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—Original Article—

Effects of Antioxidant Supplementation on Duodenal Se-Met Absorption in Ethanol-exposed Rat Offspring *In Vivo*

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Abstract. The nutritional deficiencies provoked by ethanol consumption, during gestation or lactation, can contribute to multiple birth defects in offspring. In order to improve our knowledge about selenium (Se) distribution in pups exposed to ethanol, the present study evaluated the effect of this drug on intestinal development and determined its action on duodenal absorption of selenomethionine (Se-Met). To determinate if supplementation could improve Se absorption and its serum values, we used two antioxidant supplemented regimens on dams, with selenium alone or selenium plus folic acid, and obtained six groups of pups: C (control), A (alcohol), CS (control + Se), AS (alcohol + Se), CFS (control + Se + folic acid) and AFS (alcohol + Se + folic acid). Duodenal Se-Met transport was performed using an *in vivo* perfusion method. Se levels were measured by graphite furnace atomic absorption spectrometry. The supplemented diets utilized had a positive influence on body growth, duodenal perimeter and Se content in ethanol-exposed pups. Ethanol exposure increased Se-Met duodenal absorption in all pups, supplemented or not, presenting the highest values of maximal velocity (V_{max}) compared with their control counterparts. The affinity constant (K_m) increased according to rank: A>AS>AFS groups. These results suggest that although antioxidant supplementation does not restore Se-Met absorption to normal values, it enhances the affinity of the transporters for the substrate and improves the damage caused by ethanol in the duodenal mucosa.

Key words: Ethanol, Kinetic parameters, Offspring development, Selenomethionine absorption

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The primary function of the intestinal epithelium is to absorb the small molecules produced from food digestion [1]. However, specific substances such as alcohol could alter this intestinal absorption [2, 3], producing malnutrition. Numerous studies have demonstrated that chronic ethanol consumption induces morphological and biochemical alterations in the intestinal mucosal cells [4] and that some of these changes are caused by a lipid peroxidation of duodenal membrane lipids [5]. These effects of ethanol exposure are more acute during pregnancy and lactation and can contribute to a delay in offspring growth and multiple birth defects, including fetal alcohol syndrome [6]. In this context, it is especially relevant that ethanol deregulates the homeostasis of different antioxidants such as folic acid, whose deficiency is the most common sign of malnutrition in chronic alcoholism [7]. This deficiency is associated with several alterations such as Down's syndrome and defects in neural tube closure in developing embryos [1]. According to what we have previously reported [8], the effects of ethanol exposure during gestation and lactation on folic acid intestinal absorption showed that intestinal folate absorption appears to be upregulated in suckling rats. Our research group has also found, in pups exposed to ethanol and supplemented with folic acid during the gestation and lactation periods, an increase in intestinal longitude (control, alcohol and alcohol plus folic acid: 53.56 ± 1.49, 43.7 ± 0.92 and 48.36 ± 1.69 cm, respectively) and intestinal mucosa weight (control, alcohol and alcohol plus folic acid: 15 ± 0.8, 12 ± 0.8 and 15 ± 1 mg/cm^2, respectively).

We have recently described that ethanol decreases selenium (Se) retention in dams, affecting their gestational parameters and the body weight, and the antioxidant balance of their progeny [9–12]. This essential trace element is of fundamental importance to health due to the antioxidant, anti-inflammatory and chemopreventive properties attributed to its presence in at least 25 selenoproteins such as glutathione peroxidase (GPx) [13]. In a previous experimental model with pups treated with an Se-supplemented or deficient diet, we concluded that Se was crucial for duodenal development and maturity, since Se-supplemented pups had a heavier intestinal mucosa, whereas there was a decrease in duodenum weight in Se-deficient pups'. This effect could be due to the relationship between Se and the deiodinase enzymes, which enhanced T3 levels in tissues and protein turnover, improving growth in the pups. Moreover, our results indicated that Se is related to a high number of transport proteins for Se-Methionine (Se-Met) in the duodenum [14]. As we have previously reported, exposure of pups to ethanol during gestation and lactation causes an imbalance of Se homeostasis and a depletion in Se tissue deposits related to protein and lipid oxidation [15]. In order to improve our knowledge about the effects of ethanol on Se bioavailability in pups, we will study duodenal Se absorption. As we have previously mentioned, supplementation of ethanol-exposed pups with folic acid could improve

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their intestinal development; moreover, our research group has found that Se or Se plus folic acid supplementation during gestation and lactation could prevent liver oxidative damage in the pups, thus improving their development and health [10, 11].

