METABOLIC EFFECTS

Folic Acid and Selenite during Reproduction, Gestation and Lactation Protect Against Ethanol Changed Se Bioavailability

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Abstract — Aims: Levels of antioxidants such as folic acid and selenium decrease in dams exposed to ethanol during gestation and lactation, affecting their antioxidant status, their reproductive function and consequently the health of their progeny. We will study whether a Se (0.5 p.p.m.) plus folic acid (8 p.p.m.) supplemented diet administered to ethanol-exposed dams and male rats prevents the effects provoked by ethanol in Se bioavailability and in their glutathione peroxidase (GPx) activity, thus improving the health of their offspring. Methods: Se levels in tissue were measured by graphite-furnace atomic absorption spectrometry and serum GPx activity by spectrophotometry. Results: Results show that ethanol decreases Se retention in dams, affecting their tissues’ Se deposits, decreasing serum GPx activity, gestational parameters and the weight of their progeny. Conclusions: Se plus folic acid balance Se bioavailability, something that is especially important during gestation and lactation, and as a direct result, the health of their progeny is improved.

INTRODUCTION

During the gestation and lactation period, stress factors such as alcoholism or a decrease in the intake of essential antioxidant trace elements can be especially harmful. This is a risk for both the mother and their progeny, affecting pre- and post-natal foetus development (Merlot et al., 2008), and their offspring’s vital functions, even in later life (Fowden and Forhead, 2004). Recent studies have begun to investigate the influence of alcohol on nutrition and endocrine function during pregnancy (Zhang et al., 2005).

There is increasing evidence that Se is vital for foetal and neonatal development. This is demonstrated by the embryonically lethal consequences of disruption of the gene coding for the Sec tRNA [Ser]Sec, suggesting an essential role for one or more selenoproteins in development (Bosl et al., 1997). Newborn offspring have Se deposits, but they need Se via milk to achieve an infant’s optimum Se status (Dorea, 2002). Se also has a crucial role in mammalian male fertility, being essential for embryonic development (Schneider et al., 2009).

We have found that during the gestation and suckling period ethanol-exposed pups have their hepatic antioxidant activity impaired, provoking a decrease in Se and glutathione peroxidase (GPx) activity and an increase in carbonyl groups in protein. Therapy with Se to their dams balances the activities of scavenging enzymes and reduces peroxidation protein products (Ojeda et al., 2009a). Moreover, we have recently reported (Jotty et al., 2009) that ethanol decreased Se retention in dams, affecting their tissues’ Se deposits, decreasing their GPx activity in serum, their gestational parameters and the weight of their progeny. Selenite supplementation counteracts these adverse effects.

According to different studies that defend the claim that serum Se (González-Reimers et al., 2008) and folic acid levels decrease after alcohol exposure (Kopczyńska et al., 2004), and that their presence in serum increases GPx activity (Mayer et al., 2002; Rayman, 2000) it is probable that these two antioxidants might work in the same direction against ethanol consumption-generated oxidative stress. Davis and Uthus (2003) have found that Se and folate interact to influence one-carbon metabolism, homocysteine and glutathione, and that dietary Se can modulate many of the adverse effects of folate deficiency in one-carbon metabolism [effects which are similar to those provoked by chronic ethanol exposure (Villanueva et al., 2006)]. Se and folic acid are transferred from the dam to the offspring via either the placenta or milk, probably diminishing a free radical overproduction, and the onset of different diseases.

Our purpose is to study whether a double-supplemented diet of folic acid + Se among progenitors could change the Se bioavailability in dams altered by ethanol consumption. In this context, we will evaluate their antioxidant status, the relationship with the gestational and lactating parameters and the weight of their offspring. We also want to elucidate if the co-administration of folic acid plus Se is more effective in preventing the changes caused by ethanol consumption than a Se-only supplemented diet (Jotty et al., 2009).

