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Minor age difference substantially affects renal function in conscious and anaesthetized rats*

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In this study we re-examined the postulate that in the rat age-related body metabolism and kidney function changes progress only slowly. Thus we checked whether a moderate between-animal age distance may cause any marked difference, which could affect interpretation of experimental results. Food and water consumption, renal sodium and potassium excretion and haemodynamics were lower in rats aged 15 versus 9 weeks. In turn, hematocrit, mean arterial pressure, and renal vascular resistance were significantly higher in older animals, whereas renal excretion of NO3-/NO2- and vascular endothelial growth factor A and in situ renal tissue NO signal did not differ. Evidently, an age older by 6 weeks was associated with decreased hydration, tone of systemic and renal blood vessels, and renal excretion. In conclusion, since the actual age is an important determinant of the animal's functional status, accurate age-matching of experimental groups is a necessary pre-condition for correct data interpretation.

KEY WORDS: animals' age / nitrate/nitrite / renal excretion / renal hemodynamics / Sprague Dawley

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Currently rats are the most commonly used animals in scientific experiments, accounting for about 20% of the total number of mammals used [Sengupta 2013, Jama et al. 2022]. Among other things, this is because of the relatively short period of their reproduction and rapid development in early age. They reach sexual maturity at about 6 weeks of age and social maturity 5-6 months later, while transition to adulthood begins at the age of ~9 weeks (young adult). Importantly, it is relatively easy to monitor the age of the animals on the basis of their body weight (using growth charts). At the early stage of development their weight well correlates with age, whereas later this relationship is not so definite [Sengupta 2013].

Moreover, during the aging process the genetic background, environmental and dietary factors as well as comorbidities also play a role [Martin and Sheaff 2007]. All these factors co-determine changes in anatomy, physiology and developmental processes; thus, they need to be taken into account not only when forming groups for long-term studies, but also when analyzing the research results [Sengupta 2013].

Outbred Sprague-Dawley rats are extensively used in animal models of both human and animal disorders, including diabetes, cardiovascular and renal disease [Benson *et al*. 2007]. A decrease in kidney function with age, recognized on the basis of changes in diverse parameters is well documented in humans and described in detail in various animal species (mouse, rat, dog) using an array of methodologies. The renal function status is usually taken into account when choosing a therapy for many diseases, considering the kidneys' role in removing waste products and controlling the blood level of many important molecules [Martin and Sheaff 2007].

It is commonly believed that age-related changes in the kidneys progress slowly and the renal function for a long time remains adequate for survival [Baylis and Corman 1998]. However, it has been shown that renal blood flow decreases with age, both in absolute terms and in proportion to the cardiac output. It has also been suggested that the kidney's blood flow autoregulatory capacity decreases with age and so does the sensitivity of renal arterioles to a number of vasoactive agents including nitric oxide (NO) [Martin and Sheaff 2007]. When renal regional blood flow and tissue NO were determined simultaneously, the NO effect to help maintain adequate blood perfusion was more pronounced in the medulla [Grzelec-Mojzesowicz and Sadowski 2007]. Thus, in the medulla exposed to low tissue pO_2 levels and liable to the consequences of ischaemia, maintaining the NO-mediated vasodilator influence may be of special importance. However, NO deficiency was demonstrated in aging male Sprague Dawley rats. This was found to correlate with the development of endothelial dysfunction, as well as kidney structural and functional decline [Baylis 2012].

Other substances involved in the etiology of age-related deterioration of kidney function include cytokines, growth factors, proliferation stimulants and apoptotic factors, reactive oxygen species, transcription factors, and advanced glycation end-products (AGEs). Moreover, in male rats age-dependent kidney changes may be related to androgen production. Interestingly, proteinuria is an indicator of progressive decline in renal function, but is not associated with aging *per se* [Martin and Sheaff

2007]. In general, the age difference of several months rather than several weeks was used in rodent kidney function studies that focused on the role of age [Reckelhoff *et al.* 1992, Lim *et al.* 2012].

In accordance with the experience of many researchers, including our group, the average weight of an animal taken for research was 200-330 g [*e.g.* Pflueger *et al*. 1995, Xie *et al.* 2012, Nassi *et al.* 2016, Bądzyńska *et al.* 2021, Kuczeriszka *et al.* 2022]. Such animals are sufficiently large for the surgery to be successfully performed without the need for specialized optical equipment and small enough to use small amounts of tested substances that are usually expensive. Unfortunately, in many studies the only information provided was the animals' weight rather than their age [*e.g.* Pflueger *et al*. 1995, Cardenas *et al*. 2013, Nassi *et al*. 2016], neglecting the fact that for instance male rats weighing between 250 g and 274 g may differ in age by 3 weeks [Sengupta 2013].

For our experimental study (in progress) concerning the effects of some active substances on basic metabolic parameters and renal function in Sprague-Dawley rats we needed to compare two groups of untreated rats, aged 9 and 15 weeks. In one group basic metabolic parameters in conscious animals (body weight, food and water intake, etc.), blood pressure and renal excretion were followed from the 7th to 9th week of age (Fig. 1A). In the other group similar chronic experiments were conducted from the 7th to the 15th week of age. At the end of each of the two studies renal hemodynamics was assessed in an acute experiment under anesthesia (Fig. 1B). We intended to confirm whether the slight between-group age difference could cause any marked differences in the animals' metabolism and renal function, thus potentially affecting the interpretation of the results.