At present, a limited number of studies have explored Se uptake in offspring rats [16] or infants [17, 18], and there are no reports concerning this in ethanol-exposed pups. In this context, Anan *et al.* (2009) [19] found that suckling pups mainly obtained Se presenting in breast-milk in organic form as Se-Met. As it seems that Se homeostasis is related to pup development, the aim of the present study was to elucidate whether ethanol exposure during gestation and lactation alters duodenal Se-Met absorption and duodenal development in offspring. We also studied whether supplementation of their dams with the antioxidants Se or Se plus folic acid improves these parameters in pups via the placenta and milk.

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 approximately 150–200 g were randomized into six groups and used as parents: control, alcohol, control + Se, alcohol + Se, control + Se + folic acid and alcohol + Se + folic acid. Drinking water (with or without ethanol) and diet (control or supplemented) given periods to the dams *ad libitum* during the induction (8 weeks), gestation (3 weeks) and lactation (3 weeks) periods. The week following the induction period, male (n=6) and female (n=6) rats were mated to obtain the first-generation offspring for each group. Pregnant rats were housed individually in plastic cages, and their alcoholic treatment was continued until the end of the lactation period. So, alcohol was supplied to mothers for 14 weeks. The day of parturition was designated as day 1 of lactation, and day 21 was designated as the end of the lactation period. The offspring number was reduced to 9 per mother at parturition for the *in vivo* perfusion studies. Thus, six groups of pups were obtained: group C, which was comprised of control offspring; group A, which was comprised of pups exposed to ethanol during gestation and lactation; group CS, which was comprised of pups supplemented with Se; group AS, which was comprised of alcohol offspring supplemented with Se; group CFS, which was comprised of control pups supplemented with Se and folic acid; and group AFS, which was comprised of alcohol offspring supplemented with Se plus folic acid. During the breastfeeding period, the pups had free access to the nipples. Finally, the experiments were performed on the offspring at 21 days postpartum between 0900 h and 1300 h.

During all experiments, the animals were kept at an automatically controlled temperature (22–23 C) and under a 12-h light-dark cycle (0900 h to 2100 h). Animal care complied with the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996) and was approved by the committee of animal use for research at Seville University, Spain (RD 1201/2005 of 10 October 2005).

Diets

The diets 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 (g/Kg of diet). The control diet contained 0.1 ppm of selenium and 2 ppm of folic acid, while the supplemented diets contained 0.5 ppm of Se and 8 ppm of folic acid. Se was supplemented as anhydrous sodium selenite (Panreac), and folic acid was supplemented as folic acid (Acofarma).

Ethanol treatment

Ethanol was administered in tap water at 5% v/v during the first week, 10% v/v during the second week, 15% v/v during the third week and, finally, 20% v/v during 4 additional weeks (induction period). This treatment of ethanol at 20% v/v was continued during the periods of gestation (4 weeks) and lactation (4 weeks) in accordance with the chronic treatment previously described by Ojeda *et al.* in 2008 [20].

Samples

At the end of the experimental period, the rats were fasted for 12 h and anesthetized with an intraperitoneal injection of 28% w/v urethane (0.5 ml/100 g of body weight). Blood samples were obtained by heart puncture and collected in tubes. The serum was prepared using low-speed centrifugation for 15 min at 1300 × *g*. Feces and urine samples were collected using individual metabolic cages at day 21; to avoid dehydration, the pups had free access to water.

Milk consumption

The amount of milk consumed by offspring at 21 days old was estimated by subtracting the weight of the pups obtained just prior to returning them to the dam from the weight at the end of 30 min of suckling [21]. We controlled the pups during this period; if a pup urinated or defecated during the period, the data for the pup was rejected.

Milk samples

In order to obtain the maximum amount of milk at day 21 of lactation, 3 h 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 obtained was about 1–1.5 ml per dam.

Body weight

Body weight was controlled weekly until the end of the experimental period. Each measurement was taken at 0900 h to avoid changes due to circadian rhythms.