MATERIALS AND METHODS

Animals

Male and female Wistar rats (Centre of Animal Production and Experimentation, Vice-Rector’s office for Scientific Research, University of Seville) weighing ~150–200 g were randomized into four groups: control (C), alcohol (A), alcohol + folic acid + Se (AFS) and control + folic acid + Se (CFS). Drinking water (with or without ethanol) and diet (supplemented or not) were given ad libitum during the induction period (8 weeks). Male (n = 6) and female (n = 6) rats were mated to obtain the first generation offspring for each group. After reproduction, pregnant rats were housed individually in plastic cages, and continued their alcoholic treatment until the end of the lactation period, so alcohol was supplied to mothers for 14 weeks (induction, gestation and
lactation periods); male rats were sacrificed and their testes extracted. The day of parturition was designated as Day 1 of lactation, the number of offspring being reduced to eight per mother at parturition, and Day 21 designated as the final day of the lactation period. The experiments were performed on lactating dams at Day 21 post-partum; their breastfeeding pups were also used to measure Se levels in serum and testes.

The animals were kept at an automatically controlled temperature (22–23°C) and a 12-h light-dark cycle (9:00–21:00).

All animal experiments were carried out in accordance with the European Union regulations (Council Directive 86/609/EEC of 24 November 1986) and approved by the committee of animal use for research at Seville University, Spain (RD 1201/2005 of 10 October 2005).

**Ethanol treatment**

Chronic progressive ethanol treatment (20% v/v) has previously been described by our research group (Ojeda et al., 2008).

**Diets**

The diets were prepared according to the Council of the Institute of Laboratory Animal Resources (1979). The supplemented diet contained 8 p.p.m. of folic acid and 0.5 p.p.m. of selenium versus the control diet which contained 2 p.p.m. and 0.1 p.p.m., respectively. Folic acid was supplemented as folic acid (Acofarma, Madrid, Spain) and Se as sodium selenite anhydrous (Panreac, Barcelona, Spain).

**Se intake measurement**

To know the amount of Se consumed by mothers, the food provided every day was weighed in the morning. The next morning we weighed the food again, the difference being the food consumed. Knowing Se concentration (p.p.m.) in each diet, we can calculate the amount of Se consumed.

**Samples**

At the end of the experimental period, the rats were made to fast for 12 h and anesthetized with intraperitoneal 28% w/v urethane (0.5 ml/100 g of body weight). The abdomen was opened by a midline incision and different organs were removed, debrided of adipose and connective tissue in ice-cold saline, and weighed. Samples were immediately stored at –80°C prior to biochemical determinations. Blood was collected by heart puncture and then centrifuged. Faeces and urine samples were collected using individual metabolic cages. We have used two different samples per animal for all the measurements.

**Selenium analysis**

Se levels were determined by graphite-furnace atomic absorption spectrometry. Equipment: we used a PerkinElmer Analyst™ 800 high-performance atomic absorption spectrometer with WinLab32 for AA software, equipped with a transversely heated graphite furnace with longitudinal Zeeman-effect background corrector and AS-furnace autosampler (PerkinElmer, Überlingen, Germany). The source of radiation was a Se electrodeless discharge lamp. The instrumental operating conditions and the reagents are the same that we used in the previous paper (Ojeda et al., 2009a), with slight modifications in the mineralization step: ramp time and temperature were different between tissues depending on their matrix content. Samples: serum samples were diluted five-fold in 0.2% v/v HNO₃ and 0.2% Triton X-100 solutions and urine samples were diluted 1:2 v/v. After 72 h at 100°C dry temperature, faeces and different tissue samples were weighed and digested in a sand bath heater with nitric acid during 72 h, and perchloric acid and chloridric acid (6N) were added. Nails and hair, after being washed in Milli-Q water + acetone + acetone + acetone + Milli-Q water, were treated as the rest of the tissues.

**Index**

Apparent Se absorption rate was calculated as [(I−F)/I] × 100 and apparent Se balance as I−(F + U), where I refers to Se intake, F refers to Se faecal excretion and U refers to Se urinary excretion. Gestational index (GI) was calculated as (no of successfully births/no of gestating rats) × 100 and lactation survival index (LSI) as (no of total offspring−no of died offspring/ no of total offspring) × 100.

