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MATERNAL SELENIUM STATUS IS PROFOUNDLY INVOLVED IN METABOLIC FETAL PROGRAMMING BY MODULATING INSULIN RESISTANCE, OXIDATIVE BALANCE AND ENERGY HOMEOSTASIS.

Ojeda María Luisa, **Nogales** Fátima*, **Membrilla** Alba, **Carreras** Olimpia.

Department of Physiology, Faculty of Pharmacy, Seville University, 41012 Seville, Spain.

*Correspondence to: Dra. Fátima Nogales Bueno

Department of Physiology.

Faculty of Pharmacy, Seville University.

C/ Profesor García González, nº 2.

41012. Sevilla. Spain.

Tel: +34 954556518

Fax: +34 954233765

E-mail: fnogales@us.es

Running title: Selenium involvement in metabolic fetal programming.

ABSTRACT:

Purpose: High and low levels of selenium (Se) have been related to metabolic disorders in dams and in their offspring. Their relationship to oxidative balance and to AMP-activated protein kinase (AMPK), are some of the mechanisms proposed. The aim of this study is to acquire information about how Se is involved in metabolic programming.

Methods: three experimental groups of dam rats were used: control (Se: 0.1ppm), Se-supplemented (Se: 0.5ppm) and Se-deficient (Se: 0.01ppm). At the end of lactation, the pups' metabolic profile, oxidative balance, Se levels, selenoproteins and IRS-1 hepatic expression, as well as hepatic AMPK activation were measured.

Results: The experimental groups present deep changes in Se homeostasis, selenoproteins and IRS-1 hepatic expression, oxidative balance, AMPK activation ratio and insulin levels. They do, however, have different metabolic profiles.

Conclusions: High- and low- Se diets are linked to insulin resistance, yet the mechanisms involved are completely opposite.

Key words: Dietary Selenium, metabolic programming, insulin resistance, oxidative balance, energy homeostasis.

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53 **1. INTRODUCTION:**

54 Trace elements, such as Se, Zn, Cr and Li are known to influence the endocrine regulation of
55 energy metabolism in mammals, mainly by enhancing or interfering with components of the
56 insulin signaling cascade, affecting carbohydrate and lipid metabolism and energy homeostasis [1].
57 Se plays its biological functions by forming part of 25 selenoproteins [2], among which the family
58 of the antioxidant enzymes glutathione peroxidase (GPx), and the Se plasma transporter
59 selenoprotein P (SelP) have recently been related to Insulin Resistance (IR) and Metabolic
60 Syndrome (MS). MS is one of the most important metabolic disorders affecting the world
61 population. It is defined as a cluster of risk factors including obesity, IR, raised blood pressure and
62 dyslipidemia, predisposing the sufferer to cardiovascular diseases and diabetes [3]. This syndrome
63 appears among 20% of pregnant women, affecting both mother and offspring [4], even during the
64 latter's adulthood, through metabolic fetal programming [5]. In an experimental rat MS model, Se
65 body distribution and selenoprotein activities have been found to be altered in both dams and
66 their offspring [6-7] . However, depending on the tissue under study, Se tissue deposits have been
67 both increased and decreased. This makes it difficult to understand whether Se supplementation
68 could be an effective therapy for MS development or, indeed, pose a risk [8].

69 In this context, both infra- and supra- Selenoprotein regulations have been involved in the
70 development of IR and MS [9-11]. Seale et al., [12], found that upon dietary restriction,
71 selenocysteine lyase knockout mice cannot supply Se for selenoprotein biosynthesis and that they
72 develop fatty liver, hypercholesterolemia, and glucose intolerance; GPx1 and SelP expression

73 decreased; oxidative stress appeared and insulin-signaling inhibitor protein-tyrosine phosphatase
74 1B (PTP1B) levels increased. Reddi and Bollineni, [13] found that Se deficiency impaired pancreas
75 islet function and free radical-scavenging systems in rats, resulting in a decreased insulin secretory
76 reserve. If Se levels are below 80 µg/l in the offspring of diabetic patients, Se correlates inversely
77 with insulin resistance [14]. However, no positive effect on diabetes prevention with Se was found
78 and some data even pointed towards supranutritional Se status as an unexpected risk factor in
79 potentiating IR [15].
80 As an excess of reactive oxygen species (ROS) causes damage to mitochondrial components, which
81 are involved in the pathogenesis and etiology of IR [16], the antioxidant properties of
82 selenoproteins are necessary to prevent metabolic disorders. However, when insulin reaches its
83 receptor, a small amount of ROS, necessary for the insulin to act, are liberated. These species
84 deactivate the insulin-signaling inhibitors' phosphatase protein (PTEN) and protein tyrosine
85 phosphatase 1B (PTP-1B) which contribute to the insulin signaling process. When GPx1 acts, this
86 process is prevented, thus leading to IR. In agreement with the above, Steinbrenner, [17] showed
87 that GPx1 and/or SelP inhibited phosphorylation (activation) of key mediators, such as protein
88 kinase B (Akt) and adenosine monophosphate-activated protein kinase (AMPK), in energy
89 metabolism in liver and/or skeletal muscle. AMPK is an energy status sensor that controls cellular
90 energy homeostasis and activates energy production processes by the stimulation of catabolic
91 pathways and the inactivation of processes involved in ATP consumption [18]. Supranutritional Se
92 supply also induces alterations in energy-metabolism-related molecular targets in the skeletal
93 muscle and visceral adipose tissue of pigs [19]. Recently Tajima-Shirasaki et al., [20] have found
94 that suppressing SelP may provide a novel therapeutic approach to treating type 2 diabetes in rat
95 hepatoma cell line. Using eicosapentaenoic acid, they suppressed *SelP* expression by inactivating
96 sterol regulatory element-binding protein-1c and caused a reduction in SelP expression. This
97 reduction, moreover, activates AMPK, preventing IR induction and vascular endothelial growth
98 factor in type 2 diabetes. This is, however, an *in vitro* study and Se status in different tissues such