Selenium analysis

Selenium levels in milk, serum, urine and feces were determined by graphite furnace atomic absorption spectrometry using a PerkinElmer AAnalyst™ 800 high-performance atomic absorption spectrometer with WinLab32 for AA software equipped with a Transversely Heated Graphite Furnace (THGA) with a longitudinal Zeeman-effect background corrector and an AS-furnace autosam-

Table 1. Milk intake (ml) during 30 min of suckling, Se in milk (ppm), body weight (g) and intestinal parameters in offspring

	C	A	CS	AS	CFS	AFS
Offspring weight at birth (g)	5.5 ± 0.09 $^{\beta\beta}$	5.0 ± 0.2 $^{\#\#, aaa}$	7.2 ± 0.2 $^{▲▲▲, 1}$	5.9 ± 0.2 $^{+++}$	6.4 ± 0.2 ^{cc}	6.51 ± 0.1
Offspring weight at 21 days (g)	31.6 ± 1.0 ***	22.0 ± 1.7 $^{\#\#\#,aaa}$	35.0 ± 1.1	31.9 ± 1.4	36.1 ± 1.45	30.6 ± 1.5 ^{b}
Milk intake in 30 min (ml)	0.56 ± 0.03 **	0.43 ± 0.02 $^{\#, a}$	0.61 ± 0.02	0.52 ± 0.04	0.57 ± 0.03	0.55 ± 0.04
Se milk (p.p.m)	0.12 ± 0.005 *	0.10 ± 0.004 $^{\#\#,a}$	0.12 ± 0.003	0.13 ± 0.005	0.11 ± 0.005	0.12 ± 0.004
Duodenum perimeter (cm)	0.6 ± 0.030 *	0.51 ± 0.023 $^{\#\#, a}$	0.61 ± 0.026	0.62 ± 0.019	0.65 ± 0.018	0.60 ± 0.015
Duodenum wet weight (mg/cm^2)	53.44 ± 3.54	52.64 ± 2.02	63.15 ± 3.97	51.86 ± 3.23 $^{+}$	54.48 ± 2.34	52.34 ± 2.04
Duodenum mucosa weight (mg/cm^2)	20.03 ± 1.18 *	16.88 ± 0.69 $^{\#\#, aaa}$	27.07 ± 2.48 $^{▲}$	21.14 ± 0.87 $^{+}$	27.63 ± 1.3 ^{c}	22.94 ± 0.61 ^{bb}

The results are expressed as means ± SEM and were analyzed by a multifactorial analysis of variance (one-way ANOVA) followed by Tukey's test. The number of animals in each group is 9. C: control group; A: alcohol group; CS: control Se group; AS: alcohol Se group; CFS: control Se + folic acid group; AFS: alcohol Se + folic acid group. *, ** and ***: P<0.05, P<0.01 and P<0.001 for C *vs.* A, respectively. $^{▲}$ and $^{▲▲▲}$: P<0.05 and P<0.001 for CS *vs.* C, respectively. ^{c} and ^{cc}: P<0.05 and P<0.01 for CFS *vs.* C, respectively. 1: P<0.05 for CFS *vs.* CS. $^{\#, \#\#}$ and $^{\#\#\#}$: P <0.05, P<0.01 and P<0.001 for A *vs.* AS, respectively. ^{a} and ^{aaa}: P<0.05 and P<0.001 for A *vs.* AFS, respectively. $^{+}$ and $^{+++}$: P<0.05 and P<0.001 for AS *vs.* CS. $^{\beta\beta}$: P<0.01 for AFS *vs.* C. ^{b} and ^{bb}: P<0.05 and P<0.01 for AFS *vs.* CFS, respectively.

pler (PerkinElmer, Überlingen, Germany). The source of radiation was an Se electrodeless discharge lamp (EDL). The instrumental operating conditions and reagents are the same those we used in our previous paper [10].

Serum samples were diluted fivefold in 0.2% v/v HNO_3 and 0.2% Triton X-100 solutions, and urine samples were diluted 1:2 v/v. After 72 h at a temperature of 100 C, feces and milk samples were weighed and digested in a sand bath heater (OVAN) with nitric acid for 72 h, and perchloric acid and chlorhydric acid (6 N) were added.

