This is an Accepted Manuscript of an article published by Elsevier in Journal of Trace Elements in Medicine and Biology on Epub 2020 Dec 26.available at doi: 10.1016/j.jtemb.2020.126709

METABOLIC SYNDROME DURING GESTATION AND LACTATION: AN IMPORTANT RENAL PROBLEM IN DAMS. SELENIUM RENAL CLEARANCE.

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Running title: **RENAL PROBLEM IN DAMS DURING METABOLIC SYNDROME.**

Support disclosure: Grants from Andalusian Regional Government to support CTS-193 research group.

Abstract:

BACKGROUND: Metabolic syndrome (MS) in lactating dams leads to several cardiometabolic changes related to selenium (Se) status and selenoproteins expression which produce hypertension. However, little is known about the state of these dams' kidney functions and their Se deposits.

METHODS: Two experimental groups of dam rats were used: control (Se: 0.1ppm) and MS (Fructose 65% and Se: 0.1ppm). At the end of lactation (21d postpartum) kidney weight and protein content, Se deposits, and the activity of the antioxidant selenoprotein glutathione peroxidase (GPx) were measured in dams. Kidney functional parameters: albuminuria, creatinine clearance, serum aldosterone and uric acid levels and water and electrolyte (Na^+ and K^+) balance were also evaluated. Systolic blood pressure (SBP) was measured.

RESULTS: In MS dams at the end of lactation Se deposits and GPx activity are higher in the kidney; however, lipid renal peroxidation appears, relative Se clearance increases, and the dams have lost Se by urine. MS dams have polyuria and polydipsia, high uric acid serum levels, albuminuria and high creatinine clearance, implying glomerular renal malfunction with protein loss. They also present hypernatremia, hypokalemia and hyperaldosteronemia, leading to high SBP; however, a natriuretic process is taking place.

CONCLUSION: Since these alterations appear, at least in part, to be related to oxidative stress in renal cells, Se supplementation could be beneficial to avoiding greater lipid renal oxidation during lactation.

Key words: metabolic syndrome, selenium, renal damage, systolic blood pressure.

INTRODUCTION:

Metabolic Syndrome (MS) is a general endocrine metabolic disorder that is increasing continuously. It is defined as a cluster of risk factors that include central obesity, insulin resistance (IR), raised blood pressure and dyslipidemia. These factors usually predispose individuals to cardiovascular diseases (CVD) and diabetes (DM). This syndrome affects nearly 25% of the population. It appears among pregnant women and, therefore, continues during breastfeeding [1]. As gestational diabetes (GD), this syndrome especially affects the mother during pregnancy, since this period is physiologically related to IR in tissues, due to the fact that an anabolic process is taking place [2]. In clinical studies the lactation period appears to cause an increase in metabolic load and energy needs – a fact that could counteract MS progression [3,4]. However, not all clinical studies support the protective role of lactation in the development of MS, and most are related to a future development of MS, not to the evolution of an implanted MS [5,6]. Little, therefore, is known regarding the maternal response to MS that arises during pregnancy and persists through the breastfeeding period. In experimental animal models, Zou et al., [7] found that fructose-rich diets, an experimental model of induced MS, induced fatty liver and glucose intolerance in pregnant and lactating rats in a more pronounced way than in

unmated rats and that the effects on liver were even worse during lactation. In fact, it is reported that not only does prolactin (PRL), the pituitary hormone which controls the initiation and continuity of lactation, have effects on food intake, body weight gain and IR, but also on the growth of pancreatic βcells. In non-diabetic men with high PRL levels within the physiological range, there is an association with IR induction [8], whereas in diabetic rats low-PRL improves energy and glucose metabolism by increasing hypothalamic dopamine levels [9]. Therefore, since this hormone increases during lactation, this physiological period should be specifically analyzed during an IR process such as MS.