Material and methods

Animals

The experiments were conducted on sixteen male Sprague-Dawley rats (Tac:Cmd:SD) bred in the animal house of the Mossakowski Medical Research Institute, Warsaw, Poland, aged 6 weeks, with free access to tap water and standard dry pellet rat diet (0.25% Na w/w, SSINFF GmbH, Soest, Germany). The rats were housed in groups of 2-4 animals in cages with environmental enrichment under 12:12 h light-dark cycle, and temperature 22-23°C. Before the start of observations with conscious animals (the chronic part of the study, Figure 1A) for one week the rats were accustomed to 24h observation in metabolic cages and to tail vein blood sampling, as well as the presence of the staff involved in chronic studies. We also refined the technique of rats' immobilization needed to puncture the tail vein to sample blood in order to make the procedure less stressful. The rats were immobilized in Plexiglas restrainer tubes placed in warming chambers (Model RCB II; IITC Inc., Woodland Hills, CA, USA) (32°C) for 10-15 min; this helped to dilate the veins and made them well visible, which shortened sampling time.

The animals (n = 16), aged 7 weeks, were randomly assigned to two experimental groups (Fig. 1), thus on the day of the acute experiment animals were aged 9 weeks (65±1 days, younger group) and 15 weeks (108±2 days, older group); with body weight 317±5 g and 389±8 g, respectively (Fig. 1A, right panel).

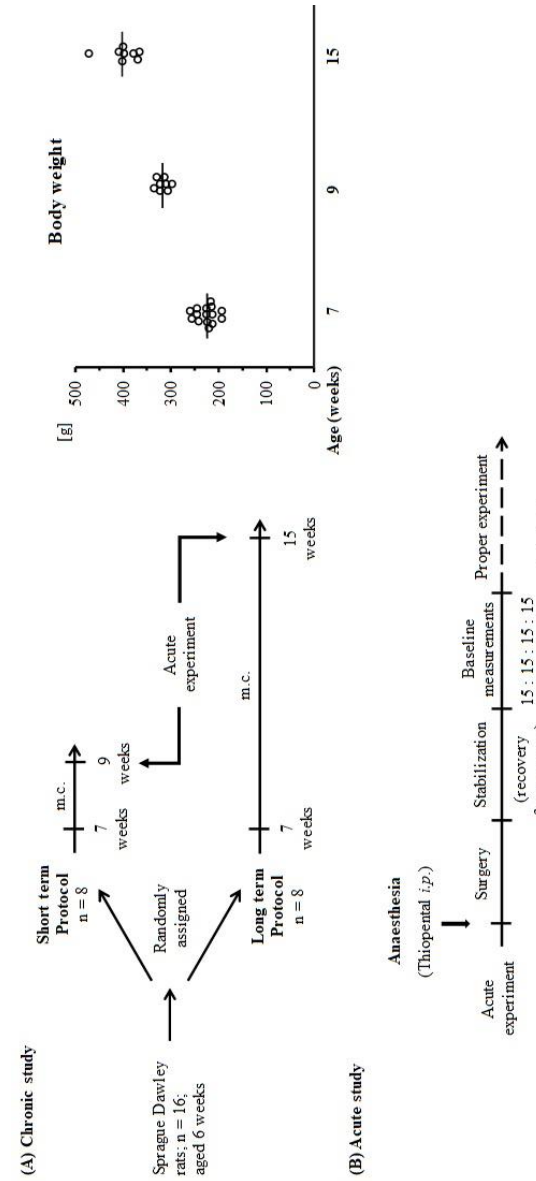

Fig. 1. The scheme of the study. A) Chronic part of the study. One week before the rats reached 7 weeks of age, they were adapted to experimental conditions and thereafter randomly assigned to short- and long term protocol. B) Acute part of the study - anaesthetized rats aged 9 and 15 weeks were surgically prepared for kidney function measurements. In the right panel, the body weight values of animals aged 7, 9 and 15 weeks are shown as a distribution of individual differences (dotplot) and age-group means (solid line). m.c. - timed 24-hour metabolic cage observation; last m.c. measurements were made 3-4 days before acute experiments in rats aged 9 and 15 weeks.

Chronic study

The animals were kept for 24 hours in metabolic cages (Tecniplast S.p.A., Buguggiate, Italy) under feeding and metabolism control measurement performed on rats aged 7 weeks and 3-4 days before the acute experiment (Fig. 1A). The rats' body weight, food and water intake and urine excretion were monitored. Urinary sodium and potassium levels were measured to determine the corresponding renal excretion rates. Thereafter, the animals were taken out of the metabolic cage and after 2-hour food deprivation their hematocrit level, plasma osmolality, and sodium and potassium concentrations were measured in tail vein blood samples. Withdrawal of the rats from the metabolic cage and blood sampling were performed between 10 *a.m*. and 1 *p.m.*

Acute study

After completing the chronic part of the study acute experiments were performed both in the younger and older groups under anesthesia (thiopental sodium, 100 mg/kg *i.p.*; Thipen, Samarth, Mumbai, India) to measure renal hemodynamics and excretion (Fig. 1B). The anesthesia and experimental preparation were started at 9-10 *a.m*.

The details of surgical preparation were described earlier [Dobrowolski *et al.* 2007, Kuczeriszka *et al.* 2019]. Briefly, the jugular vein was cannulated for fluid infusion and the carotid artery for arterial blood pressure measurement (Stoelting blood pressure meter and transducers, Wood Dale, Illinois, USA). The left kidney was exposed from a subcostal flank incision and placed in a plastic holder, similar to that used for kidney micropuncture. The ureter was cannulated for the timed urine collection to measure urine flow and to determine urine osmolality and sodium and potassium excretion. During surgical preparations and experimental procedures rats were placed on a heated surgery table and rectal temperature was maintained at about 37°C.

