SELENOPROTEINS AND RENAL PROGRAMMING IN METABOLIC SYNDROME-EXPOSED RAT OFFSPRING.

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Abstract:

Maternal metabolic syndrome (MS) during gestation and lactation leads to several cardiometabolic changes related to Selenium (Se) status and selenoproteins expression in offspring. However, little is known about kidney programming and antioxidant selenoprotein status in MS pups. To gain more knowledge on this subject, 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, the Se deposits in kidney, selenoprotein expression (GPx1, GPx3, GPx4 and selenoproteinP), oxidative balance and AMP-activated protein kinase (AMPK) and activated transcriptional factor NF-κB expression were measured. Kidney functional parameters: albuminuria, creatinine clearance, aldosteronemia, and water and electrolyte balance were also evaluated. One week later systolic blood pressure was measured. Lipid peroxidation takes place in the kidney of MS pups and Se, selenoproteins and NF-κB expression increased, while AMPK activation decreased. MS pups have albuminuria and low creatinine clearance which implies glomerular renal impairment with protein loss. They also present hypernatremia and hyperaldosteronemia, together with a high renal Na^+ reabsorption, leading to a hypertensive status, which was detected in these animals one week later. Since these alterations seem to be related, at least in part, to oxidative stress, the increase in Se and selenoproteins found in the kidney of these pups seems to be beneficial, avoiding a higher lipid oxidation. However, in order to analyze the possible global beneficial role of Se in kidney during MS exposure, more data are necessary to document the relationships between GPx4 and NF-κB, and SelP and AMPK in kidney.

Key words: metabolic syndrome, selenium, renal programming, selenoprotein, AMPK.

INTRODUCTION:

Metabolic syndrome (MS), an endocrine metabolic disorder which predisposes sufferers to insulin resistance (IR), diabetes mellitus (DM) and cardiovascular disease (CVD) (1), affects both mother and offspring by altering their early metabolic programming (2–4). The prevalence of MS is increasing worldwide, MS rates at pediatric age being slightly higher than in the past (5,6). Since MS is related to CVD, it has been demonstrated that it could contribute to the development and progression of chronic kidney disease (CKD) in the human population (7,8). Moreover, important renal programming changes have been described in rats exposed to a high-fructose-diet-induced MS model during gestation and lactation (9,10) and in pups whose mother suffers DM (11–13). In general terms, the renal programming found is associated to future programmed hypertension (HTA) by genes related to nephrogenesis, renin-angiotensin system (RAS), epigenetic regulators in the kidney and oxidative stress (OE) (9,14).

In adult animals it has been shown that MS exposure leads to renal oxidation by increasing reactive oxygen species (ROS) production and probably by decreasing the antioxidant enzymes activities of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and Glutathione Reductase (GR) (15–17). During oxidative perinatal environmental conditions, ROS elevation in the renal medulla leads to lipid peroxidation of nephron epithelial cells, interfering with membrane carrier functions such as Na+K+-ATPase activity. It leads, furthermore, to a reduction in medullary blood flow and an increase in Na+ reabsorption and causes a reduction in local nitric oxide (NO) levels that can increase Na+ reabsorption. OE also alters the interplay between aldosterone and its receptor, HTA being induced by this great increase in Na+ reabsorption (14).

The essential trace element Selenium (Se) plays its biological functions as the catalytic center of 25 different selenoproteins, such as the antioxidant family GPx or the Se plasmatic transporter Selenoprotein P (SelP), which also has antioxidant properties (18). Because Se exerts an influence on antioxidant balance, immunological cell response and cell growth it has