99 as muscle and heart are also important key factors that need to be analyzed. Zhou et al., [21]
100 found that Se-enriched exopolysaccharides alleviate adipose inflammation in diabetic mice by
101 exerting anti-diabetic effects. GPx1 also plays an important role in pancreas, stimulating different
102 molecules involved in insulin synthesis and secretion [22]. SelP did not, however, show any effect
103 on beta cell mass or insulin synthesis and secretion.
104 Chronic metabolic alterations such as IR, MS or type 2 diabetes leads to cellular oxidative
105 dysfunction, but also to endoplasmic reticulum (ER) stress and inflammation. The ER is an
106 organelle specialized in integrating cellular stress responses, and has seven ER-resident
107 selenoproteins necessary for its correct function [23]. At the present moment, therefore, there is
108 no clear understanding of the relationship between Se status and IR.
109 During healthy pregnancy, maternal organs and placenta adapt to physiological changes
110 related to hormones and energy homeostasis. Therefore, during this period, women have a
111 greater predisposition to suffering metabolic disorders, which could even, in some cases, have
112 repercussion both on them and their progeny. For this reason, the aim of the present study is
113 to analyze the repercussion of Se status in metabolic fetal programing by modulating insulin
114 resistance, oxidative balance and energy homeostasis.

115 **2. MATERIAL AND METHODS.**

116 **2.1. Animals.** Male and female Wistar rats (Centre of Production and Animal experimentation,
117 Vice-rector's Office for Scientific Research, University of Seville) weighing approximately 150-
118 200 g, were randomised into three groups: control (C), selenium supplemented (SS) and
119 selenium deficient (SD) groups. Animal care procedures and experimental protocols were
120 performed in accordance with EU regulations (Council Directive 86/609/EEC, November 24th
121 1986) and approved by the Ethics Committee of the University of Seville. All rats received
122 drinking water and diet *ad libitum* during three week before mate, and then, during gestation
123 (3 weeks) and lactation (3 weeks) periods. C, SS and SD groups received solid diets with 0.1,

124 0.5 or 0.01 ppm of Se respectively. Se was supplemented as anhydrous sodium selenite (an
125 inorganic compound; Panreac, Barcelona, Spain). The diets of these rats were prepared
126 according to The Council of the Institute of Laboratory Animal Resources (ILAR, 1979) which
127 details known nutrient requirements for most of the common laboratory animals.
128 In four week, male (n=3) and female (n=6) rats were mated to obtain the first-generation
129 offspring for each group. Pregnant female rats were inspected daily by the presence of the
130 vaginal plug, which indicated day zero of pregnancy; at this moment pregnant rats were
131 housed individually in plastic cages. The day of parturition, which occurs spontaneously three
132 weeks after coitus, was designated as day 1 of lactation. The offspring number was reduced to
133 8 per mother at parturition (four males and four females, when this was possible). The
134 experiments were performed on the offspring of all groups to 21d postpartum. In this study,
135 we have used 8 pups per group to measure all the parameters cited below. These 8 pups
136 represent all the litters, as a maximum of 2 rats per litter, and were allocated to each group
137 taking into account the sex.
138 **2.2. Nutritional controls.** Body weights of the dam rats were determined once a week while
139 that the amount of food and liquid consumed by rats were monitored daily until the end of the
140 experimental period. Se intake was calculated by multiplying the food consumed by ppm of Se
141 in the diets. Weekly, body weight and cranium-caudal length of pups was controlled, using a
142 metric calliper, until end of the experimental period, to calculate body mass index (BMI)
143 according to the formula: Body weight (g)/length^2 (cm^2). All measures were taken at 9:00 am
144 to avoid changes due to circadian rhythms.
145 **2.3. Samples.** The amount of milk consumed by the offspring at the end of the lactation period
146 (days 19 and 20) was estimated by subtracting the weight of the pups obtained immediately
147 prior to returning them to the dam from their weight after 30 minutes of suckling. In order to
148 obtain the maximum amount of milk at day 21 of lactation, 3h after removing the litters from
149 their mothers, the dams were anesthetized with urethane, and milk samples were immediately

150 collected. The milk was obtained by gently massaging the area around each of the 12
151 mammary glands and then pressing upward from the base of the gland towards the nipple.
152 The amount of milk collected was around 1 to 1.5 ml per dam.
153 At the end of the experimental period, dams and their pups were weighed and anesthetized
154 with intraperitoneal 28% w/v urethane (0.5 ml/100 g of body weight). Blood samples were
155 obtained by heart puncture and collected in tubes. The serum was prepared using low-speed
156 centrifugation for 15min. at 1300 *x* g*.* The abdomen was opened by a midline incision and
157 pancreas and liver were removed, debrided of adipose and connective tissue in ice-cold saline,
158 weighed and stored at −80◦C prior to biochemical determinations. Hepatic and pancreatic
159 somatic index (HSI and PSI) were calculated as (liver or pancreas weight / total body weight).
160 **2.4. Metabolic profile.** The metabolic profile was determined in offspring before sacrifice, in
161 blood from their tails. Glucose, triglycerides (TG) and cholesterol were determined using test
162 strips Accutrend (ROCHE, Spain). Serum insulin was determined by using a rat insulin ELISA kit
163 (BioVendor GmbH, Heidelberg, Germany) according to the manufacturer's instructions. The
164 model homeostasis assessment of insulin resistance index (HOMA-IR) was calculated according
165 to the following formula: (Fasting glucose concentration x Fasting insulin serum
166 concentration)/ 405. Creatinine and urea in serum was determined by colorimetric methods
167 using a commercial kits (BioSystems kit (Barcelona, Spain) and Randox diagnostic kit (Crumlin
168 Co., Antrim, UK) respectively).
169 **2.5. Selenium analysis.** Selenium levels were determined by graphite-furnace atomic
170 absorption spectrometry, using a PerkinElmer AAnalyst™ 800 high-performance atomic
171 absorption spectrometer with WinLab32 for AA software, equipped with a Transversely
172 Heated Graphite Furnace (THGA) with longitudinal Zeeman-effect background corrector and
173 an AS-furnace autosampler (PerkinElmer, Überlingen, Germany). The source of radiation was a
174 Se electrodeless discharge lamp (EDL). The instrumental operating conditions and the reagents
175 are the same that we have used in the previous paper [24]. Samples: serum samples were