The total renal blood flow was measured using an ultrasound non-cannulating renal artery probe (flowmeter type T106; Transonic System Inc., Ithaca, NY, USA). Perfusion of individual kidney zones was measured as laser-Doppler fluxes. For the upper cortex (cortical blood flow) a PF 407 probe was placed on the kidney surface (Periflux 5010 flowmeter, Perimed, Jarfalla, Sweden). For the outer and inner medulla the flows were measured using two PF 402 needle probes inserted to the depths of about 3-4 and 5-6 mm, respectively, the exact depth depending on the kidney size. For amperometric detection of tissue NO signal in the renal medulla a needle-shaped ISO-NOP 200 sensor (0.2 mm in diameter, 5 mm sensing length) held in a micromanipulator was inserted into the kidney to the depth of 5-7 mm from the kidney surface (for technical details see Grzelec-Mojzesowicz and Sadowski [2007]). The sensor based on a carbon fiber working electrode coated with a proprietary gas permeable selective membrane and Ag/AgCl reference electrode was connected to a Free Radical Analyzer (TBR4100, World Precision Instruments, Sarasota, Florida, USA).

At the end of the experiment the rats were euthanized and the position of the intrarenal probes was checked at the kidney cross-section (for details see Walkowska *et al*. [2015]). After completing surgical preparation and placement of the renal

probes, a min. 60-min period was allowed for stabilization of the parameters recorded. Thereafter, four 15-minute periods of urine collection (U1 to U4) were made to determine renal excretion and hemodynamics to be used as a baseline before the proper experiment was started, see Figure 1B.

Analytical procedures and calculations

To determine hematocrit, capillaries filled with blood were centrifuged at 13000 r.p.m. The remaining blood was then spun at 3000 r.p.m for 20 min and the separated plasma was used to determine plasma potassium and sodium concentrations. Urine volumes were determined gravimetrically. Urine osmolality was measured with an cryoscopic osmometer (Osmomat 030, Gonotec, Berlin, Germany). Plasma and urinary concentrations of sodium and potassium were measured using a flame photometer (BWB-XP, BWB Technologies Ltd, Newbury, UK). Excretion parameters were calculated from the usual formulas and standardized to body weight (conscious) or kidney weight (anaesthetized rats). NO_3^-/NO_2^- and vascular endothelial growth factor A (VEGFA) concentration in urine samples from metabolic cages were determined using assay kits (Nitrate/Nitrite Colorimetric Assay Kit, Cayman, Ann Arbor, Mi, USA; Rat VEGFA Elisa Kit, DLdevelop, Wuxi, China).

Statistical Analysis

The number of rats (n=8) for each group was established as the minimum needed to obtain statistical significance. Data were verified for normal distribution. Statistical significance between the experimental groups was determined applying a two-tailed Student's *t-test* for unpaired samples. $P<0.05$ value was considered statistically significant (STATISTICA, version 10.0, StatSoft Inc., USA).

Results and discussion

Only statistically significant parameter differences are presented below; other data are shown in Table 1. The data for water and food intake and sodium and potassium excretion in the chronic part of the experiment were standardized to body weight (expressed per 300 g of animal body weight). Because the focus was on the effects of age alone, normalizing by animal weight would clearly amplify the age-related effects.

The main differences between the 9-week old (younger) and 15-week old (older) rats detected during their metabolic cage observation are summarized in Figure 2. Almost all the parameters presented (food and water intake, urinary sodium and potassium excretion, as well as plasma potassium) were lower in the older than in the younger rats. For hematocrit only the change was different, the older rats had higher hematocrit than the younger animals (42.9±0.6 *vs.* 45.3±0.8%). To prove that changes in daily renal excretion parameters are age-related rather than body weight dependent an additional comparison was performed between the rats' weight and sodium and potassium excretion. It showed that alterations in renal excretion and body weight

Table 1. Parameters of blood and plasma (tail vein samples, upper section) and the data of 24 h observations in metabolic cages (lower section) for samples obtained from rats aged 7 weeks ("Initial") and 3 - 4 days before acute experiment ("Final") in male Tac:Cdm:SD rats aged 9 and 15 weeks

Item	Metabolic cage weeks	Initial	Final
		blood	
Body weight (g)	9	237±6	317±5*
	15	213±5	400±12*#
Glycaemia (mg/dL)	9	168±6	152±6
	15	174±10	168±10
Hematocrit (%)	9	43±1	43±1
	15	41±0	45±1*#
Plasma osmolality (mosmol/kg H2O)	9	295±4	298±3
	15	293±3	304±2*
Plasma sodium (mmol/L)	9	135±1	136±2
	15	133±1	133±1
Plasma potassium (mmol/L)	9	6.2±0.3	5.1±0.2*
	15	5.8±0.3	4.3±0.2*#
		metabolic cages	
Food intake (g/24 h/300 g BW)	9	31±2	22±1*
	15	29±2	15±2*#
Water intake (ml/24 h/300 g BW)	9	45±4	34±4*
	15	46±3	22±2*#
Urine flow (ml/24 h/300 g BW)	9	15±1	11±1*
	15	16±3	10±1*
Urine osmolality (mosmol/kg H_2O)	9	1947±88	2046±114
	15	1843±161	2071±125
Total solute excretion (mosmol/24 h/300 g BW)	9	25±2	21±1
	15	37±2	19±2*
Urine sodium excretion (mmol/24 h/300 g BW)	9	2.3±0.1	1.8±0.1*
	15	2.3±0.2	1.2±0.1*#
Urine potassium excretion (mmol/24 h/300 g BW)	9	7.4±0.6	5.6±0.3*
	15	7.1 ± 0.6	3.6±0.5*#

BW - body weight.
Values are means ± SEM; n = 8; *significantly different from the corresponding "Initial" metabolic cage; #significantly different from the corresponding 9th week value.

have a very low correlation coefficient (r^2= -0.324 or -0.203 for sodium or potassium excretion, respectively). Daily sodium and potassium excretion measured in metabolic cages once a week during the long-term observation is presented in Figure 3. Taken together, we demonstrated clearly that renal excretion is dependent on age rather than body weight.