MS is related to CVD, and therefore it contributes to the development and progression of chronic kidney disease (CKD) in the human population [10,11]. Its progression is, however, complex because of the interplay between systemic metabolic and vascular dysfunctions that involves both the tubular and glomerular renal systems. In adult animals, MS exposure leads to renal oxidation by increasing reactive oxygen species (ROS) production and probably by decreasing the activities of antioxidant enzymes such as the selenoprotein Glutathione Peroxidase (GPx) [12–14]. ROS elevation in kidney leads to lipid peroxidation of nephron epithelial cells, interfering with membrane carrier functions such as Na+K+-ATPase activity and increasing Na+ reabsorption; it also leads to a pro-inflammatory status related to glomerular changes that affect glomerular filtration rate (GFR) [13,15]. A renal reduction in Na+ excretion, either by a reduction in GFR or by an increase in its tubular reabsorption, causes HTA. PRL, which is specifically higher during breastfeeding, is implicated in the modulation of the activity of Na+ transporter in the kidney,

thirst and diuresis [16]. For this reason, kidney function during lactation in MS dams should be analyzed in greater depth.

Since the essential trace element Selenium (Se) is part of the catalytic center of 25 selenoproteins [17], among which is the GPx family of antioxidants, and it is documented that MS is linked to oxidative stress in different tissues [18], Se is known to be implicated in the evolution of this syndrome. However, a dual role for Se in insulin resistance and MS has been described [19–21]. In an MS rat model, used previously by this research group, Se body distribution and selenoprotein expression have been found to be both up- and down-regulated in different tissues extracted from lactating dams [22]. In the heart of MS lactating dams, for instance, Se deposits are depleted and GPx activity and expression downregulated. Since Se is necessary for a correct heart function, especially for avoiding mitochondria oxidation and apoptosis, the so-called depletion is related to cardiovascular damage in dams which have a low heart rate [23]. Moreover, with respect to kidney, the other tissue implied in Blood Pressure (BP) regulation, Se deposits are increased in lactating dams, although there is no information about their kidney physiological status. Through its antioxidant properties, Se has an important protective role in kidney function [24]. When administered as a supplement, it restores the molecular content, structure and fluidity of the kidney brush border cell membrane of diabetic rats, [25]. However, little is known about its possible role in the development and progression of MS-induced CKD in lactating dams.

In order to understand if CKD is established in Lactating MS dams and whether Se supplementation could be an effective therapy for them or not, the aim of the present study is to continue analyzing the repercussion of Se status on the MS of

breastfeeding dams and its relation to oxidative balance and kidney function as regards to BP control – analyzing albuminuria, glomerular filtration rate (GFR), electrolytes and water reabsorption.

MATERIAL AND METHODS.

Animals: Male and female Wistar rats (Centre of Production and Animal experimentation, Vice-rector's Office for Scientific Research, University of Seville) weighing approximately 150-200 g, were randomized into two groups: control (C) and metabolic syndrome (MS). Animal care procedures and experimental protocols were performed in accordance with EU regulations (Council Directive 86/609/EEC, November 24th 1986) and approved by the Ethics Committee of the University of Seville. All rats received drinking water and diet *ad libitum* during three week before mate, and then, during gestation (3 weeks) and lactation (3 weeks) periods. MS group was fed with rich fructose diet (65%) to induce MS which contained 0.1 ppm of Se; C group received solid diets with 0.1 ppm of Se. Se was supplemented as anhydrous sodium selenite (an inorganic compound; Panreac, Barcelona, Spain). The diets of these rats were prepared according to The Institute of Laboratory Animal Resources Council (ILAR, 1979), which details known nutrient requirements for most of the common laboratory animals.