been related to several diseases such as MS. Recently, in fact, changes in Se homeostasis have been related to MS, yet both infra- and supra- dietary Se interventions have been involved in its development (19–22). In a rat model with high-fructose-diet-induced MS, used previously by this research group, Se body distribution and selenoprotein expression have been found to be up- and down-regulated in different tissues from both dams and their offspring (4,23). In the liver of MS pups it is found repletion of Se together with upregulation in selenoproteins expression which seems to be related to energy hepatocyte alteration via AMP-activated protein kinase (AMPK) (24). However in the heart of pups exposed to the same MS protocol, Se deposits are depleted, since Se is necessary for a correct heart function - especially for preventing mitochondria oxidation and apoptosis, this depletion is related to cardiovascular damage in these pups (25). With respect to kidney, the other tissue implied in Blood Pressure (BP) regulation, it is known that Se deposits are repleted in MS-exposed pups (23). There is, however, no information about selenoproteins expression, oxidative balance, NF-κB activation or energy AMPK status. The most important selenoprotein produced in rodent kidney tissues is the antioxidant GPx3 (26), which acts in plasma by reducing hydrogen peroxide to water; it also acts in the proximal tubules of nephrons (27). GPx4 is the second main selenoprotein synthesized in kidney (28) and is the only GPx member that reduced hydroxyperoxides in lipoproteins, complex lipids and the phospholipids of biomembranes. Moreover, it plays an essential antioxidant role in mitochondria, modulating its intrinsic apoptotic pathway and activating the transcriptional factor NF-κB protein (29). SelP is also synthesized in kidney at even higher levels than GPx1, which has antioxidant cytosolic actions (28). SelP in plasma functions as an Se-supply protein, but it is also identified in liver as a hepatokine (30), promoting IR by inactivating AMPK, a metabolic sensor that regulates cellular energy balance. AMPK has recently been found to be deeply implicated in regulating kidney function and protein transport (31).

Therefore, due to its antioxidant properties (32) Se seems to play an important protective role in kidney function. Little, however, is known about its possible role in the development and progression of renal programming alterations induced by MS exposure. In order to understand whether Se supplementation could constitute an effective therapy for fetal MS development or pose a risk, the aim of the present study is to continue to analyze albuminuria, glomerular filtration rate (GFR), electrolytes and water reabsorption in order to analyze the repercussion of Se status on MS fetal programming by evaluating kidney Selenoprotein balance, and its relationship with oxidative balance, inflammatory and fibrotic status via NF-κB activation, energy AMPK balance and kidney function in relation to BP control.

MATERIAL AND METHODS.

Animals. 6 Male and 12 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 randomised 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 feed 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 added as anhydrous sodium selenite (an inorganic compound; Panreac, Barcelona, Spain). The diets of these rats were prepared according to The Council of the Institute of Laboratory Animal Resources (ILAR, 1979) which details known nutrient requirements for most of the common laboratory animals.

In four week, male (n=3) and female (n=6) rats per group 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 (four males and four females, when this was possible). The experiments were performed on the offspring of all groups to 21d postpartum. In this study, we have used 8 pups per group to measure all the parameters cited below. These 8 pups represent all the litters, as a maximum of 2 rats per litter, and were allocated to each group taking into account the sex. Other two groups of 8 pups of 21d old, one C and one MS, were kept one week more housed in plastic cages with control diet, in order to measure blood pressure. The blood pressure measurement in the tail of 21d old rats is impossible since the tail occlusion method used requires a minimum tail thickness.

Nutritional controls. Body weights of the dam rats were determined once a week while that the amount of food and liquid consumed by rats were monitored daily until the end of the experimental period. Se intake was calculated by multiplying the food consumed by ppm of Se in the diets. Weekly, body weight and cranium-caudal length of pups was controlled, using a balance and metric calliper, respectively, until the end of the experimental period. Additionally, glucose was determined using test strips Accutrend (ROCHE, Spain). All measures were taken at 9:00 am to avoid changes due to circadian rhythms.

Samples. The amount of milk consumed by the offspring at the end of the lactation period (days 19 and 20) was estimated by subtracting the weight of the pups obtained immediately prior to returning them to the dam from their weight after 30 minutes of suckling. In order to obtain the maximum amount of milk at day 21 of lactation, 12h after removing the litters from their mothers, the dams were anesthetized with urethane, and milk samples were immediately collected. The milk was obtained by gently massaging the area around each of the 12 mammary glands and then pressing upward from the base of the gland towards the nipple. The amount of milk collected was around 1 to 1.5 ml per dam.