It is noteworthy that the above between-group differences were not associated with any difference in daily renal NO_3^-/NO_2^- excretion, which reflects mostly NO production in the kidney. Moreover, we did not observe differences in the level of VEGFA excretion during the chronic study, which would reflect no age-related changes in renal angiogenesis (Fig. 4A). Interestingly, body weight increased despite a progressing decrease in food intake, which indicates improving food conversion (see Fig. 5).

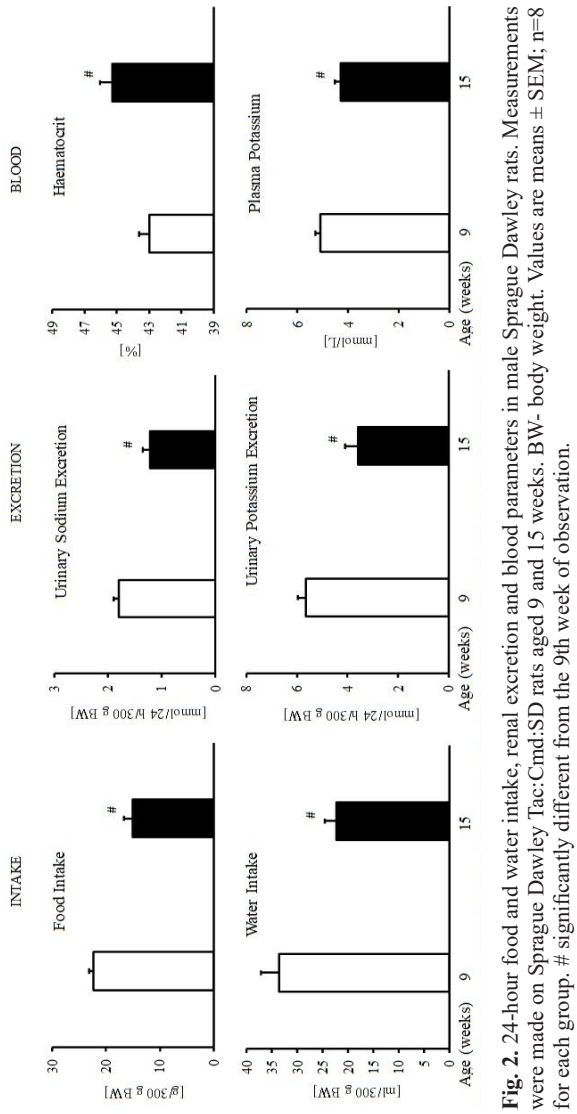

Fig. 2. 24-hour food and water intake, renal excretion and blood parameters in male Sprague Dawley rats. Measurements were made on Sprague Dawley Tac:Cmd:SD rats aged 9 and 15 weeks. BW- body weight. Values are means ± SEM; n=8 for each group. # significantly different from the 9th week of observation.

In anaesthetized animals (note: data standardized to kidney weight) the renal excretion depended on age, similarly as seen in conscious rats (Fig. 6A, Tab. 2). Only urine osmolality was clearly lower in younger rats, whereas both urinary sodium and potassium excretion and urine flow were higher. Interestingly, the time required for the stabilization of most parameters was longer in the younger than in older animals. Of some interest was the between-group difference in the pattern of changes in the medullary amperometric NO signal: in the 15-week old group it increased from the

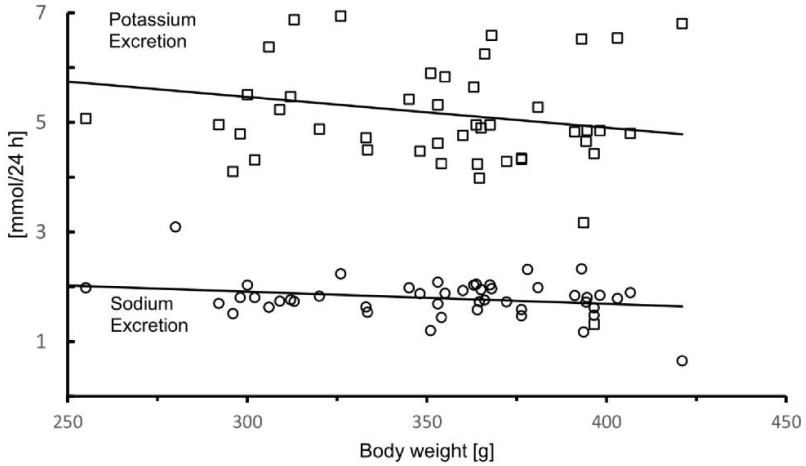

Fig. 3. Body weight and 24-hour renal excretion parameters in male Sprague Dawley Tac:Cmd:SD rats during six weeks of observation. Values are raw data of daily urinary sodium (o) or potassium (□) excretion for eight rats. Measurements were made during weekly metabolic cage observations on rats aged between 9 and 15 weeks. R^2 was -0.263 or -0.185 for sodium or potassium excretion (both NS), respectively.