176 diluted fivefold in 0.2% v/v HNO_3 and 0.2% Triton X-100 solutions and urine samples were
177 diluted 1:2 v/v. After 72h at 100ºC dry temperature, pancreas, liver and milk samples were
178 weighed and digested in a sand bath heater (OVAN, Badalona, Spain) with nitric acid for 72h.,
179 and perchloric acid and chlorhydric acid (6N) were added.

180 **2.6. Antioxidant enzymes and oxidative stress markers.** In order to measure the activity of
181 antioxidant enzymes (SOD, CAT, GPx and GR) as well as lipid and protein oxidation (levels of
182 MDA and carbonyl group (CG) respestively), liver tissue samples were homogenized (100 x g
183 for 1min, 1:4 w/v) using a Potter homogenizer (Pobel 245432, Madrid, Spain) in a sucrose
184 buffer (15 mM Tris/HCl, pH 7.4, 250 mM sucrose, 1 mM EDTA and 1 mM dithiothreitol) in an
185 ice bath. The homogenate was centrifuged at 900 x g for 10min at 4 ºC. The resulting
186 supernatant was employed for the biochemical assay according to techniques described in
187 [25].

188 **2.7. Immunoblotting assays.** The expression of total and phosphorylated AMPK (AMPK and
189 pAMPK), IRS-1, SelP and GPx1 were determined in hepatic homogenates (with Protease
190 Inhibitor (ROCHE) and also Phosphatase Inhibitor (ROCHE, Spain) to pAMPK) according to
191 method deeply described in (Nogales et al., (2017). The samples utilized, which contained 100
192 µg of protein, were incubated overnight at 4ºC with specific primary antibodies (rabbit
193 polyclonal IgG, Santa Cruz Biotechnology) in dilutions: AMPK and pAMPK (1:4000), IRS-1
194 (1:1000), SelP and GPx1 (1:20000); and secondary antibody (anti-rabbit IgG HRP conjugate,
195 Santa Cruz Biotechnology) in dilutions: AMPK α½ and pAMPK α½ (1:4000, 1:2000), and IRS-1
196 (1:2500), SelP and GPx1 (1:5000). Monoclonal mouse anti β-actin (IgG1, Sigma-Aldrich, Spain)
197 (1:20000) was used to detect β-actin as a loading control, and a secondary antibody (anti-
198 mouse IgG HRP conjugate, Sigma-Aldrich, Spain) in dilutions 1:8000. The quantification of the
199 blots was performed by densitometry with PCBAS 2.08e software analysis (Raytest Inc,
200 Germany). The results were expressed as percent arbitrary relative units referred to values in
201 control pups which were defined as 100%. The samples from experimental and control animals

202 run on the same gel. Activation of AMPK was determined by the ratio pAMPK/ tAMPK, which
203 was calculated dividing pAMPK expression by AMPK expression values.

204 **2.8. Statistical Analysis.** The results are expressed as means ± standard error of the mean
205 (SEM). The data were analysed using a statistical program (GraphPad InStat 3, CA, USA) by
206 analysis of variance (one-way ANOVA). The statistical significance was established at $p<0.05$.
207 When ANOVA resulted in differences, multiple comparisons between means were studied by
208 the Tukey-Kramer test.

209 **3. RESULTS:**

210 **3.1. Effects of selenium status on food intake, morphological changes and Se body**
211 **distribution.** Table 1 shows that SS dams intake a higher amount of solid diet than
212 controls, reporting an increase in weight gain during lactation. SD dams, unexpectedly,
213 present the highest solid intake figures; however, these dams have lower increase in body
214 weight, which is consistent with the lowest Se intake. The same pattern appears in their
215 offspring, SD pups have lower body weight gain and cranium-caudal length than controls,
216 but their BMI is not altered. On the contrary, SS pups have higher body weight increase and
217 BMI than control ones.

218 Milk Se concentration decreases in SD dams; their pups, however, present normal Se
219 serum values, yet drastically low Se levels in liver and pancreas, presenting this last tissue
220 a poor development. SS offspring receive higher amount of Se via milk, they have higher
221 serum and hepatic Se levels than control pups, along with hepatomegaly. However
222 pancreas Se deposits are not altered.

223 **3.2. Selenium status and hepatic oxidative balance.** With respect to control animals, the
224 pups which received a Se-deficient diet have higher SOD activity, lower CAT and GPx
225 activity and a lower antioxidant enzymes ratio, consistent with protein oxidation. SS

226 offspring have higher activity of the three antioxidant enzymes but a normal antioxidant
227 enzymes ratio, with no lipid or protein oxidation (Table 2).

228 **3.3. Selenium status on serum triglycerides, glucose, cholesterol, creatinine and urea**
229 **values.** The Se-supplemented diet greatly increases serum TG levels in pups with respect
230 to control ones; they also have lower creatinine serum levels. The Se-deficient diet leads
231 to an extremely significant increase in serum TG, cholesterol and glucose levels in pups
232 ($p<0.001$) and to an increase in serum creatinine and urea ($p<0.05$ and $p<0.001$,
233 respectively) compared to control pups (Figure 1).