**Biochemical analysis**

Serum samples were used to measure GPx activity. GPx activity was determined by spectrophotometry using the method of Lawrence and Burk (1976) that based on GPx, catalyses the oxidation of glutathione by hydrogen peroxide. The protein content of the samples was determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

**Statistical methods**

Differences in means were studied by using variance analyses (one-way ANOVA), statistical significance being established at P < 0.05 (Instat software program). When ANOVA resulted in differences, multiple comparisons between means were studied by a Tukey–Kramer test. The sample size used was n = 12 for adult rats and n = 16 for offspring.

**RESULTS**

A dams consume significantly less Se than their control counterparts during lactation (P < 0.05; Table 1). The same relationship was found to exist among supplemented rats (P < 0.001). Supplemented rats (AFS and CFS) showed significantly higher selenium intakes than non-supplemented rats (P < 0.001). The Se excretion pattern was affected by chronic ethanol exposure. Therefore, A dams eliminated more Se via urine than C dams (P < 0.05) and less Se via faeces (P < 0.001; Table 1). Supplementation to ethanol dams does not affect Se excretion via faeces but increased the Se elimination via urine (P < 0.001). Ethanol-supplemented dams excreted less Se via faeces (P < 0.001) and urine (P < 0.001) than CFS. Ethanol exposure during gestation and lactation does not affect apparent Se absorption rate, but it significantly decreases the apparent Se balance (P < 0.001). However, serum Se levels were similar in all groups (Table 1). In supplemented dams, the effect was similar;
ethanol-receiving rats (AFS) had a similar apparent absorption as CFS, but significantly lower apparent balance values ($P<0.001$). Folic acid plus Se supplementation increases apparent Se absorption and balance with respect to their non-supplemented counterparts ($P<0.001$). In Fig. 1, we observe that ethanol rats excrete less Se via hair than control ones ($P<0.001$). Supplemented dams (AFS and CFS) excrete more Se via nails, hair and lung ($P<0.001$) than non-supplemented ones (A and C). AFS rats excrete less Se via nails ($P<0.01$) and more via lung ($P<0.001$) than CFS.

Ethanol consumption significantly diminished the serum antioxidant capacity of the dams via GPx ($P<0.01$). There were no differences among the rests of the groups (Fig. 2).

Figure 3 shows dams’ weight gain during the induction, gestation and lactation periods. The lowest weight gain was found in ethanol dams during induction ($P<0.01$ versus C and $P<0.001$ versus AFS), gestation ($P<0.01$ versus C and AFS) and lactation ($P<0.001$ versus C and AFS). Control supplemented rats had a higher weight gain during induction than C ($P<0.05$) and during gestation than C and AFS ($P<0.001$).

In Table 2, we observe that chronic ethanol consumption by dams during gestation and lactation alters Se deposits in tissue with respect to control ones, the former having significantly higher Se levels in liver ($P<0.05$), heart ($P<0.01$) and spleen ($P<0.05$); and significantly lower Se levels in muscle ($P<0.01$) and cortex ($P<0.001$). The treatment with folic acid plus Se to ethanol rats decreased Se deposits in spleen ($P<0.05$) and heart ($P<0.01$); and significantly increased Se concentration in liver and muscle ($P<0.01$). AFS dams had the lowest Se deposit in cortex, significantly lower than in CFS dams ($P<0.001$). Control supplemented rats had higher Se levels in liver ($P<0.01$) and muscle than control ones ($P<0.001$).

Ethanol consumption affects gestational parameters, such as GI that is reduced by 44% with respect to the rest of the groups and LSI which suffered a 10.7% decrease (Table 3). Ethanol-receiving dams (A and AFS) had significantly less offspring per litter than their control counterparts (C and CFS; $P<0.05$). The offspring’s weight at birth was similar in A and C groups, and significantly higher in the supplemented groups CFS versus C ($P<0.01$) and AFS versus A ($P<0.001$). However, at the end of the lactation period, A offspring have a lower weight than control ones. After supplementation, AFS pups reach control weight, despite it having been lower than that presented by CFS offspring (Table 3). In this table, we also observe that ethanol consumption by male adult rats does not affect Se levels in their

### Table 1. Se homeostasis: Se intake (μg/d) during the induction, gestation and lactation period. Se levels in faeces, urine and serum. Se apparent absorption and balance