154 At the end of the experimental period, pups were separated from their mother and fasted for
155 12 h to get urine samples using individual metabolic cages, then urinary flow was calculated.
156 The next morning, dams and their pups were weighed and anesthetized with intraperitoneal
157 28% w/v urethane (0.5 ml/100 g of body weight). Blood samples were obtained by heart
158 puncture and collected in tubes. The serum was prepared using low-speed centrifugation for
159 15min. at 1300 *x g.* The abdomen was opened by a midline incision and kidneys were removed,
160 debrided of adipose and connective tissue in ice-cold saline, weighed and stored at −80◦C prior
161 to biochemical determinations. Kidney somatic index (KIS) was calculated as kidney weight /
162 total body weight.
163 **Selenium analysis.** Selenium levels were determined by graphite-furnace atomic absorption
164 spectrometry, using a PerkinElmer AAnalyst™ 800 high-performance atomic absorption
165 spectrometer with WinLab32 for AA software, equipped with a Transversely Heated Graphite
166 Furnace (THGA) with longitudinal Zeeman-effect background corrector and an AS-furnace
167 autosampler (PerkinElmer, Überlingen, Germany). The source of radiation was a Se
168 electrodeless discharge lamp (EDL). The instrumental operating conditions and the reagents
169 are the same that we have used in the previous paper Ojeda et al. (33). Samples: serum
170 samples were diluted fivefold in 0.2% v/v HNO_3 and 0.2% Triton X-100 solutions and urine
171 samples were diluted 1:2 v/v. After 72h at 100ºC dry temperature, kidneys and milk samples
172 were weighed and digested in a sand bath heater (OVAN, Badalona, Spain) with nitric acid for
173 72h., and perchloric acid and chlorhydric acid (6N) were added.
174 **Antioxidant enzymes and oxidative stress markers.** In order to measure the activity of
175 antioxidant enzymes (SOD, CAT, GPx and GR) as well as lipid oxidation, kidney tissue samples
176 were homogenized (100 x g for 1min, 1:4 w/v) using a Potter homogenizer (Pobel 245432,
177 Madrid, Spain) in a sucrose buffer (15 mM Tris/HCl, pH 7.4, 250 mM sucrose, 1 mM EDTA and
178 1 mM dithiothreitol) in an ice bath. The homogenate was centrifuged at 900 x g for 10min at 4

ºC. The resulting supernatant was employed for the biochemical assay according to techniques described in Ojeda et al. (34).

Immunoblotting assays. The expression of the selenoproteins GPx1, GPx3, GPx4 and SelP as well as NF-κB p65, p-AMPK and AMPk total were determinate in kidney of pups rats. The samples utilized contained 100 µg of protein. Proteins were separated on polyacrylamide gel and were transferred onto a nitrocellulose membrane (Immobilon-P Transfer Membrane, Millipore, Billerica, MA, USA) using a blot system (Transblot, BioRad CA, USA). Nonspecific membrane sites were blocked for one hour with a blocking buffer: TTBS (50 mM Tris-HCl1, 150 mM NaCl1, 0.1% (v/v) Tween 20, pH 7.5) and milk powder 3% (BioRad CA, USA), and thereafter they were probed overnight at 4ºC with specific primary antibodies (rabbit polyclonal IgG, Santa Cruz Biotechnology) dilutions: GPx1 (1:5000), GPx3 (1:5000), GPx4 (1:2500), SelP (1:2500), NF-κB p65 (1:1000), AMPK-t (1:2000) and p-AMPK (1:2000). Secondary antibody (anti-rabbit IgG HRP conjugate, Santa Cruz Biotechnology) was utilized in dilutions of 1:5000 for GPx1, GPx3 and SelP, and 1:2500 for GPx4, NF-κB p65, AMPK-t and p-AMPK. Monoclonal mouse anti β-actin (IgG1 A5441, Sigma-Aldrich, Spain) was used to detect β-actin as a loading control, with a dilution of 1:20000, and a secondary antibody anti-mouse IgG Peroxidase conjugate (A9044, Sigma-Aldrich, Spain) was used in a dilution of 1:8000. The membrane was incubated for one minute with the commercial developer solution Luminol ECL reagent (GE Health Care and Lumigen INC Buckinghamshire, UK). The quantification of the blots was performed by densitometry using the ImageJ analysis software (NIH).The results were expressed as percent arbitrary relative units, referring to values in control animals which were defined as 100%.