234 **3.4. Expression of hepatic selenoproteins related to insulin resistance: GPx1 and SelP.** SS
235 offspring have higher GPx1 and SelP expressions than control ones. Those pups which
236 received a low Se diet present an almost insignificant GPx1 expression and very
237 significantly low SelP values ($p<0.001$) (Figure 2).

238 **3.5. Hepatic expression and activation of AMPK, the controller of cellular energy**
239 **homeostasis.** Those pups whose mothers were exposed to a high Se diet have a lower
240 expression of pAMPK and of the activation pAMPK/tAMPK ratio. The Se-deficient diet
241 leads to an extremely low hepatic expression of tAMPK and pAMPK. The functional ratio,
242 however, was up-regulated (Figure 3).

243 **3.6. Insulin response profile: hepatic expression of IRS-1, serum insulin levels and**
244 **HOMA-IR value.** The two experimental groups studied have a lower expression of hepatic
245 IRS-1, especially the Se-supplemented group, coinciding with higher insulin serum levels
246 and a higher HOMA-IR value. SD pups, however, have extremely low insulin levels and
247 HOMA-IR values (Figure 4).

248 **4. DISCUSSION:**

249 Dams exposed to a high-Se diet during gestation and lactation intake a greater amount of food
250 and obviously of Se, thus showing an increased gain in body weight. Se concentration in milk
251 was, however, unaltered, maybe due to the effort that mothers make in order to maintain Se
252 homeostasis. Selenoproteins have been intimately related to obesity in a porcine model [26].
253 In this model, 12 selenoprotein genes were upregulated in six tissues and 13 were
254 downregulated in seven tissues during obesity. These selenoprotein changes were mainly
255 correlated with body weight and circulating TGs. Rat SS offspring, like their mothers, intake a
256 greater amount of milk and present a higher increase in body weight and BMI, showing a
257 tendency to obesity, also a sign found in human newborns whose mothers suffer gestational
258 diabetes [27]. In SS rat pups serum Se levels are high and they present higher Se liver deposits
259 and hepatomegaly. It is known that SS pups deposit their excessive Se levels in tissues,
260 especially liver and kidney, the liver being the body's main Se reservoir [28].

261 The repletion of Se found in the liver of SS pups is related to a higher activity of the antioxidant
262 selenoprotein GPx1, which is known to be necessary for preventing the oxidative stress
263 generated during metabolic dysfunctions such as MS [29]. Moreover, these pups also present
264 higher SOD and CAT antioxidant enzyme activity; their final oxidative balance remaining
265 unaltered, thus preventing hepatic oxidation. This general antioxidant upregulation is of great
266 importance, since despite the fact that GPx1 increases in MS pups, the enzyme SOD decreases
267 and liver oxidation takes place [7]. The higher levels of hepatic Se deposits in SS pups are in
268 consonance with a greater expression of GPx1 and SelP which are also involved in IR genesis,
269 albeit through different mechanisms. As mentioned previously, GPx1 acts by decomposing the
270 oxidative radical H_2O_2. This effect, however, also has an undesirable aspect: H_2O_2 is a necessary
271 compound for the insulin signaling pathway, while SelP acts by decreasing the key energy
272 factor AMPK [9, 30]. These pups therefore present high SelP and a low AMPK/AMPKt
273 activation ratio. AMPK activates the energy production process by catabolic pathways and its
274 decrease should produce a tendency to anabolic processes, such as lipogenesis.

275 In fact, these pups have extremely high serum TG levels which, along with the hepatomegaly
276 detected, could be an indicator of hepatic steatosis. Recently, moreover, AMPK activation has
277 been reported as being involved in the insulin signaling cascade since, by inhibiting the
278 mammalian target of rapamycin (mTOR), it amplifies the cascade [31-32]. Its decrease is,
279 therefore, related to an increase in IR. The high serum TG levels found in SS pups might also
280 be due to other mechanisms and not only to changes in liver insulin metabolism.

281 It has been shown [19] that supranutritional Se induces alterations in gene expression and
282 protein phosphorylation related to energy metabolism in the skeletal muscle and the visceral
283 adipose tissue of adult pigs. Specifically, in these pigs' visceral adipose tissue, mRNA levels of
284 Sterol Regulatory Element Binding Transcription Factor 1 (REBF1) increased. This factor is
285 required for lipid homeostasis and regulates transcription of the LDL receptor gene, as well as
286 the fatty acids and, to a lesser degree, the cholesterol synthesis pathway.

287 Since SS offspring present obesity, this theory is also plausible, and will correlate with the
288 extremely high TG levels found. Pinto et al. [19] also found a lower relative AMPK activation
289 expression in adipocytes, which was also related to adipose tissue metabolism and the pro-
290 inflammatory environment that appears in hypertrophic adipocytes [33]. It is known that
291 AMPK activation in adipocytes inactivates the lipogenic action of Acetyl-CoA carboxylase (ACC),
292 the main enzyme involved in body TG synthesis. A hypothetical decrease in AMPK activity in
293 adipocytes might be taking place in SS pups and could be the cause of the large amount of
294 circulating TG found. Moreover, in addition to glucose transport, lipid and protein synthesis,
295 AMPK regulates different factors that have been linked to IR, including inflammation, oxidative
296 stress and ER stress [34].

297 Therefore, in relation to Se levels and selenoprotein expression in liver, SS pups present an
298 increase in GPx1 and SelP, a decrease in p-AMPK and IRS-1 expression, which are all IR genesis-
299 related factors. Despite the fact that SS pups have normal pancreatic Se deposit values, they

300 have high serum insulin and HOMA-IR values and a high BMI consistent with a type 2 diabetes or
301 gestational diabetes process.