Selenium absorption

This set of experiments was performed on the offspring 21 days after birth. Pups were anesthetized with a subcutaneous injection of 28% w/v urethane (0.5 ml/100 g animal weight). The abdominal cavity was opened by a longitudinal incision, and the duodenum was cannulated with polyethylene tubing. Inflow and outflow cannulas were tied into the intestine. After cannulation, the loop was rinsed with 0.9% NaCl solution, replaced inside the body wall and perfused as previously described by Tavares *et al.* (1998) [22]. A flow rate of 3.0 ml/min during a period of 5 min was used for each concentration in all animals. Pups were maintained at a controlled temperature (37 C) with a heating pad.

Se in the form of Se-Met was dissolved in a Tyrode's medium (pH 7.4) composed (in g/l) of NaCl (7.36), KCl (0.2), $CaCl_2.2H_2O$ (1.36), glucose (1.36), HEPES (2.4), HEPES-Na (1.3) and Se-Met at final concentrations of 2, 5, 10, 25, 75 and 150 µM. This solution was prewarmed (37 C) before perfusion. Se-Met uptake was measured in different experimental animals. The perfusion time for all the pups studied was divided into an equilibrium period of 5 min at the beginning of the experiment and one period of 5 min for each substrate concentration.

Se-Met absorption was determined as the difference between the initial and the final amount of substrate obtained in the perfusates and expressed as $\mu moles/cm^2/5$ min. Analyses of Se-Met concentrations in the perfusates were carried out by graphite furnace atomic absorption spectrometry.

Morphometric intestinal evaluation

At the end of perfusions, pups were sacrificed, and the perfused loops were removed to determine their wet weight. After this, the mucosa was obtained by scraping the duodenum with two slides, and then it was weighted. To calculate the total serosa area, the duodenum perimeter was measured according to the method of Winne (1976) [23].

Kinetic analysis

Total Se-Met absorption from at least nine independent rats was analyzed by nonlinear regression using the EnzFitter software (Biosoft, Cambridge, UK). As errors associated with experimental intestinal absorption values were roughly proportional to their values, applying a proportional weighting to the data was considered appropriate. The kinetic parameters were calculated considering a model equation comprised of one saturable Michaelis-Menten component.

Statistical analysis

The results are expressed as means ± standard error of the mean (SEM). The data were analyzed using the GraphPad InStat 3 statistical software by multifactorial analysis of variance (one-way ANOVA). Statistical significance was established at P<0.05. When ANOVA resulted in differences, multiple comparisons between means were studied by the Tukey-Kramer test.

Results and Discussion

Selenium intake. Body and intestinal development

Although ethanol exposure in dams produced a significant decrease in Se in their milk (P<0.05) compared with to the control group, administration of one or both supplements to ethanol-exposed dams restored the Se levels in milk to control values (Table 1). This is in agreement with other studies performed by our research group [9, 12] where ethanol-exposed dams had a lower Se retention, which in turn altered Se deposits in several tissues, such as the mammary gland. Our supplementation of the diet of ethanol dams increases the Se levels in milk to control values and is an effective treatment to correct the lower Se concentration found in

mammary glands.

Similarly, ethanol also modified the amount of milk consumed during the suckling period. In this regard, we found that A pups consumed a smaller amount of milk, about 30% less, than the control ($P<0.01$) and supplemented pups ($P<0.05$). Previous results [24] have demonstrated that alcohol consumption by gestating and nursing dams reduced milk intake by the suckling pups; that study found that this decrease is a consequence of the effects of ethanol and not of maternal malnutrition. However, the supplemented diets used in this study increased the milk intake among pups, and this value did not significantly differ from that of the control pups.

The antioxidant diets utilized, diets supplemented with Se or with Se plus folic acid, had a positive influence on pups body weight, since, as shown in Table 1, all animals born of dams supplemented and treated with or without ethanol had significantly higher weights at birth than their nonsupplemented counterparts ($P<0.001$); in fact, even the body weights of the pups in the AFS group were higher than those in the C group ($P<0.01$). This finding is similar to the results of Vonnahme *et al.* (2010) [25], who found that there was an effect of Se on body weight at birth. Recently, we argued [14] that Se is an essential micronutrient with direct effects on offspring development, since it is needed to synthesize deiodinase enzymes that enhance T3 levels in tissues, specifically in the hypothalamus, where it produces an increase of the hypothalamic neuropeptide Y [26], causing hunger and hyperphagia.