Clearance and urine determinations. Creatinine and uric acid in both serum and urine were determined by colorimetry using the commercial kits Creatine BioSystems ref. 11802 (Barcelona, Spain) and Uric Acid Assay Kit Sigma-Aldrich (Madrid, Spain) respectively. For urine, the sample was previously diluted with bidistilled water to a ratio of 1/50. The Na^+ and K^+ were

205 determined by GDV flame photometry (DV 710 model, Italy). The samples were diluted 1/100
206 in a working solution supplied by the manufacturer before measuring. The characteristic light
207 wavelengths of sodium and potassium are 589 nm and 766.5 nm, respectively. Aldosterone
208 levels in serum was determined using Aldosterone ELISA Kit (Enzo Life Sciences, Inc.
209 Switzerland) based on the binding of aldosterone to a specific antibody that is immobilised on
210 the wall of plate of 96 wells. The albumin levels in serum and urine were
211 spectrophotometrically determined using commercially available kits (Tietz, 1991). The serum
212 ratio albumin/creatinin was also estimated. Serum and urine urea levels were measured using
213 a Randox diagnostic kit (Crumlin, Co., Antrim, UK). Urinary and plasma osmolality were
214 determined by the technique pressure steam with the osmometer model 5100C (Wescor Inc.,
215 USA).
216 Na^+, K^+, Se, urea, and creatinine clearances were calculated from the standard formula:
217 clearance (CL) = U * V/P, where U is the level in urine of the substance to be cleared, V the
218 volume of urine collected in 24 h, and P the level of the substance studied in plasma. The ratios
219 between Na^+, K^+, Se or urea in relation to creatinine were calculated as CLx/CLCreatinine × 100,
220 where x is the substance to be compared. Fractional excretion of sodium (FENa) and
221 transtubular potassium gradient (TTKG) were calculated by standard formulae (FENa = UNa+ ·
222 PCr/PNa+ · UCr); TTKG = (POsm x UK+)/(PK+ x UOsm); where U is the level in urine of the
223 substance, P the level of the substance studied in plasma; and Osm is the osmolality.
224 **Systolic Blood pressure (mm Hg).** Systolic blood pressure (SBP) was monitored with pressure
225 meter (NIPREM 645, CIBERTEC, Spain) using the indirect tail occlusion method. Measurements
226 were taken in 28d old rats. The signals collected were treated with an IT support via a data
227 acquisition system coupled to the pressure meter. Each animal was measured 4-5 times
228 successively in order to calculate the arithmetical mean, this being the value used.
229 **Statistical Analysis.** The results are expressed as means ± standard error of the mean (SEM).
230 The data were analysed 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:

Kcal intake, body weight, glycemia and SBP in dams. Table 1 shows that MS dams intake less kcal and Se than control ones during lactation only, presenting a lower increase in body weight at the end of experimental procedure. During gestation and breastfeeding, MS dams have high SBP values while also presenting hyperglycemia during gestation.

Selenium and kidney development in pups. MS offspring intake less milk and Se by milk than control ones, having a lower body and kidney weight. However, relative kidney weight and Se deposits increased significantly. Kidney protein content was lower. One week later MS pups have significantly high SBP and normal glycemia (Table 1).

Kidneys selenoprotein expression. Figure 1 shows that the expression of the four selenoproteins measured (GPx1, GPx3, GPx4 and SelP) is significantly higher in MS pups with respect to control ones.

Kidneys oxidative balance. MS pups have low GR and CAT activities and high GPx activity. The lipid oxidation marker (MDA) increases in MS pups (Figure 2).

Kidneys AMPK and d NF-κB activation. Figure 3 shows that the expression of the energy status marker, AMPK-t is higher in MS pups. Its activation however is significantly lower in these pups with respect to control ones. The expression of NF-κB p65 (the active form of NF-κB) is significantly higher in MS pups.

Serum, urine and clearance values: in serum, creatinine, Na+, and urea are significantly higher in MS pups with respect to C ones. In urine all of the parameters measured, except creatinine

254 are higher. MS pups present low urinary flow and therefore high osmolality in urine. Relative
255 Na+ and Se clearances are lower and K+ is higher. (Table 2)

256 **Renal functional parameters:** MS-exposed pups have low creatinine clearance and fractional
257 excretion of Na+ (FENa), and high albumin/creatinine ratio, as well as high transtubular
258 potassium gradient (TTKG) and aldosterone serum levels (Figure 4).