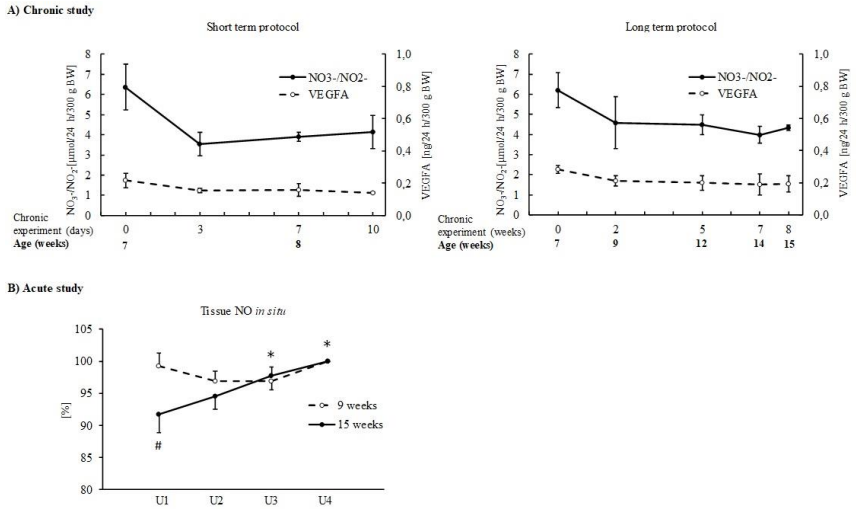

Fig. 4. NO_3^-/NO_2^- and VEGFA excretion during chronic observation and renal tissue NO measured in anaesthetized rats. Measurements were made on male Sprague Dawley Tac:Cmd:SD rats aged 9 and 15 weeks. (A) Measurements were made in urine samples using Cayman Elisa Kit (NO3-/NO2-) and DLdevelop Elisa Kit (VEGFA). (B) The signal of NO selective probes placed in renal tissue was recorded simultaneously with renal hemodynamics and excretion for one hour (four periods, 15 min each, U1-U4). BW - body weight. Values are means ± SEM, n = 8 for each group; * significantly different from U1, # significantly different from the 9th week value.

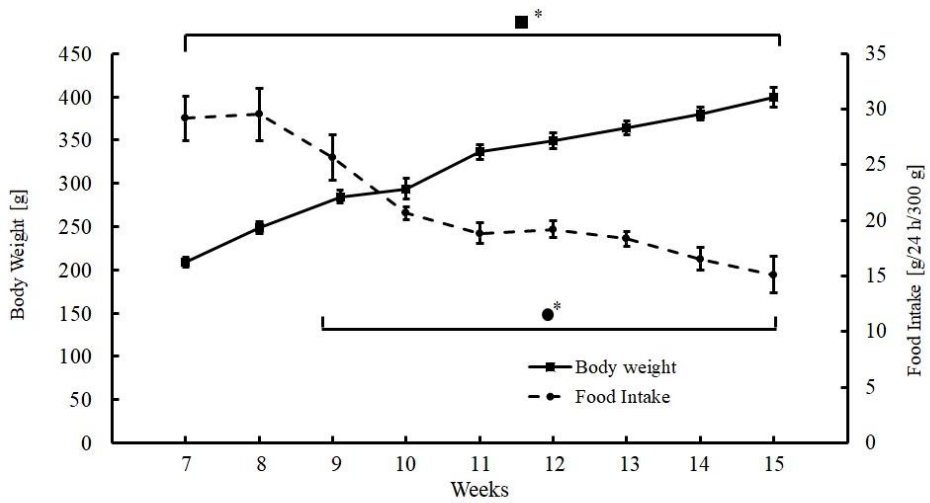

Fig. 5. Changes of body weight (left Y-axis) and 24 hour food intake (right Y-axis) in male Sprague Dawley Tac:Cmd:SD rats. Values are means ± SEM; n = 8 for each time point. ●*, ■* each point value on the curves significantly different from the corresponding 7th week value.

very beginning and in the 9-week old group an ultimate increase was preceded by a decreasing tendency (Fig. 4B).

In contrast to age-dependent fluctuations in renal excretion, hemodynamic parameters remained stable throughout the urine collection time (Fig. 6B, Tab. 2). Interestingly, the renal (whole kidney) and (upper) cortical blood perfusion rates were lower in the older rats; nonetheless, no difference between the age groups was shown for medullary perfusion both in the outer and the inner medullae. Since older rats had lower renal blood flow, but higher mean arterial blood pressure, the calculated renal vascular resistance was substantially elevated compared with younger rats.

Our results demonstrate that even though the parameter values were determined at not too distant time interval (6 weeks), a number of physiological and metabolic variables showed distinct age-dependent differences. More specifically, there was an age-dependent reduction of food and water intake and the renal excretion of sodium and potassium.

Interestingly, despite free access to water, older rats showed hemoconcentration (higher hematocrit). Thus, the association of enhanced hematocrit with a lowered plasma potassium concentration may suggest that among other changes in our rats vasopressin levels decreased with age. A similar tendency was reported by the Ihedioha group [2004] in another Sprague-Dawley breed; however, their study focused on the difference between age-matched males and females over a period of 3 to 72 weeks of age. On the other hand, Petterino and Argentino-Storino [2006] found no age-related differences in hematocrit or plasma potassium concentration between rats aged 4 and 13 weeks. However, it could be so that age related hemoconcentration shown in our study