In four weeks, male (n=3) and female (n=6) rats were mated to obtain the first-generation offspring for each group. Pregnant female rats were inspected daily by the presence of the vaginal plug, which indicated day zero of pregnancy; at this moment, pregnant rats were housed individually in plastic cages. The day of parturition, which occurs spontaneously three weeks after coitus, was designated as day 1 of lactation. The offspring number was reduced to 8 per mother at parturition in order to provoke the same lactating stimulus in all the dams. The

146 experiments were performed on the lactating dams at the end of the breastfeeding
147 period (21d postpartum).
148 ***Nutritional controls:*** Body weights of the dam rats were determined once a
149 week, whereas the amount of food and liquid consumed by rats were monitored
150 daily until the end of the experimental period. Se intake was calculated by
151 multiplying the food consumed by ppm of Se in the diets. Weekly body weight was
152 measured until the end of the experimental period, at 9:00 am to avoid changes
153 due to circadian rhythms.
154 ***Samples:*** 12 h before the end of the experimental period, dams were separated
155 from their offspring and fasted for 12 h to collect urine samples using individual
156 metabolic cages. Urinary flow was calculated as ml of urine excreted in 24h. At the
157 end of the experimental period, in order to calculate the body max index (BMI),
158 cranium-caudal length of lactating dams was controlled using a metric caliper; they
159 were also weighed and anesthetized with intraperitoneal 28% w/v urethane (0.5
160 ml/100 g of body weight). Blood samples were obtained by heart puncture and
161 collected in tubes. The serum was prepared using low-speed centrifugation for
162 15min. at 1300 x g. The abdomen was opened by a midline incision and kidneys
163 were removed, debrided of adipose and connective tissue in ice-cold saline,
164 weighed and stored at −80◦C prior to biochemical determinations. Kidney-somatic
165 index (KSI) was calculated as kidney mass/ total body mass.
166 ***Selenium analysis***: Selenium levels were determined by graphite-furnace atomic
167 absorption spectrometry, using a PerkinElmer AAnalyst™ 800 high-performance
168 atomic absorption spectrometer with WinLab32 for AA software, equipped with a
169 Transversely Heated Graphite Furnace (THGA) with longitudinal Zeeman-effect
170 background corrector and an AS-furnace auto sampler (PerkinElmer, Überlingen,

171 Germany). The source of radiation was a Se electrodeless discharge lamp (EDL).
172 The instrumental operating conditions and the reagents are the same that we have
173 used in the previous paper Ojeda et al. [26]. Samples: serum samples were diluted
174 fivefold in 0.2% v/v HNO_3 and 0.2% Triton X-100 solutions and urine samples were
175 diluted 1:2 v/v. After 72h, at 100ºC dry temperature, kidneys samples were
176 weighed and digested in a sand bath heater (OVAN, Badalona, Spain) with nitric
177 acid for 72h., and perchloric acid and hydrochloric acid (6N) were added.
178 ***Antioxidant enzymes and oxidative stress markers:*** In order to measure the
179 activity of antioxidant enzyme GPx as well as lipid oxidation, kidney tissue samples
180 were homogenized (100 x g for 1min, 1:4 w/v) using a Potter homogenizer (Pobel
181 245432, Madrid, Spain) in a sucrose buffer (15 mM Tris/HCl, pH 7.4, 250 mM
182 sucrose, 1 mM EDTA and 1 mM dithiothreitol) in an ice bath. The homogenate was
183 centrifuged at 900 x g for 10min at 4 ºC. The resulting supernatant was employed
184 for the biochemical assay according to techniques described in Ojeda et al., [27].
185 ***Clearance Measurement:*** The creatinine and uric acid in both serum and urine
186 were determined by colorimetry, using commercial kits (Creatinine BioSystems ref.
187 11802 (Barcelona, Spain) and Uric Acid Assay Kit Sigma-Aldrich (Madrid, Spain)).
188 For urine, the sample was previously diluted with bidistilled water to a ratio of 1/50.
189 The Na+ and K+ were determined by GDV flame photometry (DV 710 model,
190 Italy). The samples were diluted 1/100 in a working solution supplied by the
191 manufacturer before measuring. The characteristic light wavelengths of sodium
192 and potassium are 589 nm and 766.5 nm, respectively. Aldosterone levels in
193 serum was determined using Aldosterone ELISA Kit (Enzo Life Sciences, Inc.
194 Switzerland), which is based on the binding of aldosterone to a specific antibody
195 that is immobilized on the wall of the 96-wells plate. The albumin levels in serum