302 The dams exposed to a low-Se diet during gestation and lactation intake an extremely large amount
303 of food, but they present the lowest weight gain together with a low Se intake. Se deficiency is,
304 therefore, intimately related to the modulation of solid intake and body weight, since SD dams
305 intake a sufficient amount of other nutrients. Taking into account their body weight SD pups, like
306 their mothers, also intake a greater amount of milk and like them, present a lower body weight as
307 well as being shorter in length. This, as other authors have pointed out [35], indicates that SD pups
308 suffer severe developmental problems and that correct Se levels are necessary for normal growth
309 and development in offspring. Se is necessary for correct thyroid hormones synthesis, insulin-like
310 growth factor-I (IGF-1) regulation, and for a correct oxidative balance – all factors that are intimately
311 related to normal growth and development [25]. In this context Selenoprotein T (SelT) has recently
312 been characterized as a protein whose expression is very high during development; it is confined to
313 endocrine tissues and is required for adapting to stressful endocrine situations. SelT is expressed on
314 the ER membrane in all hormone-producing pituitary cell types and is essential to endocrine
315 regulation [36]. SD pups probably have low levels of this selenoprotein. Furthermore, GPx4 knockout
316 mice are non-viable, since GPx4 is the only selenoprotein which protects cellular membranes and
317 mitochondria from oxidation and it plays an important role in apoptosis regulation [37]. SD pups
318 have a profound depletion of Se in liver, but their livers are correctly developed. These pups also
319 have undetected Se deposits in pancreas, since Se is necessary for correct insulin synthesis. This
320 depletion could be related to the extremely low insulin serum levels found, and also to their
321 underdeveloped pancreas.

322 The depletion of Se found in the liver of SD pups is related to a significantly low GPx antioxidant
323 activity. SD offspring have a higher SOD activity and lower antioxidant activity ratio, leading to
324 protein oxidation. This increase in SOD activity during Se-deficient periods has been reported

325 previously and is probably due to the high amount of superoxide anion generated by mitochondrial
326 dysfunction [38]. In consonance with their low Se deposits, SD pups have an extremely low GPx1 and
327 SelP expression in liver. This low SelP expression is inversely proportional to the relative
328 phosphorylation of AMPK, favoring a catabolic state. In fact, metabolic serum parameters are
329 profoundly altered in these offspring. SD pups have high TG levels in serum, but also high levels of
330 cholesterol and glucose. All of the body energy sources measured in these pups indicated that Se
331 deficiency leads to a general biomolecular catabolism. In this context, He et al., [39] found similar
332 results in adult rats exposed to a Se-deficient diet and also found that non-esterified fatty acids and
333 total amino acids were significantly higher in serum. Serum insulin levels were, however, drastically
334 lower. The catabolic energy upregulation is also confirmed by the high levels of serum creatinine
335 and urea, both markers of muscle and protein catabolism, that were found. This energy-wasting
336 process is intimately related to the lower development and the high food intake observed in SD
337 offspring. AMPK phosphorylation inhibits mTOR activation, which in turn decreases protein
338 anabolism; it increases appetite and acts as a catabolic signal in skeletal muscle mass leading to
339 muscle wastage [40]. It is known that ROS stress and ER stress significantly impact the neural
340 regulation of the hypothalamic nucleus which regulates global energy metabolism [41]; both ROS
341 and ER stress homeostases are influenced by multiple hypothalamic selenoproteins which depend
342 on dietary Se intake. This global energy metabolism is deeply related to AMPK regulation and food
343 intake. The Se restriction provoked in SD pups probably alters hypothalamic selenoprotein
344 expression and function, profoundly disrupting energy balance.

345 When IRS-1 hepatic expression is analyzed it is low. Different authors have found pancreatic
346 atrophy, hypoinsulinemia and lower IRS-1 expression in different models of seleno-deficient animals
347 [42-43]. Moreover, in this study SD pups present extremely low insulin in serum and HOMA-IR
348 values, it seems that both insulin signaling and insulin secretion are linked to the cellular redox
349 state, and therefore to selenoproteins and Se homeostasis.

350 **In conclusion**, SS offspring present hepatomegaly and probably steatosis, repletion of Se in liver
351 related to higher GPx1 and SelP expressions, lower AMPK activation, lower IRS-1 expression, and
352 high serum TGs levels. They present a high BMI, high insulin secretion and no hepatocytes oxidation.
353 SS pups therefore present a metabolic profile more similar to those of offspring whose mothers
354 suffer gestational or type 2 diabetes. However, SD pups present lower body weight, lower
355 pancreatic development, protein oxidation in liver, high levels of TGs, creatinine and urea in serum,
356 and low insulin levels and expression of hepatic IRS-1. Therefore SD pups present a metabolic profile
357 more similar to that of MS pups than to SS ones, since this profile is more closely-related to type 1
358 diabetes with extremely low insulin secretion and renal damage. SD pups also present an extremely
359 high catabolic energy profile with high levels of serum Se, cholesterol and glucose – all related to
360 very low Se and selenoproteins tissular deposits and an increase in relative AMPK hepatic activation.
361 It could be concluded that high- and low-Se diets led to insulin resistance. However, the mechanisms
362 involved are completely the opposite; one is related to repletion of Se in liver, a correct oxidative
363 balance and anabolic process, while the other is related to a depletion of Se in liver and pancreas,
364 oxidation and an extremely catabolic energy balance, lactating SD pups being the most affected.
365 Therefore, depending on the dams' metabolic profile, it will be of interest – or not, as the case may
366 be – to supply Se during gestation and lactation. Moreover, since Se deposits increase or decrease in
367 different MS pups' tissues, it would be interesting to redirect the Se provided to target tissues.