259 **DISCUSSION:**

260 At the end of the experimental procedure, the dams which suffered high-fructose diet-induced MS
261 during gestation and lactation have a lower body weight gain. However, they intake less kcal and
262 Se during the lactation period only and therefore their pups could be exposed to caloric restriction
263 during breastfeeding. Despite these dams having high systolic blood pressure (SBP) during
264 gestation and lactation, hyperglycemia takes place during gestation only, the fetuses being
265 exposed to a hyperglycemic uterine environment. The high SBP suffered by the dams is probably
266 related to the high SBP that their pups suffer at 28 days old (25). Interestingly, during lactation the
267 dams make an effort to supply a sufficient amount of Se through milk (4), but MS pups have a
268 lower appetite, intake less Se via milk; have a lower body weight and lower kidney weight.
269 However relative kidney weight increases, probably indicating that fibrosis is taking place in
270 kidneys. This fact, together with the lower protein content found, implies that kidneys in pups are
271 probably affected by MS.

272 It is important to point out that these animals have replete kidney Se deposits despite the fact
273 that they intake less Se during breastfeeding and they have unaltered serum Se levels. It is known
274 that Se deposits in kidney are age-dependent; they significantly increase during the postnatal
275 period (35). Moreover, the kidney is the principal route of Se excretion and it therefore
276 accumulates a high amount of Se. In this study MS pups have decreased their relative Se clearance
277 value, indicating that a tubular Se reabsorption process is taking place. Kidney is the main
278 producer of plasmatic GPx3, since MS pups have high kidney GPx3 expression. The kidney derives

279 Se in the form of GPx3 to plasma, avoiding in part plasmatic Se depletion and oxidation (36). This
280 derivation of GPx3 to plasma is important, since different clinical trials in adults and children with
281 MS and documented CVD have detected a significant decrease in circulating levels of the main Se
282 plasmatic transporter: SelP (37,38).

283 In fact, in kidney MS pups present an increase in the expression of the four selenoproteins
284 studied: GPx1, GPx3, GPx4 and SelP. The higher antioxidant activity of GPx measured in kidney
285 corresponded to GPx1 and GPx3. In other studies a high activity of GPx in proximal and distal
286 nephrons tubules and in renal arteries has been detected (39), GPx3 being detected weakly in
287 kidney proximal tubules and GPx1 being detected extensively in kidney tubular epithelial cells
288 (40). This increase in renal GPx activity seems to be necessary since lipid oxidation is taking place
289 in the kidney of MS pups, these latter suffering from significantly smaller CAT and GR systems. In
290 consonance with the hypernatremia found in the MS pups from this study, renal lipid peroxidation
291 leads to changes in membrane composition. This interferes with carrier functions such as Na^+K^+-
292 ATPase activity, increasing Na^+ reabsorption and K^+ excretion(41), especially in the renal papillary
293 collecting duct cells(42). Lipid oxidation also leads to progressive tissue injury, since ROS promotes
294 disease by activating different signaling pathways and transcriptional factors such as NF-κB,
295 stimulating inflammation and epithelial to myofibroblast transdifferentiation (15,40). In fact MS
296 pups also have a high amount of activated NF-κB in their kidneys.

297 With regard to GPx4 expression, GPx4 is the only selenoprotein associated with the
298 antioxidant protection of biomembranes and therefore of mitochondria (43). In studies with
299 transgenic mice, it has been shown that GPx4 inhibits oxidative-stress-induced cyt. c release
300 from mitochondria and the induction of apoptosis (44). Moreover, there seems to be a
301 relationship between GPx4 and NF-κB; in the liver and kidney of adolescent rats exposed to
302 acute ethanol oxidative insult, GPx4 and NF-κB activation seems to be directly and inversely
303 related to apoptosis(45,46). NF-κB plays a crucial role in regulating inflammation, fibrosis and

apoptosis. Similarly, in this study both GPx4 and NF-κB expression are directly related and their presence is greater in the kidneys of MS pups than in control pups.