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>A</th>
<th>CFS</th>
<th>AFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se intake during lactation (μg/d)</td>
<td>3.12 ± 0.31*</td>
<td>2.23 ± 0.13†</td>
<td>21.4 ± 3.01†</td>
<td>12.85 ± 1.03*</td>
</tr>
<tr>
<td>Se (μg/d) in faeces</td>
<td>0.26 ± 0.02***</td>
<td>0.14 ± 0.001</td>
<td>0.28 ± 0.01</td>
<td>0.12 ± 0.01†</td>
</tr>
<tr>
<td>Se (μg/d) in urine</td>
<td>0.18 ± 0.01†</td>
<td>0.23 ± 0.02‡</td>
<td>0.55 ± 0.02†</td>
<td>0.41 ± 0.01*</td>
</tr>
<tr>
<td>Apparent Se absorption rate (%)</td>
<td>91.6 ± 0.74</td>
<td>93.3 ± 0.64*</td>
<td>98.7 ± 0.94†</td>
<td>99.0 ± 0.87</td>
</tr>
<tr>
<td>Apparent Se balance (μg/d)</td>
<td>2.67 ± 0.07***</td>
<td>1.85 ± 0.008‡</td>
<td>20.6 ± 1.6†</td>
<td>12.3 ± 0.41*</td>
</tr>
<tr>
<td>Se (μg/l) in serum</td>
<td>245 ± 15.2</td>
<td>259 ± 21.6</td>
<td>261 ± 15.7</td>
<td>284 ± 13.2</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SEM and analysed by a multifactorial ANOVA followed by Tukey’s test. The number of samples in each group is 12. Apparent Se absorption was calculated as $\frac{(I-F(2+U))}{I} \times 100$ and apparent Se balance as $I-F$. I refers to Se intake, F refers to Se faecal excretion and U refers to Se urinary excretion.

C, control group; A, alcohol group; CFS, control + folic acid + Se group; AFS, alcohol + folic acid + Se group.

Signification: C versus A *$P<0.05$, ***$P<0.001$; A versus AFS †$P<0.001$; C versus CFS ‡$P<0.001$; AFS versus CFS ††$P<0.001$. The offspring per litter than their control counterparts (C and AFS) and significantly higher in the supplemented groups CFS versus C ($P<0.01$) and AFS versus A ($P<0.001$).
testes; however, CFS rats have the highest Se concentration ($P < 0.05$). In pups, Se levels in testes were decreased by ethanol consumption ($P < 0.01$), whereas our supplemented diet to their dams restored these levels. Testes weights of adult and pups do not present difference between groups, the values expressed as organ weight/g body weight (%) are in adults: C ($0.47 \pm 0.01$), A ($0.44 \pm 0.01$), CFS ($0.44 \pm 0.01$) and AFS ($0.42 \pm 0.01$) and in pups: C ($0.67 \pm 0.03$), A ($0.71 \pm 0.02$), CFS ($0.68 \pm 0.02$) and AFS ($0.73 \pm 0.02$).

In Fig. 4, we found that ethanol dams have the lowest Se levels in mammary gland, significantly lower than in C and AFS ($P < 0.01$). However, serum Se levels in alcoholic progeny (A and AFS) were higher than in their control counterparts (C and CFS; $P < 0.05$). Pups of supplemented dams (CFS and AFS) had significantly higher serum Se levels than non-supplemented ones ($P < 0.001$).

**DISCUSSION**

According to Pappas et al. (2008), the future health of her progeny depends on the dam’s nutritional and antioxidant status during gestation and lactation. We have confirmed that ethanol consumption during the breastfeeding period (A and AFS) reduced the amount of Se consumed with respect to their control counterparts (C and CFS), affecting Se balance but not Se apparent absorption or serum Se levels. Despite the four groups having similar Se levels in serum, serum GPx activity is significantly reduced in ethanol dams, and a supplemented diet with Se and folic acid does not increase this activity. Probably the interaction between Se and folic acid, increasing GPx activity, only occurs in the liver where the one-carbon cycle is taking place. Previously, we have found an exacerbated increase in hepatic GPx activity in pups exposed to ethanol and supplemented with folic acid plus Se (Ojeda et al., 2009b).