368 **Author contributions:** MLO and FN were responsible for the study concept and design. AM and FN
369 were responsible for acquisition of animal data. MLO was responsible for data analysis and
370 interpretation of findings. MLO drafted the manuscript. FN and OC provided critical revision of the
371 manuscript. All authors critically reviewed content and approved final version for publication. OC
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379 **REFERENCES.**

380 [1] Wiernsperger N, Rapin J (2010) Trace elements in glucometabolic disorders: an update.
381 Diabetol Metab Syndr 2:70.

382 [2] Carreras O, Ojeda ML,Nogales F (2016) Selenium Dietary Supplementation and
383 Oxidative Balance in Alcoholism In: Patel V (ed) Molecular Aspects of Alcohol and
384 Nutrition, 1st edn. Elsevier, UK, pp. 133–142.

385 [3] Day C (2007) Metabolic syndrome, or What you will: definitions and epidemiology. Diab
386 Vasc Dis Res 4:32.

387 [4] Zou M,Arentson EJ, Teegarden D et al (2012) Fructose consumption during pregnancy
388 and lactation induces fatty liver and glucose intolerance in rats. Nutr Res 32:588–598.

389 [5] Fowden AL, Forhead AJ (2004) Endocrine mechanisms of intrauterine programming.
390 Reproduction 127: 515–526.

391 [6] Nogales F, Ojeda ML, Muñoz del Valle P, Serrano A, Murillo ML, Carreras O (2017)
392 Metabolic syndrome and selenium during gestation and lactation. Eur J Nutr 56:819–
393 830.

394 [7] Ojeda ML, Nogales F, Muñoz Del Valle P, Díaz-Castro j, Murillo ML,Carreras O (2016)
395 Metabolic syndrome and selenium in fetal programming: Gender differences. Food
396 Funct 7: 3031-3038.

397 [8] Serrano A, Nogales F, Sobrino P, Murillo ML, Carreras O, Ojeda ML (2016) Heart
398 selenoproteins status of metabolic syndrome-exposed pups: A potential target for
399 attenuating cardiac damage. Mol Nutr Food Res 60: 2633-2641.

400 [9] Zhou J, Huang K, Lei XG (2013) Selenium and diabetes—Evidence from animal studies.
401 Free Radic Biol Med 65:1548–1556.
402 [10] Labunskyy VM, Lee BC, Handy DE et al (2011) Both maximal expression of
403 selenoproteins and selenoprotein deficiency can promote development of type 2
404 diabetes-like phenotype in mice. Antioxid Redox Signal 14:2327–2336.
405 [11] Wang X, Zhang W, Chen H et al (2014) High selenium impairs hepatic insulin sensitivity
406 through opposite regulation of ROS. Toxicol Lett 224:16–23.
407 [12] Seale LA, Hashimoto AC, Kurokawa S et al (2012) Disruption of the Selenocysteine
408 Lyase-Mediated Selenium Recycling Pathway Leads to Metabolic Syndrome in Mice.
409 Mol Cell Biol 32:4141–4154.
410 [13] Reddi AS Bollineni JS (2001) Selenium-deficient diet induces renal oxidative stress and
411 injury via TGF-β1 in normal and diabetic rats. Kidney Int 59:1342–1353.
412 [14] Ozkaya M, Sahin M, Cakal E et al (2009) Selenium Levels in First-Degree Relatives of
413 Diabetic Patients. Biol Trace Elem Res 128:144–151.
414 [15] Stranges S, Marshall JR, Natarajan R (2007) Effects of long-term selenium
415 supplementation on the incidence of type 2 diabetes: a randomized trial. Ann Intern
416 Med 147:217–223.
417 [16] Houstis N, Rosen ED, Lander ES (2006) Reactive oxygen species have a causal role in
418 multiple forms of insulin resistance. Nat 440: 944–948.
419 [17] Steinbrenner H (2013) Interference of selenium and selenoproteins with the insulin-
420 regulated carbohydrate and lipid metabolism. *F*ree Radic Biol Med 65:1538–1547.
421 [18] Hardie DG (2015) AMPK: positive and negative regulation, and its role in whole-body
422 energy homeostasis. Curr Opin Cell Biol 33:1–7.
423 [19] Pinto A, Juniper DT, Sanil M (2012) Supranutritional selenium induces alterations in
424 molecular targets related to energy metabolism in skeletal muscle and visceral adipose
425 tissue of pigs. J Inorg Biochem 114:47–54.

426 [20] Tajima-Shirasaki N, Ishii KA, Takayama H et al (2017) Eicosapentaenoic acid down-
427 regulates expression of the selenoprotein P gene by inhibiting SREBP-1c protein
428 independently of the AMP-activated protein kinase pathway in H4IIEC3 hepatocytes. J
429 Biol Chem 292:10791–10800.
430 [21] Zhou X, Wang F, Yang H et al (2014) Selenium-enriched exopolysaccharides produced
431 by Enterobacter cloacae Z0206 alleviate adipose inflammation in diabetic KKAy mice
432 through the AMPK/SirT1 pathway. Mol Med Rep 9:683–688.
433 [22] Pepper MP, Vatamaniuk MZ, Yan X et al (2011) Impacts of Dietary Selenium Deficiency
434 on Me tabolic Phenotypes of Diet-Restricted GPX1-Overexpressing Mice. Antioxid
435 Redox Signal 14:383–390.
436 [23] Addinsall AB, Wright CR, Andrikopoulos S, van der Poel C, Stupka N (2018) Emerging
437 roles of endoplasmic reticulum-resident selenoproteins in the regulation of cellular
438 stress responses and the implications for metabolic disease. Biochem J 475:1037-1057.
439 [24] Ojeda ML, Nogales F, Vázquez B et al (2009)Alcohol, Gestation and Breastfeeding:
440 Selenium as an Antioxidant Therapy," Alcohol Alcohol. 44:272–277.
441 [25] Nogales F, Ojeda ML, Fenutría M et al (2013) Role of selenium and glutathione
442 peroxidase on development, growth, and oxidative balance in rat offspring
443 Reproduction 146: 659-667.
444 [26] Zhao H, Li K, Tang JY et al (2015) Expression of Selenoprotein Genes Is Affected by
445 Obesity of Pigs Fed a High-Fat Diet. J Nutr 145:1394–1401.
446 [27] Metzger BE, Buchanan TA, Coustan DR et al (2007) Summary and Recommendations of
447 the Fifth International Workshop-Conference on Gestational Diabetes Mellitus.
448 Diabetes Care 30:S251–S260.
449 [28] Ojeda ML, Jotty K, Nogales F et al (2010) Selenium or selenium plus folic acid intake
450 improves the detrimental effects of ethanol on pups' Selenium balance. Food Chem
451 Toxicol 48: 3486-3491.