At the end of the lactation period, we found that offspring from ethanol-fed dams presented a lower body weight than pups from control dams ($P<0.001$) and a much lower weight than their supplemented counterparts ($P<0.001$). Once more, the antioxidant supplemented diets used restored this parameter to control levels. The lower Se contents in the milk of the ethanol-exposed dams, together with a decrease in the amount of milk consumed during the breastfeeding process, were probably responsible for the decrease in body weight in the ethanol-exposed pups. These results are in agreement with those of Bhalla *et al.* (2004) [6] and were reversed by the supplements used, as there were no differences between the AS or AFS group versus the C group.

Furthermore, it is widely accepted that chronic ethanol intake induces morphological and biochemical alterations in the mucosal intestinal cells that affect the absorption of several nutrients, including sugar, vitamins and other elements [3]. Consequently, we also determined intestinal parameters at the end of the lactation period, finding that A pups presented a smaller perimeter and mucosa wet weight in the duodenum than the control pups ($P<0.05$); however, these offspring did not present significant differences in wet duodenum weight. Previous studies had found that pups prenatally exposed to ethanol showed a decrease in intestinal weight and length at birth [27] and also during postnatal development [6]. Murillo-Fuentes *et al.* (2003) [28] found a decrease in intestinal area in litters exposed to ethanol during the gestation or lactation period, although this effect was more significant in the group of pups exposed to ethanol during lactation only.

The antioxidant supplement regimens employed improved the duodenum perimeter value and the weight of the mucosa in the AS and AFS pups compared with the ethanol-exposed pups, showing similar values to control pups. Moreover, these two supplemented diets increased the mucosa weight in the CS and CFS pups compared with the control pups ($P<0.05$). Finally, we observed significant differences between the supplemented control pups and their ethanol counterparts not in the duodenum perimeter but in the duodenum mucosa weight (AS *vs.* CS, $P<0.05$, and AFS *vs.* CFS, $P<0.01$). Therefore, the supplement regimens used avoid the effects of ethanol on the perimeter of the duodenum and weight of the mucosa but also have an effect on the control status improving mucosa development of the duodenum. In the case of CS pups, we also found an increase in the duodenum wet weight that was significant compared with the AS pups ($P<0.05$). It appears that supplementation of dams with Se can alter fetal responses and increase small intestinal mass [14, 29]. Recently, we concluded that Se is crucial for the development and maturity of the duodenum because Se-supplemented pups had a heavier intestinal mucosa, while Se-deficient pups had a lower duodenum weight [14]. In addition, we think that this effect could be due to the fact that Se is part of the glutathione peroxidase 2 enzyme (GPx2) expressed in the gastrointestinal tract. Therefore, Se supplementation could protect the intestinal epithelium from oxidative stress [30] and induce more cell proliferation than in offspring fed an adequate or low Se diet. However, despite the fact that folic acid improves DNA synthesis and intestinal development, there were no differences between AS and AFS pups or between CS and CFS pups in terms of duodenum maturity.

Selenium levels in serum, urine and feces

In the ethanol-exposed pups, the serum Se levels were greater than in the control pups ($P<0.05$) despite the fact that they ingested a smaller amount of Se during the suckling period (Fig. 1); however, the elimination of this micronutrient in urine and feces was similar. It appears that these pups preserved the serum Se levels above the control values in order to maintain high glutathione peroxidase activity in serum [10], which protects against oxidative damage.

Despite the fact that AS and AFS offspring also received ethanol, the supplemented diets administered to the control or ethanol exposed pups increased the serum Se levels compared with the A and C groups ($P<0.001$), and these groups excreted more Se via urine and feces (see statistical significance in Fig. 1). We were able to determine that the ethanol-supplemented pups had a similar Se content in serum and urine to their respective controls, although they excreted a lower amount of Se via feces ($P<0.001$). This could indicate that pups from ethanol-supplemented dams presented a higher absorption of Se, since they consumed the same quantity of milk as their control counterparts, yet they excreted less selenium in feces. Ethanol probably alters intestinal permeability and promotes nutrient absorption [7]. In addition, offspring from mothers supplemented with Se plus folic acid had lower serum and urine Se levels than Se-only supplemented pups and had higher Se levels in feces. It appears that this might be due to an interaction between folic acid and Se intestinal absorption related perhaps to the LDL receptors required for Se and folic acid uptake, such as megalin [31].