Supplemented dams have higher apparent Se absorption and balance than non-supplemented ones, because despite excreting more Se via urine, nails, hair and lung, they consume much more Se in their diet, so they apparently retain nearly seven times more Se in their tissues. This difference is observed in their weight gain and their Se deposits in different organs. Our double-supplemented diet seems to be a good nutritional strategy to increase the lower weight gain caused by ethanol consumption in dams. The same supplementation to C dams also increases their weight, especially during the gestation period. This might explain why these rats have the highest liver weight [g/g body weight (%)] at the end of the experiment ($8.24 \pm 0.31\%$, $P < 0.001$). Among the different groups, the rest of the studied organs have a similar weight (%), so we can conclude that ethanol and/or a dietary Se plus folic acid intake, do not change dams’ organ weights (data not shown). However, Se deposits in the different tissues studied are indeed affected by these treatments.

Ethanol consumption decreases Se levels in the cortex, muscle and mammary gland, and sequesters Se to heart, liver and spleen, probably to decrease these organs’ ethanol-generated oxidation. Our dietary treatment restores all of these values to control levels, except in the liver where Se concentration is still higher (even than in CFS), and in the cortex, where Se values remain low. It is known that a dietary Se supplementation with selenite mainly increases GPx. However, Se supplementation with selenomethionine results in an increase of Selenoprotein P (Payne and Southern, 2005a). Hoffmann et al. (2007) have found that Se levels in the cortex depend on Selenoprotein P concentration, and therefore ethanol decreased this selenoprotein, meaning that a selenite supplementation cannot restore this value. Supplementation with selenomethionine would be effective in increasing cortex Se levels.

If we compare this double supplementation therapy to C dams, we do not find, in doubled supplemented ethanol

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**Table 2. Dam’s selenium deposits in different tissues (μg/g dry weight)**

<table>
<thead>
<tr>
<th>Se levels (μg/g dry weight)</th>
<th>C</th>
<th>A</th>
<th>CFS</th>
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<tbody>
<tr>
<td>Liver</td>
<td>0.21 ± 0.02*</td>
<td>0.28 ± 0.02**</td>
<td>0.38 ± 0.03†</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.66 ± 0.04</td>
<td>0.63 ± 0.04</td>
<td>0.61 ± 0.01</td>
<td>0.55 ± 0.07</td>
</tr>
<tr>
<td>Heart</td>
<td>0.22 ± 0.03**</td>
<td>0.27 ± 0.05**</td>
<td>0.20 ± 0.01</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.28 ± 0.01*</td>
<td>0.37 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.15 ± 0.01***</td>
<td>0.12 ± 0.003**</td>
<td>0.25 ± 0.03†</td>
<td>0.28 ± 0.02</td>
</tr>
<tr>
<td>Cortex</td>
<td>0.09 ± 0.004***</td>
<td>0.07 ± 0.002</td>
<td>0.09 ± 0.003</td>
<td>0.06 ± 0.004*</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SEM and analysed by a multifactorial ANOVA followed by Tukey’s test. The number of samples in each group is 12. Groups: C, control group; A, alcohol group; CFS, control + folic acid + Se group; AFS, alcohol + folic acid + Se group.

Signification: C versus A $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; A versus CFS $^{4}P < 0.05$, $^{5}P < 0.01$; C versus AFS $^{6}P < 0.05$, $^{7}P < 0.01$; AFS versus CFS $^{8}P < 0.001$. 

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**Table 3. Pups’ selenium deposits in different tissues (μg/g dry weight)**

<table>
<thead>
<tr>
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<td>0.25 ± 0.03†</td>
<td>0.28 ± 0.02</td>
</tr>
<tr>
<td>Cortex</td>
<td>0.09 ± 0.004***</td>
<td>0.07 ± 0.002</td>
<td>0.09 ± 0.003</td>
<td>0.06 ± 0.004*</td>
</tr>
</tbody>
</table>
Number of offspring/litter
Offspring weight (g) at birth
Offspring weight (g) at 21 days
Testes Se levels (μg/g dry weight) in progenitors
Testes Se levels (μg/g dry weight) in pups of 21-day old

The results are expressed as mean ± SEM and analysed by a multifactorial ANOVA followed by Tukey’s test. The number of sample used per group was 12 in dams and 16 in offspring. Gestational index (GI) was calculated as (no of successfully births/no of gestating rats) × 100, and lactation survival index (LSI) as (no of total offspring–no of died offspring/no of total offspring) × 100. The number of animals in each group is n.

