Nutritional Neuroscience, Vol. 3, pp. 331–337
Reprints available directly from the publisher
Photocopying permitted by license only

© 2000 OPA (Overseas Publishers Association) N.V.
Published by license under
the Harwood Academic Publishers imprint,
part of The Gordon and Breach Publishing Group.
Printed in Malaysia.

Ethanol Consumption by Lactating Rats Induces Changes in Pup's Fatty Acid Profiles*

KELSE TIBAU DE ALBUQUERQUE[a], MARIA DAS GRAÇAS TAVARES DO CARMO[a,**] and EMÍLIO HERRERA[b]

[a]*Instituto de Nutrição, Universidade Federal do Rio de Janeiro, RJ, Brasil;*
[b]*Facultad de Ciencias Experimentales y Técnicas, Universidad San Pablo-CEU, Madrid, Spain*

(Received 27 October 1999)

From parturition, lactating Wistar rats were given 20% ethanol in drinking water and solid diet *ad libitum* (ET group) or were pair-fed to the ET group (PF group) or were given water and solid diet *ad libitum* (control group, C). Animals were studied on day 12 of lactation and/or treatment, when dams were separated from their litters and 2–4 hours latter they were i.p. injected with oxytocin for milk collection under anaesthesia. Maternal food intake, weight gain and pup's body weight were lower in ET than in C rats. When compared to C rats, milk of ET dams had a decreased proportion of C14:0 and C22:6 *n*-3 fatty acids while an increase in C18:0, C16:1 and C18:1 *n*-9 was detected, whereas when compared to PF it had higher C8:0, C10:0, C18:0, C18:1 *n*-9, C18:2 *n*-6 and lower C20:5 *n*-3 and C22:6 *n*-3. Body weight was lower in pups from ET than in those from C or PF, and whereas brain weight and brain lipid content was lower in ET and PF pups than in C, total phospholipid (PL) brain content was similar among the groups. The ratio of C20:3 *n*-9 to C20:4 *n*-6 in brain PL was higher in ET pups than in either C or PF, indicating an essential fatty acid deficiency in the formers. Ethanol treatment also decreased the proportional amount of C18:2 *n*-6, C18:3 *n*-3, C20:4 *n*-6, C20:5 *n*-3 and C22:6 *n*-3 in brain PL as compared to C, whereas from these fatty acids only C18:3 *n*-3, C20:5 *n*-3 and C22:6 *n*-3 were decreased in PF. On the other hand, the proportion of C22:6 *n*-3 was significantly lower in the pups of ET group than in PF animals. Present results show that maternal intake of ethanol during lactation in the rat modifies milk lipid composition and that these effects are not caused by the undernutrition condition of the animals. These effects alter fatty acid composition of brain PL in pups, and such effect may contribute to its abnormal development.

Keywords: Ethanol, Lactation, Fatty acids, Phospholipids, Pup's growth, Brain, Rat, Breast milk

INTRODUCTION

During suckling, milk lipids supply about 50% of the energy intake of various species, including humans and rodents (Neville and Picciano, 1997;

*Supported by: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Universitária José Bonifácio (FUJB)

**Corresponding author. Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Instituto de Nutrição Bloco J-2° andar 21.9415.90, Rio de Janeiro, Brasil. Fax: +55 21 280 83 43. E-mail: tcarmo@ibm.net

Girard *et al.*, 1992). Medium-chain fatty acids (C8:0–12:0) are synthesized de novo in the mammary gland and represent an easily absorbable energy source for breast-fed infants. They are also rapidly transported to tissues for oxidation or used by liver for ketogenesis (Girard *et al.*, 1992). Essential fatty acids (EFA) and their longer chain, more unsatured derivatives, provide the precursors for eicosanoids (arachidonic acid, 20:4 *n*-6, AA) and are important constituents of cell membranes, especially those in the brain tissue (docosahexaenoic acid, 22:6 *n*-3) (Innis, 1986; Martinez, 1992; Dutta-Roy, 1994; Suárez *et al.*, 1996).