and urine were spectrophotometrically determined using commercially available kits (Tietz, 1991). The serum ratio albumin/creatinine was also estimated. Serum and urine urea levels were measured using a Randox diagnostic kit (Crumlin, Co., Antrim, UK). Urinary and serum osmolality was determined by the technique pressure steam in the osmometer model 5100C (Wescor Inc., USA).

Na+, K+, Se, urea, and creatinine clearances were calculated from the standard formula: clearance (CL) = U * V/P, where U is the level in urine of the substance to be cleared, V the volume of urine collected in 24 h, and P the level of the substance studied in plasma. The ratios between Na+, K+, Se or urea in relation to creatinine were calculated as CLx/CLCreatinine × 100, where x is the substance to be compared. Fractional excretion of sodium (FENa) and transtubular potassium gradient (TTKG) were calculated by standard formulae (FENa = UNa+ · PCr/PNa+ · UCr); TTKG = (POsm x UK+)/(PK+ x UOsm); where U is the level in urine of the substance, P the level of the substance studied in plasma; and Osm is the osmolality.

Blood pressure (mm Hg): Systolic and Diastolic blood pressure were monitored with pressure meter (NIPREM 645, CIBERTEC, Spain) using the indirect tail occlusion method. Measurements were taken one day before the anesthesia protocol; therefore, at day 20 after parturition. The signals collected were treated with an IT support via a data acquisition system coupled to the pressure meter. Each animal was measured 4-5 times successively in order to calculate the arithmetical mean, this being the value used. Median Blood Pressure (MBP) was calculated.

Statistical Analysis: The results are expressed as means ± standard error of the mean (SEM). The data were analyzed using a statistical program (GraphPad

InStat 3, CA, USA). Student's t-test (unpaired t-test) was used to compare the difference between two experimental groups (C and MS), considering statistically significant differences at $p < 0.05$. The Kolmogorov–Smirnov test was used to validate the assumption of normality.

RESULTS:

Nutritional Parameters, water balance and blood pressure: Table 1 shows that lactating MS-exposed dams intake fewer kcal and Se than control ones, presenting a lower increase in body weight and BMI at the end of experimental procedure. MS dams intake higher quantities of water, presenting a higher urinary flow. This urine has an extremely low osmolality. MS dams present a significant higher MBP value.

Morphological and oxidative renal parameters: lactating MS dams have higher relative kidney weight, with higher Se deposits and GPx activity; they do, however, also have higher renal lipid oxidation (Table 2).

Serum, urine and clearance values: in serum, Na+ and urea are significantly higher in MS dams with respect to C ones. In urine all the parameters measured are lower, except for Na+, which is higher, and K+ which remains unaffected. Relative Na+, K+ and Se clearances are higher. (Table 3)

Renal functional parameters: MS-exposed dams have significantly higher creatinine clearance, albumin/creatinine ratio, serum acid levels, serum aldosterone, fractional excretion of Na+ (FENa) and transtubular K+ excretion (TTKG) values (Figure 1).

DISCUSSION:

At the end of breastfeeding, the dams which suffered MS induced by a high-fructose diet during gestation and lactation intook less food and therefore less Se, presenting lower body weight gain and BMI. However, during gestation they intook the same quantity of kcal as control dams [22]. This implies that an energy balance disruption has taken place during lactation period in MS-exposed rats. As Zou et al., [7] comment and Nogales et al., [22] describe, lactating dams exposed to a high-fructose diet have low insulin serum levels and β-cell functions, since leptin is inversely related to insulin [28]. Like their pups, which present high serum leptin and low insulin levels, appetite and weight [29], the dams' leptin levels might have raised. PRL, the most important hormone during the lactation period, orchestrates this process and is intimately related to leptin [30]. When PRL levels during lactation are physiologically decreased, serum leptin levels increase, and their progeny present low weight and a tendency to develop MS [31].

