis of renal anemia by decreasing EPO production and sensitivity or the available iron for hematopoiesis.

We performed 5/6Nx in female marmosets and observed the pathophysiological conditions of renal disorder and chronic renal failure for 26 weeks. The 5/6Nx marmosets showed a significant increase in serum parameters correlated with the renal disorder and renal failure immediately after the 5/6Nx surgery. The changes in these renal disorder parameters occurred slowly. In the remnant kidneys, tubulointerstitial changes comprised degenerative or atrophic renal tubules surrounded by an interstitial fibrosis area with mononuclear cell infiltration remained for a long time.

There were no progressive glomerular changes including glomerulosclerosis. In addition, hyperplastic parathyroid glands, a high turnover state of osteodystrophy in the femoral bone with higher serum ALP activity and anemia with slight hypocellularity of bone marrow were evident. During the progression of CRF, the state, intensity or grade of complicating morbidity is influenced by many factors such as systemic hypertension, inflammation, uremic toxins, physical condition, aging and nutritional condition. By regulating or applying the loads of these factors, 5/6Nx marmosets may develop a pathophysiological condition more similar to the clinical state of human CRF patients.

The 5/6Nx marmosets showed a stable CRF condition for a long time and showed some characteristic disorders similar to those in human CRF patients. These diagnostic aspects might be a species-specific anatomical and physiological signature reflecting the nutritional condition. Although it is necessary to study and obtain more detailed information about biological features including age and gender, the CRF model using 5/6Nx marmosets might become a useful method of evaluating the unique mechanism of CRF development.

Declaration of Conflicting Interest: There are no conflicts of interest to declare or financial interests to disclose.

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