Signification: C versus A * P < 0.05; C versus CFS ** P < 0.01, *** P < 0.001; A versus AFS † P < 0.05, ‡ P < 0.01, ‡‡ P < 0.001; C versus CFS †† P < 0.05, ††† P < 0.001; AFS versus C ‡‡‡ P < 0.001; C versus CFS ▴ ▴ ▴ P < 0.001; A versus AFS ▴ ▴ ▴ ▴ P < 0.001; △ △ △ P < 0.001.

Fig. 4. (A) Se concentration (μg/l) in the serum of offspring. (B) Se concentration (μg/g dry weight) in mammary glands. The results are expressed as mean ± SEM and analysed by a multifactorial ANOVA followed by Tukey’s test. The number of sample used per group was 12 in dams and 16 in offspring. Groups: C: control group, A: alcohol group, CFS: control + folic acid + Se group, AFS: alcohol + folic acid + Se group.

Signification: C versus A * P < 0.05, ** P < 0.01, *** P < 0.001; A versus AFS † P < 0.01, ‡ P < 0.001; C versus CFS † P < 0.05, †† P < 0.01; AFS versus C ‡ P < 0.05.

dams, any alterations in spleen and heart Se deposits. However, these levels were greatly increased with a single Se-supplemented diet (Jotty et al., 2009). This is to be expected because, with our supplementation therapy, folic acid deposits can increase in these tissues (Mason and Choi, 2005). As this nutrient also acts as an antioxidant element via glutathione (Villanueva et al., 2006), the need to have higher Se levels to counteract the oxidation generated in these organs is reduced. Although these two trace elements could act together against the oxidative stress causes by ethanol (Davis and Uthus, 2003)—and we are supplementing our ethanol dams with both nutrients—, Se levels remain high. This is probably because the oxidative damage in this organ still persisted. However, with respect to a Se-only supplemented diet, Se hepatic levels are 60% lower (Jotty et al., 2009), confirming that these two antioxidants act jointly against exposure to ethanol.

Dietary Se plus folic acid treatment increases Se deposits in the greatest Se tissue reservoirs: liver and muscle (Daniels, 1996), confirming the effects of our therapy. Another important difference between a double or a single supplementation to ethanol dams is that the Se concentration in muscle of rats administered a Se-only supplemented diet is 18% lower than in those supplemented with folic acid plus Se (Jotty et al., 2009). This is also according to our hypothesis because in double-supplemented dams Se is stored in muscle instead of increasing in other ethanol-affected tissues. Something similar occurs in Se kidney concentration; with Se-only supplementation, the values are 50% higher, something that is probably due to an increase in renal Se reabsorption to eliminate less Se via urine (Jotty et al., 2009). These data demonstrate that the Se deposits for counteracting ethanol oxidation increase if the levels of another antioxidant such as folic acid decrease, something that is always associated with ethanol exposure (Romero et al., 1981).

In supplemented C dams, the only difference in Se deposits was found in the storage organs: liver and muscle (Daniels, 1996). Therefore, this therapy is only effective when Se levels are altered, as in the case of ethanol exposure.

Alcohol consumption during gestation and lactation clearly affects the outcome of the progeny, so the number of successful births decreased after ethanol consumption during gestation while the number of offspring deaths during the suckling period was also negatively affected. Folic acid and Se act together to improve these parameters. Ethanol consumption also decreases the number of offspring per litter. In this case, however, our supplementation therapy does not increase this ratio. Our dietary treatment of the dams is intimately linked to the health of their progeny, increasing the offspring’s weight after the suckling period, but especially at birth. However, with a Se-only supplemented diet, the weight at the end of gestation does not differ from those of ethanol pups (Jotty et al., 2009). In terms of offspring, it would appear that this therapy is especially efficient during the gestation period when the folic acid levels are more compromised (Xu et al., 2006). According to these dates, our treatment could improve the antioxidant communication via the placenta; in fact, recent studies made by our research group measuring GPx activity and its presence in placenta, corroborates this hypothesis.