This research group had previously, detected a significant reduction in the apparent Se balance at the end of lactation in MS dams [22]. It is also known that low Se exposure during lactation is related to low insulin, high leptin and catabolic processes [19,32], since Se is necessary for a correct endocrine pancreatic function, but also because it is related to the neural population in the hypothalamic arcuate (ARC) nucleus, which regulates global energy metabolism and appetite [33]. Despite the fact that PRL levels are not measured in this work, they are probably lower in MS dams in respect of control dams, because it is known that Se affects the expression of gene response for PRL in pituitary and for PRL levels in serum [34]. Moreover, previous studies in our lab have demonstrated that they synthetize a smaller quantity of milk, since their pups intake less milk during the breastfeeding process [23].

With respect to water balance, lactating MS dams have a significantly higher urinary flow rate, and, since they also have to synthetize milk, they intake more water in order to avoid dehydration. MS dams, therefore, excrete a larger quantity of diluted urine. MS rats have duplicated their urinary flow rate with respect to control ones. However, when serum osmolality was measured, it appears that volemia is balanced, since MS and C animals present similar values. In this context, MS dams present higher MBP values, but these higher values do not appear to be related to a greater tendency to increase their volemia. Lactating MS dams have hypernatremia and this increase in serum Na+ levels could be involved in the high BP found.

From a physiological point of view, the polyuria found in MS dams does not appear to have beneficial consequences. It is, moreover, one of the first renal symptoms found in DM. This fact redirected us to analyze kidney function. The first sign that something is occurring in this tissue is the higher KIS found in MS dams, which could indicate a fibrotic process. Uremia, another biochemical parameter used to determine renal damage, is also higher in these dams. Moreover, and despite the increase in Se and the antioxidant selenoprotein GPx activity in kidney, lipid oxidation is also taking place in renal cells. The kidney is the principal Se excretion route [35], and, therefore, it accumulates a great quantity of Se by modulating Se renal clearance. In this study MS dams have their relative Se clearance value increased, indicating that the Tubular Se reabsorption process is inefficient. Lactating MS dams intake less Se by diet and excrete more by kidney; despite this organ's efforts to reabsorb it, Se availability is reduced in breastfeeding dams. Maybe for this reason, and despite the Se repletion in kidneys, available Se is insufficient and lipid peroxidation takes place. Renal lipid peroxidation leads to

294 changes in membrane composition, interfering with carrier functions such as
295 Na^+K^+-ATPase activity [36], and leads to progressive renal injury by activating
296 different signaling pathways and transcriptional factors such as NF-kB [12,37].
297 Renal oxidative stress also reduces local NO levels [38] and alters the interplay
298 between aldosterone and its receptor [39]. In diabetic kidney brush border cell
299 membrane in rats, a greater quantity of lipid peroxidation end products, a decrease
300 in saturated lipid content, a disruption in lipid order and a decrease in membrane
301 dynamics were found: all these are conditions that are improved by Se
302 supplementation [25]. In the same context, Se supplementation in rats with renal
303 damage caused by ethanol pro-oxidative actions improves their renal oxidative,
304 inflammatory and apoptotic balance, improving their kidney functions – principally
305 those functions related to electrolyte balance and aldosterone homeostasis [40].
306 Perhaps this is the reason why Se levels increase in the kidney of lactating MS
307 dams, in spite of the fact that its body availability is lower.