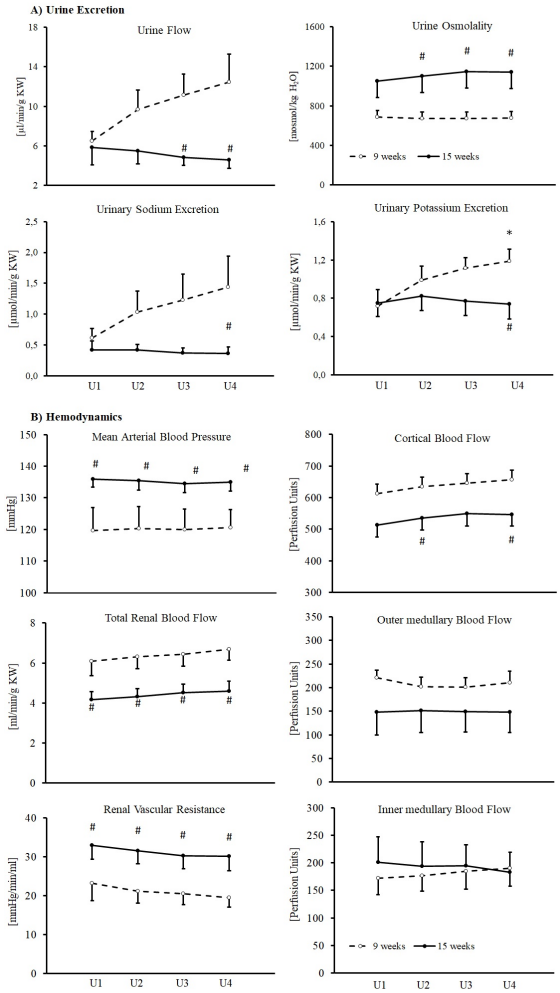

Fig. 6. Changes of renal excretion and systemic and kidney hemodynamics measured in anaesthetized rats. Measurements were made on male Sprague Dawley Tac:Cmd:SD rats aged 9 and 15 weeks. Mean arterial blood pressure and renal function measurements were made simultaneously for one hour (four 15-min periods, U1-U4). KW – kidney weight. Values are means ± SEM, n = 8. * significantly different from U1. # significantly different from the group of rats aged 9 weeks.

was a direct consequence of lower water intake, whereas separate decreased plasma potassium levels, without changes in plasma sodium and osmolality, could have resulted from separate alterations in this individual ion movement between cells, the interstitium and plasma. Nevertheless, it may not be completely excluded that the changes found in the current study are, at least in part, the effect of the differences in breeding (e.g. genetic drift) between our and other animal houses [Brower *et al.* 2015].

Table 2. Parameters of renal excretion and systemic and kidney hemodynamics measured in anesthetized male Sprague-Dawley Tac:Cmd:SD rats aged 9 and 15 weeks

Item	Weeks	U1	U2	U3	U4
		Hemodynamics			
Mean arterial blood pressure (mmHg)	9	120±7	120±8	120±6	120±7
	15	136±3#	135± #	134±3#	135±3#
Renal blood flow (ml/min/g Kw)	9	6.1±1	6.3±1	6.4±1	6.7±1
	15	4.2±0#	4.3±0#	4.5±0#	4.4±0#
Renal vascular resistance (mmHg/min/ml)	9	23±4	21±3	20±3	19±2
	15	33±4#	32±3#	30±3#	30±4#
Cortical blood flow (Perfusion Units)	9	612±30	635±29	645±30	657±30
	15	514±38	535±28#	549±39	546±37#
Outer medullary blood flow (Perfusion Units)	9	221±16	202±20	201±20	210±24
	15	148±49	151±47	150±44	148±43
Inner medullary blood flow (Perfusion Units)	9	172±30	177±29	185±33	191±33
	15	201±46	194±45	195±38	183±37
		Urine excretion			
Urine flow (µl/min/g KW)	9	6.5±1	9.7±2	11±2	12±3
	15	5.8±2	5.5±1	4.8±1#	4.6±1#
Urine osmolality (mosmol/kg H_2O)	9	688±56	675±66	675±67	679±66
	15	1051 ±188	1102±184#	1146±159#	1110±167#
Total solute excretion (µosmol/min/g KW)	9	4.4±1	6±1	7±1	7±1
	15	10±6	5±1	10±5	5±1
Urine sodium excretion (µmol/min/g KW)	9	0.6±0.2	1.0±0.3	1.2±0.4	1.1±0.3
	15	0.4±0.1	0.4±0.1	0.4 ± 0.1	0.4 ± 0.1#
Urine potassium excretion (µmol/min/g KW)	9	0.7±0.2	1.0±0.1	1.1±0.1	1.2±0.1
	15	0.8±0.1	0.8±0.2	0.8±0.2	0.7±0.2*#

Mean arterial blood pressure and renal function measurements were made simultaneously for one hour (four 15-min periods, U1-U4). KW – kidney weight.
Values are means ± SEM; n = 8; *significantly different from U1; #significantly different from the group assessed at the 9th week.

Higher blood pressure and lower total renal blood flow measured here in older rats compared with their younger counterparts indicate systemic and renal cortical vasoconstriction in the former, in accordance with the report by Martin and Sheaff [2007], with the difference in hemodynamics being associated with lower renal excretion. In contrast, in a study conducted on conscious male Sprague-Dawley rats (Harlan Sprague-Dawley) aged 3-5 months (young) *vs.* 18-20 months (old) no differences were found in baseline (control) arterial blood pressure and urinary sodium excretion [Baylis *et al*. 1997]. However, caution is required when comparing the results obtained in short-lasting experiments under anesthesia, as in our study, with the changes that develop in conscious animals over several weeks.

Regarding the renal glomerular structure and function the changes developing with age are not well understood. A progressive decline in glomerular function was reported and this was intensified by the increase in mean arterial pressure [Martin and Sheaff 2007]. In a histological evaluation including magnetic resonance histology [Xie *et al*. 2012] the glomeruli in younger (8 weeks old) compared to older rats (54 weeks old) were found to be smaller and more densely packed. The older kidneys

were more heterogeneous in structure, with signs of chronic progressive nephropathy (fibrosis and glomerulosclerosis). Our study shows that the initial changes in renal cortical circulation were visible already at the age of 15 weeks: the blood flow through the kidney cortex was reduced compared to that measured at the age of 9 weeks. Recently it was shown that similarly to the changes seen in systemic vessels of aging rats the intrarenal arteries could also develop arteriolosclerosis and these vascular changes might cause outer cortical glomerulosclerosis, with local tubular atrophy and interstitial fibrosis. In addition to morphological changes, the enhanced renal vasoconstriction is considered to be the effect of increased local acting factors involving angiotensin II, renal nerve activity, nitric oxide and endothelin. However, these age-related changes were compared between age-matched rats over a period much longer than in our study [Martin and Sheaff 2007].