Duodenal absorption of selenomethionine

We have previously found that, as a direct effect of ethanol exposure, an imbalance on Se homeostasis occurs and that there is a de-

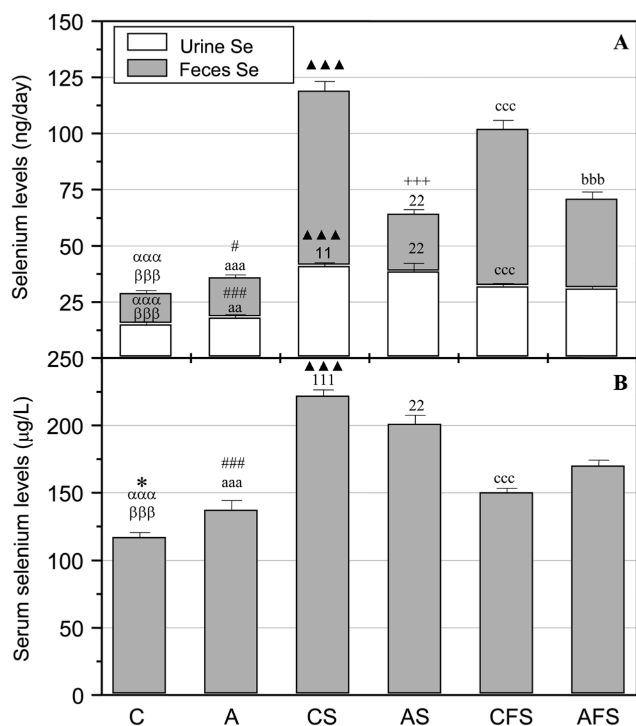

Fig. 1. Selenium levels excreted in urine and feces (ng/day) (A) and serum selenium levels (μg/l) (B) in offspring. The results are expressed as means ± SEM and were analyzed by a multifactorial analysis of variance (one-way ANOVA) followed by Tukey's test. The number of animals in each group is 9. C: control group; A: alcohol group; CS: control Se group; AS: alcohol Se group; CFS: control Se + folic acid group; AFS: alcohol Se + folic acid group. *: P<0.05 for C *vs*. A. ▲▲▲: p<0.001 for CS *vs*. C. ccc: P<0.001 for CFS *vs*. C. 11 and 111: P<0.01 and P<0.001 for CFS *vs*. CS, respectively. # and ###: P<0.05 and P<0.001 for A *vs*. AS, respectively. aa and aaa: P<0.01 and P<0.001 for A *vs*. AFS, respectively. ααα: P<0.001 for AS *vs*. C. +++: P<0.001 for AS *vs*. CS. 22: P<0.01 for AS *vs*. AFS, respectively. βββ: P<0.001 for AFS *vs*. C. bbb: P<0.001 for AFS *vs*. CFS.