Ethanol consumption in adult rats has been shown to exert profound effects on cell membranes, including modification of the PUFA composition (Goldstein and Chin, 1981; Reitz, 1993; Zheng *et al.*, 1996), decreased levels of arachidonic acid and inhibition of both *n*-6 and *n*-5 desaturase enzymes (Wang and Reitz, 1983; Reitz, 1993).

Several studies in the rat reported that maternal alcohol consumption during pregnancy results in negative effects on offspring development which persist after birth (Lee and Leichter, 1980; Ludeña, *et al.*, 1983; Vilaró *et al.*, 1987). These effects are not a consequence of maternal undernutrition, since they are evident even in control animals that received the same daily caloric intake than the ethanol treated rats (Jones *et al.*, 1981; Testar *et al.*, 1986; López-Tejero *et al.*, 1986).

Previous studies from our laboratory have shown that ethanol intake during lactation in rat increases both the rate of in vivo lipogenesis in mammary gland (Tavares do Carmo, *et al.*, 1996) and milk triacylglyceride concentration (Vilaró *et al.*, 1987), and impairs milk production and suckling pups' growth (Vilaró *et al.*, 1987; Tavares do Carmo and Nascimento Curi, 1990; Tavares do Carmo *et al.*, 1999). Present study was addressed to determine whether ethanol intake during lactation in the rat also affects milk fatty acid composition, and the work was extended to determine brain phospholipid fatty acids in offspring.

MATERIALS AND METHODS

Animals and Procedures

Adult virgin female Wistar rats were housed in groups of 4–5 animals per cage and maintained under controlled conditions of light/dark (12/12 h) and temperature (24 ± 1 °C). After mating, pregnant rats were housed in individual cages and fed laboratory chow pellets (Purina, Labina, Ltda, Brasil) with free access to water. On the day of parturition, considered day 0 of lactation, litters were weighed and adjusted to 8 pups per dam. No gender differentiation was done. On the first day postpartum, lactating dams were divided into three groups: 1) Ethanol-treated rats which received 20% ethanol diluted in drinking water and food *ad libitum* until sacrifice. 2) Pair-fed rats, used as a nutritional control, that received the same solid diet per day and per 100 g of body weight as that being fed to the ethanol-treated rats and tap water *ad libitum*. To avoid immediate consumption of the diet, the daily dose was divided in two equal portions for the dark and light periods. 3) Control animals that received a solid diet and tap water *ad libitum*, and were handled in the same way as the ethanol rats. All animals were fed laboratory chow (Purina, Labina, Ltda, Brasil) containing 20% protein; 68% carbohydrate, 4% cellulose; 1% salt mix (AIN-93); 2% vitamin mix (AIN-93) and 5% (w/w) total fat (containing an oil mix, including fish oil, according to the supplier). The fatty acids of laboratory chow were analyzed by gas chromatography on a Perkin Elmer as described below, having 19.7% saturated fatty acids, 16.8% monounsaturated fatty acids, 51.1% linoleic acid (18:2 *n*-6), 5.7% linolenic (18:3 *n*-3), 0.9% arachidonic acid (20:4 *n*-6), 1.0% docosahexaenoic acid (22:6 *n*-3) and 2% eicosapentaenoic acid (20:5 *n*-3). Daily food and liquid intake and body weight were measured throughout the treatment period (days 1–12 of lactation).

Collection of Milk, Blood and Brain

Milk samples were collected from dams on day 12 of lactation in the morning. After being separated from their litters for 2–4 hours, they were intramuscularly injected with Ketalar Parke-Davis (0.5 ml/kg body weight) and 5 units oxytocin (Sandoz Products Ltda.). Fifteen minutes later, milk was obtained by gentle hand stripping of the teat. After milking, dams were decapitated and blood collected in heparinized tubes. Plasma was obtained by centrifugation and aliquots were used to measure maternal alcohol levels. Pups were decapitated, their brains were removed and weighed, pooled for each litter, and then frozen in liquid nitrogen and kept at −80 °C until analysis. Since determinations were conducted on the pooled brains from a litter, the litter represents the unit of analysis.