Ethanol alters maternal-offspring Se communication via milk, because maternal alcohol intake during lactation...
greatly impairs milk production (Tavares do Carmo et al., 1999), and moreover, ethanol exposure during the suckling period produces distinctive changes in the infants’ feeding patterns (Mennella, 2001). As well as these facts, in this study, we have also found lower Se levels in dams; we might, therefore, conclude that these offspring received less Se via milk. Our supplemented diet also counteracts this effect because it increases Se mammary deposits to control levels.

It is known that ethanol intake causes drastic changes in sperm and in testosterone and fructose levels; but the co-administration of Se with alcohol restores these parameters to normal levels (Swathy et al., 2006). Moreover, it is known that Se deficiency also gives rise to structural and functional testicular disorders similar to those caused by alcohol (Bekpinar and Tugrul, 1995). Taking into account that the previous studies reported have used a higher ethanol concentration than ours (20% for 8 weeks), we have found that male progenitors had similar Se testes levels to their control and supplemented counterparts and that their testes weights were similar to the rest of the groups (data not shown). However, our dietary supplementation to male C rats increases Se concentration without altering their testes weight. Therefore, ethanol treatment does not alter Se concentration in the testes of the adult males, but their progeny’s testes are indeed affected by ethanol consumption. Alcohol-exposed pups had nearly 40% less Se in their testes than C pups. Our dietary therapy counteracts this effect, indicating a protective effect of Se + folic acid via placenta and milk.

Despite all of these obstacles, ethanol-exposed pups had higher Se levels in serum and, according to our previous work, higher GPx activity (Ojeda et al., 2009a). Something similar occurred in supplemented pups. Although dams had similar Se serum levels in the four experimental groups, it would appear that in offspring ethanol sequesters Se to the blood, perhaps in part, to maintain high GPx activity in serum, something that might play an important role in the first line of defence against ethanol-provoked oxidative stress. This hypothesis is according to Payne and Southern (2005b), who defend the claim that Se stored in tissues —and not only in liver— plays a survival role in the first line of defence against ethanol-provoked oxidative stress. This hypothesis is according to Payne and Southern (2005b), who defend the claim that Se stored in tissues —and not only in liver— plays a survival role in the first line of defence against ethanol-provoked oxidative stress.

REFERENCES

Bekpinar S, Tugrul Y. (1995) Influence of selenium supplemen-


tation in non-toxic doses on testis lipid peroxide and antioxidant


Bosil MR, Takadu K, Oshima M et al. (1997) Early embryonic leth-


ality caused by targeted disruption of the mouse selenocysteine


davis CD, Uthus EO. (2003) Dietary folate and selenium affect


dimethyldihydrine-induced aberrant crypt formation, global


DNA methylation and one-carbon metabolism in rats. J Nutr


133:2907–14.


Fowden AL, Forhead AJ. (2004) Endocrine mechanisms of intrau-


González-Reimers E, Galindo-Martín L, Santolaria-Fernández F


et al. (2008) Prognostic value of serum selenium levels in alco-


Hoffmann PR, Höge SC, Li PA et al. (2007) The selenoproteome


exhibits widely varying, tissue-specific dependence on seleno-


Jotty K, Ojeda ML, Nogales F et al. (2009) Selenium tissue


distribution changes after ethanol exposure during gestation and


Kopeytsya E, Zidlovsky M, Jendrzych-Mackiewicz E et al.


(2004) The concentrations of homocysteine, folic acid and


vitamin B12 in alcohol dependent male patients. Psychiatr Pol


Lawrence RA, Burk RF. (1976) Glutathione peroxidase activity in


selenium-deficient rat liver. Biochem Biophys Res Commun


7:952–8.


Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951) Protein


measurement with the Folin phenol reagent. J Biol Chem 1:


265–75.


235–41.


Ojeda ML, Delgado-Villa MJ, Llopis R et al. (2008) Lipid metabol-


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