308 The biomolecular changes caused by oxidative stress in the kidneys of lactating
309 MS dams are reflected in kidney functional alterations. Consistent with an initial
310 stage of Diabetic Nephropathy (DN) [41,42], hyperfiltration measured as high
311 creatinine CL, microalbuminuria (high albumin/creatinine ratio), a well-known
312 marker of size loss and charge permselectivity at the glomerulus, are observed in
313 lactating MS dams. This implies that there is a supraphysiologic increase in whole
314 kidney GFR and that there are functional changes at the glomerulus. Podocytes
315 (glomerular epithelial cells) cannot regenerate when they suffer from injury, so their
316 damage and/or apoptosis could result in the destruction of the glomerular filtration
317 membrane. It is known that oxidation takes place in podocyte after high glucose
318 insults, and it is also implicated in the induction of caspase-3 via mitochondria,

319 inducing podocytes apoptosis [43]. In line with the above, it is reported that
320 administering adiponectin improves podocyte permeability to albumin through the
321 inhibition of NADPH oxidase activity, which prevents oxidation [44].

322 Uric acid levels are found to predict an increased risk of developing renal disease.
323 Among other inflammatory and oxidative effects, it reduces NO levels in the
324 endothelium and in the dense macula, and stimulates the renin-angiotensin-
325 aldosterone system (RAS), thus having repercussions in kidney [45]. Moreover,
326 uric acid may induce renal damage without urate renal deposition within a context
327 of systemic and glomerular hypertension associated with renal arteriolopathy [46].
328 The model used in this work to develop MS uses a high quantity of fructose in rat
329 diet. Rapidly intaken fructose raises uric acid levels; it activates fructokinase, which
330 in turn increases the AMP/ATP ratio. This increase in AMP leads to a catabolism
331 process in the purines' cycle, incrementing the formation of uric acid [47]. This is
332 the reason for the great raise in uric acid levels in lactating MS dams, which affects
333 their endothelial and renal functions. However, in other experimental MS induction
334 models, uric acid is also found to be higher.

335 Moreover, MS-exposed pups also present hypernatremia and
336 hyperaldosteronemia, together with a high renal Na^+ and K+ excretion, measured
337 as high FENa and TTKG. DN is associated with RAS activation, hypernatremia
338 and high blood pressure. However, the natriuretic effect found in this work is
339 strange, since despite the fact that GFR increases, there are high serum
340 aldosterone levels and hypernatremia. It is known that PRL affects hydric and
341 electrolytic balance and osmoregulation in mammals; specifically PRL acts by
342 modulating rather than directly controlling these actions [48]. When PRL was
343 administered in a rat model of Cholestasis of Pregnancy with well-defined renal

problems, this hormone caused diuretic and natriuretic effects, decreasing sodium renal reabsorption [16]. This suggests that PRL contributes to modulating sodium transporters activity, avoiding Na+ reabsorption when the kidney is not working properly. Fidchenko et al., [16] suggest that PRL could modulate Na/K/ATPase or Na/K/2Cl cotransporter functions in kidney. For this reason, the breastfeeding period has its own particular physiological renal changes and regulation, which have to be analyzed in greater depth in order to avoid future renal damage in females.

In conclusion, since during lactation there is an own hormonal status caused by PRL which affects the modulation of water and electrolytic balance, and on the basis of the results obtained, MS appears to specifically affect the dams' kidneys function during this period. MS profoundly affects Se homeostasis in lactating dams: they intake a low quantity of Se by diet and excrete more Se by its main excretion route (urine). Yet these dams have higher Se renal deposits and antioxidant GPx activity in order to combat against lipid renal peroxidation, related to renal damage. Despite this increase in Se deposits, lipid peroxidation takes place and contributes to functional changes in the glomerulus and tubular cells such as hyperfiltration, microalbuminuria, and alterations in electrolytes reabsorption. MS dams also present hypernatremia and hyperaldosteronemia, which are probably related to their high MBP. All of these changes are strictly related to the oxidative process. For this reason, Se supplementation could be administered to these dams during lactation.

Declaration of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Table 1. Nutritional parameters in dams at the end of breastfeeding.