Assays of Maternal Plasma Ethanol Levels

Blood ethanol concentrations were assayed by an alcohol dehydrogenase method using a commercial ethanol diagnostic Kit (Sigma, St. Louis, MO).

Lipid Extraction and Analysis

The brains were homogenized in saline and homogenized aliquots were used for lipid extraction with chloroform/methanol (2:1, v/v) containing 0.02% BHT (w/v) as described by Kates (1972). Lipids were then fractionated by thin-layer chromatography (TLC) on g-silica gel plates using chloroform/methanol/HCl (100:200: 0.75 v/v/v). Phospholipid fatty acids were analyzed after derivation with boron trifluoride-methanol as described by Morrison and Smith (1964). Dietary and milk fatty acid methyl esters (FAME) were directly prepared by the transesterification method of Lepage and Roy (1986), using a mixture of benzene:methanol (1:4) and acetylchloride (Sigma, St.Louis, Mo., USA). Fatty acid methyl esters were measured in a Perkin Elmer cromatograph autosystem provided with a hydrogen flame ionization detector and a capillary column (60 m × 0.30 mm i.d.) packed with 10% SP 2330 (Supelco Inc, Bellefonte, PA) as stationary phase. Nitrogen was used as carrier gas. Injector and detector temperatures were set at 250 °C , whereas oven temperature was programmed to 5 °C/min from 40 to 225 °C. Esters were identified comparing retention times with known standards (Sigma), and quantification was done calculating peak areas with an integrator. Results were expressed as a weight percentage (g/100 g total fatty acids).

Statistical Analysis

Results were expressed as mean ± SEM and statistical comparisons were performed using analysis of variance. When the ANOVA showed that a statistically significant difference existed, Duncan's test was used to determine statistical significance ($p < 0.05$).

RESULTS

Food Intake, Alcohol Consumption and Body Weight Changes

Maternal body weight, food, fluid and caloric intake at day 12 of lactation are summarized in Table I. When compared to control rats, ethanol treatment greatly decreased body weight and solid food intake of dams during lactation without modifying fluid intake. Ethanol treatment also decreased daily energetic intake, and this is so even when considering the calories from food plus those from ethanol. Although total daily energetic intake (from food plus ethanol) was higher in ethanol treated rats than in pair fed ones body weight did not differ between these two groups (Table I).

Blood ethanol concentration was found to be 113.5 ± 6.5 mg/dL (mean ± SEM) in the ethanol treated rats.

TABLE I Effect of feeding 20% ethanol in the drinking water on food, fluid and total Kjoules intake in rats on day 12 of lactation

	Control	Ethanol	Pair-Fed
Body weight, g	230±3	190±2*	185±2*
Food, g/100 g BW/day	22.9±1.6	8.82±0.97*	9.05±0.22*
Fluid, ml/100 g BW/day[1]	28.0±0.9	29.6±0.9	31.3±0.7
KJoules/100 g BW/day			
As food	376±10	160±15*	168±4*
As ethanol	—	72.0±2.4	—

Each value represents the mean ± SEM of 6–8 samples. [1] 20% ethanol. Values that are significantly different from those of control rats are shown by * ($p < 0.05$). No significant differences were found between pair-fed and ethanol groups for any variable in this Table ($p > 0.05$).