452 [29] Feoli AM, Macagnan FE, Piovesan CH (2014) Xanthine Oxidase Activity Is Associated
453 with Risk Factors for Cardiovascular Disease and Inflammatory and Oxidative Status
454 Markers in Metabolic Syndrome: Effects of a Single Exercise Session. Oxid Med Cell
455 Longev 2014:587083.
456 [30] Misu H, Takamura T, Takayama H et al (2010) A Liver-Derived Secretory Protein,
457 Selenoprotein P, Causes Insulin Resistance. Cell Metab 12:483–495.
458 [31] Tanti JF, Jager J (2009) Cellular mechanisms of insulin resistance: role of stress-
459 regulated serine kinases and insulin receptor substrates (IRS) serine phosphorylation.
460 Curr Opin Pharmacol 9:753–762.
461 [32] Sonntag AG, Dalle Pezze P, Shanley DP, Thedieck K (2012) A modelling-experimental
462 approach reveals insulin receptor substrate (IRS)-dependent regulation of adenosine
463 monosphosphate-dependent kinase (AMPK) by insulin. FEBS J: 279:3314–3328.
464 [33] Bijland S, Mancini SJ, Salt IP (2013) Role of AMP-activated protein kinase in adipose
465 tissue metabolism and inflammation. Clin Sci 124:491–507.
466 [34] Ruderman NB, Carling D, Prentki M, Cacicedo JM (2013) AMPK, insulin resistance, and
467 the metabolic syndrome. J Clin Invest 123:2764-2772.
468 [35] Mistry HD, Broughton Pipkin F et al (2012) Selenium in reproductive health. Am J
469 Obstet Gynecol 206:21–30.
470 [36] Hamieh A, Cartier D, Abid H, et al. (2017) Selenoprotein T is a novel OST subunit that
471 regulates UPR signaling and hormone secretion. EMBO Rep 18:1935-1946.
472 [37] Imai H, Hirao F, Sakamoto T et al (2003) Early embryonic lethality caused by targeted
473 disruption of the mouse PHGPx gene. Biochem Biophys Res Commun 305:278–86.
474 [38] Shi D, Guo S, Liao S et al (2012) Influence of Selenium on Hepatic Mitochondrial
475 Antioxidant Capacity in Ducklings Intoxicated with Aflatoxin B^1. Biol Trace Elem Res
476 145:325–329.
477 [39] He S, Guo X, Tan W et al (2016) Effect of Selenium Deficiency on Phosphorylation of the

478 AMPK Pathway in Rats. Biol Trace Elem Res. 169:254–260.
479 [40] Yoon MS (2017) mTOR as a Key Regulator in Maintaining Skeletal Muscle Mass. Front
480 Physiol 8:788.
481 [41] Gong T, Torres DJ, Berry MJ, Pitts MW (2018) Hypothalamic redox balance and leptin
482 signaling - Emerging role of selenoproteins. Free Radic Biol Med S0891-5849:30103-
483 30105.
484 [42] Yang J, Hamid S, Cai J et al (2017) Selenium deficiency-induced thioredoxin suppression
485 and thioredoxin knock down disbalanced insulin responsiveness in chicken
486 cardiomyocytes through PI3K/Akt pathway inhibition. Cell Signal 38:192–200.
487 [43] Xu J, Wang L, Tang J et al (2017) Pancreatic atrophy caused by dietary selenium
488 deficiency induces hypoinsulinemic hyperglycemia via global down-regulation of
489 selenoprotein encoding genes in broilers. PLoS One 12:e0182079.
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506 **Table 1. Nutritional parameters in dams and offspring at end of lactation**

		C	SS	SD
DAMS	**Solid intake** (g/day)	31.24 ± 3.2	47.8 ± 4.0 *cc*	52.93 ± 3.4 *ccc*
	Se intake (µg/day)	3.12 ± 0.32	23.9 ± 2.0 *ccc*	0.53 ± 0.03 *c, sss*
	Weight gain (g)	42.5 ± 1.06	53.6 ± 3.49 *cc*	36.48±1.85 *c, sss*
	Se in milk (µg/ml)	0.124 ± 0.005	0.126 ± 0.003	0.102 ± 0.003 *cc, sss*
OFFSPRING	**Milk intake** (g/30 min sucklig)	0.39 ± 0.03	0.61 ± 0.02 *cc*	0.37 ± 0.03 *ss*
	Milk intake/body weight	1.2 ± 0.09	1.7 ± 0.12 *cc*	1.8 ± 0.10 *cc*
	Weight gain (g)	26.3± 0.9	28.2±0.9 *c*	16±0.9 *ccc, sss*
	Cranium-caudal length (cm)	10.95± 0.122	10.86 ± 0.204	8.95±0.204 *ccc, sss*
	Body Mass Index (BMI) (kg/m^2)	2.66 ± 0.047	2.9 ± 0.05 *cc*	2.6 ± 0.03 *sss*
	Se in serum (ng/mL)	117 ± 4.1	217 ± 3.7 *ccc*	*109*± 5.1 *sss*
	Se in liver (µg/g dry weight)	0.38 ± 0.03	0.46 ± 0.02 *c*	0.05 ± 0.003 *ccc, sss*
	HSI (g/g body weight (%))	3.3 ± 0.1	3.94 ± 0.05 *ccc*	3.4 ± 0.05 *sss*
	Se in pancreas (µg/g dry weight)	0.225 ± 0.01	0.23 ± 0.01	No detected
	PSI (g/g body weight (%))	0.41 ± 0.02	0.41 ± 0.03	0.34 ± 0.02 *c, s*