LACTATING DAMS	C	MS	*P* value
Solid kcal intake (kcal/day)	201 ± 13	176 ± 10	*
Se intake (µg/day)	5.1 ± 0.3	4.3 ± 0.2	*
Body mass gain (g)	53.4 ± 3.2	42.3 ± 2.0	*
Body Mass Index (BMI)	4.9 ± 0.2	4.3 ± 0.1	*
Liquid intake (ml/day)	74 ± 7	121 ± 12	**
Urinary flow (ml/day)	14.5 ± 0.6	28.5 ± 0.4	***
Urine osmolality (mOsm/l)	2.0 ± 0.21	0.8 ± 0.08	***
Serum osmolality (mOsm/l)	314± 29	323± 33	
Median Blood Pressure (mmHg)	109 ± 3.1	120 ± 2.9	*

The results are expressed as mean ± SEM and analysed by Student´s t-test. The number of animals in each group of dams is 6. Statistic difference between groups was expressed as *P* value: C vs MS: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 2. Morphological and oxidative renal parameters.

LACTATING DAMS	C	MS	*P* value
Kidney mass (g)	2.21± 0.08	2.24± 0.19	
Kidney-somatic index (KSI) (%)	1.04± 0.04	1.15 ± 0.04	*
Renal protein content (mg/ml)	7.18 ± 0.41	6.2 ± 0.41	
Renal Se levels (µg/g dry weight)	0.51 ± 0.02	0.61±0.03	*
Renal GPx activity (mU/mg protein)	0.41± 0.03	0.49± 0. 04	*
Renal MDA levels (mU/mg protein)	0.11± 0.01	0.14± 0.01	*

The results are expressed as mean ± SEM and analysed by Student´s t-test. The number of animals in each group of dams is 6. Statistic difference between groups was expressed as *P* value: C vs MS: * p<0.05.

Table 3. Serum and urine measurement: Creatinine, Na+, K+, Urea and Selenium. Relative clearances.

Parameters		C	MS	*P* value
SERUM	**Creatinine** (mg/dL)	0.59 ± 0.02	0,53 ± 0,02	
	Na^+ (mmol/L)	136 ± 0.6	138 ± 0,6	*
	K^+ (mmol/L)	6.7 ± 0.32	7.2 ± 0.35	
	Urea (mg/dl)	50.2 ± 1.8	58.6 ± 1.3	**
	Selenium (µg/L)	317± 6.4	306 ± 8.9	
URINE	**Creatinine** (mg/dL)	50.7 ± 3.2	29.8 ± 2.1	***
	Na^+ (mmol/L)	42.6 ± 3.3	49.1 ± 0.7	**
	K^+ (mmol/L)	85 ± 2.5	81 ± 0.9	
	Urea (mg/dl)	4021 ± 318	2850± 101	***
	Selenium (ng/day)	269 ± 3	229± 12	*
RELATIVE CLEARANCES	**CL Na^+ /CL creatinine**	0.0035 ± 0.00033	0.0062 ± 0.00059	**
	CL K^+/CL creatinine	0.112 ± 0.011	0.205 ± 0.02	**
	CL Urea/CL creatinine	0.89 ± 0.04	0.81 ± 0.03	
	CL Se/CL creatinine	0.0099 ± 0.0009	0.013 ± 0.001	*

The results are expressed as mean ± SEM and analysed by Student´s t-test. The number of animals in each group is 8. Statistic difference between groups was expressed as *P* value: C vs MS: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Figure captions

Figure 1. Renal functional parameters: creatinine clearance, albumin/creatinine ratio, serum uric acid, fractional excretion of Na^+ (FENa), transtubular K^+ excretion (TTKG) and serum aldosterone levels. The results are expressed as mean ± SEM and analysed by Student´s t-test. The number of animals in each group is 8. Statistic difference between groups was expressed as *P* value: C vs MS: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.