In a normal kidney, VEGFA is constitutively expressed by glomerular podocytes and the cells of the proximal tubule and those of the medullary thick ascending limb. Changes in VEGFA expression have been reported in a variety of kidney diseases, possibly leading to excessive angiogenesis, glomerular dysfunction (hypertrophy) and proteinuria. VEGFA has been suggested to play a key role in the maintenance of renal integrity, including preservation of the microvasculature (architecture and function) [Kang and Johnson 2003, Patel and Thaker 2014]. In our study VEGFA excretion did not alter with age, which may suggest that a longer observation period is needed to see any age-dependent changes, and that VEGF was not involved in the lowering of cortical blood perfusion in the group of older rats.

Regarding the renal regional perfusion, we found no consistent age dependence for both the outer and inner medullary blood perfusion. This suggests that in the renal medulla, in contrast to the cortex, normal blood circulation is specifically protected within the age interval studied. One of the responsible factors could be the preserved NO status. As reviewed by Prabhakar [2005], under normal circumstances the synthesis of NO is much greater in the renal medulla than in the cortex. In the kidney, NO is a very important regulator of blood flow and it helps maintain body sodium and water homeostasis [Prabhakar 2005]. In the current work we noticed no differences in renal NO_3^-/NO_2^- excretion or NO availability in the medullary tissue *in situ* between the younger and older rats, which may indicate preservation of the NO status in the renal medulla. This would help maintain adequate blood perfusion at this age. Recently, distinct changes in NO_3^-/NO_2^- excretion were reported, but this was seen at the rats' age interval of several months and not weeks [Baylis 2012]. However, the data available here do not justify any attempts to assess the mechanism(s) of such protection.

We observed here that a small age difference not only affected actual renal function; interestingly, it was seen that younger animals needed more time to obtain stable baseline renal excretion measurements. This was shown despite the time of surgery and recovery period not differing between the animals. Apparently, stabilization of the measured parameters after surgical intervention may vary with age. One possible

explanation could be that the pharmacodynamics of the given anesthetic may differ depending on different body constitution related to the animals' age, despite the same dose being given to all animals (body weight calculated). In younger (lean) animals all of the intraperitoneal given anesthetic could be absorbed into the blood vessels, whereas in older (fatter) rats the drug could be distributed at first between the accumulated fat tissue and vasculature. In consequence, in younger animals distinctly higher plasma anesthetic peak after injection compared to that in older rats could be expected and the response to anesthesia and then the surgery stimulus may be greater and take a longer, and more variable time to stabilize compared with older rats.

When designing the experimental setting, the actual phase of growth should be taken into account: whether the animals are in the adolescence period, i.e. after the eighth week of life, or in the aged phase, which starts between 15 and 20 months of life [Sengupta 2013]. It is noteworthy that in the course of long studies small differences may be blurred; however, much of the research related to kidney function was done within a relatively narrow age range, where even small differences may become significant. It is of utmost importance which parameter is analyzed: this crucially determines the presence, direction and extent of the age-related changes. In our young adults, 9- and 15-week old rats, the age difference of 6 weeks perceptibly changed some parameters, such as food and water intake, renal sodium and potassium excretion or renal total and cortical blood flow. Some other parameters, e.g. outer and inner medullary blood flow or renal NO_3^-/NO_2^- excretion, did not differ.

Conclusions

We showed that in our outbred Sprague-Dawley rats even a slight difference in their age resulted in significant differences in most metabolic and renal function parameters. Therefore, analysis and interpretation of the experimental data derived from rats with different duration of observations (for example 6 weeks) should be made with caution, and take into account the effect of both factors: duration of the disease and the actual age. The risk of misinterpretation critically depends on the actual parameter studied. Nevertheless, it seems unlikely that the mechanism of the age dependent differences is related to renal NO and/or VEGF alterations.

In summary, we would like to emphasize here the importance of appropriate planning for the characteristics of each control and experimental group. In addition to the animals' genetic background (strain/stock), sex, and body mass, the exact age of the animals should also be clearly defined and adequately reported.

Ethics approval and consent to participate

The experimental procedures were approved by the First Local Ethical Committee, Warsaw, Poland, and were following the European Union Directive 63/2010, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