Despite the fact that all of the effects of GPxs on kidney seem to be positive for a correct renal function, a situation could arise where, like in liver, high antioxidant GPx activity will, by preventing H_2O_2, have detrimental efects. It is known, for instance, that low levels of H_2O_2 are necessary for a correct insulin signalling process in liver (47), as well as for stimulating AMPK in kidneys, mainly in podocytes (48). In kidneys AMPK plays a unique role at the crossroads of energy metabolism, ion and water transport, inflammation and stress (31). In fact, AMPK activation protecs against inflammation and fibrosis in diabetic nephropathy (DN) models and it is suggested that low AMPK activity in DM could be part of DN progression etiology (49). Therefore the low renal AMPK activation found in MS-exposed pups could be related to renal damage. This low activation could be due to a lower amount of H_2O_2 caused by GPx, but what is worse is that is could be due to the higher expression of SelP found, since in liver it is well-documented that SelP inactivates AMPK. However little is known about this relationship in kidneys. These facts return us to the famous question: is Se a good theraphy for MS pups? However, it is important to remember that the general endocrine response in MS-exposed pups is more similar to those offspring exposed to Se deprivation during gestatoin and lactation, since they developed DM I and present renal damage (50).

The biomolecular changes found in the kidneys of MS pups are reflected in functional alterations in kidney. Consistent with well-established DN in the later stages (51), MS pups have albuminuria (high albumin/creatinine ratio) and low GFR which implies glomerular renal impairment with protein loss, and low urinary flow with highly concentrated urine. These results also indicate that a profound water reabsorption process is taking place, and that there is an increase in volemia – and probably BP. Moreover, MS-exposed pups also present hypernatremia and hyperaldosteronemia, together with a high level of renal Na^+ reabsorption, leading to a hypertensive status, detected in these animals one week later. Similar data

relative to renal programming changes focused on the effects of maternal nutritional status during intrauterine development have been found (9–12,14,52). All point to a glomerular hypertrophy with podocyte damage and to inappropriate sodium retention as the main cause of renal injury and hypertension. As mentioned in the introduction, the oxidative balance is important in both alterations, since the administration of adiponectin has been reported to improve podocyte permeability to albumin through the inhibition of NADPH oxidase activity (53), superoxide action reduce local NO levels in kidney (54) and oxidative stress alters the interplay between aldosterone and its receptor (55) which also activates NADPHoxidase (56). For these reasons it could be supposed that the high amount of Se found in the kidney of MS pups should have beneficial antioxidant roles through the selenoproteins. In this context, in a study of adolescent rats with renal damage caused by acute ethanol exposure, Se was found to be depleted in kidneys. Se supplementation, by increasing GPxs activity inflammatory balance and apoptosis, improved kidneys functions – mainly those related to electrolyte balance, since it partially decreases Na^+ and serum Aldo levels by increasing Aldo renal clearance (46).

In conclusion, MS pups suffer oxidative stress-related renal programing alterations which are deeply implicated in later HTA development. Se renal deposits and antioxidant selenoproteins in these pups increased significantly, avoiding in part lipid renal oxidation. However, more data are necessary to document the relationship between GPx4 and NF-κB, and SelP and AMPK in kidney in order to analyze the possible beneficial role of Se in kidney. Once more, since Se deposits in MS pups are up- or down-regulated in HTA control-related tissues such as kidney and heart respectively, a controlled Se supplementation redirected to heart, perhaps using nanotechnology, should be considered in any future study if, during early programming process, there are suspicions of HTA status, such as MS.

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Author contributions statements: MLO and FN were responsible for the study concept and design. ASR and FN were responsible for acquisition of animal data. FNB and MLO were responsible for data analysis and interpretation of findings. MLO drafted the manuscript. OC and MLM provided critical revision of the manuscript. All authors critically reviewed content and approved final version for publication. OC was responsible to find financing for the study.