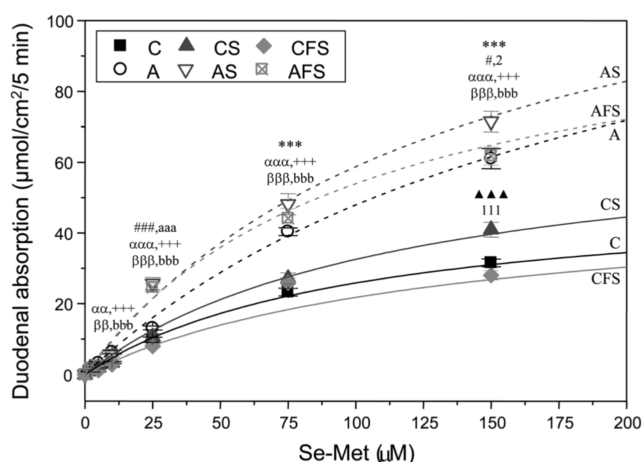

Fig. 2. Selenomethionine duodenal absorption (μmol/cm^2/5 min). The results are expressed as means ± SEM and were analyzed by a multifactorial analysis of variance (one-way ANOVA) followed by Tukey's test. The number of animals in each group is 9. C: control group; A: alcohol group; CS: control Se group; AS: alcohol Se group; CFS: control Se + folic acid group; AFS: alcohol Se + folic acid group. ***: P<0.001 for C *vs*. A. ▲▲▲: P<0.001 for CS *vs*. C. 111: P<0.001 for CFS *vs*. CS. # and ###: P<0.05 and P<0.001 for A *vs*. AS, respectively. aaa: P<0.001 for A *vs*. AFS. αα and ααα: P<0.01 and P<0.001 for AS *vs*. C, respectively. +++: P<0.001 for AS *vs*. CS. 2: P<0.05 for AS *vs*. AFS. ββ and βββ: P<0.01 and P<0.001 for AFS *vs*. C, respectively. bbb: P<0.001 for AFS *vs*. CFS.

pletion of this element in different tissues in pups [15]. To increase our knowledge about Se bioavailability after ethanol exposure, we have studied duodenal Se absorption.

Since suckling pups obtain their Se from organic forms found in breast milk, such as Se-Met [19], in this study, we determined intestinal Se-Met absorption using a different concentration of this amino acid and the kinetic parameters of the processed saturable, which obey the Michaelis-Menten equation (Fig. 2).

Ethanol exposure during gestation and lactation increased Se-Met absorption in comparison with the control group. We found significant differences between these two groups when the substrate concentrations in the perfusate were 75 and 150 μM (P<0.001). Similarly, we observed a high level of Se-Met absorption in supplemented ethanol pups compared with the supplemented control and control pups. In this case, we found significant differences (P<0.001) between group AS and groups CS and C, and between group AFS and groups CFS and C, when we utilized Se-Met concentrations of 25, 75 and 150 μM. It is probable that ethanol temporarily destabilizes the intercellular junctions of the epithelium [7] and thus promotes the absorption of a high amount of Se. Different experiments with animals have shown that chronic alcohol administration damages the intestinal cells [27, 32] and increases the space between the cells, and this may cause an increase in passive paracellular absorption [33]. This could be one of the reasons explaining the high values of V_{max} in the three ethanol groups studied, despite their duodenal development being equal to, or less than, that of the control and their control counterparts (Table 2). Thus, although ethanol pups showed the highest reduction in duodenal perimeter and mucosa weight, these pups presented a high maximal transport velocity that was similar to that found in the AS group. In the case of the AFS offspring, V_{max} was lower (P<0.01) than in the A and AS offspring. We think that duodenal Se-Met absorption decreases as a result of specific competition between Se and folic acid for the megalin receptor [31].

This receptor is expressed in several absorptive epithelia, including those of the intestine and kidney proximal tubule [34], two points where Se absorption decreases when both supplements are administered compared with when one is administered, as deduced by studying the selenium levels in urine and feces.

The apparent constant (K_m) was different in the ethanol-exposed groups; it was highest in pups from dams exposed to ethanol only (197.6 ± 7.7 μM), followed by the AS and AFS groups (139.2 ± 8.3 and 101.3 ± 4.5, respectively; Table 2). These results indicate that the affinity of Se-Met transporter proteins in A pups, was lower compared with the supplemented ethanol pups and control pups (P<0.001). It is likely that ethanol alters the permeability of the

Table 2. Kinetic parameters of selenomethionine absorption in the duodenum of offspring

	C	A	CS	AS	CFS	AFS
Vmax (µmol/cm^2/5 min)	51.7 ± 0.8 $^{***,\alpha\alpha\alpha,\beta\beta\beta}$	142.5 ± 8.1 ^{aa}	70.4 ± 3.8 $^{▲▲▲,111}$	140.5 ± 8.18 $^{+++,22}$	49.9 ± 2.7	108.5 ± 6.5 ^{bbb}
K_m (µM)	100.1 ± 7.5 $^{***,\alpha\alpha}$	197.6 ± 7.7 $^{\#\#\#,aaa}$	116.6 ± 5.4	139.2 ± 8.3 ^{22}	129.8 ± 6.2 ^{c}	101.3 ± 4.5 ^{b}