REFERENCES

1. BAYLIS C., ENGELS K., HYMEL A., NAVAR L.G., 1997 – Plasma renin activity and metabolic clearance rate of angiotensin II in the unstressed aging rat. ***Mechanisms of Ageing and Development*** 97, 163-71.
2. BAYLIS C., CORMAN B., 1998 – The Aging Kidney: Insights from Experimental Studies. ***Journal of American Society of Nephrology*** 9(4), 699-709.
3. BAYLIS C., 2012 – Sexual dimorphism: the aging kidney, involvement of nitric oxide deficiency, and angiotensin II overactivity. ***Journals of Gerontology Series A: Biological Sciences Medical Sciences*** 67(12), 1365-72.
4. BĄDZYŃSKA B., VANECKOVA I., SADOWSKI J., HOJNÁ S., KOMPANOWSKA-JEZIERSKA E., 2021 – Effects of systemic and renal intramedullary endothelin-1 receptor blockade on tissue NO and intrarenal hemodynamics in normotensive and hypertensive rats. ***European Journal of Pharmacology*** 910, 174445.
5. BENSON V.L., MCMAHON A.C., LOWE H.C., KHACHIGIAN L.M., 2007 – The streptozotocin-treated Sprague-Dawley rat: a useful model for the assessment of acute and chronic effects of myocardial ischaemia reperfusion injury in experimental diabetes. ***Diabetes and Vascular Disease Research*** 4(2), 153-154.
6. BROWER M., GRACE M., KOTZ C.M., KOYAL V., 2015 – Comparative analysis of growth characteristics of Sprague Dawley rats obtained from different sources. ***Laboratory Animal Research*** 31(4), 166-173.
7. CARDENAS A., TOLEDO C., OYARZUN C., SEPULVEDA A., QUEZADA C., GUILLÉN-GÓMEZ E., DÍAZ-ENCARNACIÓN M.M., PASTOR-ANGLADA M., MARTÍN R.S., 2013 – Adenosine A2B receptor-mediated VEGF induction promotes diabetic glomerulopathy. ***Laboratory Investigation*** 93, 135-44.
8. DOBROWOLSKI L., KOMPANOWSKA-JEZIERSKA E., WALKOWSKA A., SADOWSKI J., 2007 – Sodium intake determines the role of adenosine A2 receptors in control of renal medullary perfusion in the rat. ***Nephrology Dialysis Transplantation*** 22 (10), 2805-09.
9. Grzelec-Mojzesowicz M., Sadowski J., 2007 – Renal tissue NO and intrarenal haemodynamics during experimental variations of NO content in anaesthetised rats. ***Journal of Physiology and Pharmacology*** 58, 149-63.
10. IHEDIOHA J.I., OKAFOR C., IHEDIOHA T.E., 2004 – The haematological profile of the Sprague-Dawley outbred albino rat in Nsukka, Nigeria. ***Animal Research International*** 1(2), 125-132.
11. JAMA H.A., MURALITHARAN R.R., XU C., O'DONNELL J.A., BERTAGNOLLI M., BROUGHTON B.R.S., HEAD G.A., MARQUES F.Z., 2022 – Rodent models of hypertension. ***British Journal of Pharmacology*** 179(5), 918-37.
12. KANG D.H., JOHNSON R.J., 2003 – Vascular endothelial growth factor: a new player in the pathogenesis of renal fibrosis. ***Current Opinion in Nephrology and Hypertension*** 12, 43-9.
13. KUCZERISZKA M., WALKOWSKA A., OLSZYNSKI K.H., RAFALOWSKA J., SADOWSKI J., KOMPANOWSKA-JEZIERSKA E., 2019 – Arginine and tetrahydrobiopterin supplementation in rats with salt-induced blood pressure increase: minor hypotensive effect but improvement of renal haemodynamics. ***Journal of Physiology and Pharmacology*** 70 (2), 219-27.
14. KUCZERISZKA M., DOBROWOLSKI L., WALKOWSKA A., BARANOWSKA I., SITEK J.D., KOMPANOWSKA-JEZIERSKA E., 2022 – Role of Ang1-7 in renal haemodynamics and excretion in streptozotocin diabetic rats. ***Clinical and Experimental Pharmacology and Physiology*** 49(3), 432-41.
15. LIM J.H., KIM E.N., KIM M.Y., CHUNG S., SHIN S.J., KIM H.W., YANG C.W., KIM Y.S., CHANG Y.S., PARK C.W., CHOI B.S., 2012 – Age-associated molecular changes in the kidney in aged mice. ***Oxidative Medicine and Cellular Longevity***. Article ID 171383. doi: 10.1155/2012/171383

16. MARTIN J.E., SHEAFF M.T., 2007 – Renal ageing. ***Journal of the Pathological Society of Great Britain and Ireland*** 211(2), 198-205.
17. NASSI A., MALORGIO F., TEDESCO S., CIGNARELLA A., GAION R.M., 2016 – Upregulation of inducible NO synthase by exogenous adenosine in vascular smooth muscle cells activated by inflammatory stimuli in experimental diabetes. ***Cardiovascular Diabetology*** 15(1), 1-11.
18. PATEL L., THAKER A., 2014 – The effects of adenosine A2B receptor inhibition on VEGF and nitric oxide axis-mediated renal function in diabetic nephropathy. ***Renal Failure*** 36(6), 916-24.
19. PETTERINO C., ARGENTINO-STORINO A., 2006 – Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. ***Experimental and Toxicologic Pathology*** 57(3), 213-19.
20. PFLUEGER A.C., SCHENK F.R., OSSWALD H.A., 1995 – Increased sensitivity of the renal vasculature to adenosine in streptozotocin-induced diabetes mellitus rats. ***American Journal of Physiology Renal Physiology*** 269(4), F529-35.
21. PRABHAKAR S.S., 2005 – Pathogenic role of nitric oxide alterations in diabetic nephropathy. ***Current Diabetes Reports*** 5(6): 449-54.
22. RECKELHOFF J.F., SAMSELL L., DEY R., RACUSEN L., BAYLIS C., 1992 – The effect of aging on glomerular hemodynamics in the rat. ***American Journal of Kidney Disease*** 20(1), 70-75.
23. SENGUPTA P., 2013 – The laboratory rat: Relating its age with human's. ***International Journal of Preventive Medicine*** 4(6), 624-30.
24. WALKOWSKA A., KUCZERISZKA M., SADOWSKI J., OLSZYNSKI K.H., DOBROWOLSKI L., ČERVENKA L., KOMPANOWSKA-JEZIERSKA E., 2015 – High salt intake increases blood pressure in normal rats: putative role of 20-HETE and no evidence on changes in renal vascular reactivity. ***Kidney and Blood Pressure Research*** 40(3), 323-334.
25. XIE L., CIANCIOLO R.E., HULETTE B., LEE H.W., QI Y., COFER G., JOHNSON G.A., 2012 – Magnetic resonance histology of age-related nephropathy in the Sprague Dawley rat. ***Toxicologic Pathology*** 40(5), 764-778.