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		C	MS
AN	**Initial body weight** (g)	194.1 ± 6.1	190.9 ± 5.7

	Solid Kcal intake (Kcal/day)	74.1 ± 1.7	73.2 ± 4.3
	Se intake (µg/day)	1.85 ± 0.04	1.83 ± 0.11
	Serum glucose (mg/dl)	124.8 ± 2.4	327.5 ± 6.9***
	Systolic Blood Pressure (mmHg)	110.2 ± 3.3	133.6 ± 4.2***
LACTATING DAMS	**Solid kcal intake** (kcal/day)	179.7 ± 11.8	145.7 ± 9.3 *
	Se intake (µg/day)	5.1 ± 0.3	4.3 ± 0.2 *
	Weight gain (g)	49.4 ± 2.9	40.3 ± 2.1 *
	Serum glucose (mg/dl)	214.8 ± 6.3	221.8 ± 5.9
	Systolic Blood Pressure (mmHg)	116.42 ± 3.3	122.56 ± 0.9*
OFFSPRING 21day old			
	Milk intake (µg/30 min sucklig)	0.51 ± 0.03	0.39 ± 0.03**
	Se intake by milk (µg/30 min sucklig)	0.132± 0.002	0.102 ± 0.003**
	Weight (g)	34.7 ± 0.7	26.50± 0.5 ***
	Kidney weight (g)	0.53 ± 0.07	0.46± 0.03
	Kidney somatic index (KSI)	1.52± 0.05	1.71± 0.06 *
	Protein in kidney (mg/ml)	7.2± 0.4	6.1± 0.3 *
	Se in kidney (µg/g dry tissue)	0.59± 0.03	0.91± 0.03***
	Serum glucose (mg/dl) Pups 28 days old	148.4 ± 6.4	156.5 ± 5.9
	Systolic Blood Pressure (mmHg) Pups 28 days old	109.2 ± 2.3	116.6 ± 1.2*

Table 1. Nutritional parameters in dams and pups. 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, and in pups is 8. Statistic difference between groups was expressed as *p* value: C vs MS: * p<0.05, ** p<0.01, *** p<0.001.

Parameters		C	MS
SERUM	Creatinine (mg/dL)	0.19 ± 0.01	0.33 ± 0.02 ***
	Na^+ (mmol/L)	140.5 ± 0.7	143.3 ± 0.9 *

	K^+ (mmol/L)	6.4 ± 0.4	6.6 ± 0.6
	Urea (mg/dL)	25.9 ± 2.8	49.1 ± 4.9 **
	Selenium (µg/L)	314.6 ± 18.1	304.3 ± 25.1
	Osmolality (mosm/L)	301.3 ± 30.1	306.6 ± 30.6
URINE	Urinary flow (ml/day)	4.5 ± 0.4	2.1 ± 0.2 ***
	Creatinine (mg/dL)	24.9 ± 2.3	28.7 ± 1.1
	Na^+ (mmol/L)	20.1 ± 0.6	8.5 ± 0.4 ***
	K^+ (mmol/L)	28.5 ± 1.9	51.6 ± 0.9 ***
	Urea (mg/dL)	1567 ± 105	3807 ± 201 ***
	Selenium (ng/day)	10.7 ± 0.6	3.1 ± 0.3 ***
	Osmolality (mosm/L)	0.19 ± 0.02	0.41 ± 0.04 ***
	Albumin (mg/dL)	16.6 ± 1.4	23.4 ± 1.4 **
RELATIVE CLEARANCES	CL Na^+ /CL creatinine	0.122 ± 0.011	0.045 ± 0.004 ***
	CL K^+/CL creatinine	3.8 ± 0.4	5.5 ± 0.6 *
	CL Urea/CL creatinine	52 ± 5	69 ± 7
	CL Se/CL creatinine	0.059 ± 0.006	0.014 ± 0.001 ***

Table 2. Results in 21d old pups: Creatinine, Na+, K+, Urea and Selenium in serum and urine, and their relative clearances. Urinary flow, osmolality and serum osmolality. 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 caption:

Fig. 1 Expression of selenoproteins (GPx1, GPx3, GPx4 and SelP), in kidney of 21d old pups. Representative western blots of these proteins (normalized to β-actin). 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.

Fig. 2 Oxidative balance in the kidneys of 21d old pups: SOD activity, CAT activity, GPx activity , GR activity and lipid oxidation. 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.

Fig. 3 Kidneys energetic and inflammatory profile in 21d old pups. Expression of AMPK-t, AMPK-p and active NFkB. Representative western blots of these proteins (normalized to β-actin). 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.

Fig. 4 Results in 21d old pups: renal function 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<0.001.