The results are expressed as means ± SEM and were analyzed by a multifactorial analysis of variance (one-way ANOVA) followed by Tukey's test. The number of animals in each group is 9. C: control group; A: alcohol group; CS: control Se group; AS: alcohol Se group; CFS: control Se + folic acid group; AFS: alcohol Se + folic acid group. ***: P<0.001 for C *vs.* A. ▲▲▲: P<0.001 for CS *vs.* C. ^{c}: P<0.05 for CFS *vs.* C. ^{111}: P<0.001 for CFS *vs.* CS. $^{\#\#\#}$: P<0.001 for A *vs.* AS. ^{aa} and ^{aaa}: P<0.01 and P<0.001 for A *vs.* AFS, respectively. $^{\alpha\alpha}$ and $^{\alpha\alpha\alpha}$: P<0.01 and P<0.001 for AS *vs.* C, respectively. $^{+++}$: P<0.001 for AS *vs.* CS. ^{22}: P<0.01 for AS *vs.* AFS. $^{\beta\beta\beta}$: P<0.001 for AFS *vs.* C. ^{b} and ^{bbb}: P<0.05 and P<0.001 for AFS *vs.* CFS, respectively.

lipid membrane and destabilizes transport proteins. Maturu *et al.* (2010) [35] reported that ethanol altered the lipid packing, structure and membrane organization and the consequent changes in the functioning and orientation of membrane proteins. In the present study, our diets containing one or two supplementes enhanced the Se-Met affinity of the transporters after ethanol exposition; however, this effect was greater after administration of two supplements (P<0.01 for AS *vs.* AFS, P<0.01 for AS *vs.* C, and not significant for AFS *vs.* C). These antioxidant supplementations probably increase the activity of duodenal antioxidant enzymes, such as GPx, which it is known to be decreased in the gastrointestinal tract of ethanol-exposed rats [36], changing lipid peroxidation profile and the structure of membrane cells [5]. Furthermore, the highest affinity found in the AFS offspring could be explained by an extra protective effect of folic acid on lipid membranes. Ojeda *et al.* (2008) [20] demonstrated different lipid alterations in adults and in pups whose dams consumed ethanol and that folic acid contributes to alleviation of the adverse effects in these cases.

Se-Met absorption was lower in all the control groups studied compared with their alcohol counterparts, probably because paracellular absorption is reduced, since Se-Met, in terms of absorption, acts as an amino acid. We only found differences in absorption of this amino acid in CS pups compared with C and CFS pups when the Se-Met concentration in the perfusate was 150 µM (P<0.001). With respect to kinetic parameters, we found a higher V_{max} in CS offspring (P<0.001) compared with C and CFS offspring, while the apparent constant increased only in the CFS group (P<0.05) versus the C group. Therefore, taking into account the high duodenal mucosa weight found in the supplemented control group, our results with respect to the V_{max} and K_m values indicated that CS pups could present a higher number of Se-Met transporter proteins than C, but with a similar affinity for this substrate. This suggests that the transporter protein was the same in both groups [14]. In the CFS group, we found that the weight of duodenal mucosa was similar to that of the CS offspring, indicating that pups had the same intestinal development. Nevertheless, the transport protein velocity and affinity were lower in the CFS pups, probably due to the competition that exists for the megalin receptor in the intestinal wall. However, the antioxidant supplement regimens used affected the parameters studied (especially the Km) in a different way depending on basal or ethanol status, and more studies in this direction should been performed.

In conclusion, ethanol affects pup growth and their duodenal development during gestation and lactation, altering duodenal Se-Met absorption and Se bioavailability. Although Se and Folic acid antioxidant supplementations do not restore Se-Met absorption to normal values, they do enhance transporter affinity for substrates and ameliorate the damage caused by ethanol in the duodenal mucosa, perhaps by increasing GPx 2 activity, which prevents membrane lipid peroxidation. However, duodenal Se-Met absorption and Se bioavailability are higher after Se-only supplementation than after Se plus folic acid supplementation, and this may be due to an interaction between folic acid and Se intestinal absorption related perhaps to the LDL receptors required for their uptake, such as megalin.

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