526 The results are expressed as mean ± SEM and analysed by a multifactorial analysis of variance
527 (one-way ANOVA) followed by the Tukey´s test. The number of animals in each group is 8.

Groups: C: control group, SS: selenium supplemented group, and SD: selenium deficient group. Statistic difference between groups was expressed as *p* value; and was indicated as *c (p<0.05), cc (p<0.01)* and *ccc (p<0.001)* to control group, and s (p<0.05), ss (p<0.01) and sss *(p<0.001)* to Se supplemented group.

Table 2. Hepatic oxidative balance in offspring.

		C	**SS**	**SD**
OFFSPRING	**SOD** (U/mg protein)	1.96 ± 0.16	2.75 ± 0.20 *c*	4.08 ± 0.16 *ccc, sss*
	CAT (U/mg protein)	187.1 ± 6.6	251.3 ± 8.9 *ccc*	125.4± 4.0 *ccc, sss*
	GPx (mU/mg protein)	114.9 ± 4.5	144.2 ± 6.1 *ccc*	45.5 ± 2.7 *ccc, sss*
	MDA (mol/mg protein)	0.42 ± 0.008	0.38 ± 0.017	0.32 ± 0.015 *ccc*
	(CAT+GPx1)/SOD	117.1 ± 6.4	143.3 ± 8.4	41.9 ± 2.9 *ccc, sss*
	CG (nmol/mg protein)	4.19 ± 0.21	4.19 ± 0.22	5.39 ± 0.27 *cc, ss*

The results are expressed as mean ± SEM and analysed by a multifactorial analysis of variance (one-way ANOVA) followed by the Tukey´s test. The number of animals in each group is 8. Groups: C: control group, SS: selenium supplemented group, and SD: selenium deficient group. Statistic difference between groups was expressed as *p* value; and was indicated as *c (p<0.05), cc (p<0.01)* and *ccc (p<0.001)* to control group, and s *(p<0.05)*, ss *(p<0.01)* and sss *(p<0.001)* to Se supplemented group.

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553 **FIGURE LEGENDS.**

554 **Figure 1. Metabolic parameters in offspring.** The results are expressed as mean ± SEM and
555 analysed by a multifactorial analysis of variance (one-way ANOVA) followed by the Tukey´s
556 test. The number of animals in each group is 8. Groups: C: control group, SS: selenium
557 supplemented group, and SD: selenium deficient group. Statistic difference between groups
558 was expressed as *p* value; and was indicated as *c* $(p<0.05)$ and *ccc* $(p<0.001)$ to control group,
559 and s $(p<0.05)$ and *s* $(p<0.05)$, *ss* $(p<0.01)$ and sss $(p<0.001)$ to Se supplemented group.

560 **Figure 2. Expression of GPx1 (A) and SelP (B) in liver of offspring. Representative western**
561 **blots of proteins (normalized to β-actin) (C).** The results are expressed as mean ± SEM and
562 analysed by a multifactorial analysis of variance (one-way ANOVA) followed by the Tukey´s
563 test. The number of animals in each group is 8. Groups: C: control group, SS: selenium
564 supplemented group, and SD: selenium deficient group. Statistic difference between groups
565 was expressed as *p* value; and was indicated as *c* $(p<0.05)$ and *ccc* $(p<0.001)$ to control group,
566 and s $(p<0.05)$ and sss $(p<0.001)$ to Se supplemented group.

567 **Figure 3. Expression of AMPK (A), p-AMPK (B) and its ratio (C) in liver of offspring.**
568 **Representative western blots of proteins (normalized to β-actin) (D).** The results are
569 expressed as mean ± SEM and analysed by a multifactorial analysis of variance (one-way
570 ANOVA) followed by the Tukey´s test. The number of animals in each group is 8. Groups: C:
571 control group, SS: selenium supplemented group, and SD: selenium deficient group. Statistic
572 difference between groups was expressed as *p* value; and was indicated as *c* $(p<0.05)$ and *ccc*
573 $(p<0.001)$ to control group, and sss $(p<0.001)$ to Se supplemented group.

574 **Figure 4. Expression of IRS-1 (A) in liver of offspring. Representative western blots of**
575 **proteins (normalized to β-actin) (B). Serum insulin levels (C) and HOMA-IR Index (D).** The
576 results are expressed as mean ± SEM and analysed by a multifactorial analysis of variance
577 (one-way ANOVA) followed by the Tukey´s test. The number of animals in each group is 8.
578 Groups: C: control group, SS: selenium supplemented group, and SD: selenium deficient group.

579 Statistic difference between groups was expressed as *p* value; and was indicated as *c* $(p<0.05)$,

580 *cc* $(p<0.01)$ and *ccc* $(p<0.001)$ to control group, and *s*ss $(p<0.001)$ to Se supplemented group.

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