Fatty Acid Profile in Dams' Milk

As shown in Table II, by day 12 of lactation, the proportion of C14:0 fatty acid in milk was lower in ethanol group than in controls, whereas the proportion of C18:0, 16:1 *n*-7 and 18:1 *n*-9 fatty acids was higher in the formers. Despite these differences, the proportion of other fatty acids, including linoleic acid (18:2 *n*-6) and α-linolenic acid (18:3 *n*-3) were similar in both groups. Furthermore, a low proportion of DHA (C22:6 *n*-3) was observed in milk from ethanol treated dams as compared to controls. Milk of pair fed rats had lower proportion of C8:0, 10:0, 18:1 *n*-9 and 18:2 *n*-6 fatty acids than both control and ethanol treated rats, whereas C18:4 *n*-3 and C20:5 *n*-3 (EPA) were higher. The proportion of C14:0 fatty acid was lower and of C18:0 was higher in milk of pair-fed rats than of controls whereas C22:6 was higher in pair-fed than in ethanol treated rats (Table II).

TABLE II Effect of ethanol intake during lactation on relative fatty acid composition (% total fatty acids) of rat milk

Fatty acids (wt%)	Controls	Ethanol	Pair-fed
C8:0	3.41±0.70	4.10±0.15	1.78±0.39*†
C10:0	12.12±0.78	11.31±0.65	6.86±0.76*†
C12:0	10.51±0.34	8.17±0.51	9.97±1.80
C14:0	10.56±0.43	7.35±0.72*	8.10±0.24*
C16:0	21.50±1.15	22.25±1.31	22.74±2.75
C18:0	2.01±0.23	4.00±0.17*	2.72±0.51
C16:1 *n*-7	1.09±0.06	2.07±0.07*	1.34±0.02
C18:1 *n*-9	13.06±0.71	18.03±0.64*	8.34±0.52*†
C18:2 *n*-6	14.72±0.44	16.97±0.89	9.51±1.45*†
C18:3 *n*-3	0.84±0.02	0.86±0.05	1.27±0.30
C18:4 *n*-3	0.31±0.03	0.30±0.02	0.44±0.03*†
C20:4 *n*-6 (AA)	0.43±0.04	0.40±0.02	0.84±0.14
C20:5 *n*-3 (EPA)	1.27±0.14	1.45±0.09	2.24±0.39*†
C22:6 *n*-3 (DHA)	0.55±0.11	0.20±0.03*	0.46±0.01†

The results are means ± SEM of 6–8 samples. EPA – Eicosapentaenoic acid; DHA–Docosahexaenoic acid; AA – Arachidonic. Values that are significantly different from those for control rats are shown by * ($p < 0.05$) those between pair-fed and ethanol groups by † ($p < 0.05$).

Body and Brain Weights and Brain Lipids in Pups

As shown in Table III, maternal consumption of ethanol is associated with a significant reduction in body and brain weight of the pups. Body weight in suckling pups from ethanol treated mothers was even lower than in pair-fed rats, whereas brain weight did not differ between these two groups. Regarding brain lipids neither total lipid or phospholipid concentrations differed among the three groups, although total lipid content per brain was lower in pups suckled from either ethanol or pair-fed mothers than from control dams. However, as shown in Table IV, brain fatty acid phospholipid composition of pups substantially differed among the three groups: the proportion of C20:4 *n*-6 (AA) was significantly lower and that of 20:3 *n*-9 (mead acid) was higher in pups from ethanol

TABLE III Effect of maternal feeding 20% ethanol in the drinking water on final body weights, brain weights, brain total lipids and brain total phospholipid (PL) of pups on day 12 of lactation

	Control	Ethanol	Pair-fed
Body weight, g	22.9±0.6	14.2±1.4*	18.0±1.0*†
Brain weight, g	1.05±0.02	0.65±0.05*	0.62±0.06*
Brain Lipid			
mg/g	30.5±1.4	32.4±2.7	32.8±2.3
mg/brain	32.0±1.4	21±3*	20.3±2.3*
Brain PL			
mg/g	6.80±0.44	7.48±0.80	7.15±1.05
mg/brain	7.14±0.47	4.86±0.82	4.43±1.05

Each value represents the mean ± SEM of 6–8 samples. Statistical comparisons versus controls are shown by * ($p < 0.05$) whereas those between pair-fed and ethanol groups by † ($p < 0.05$).

TABLE IV Effect of ethanol ingestion during lactation on suckling pup brain phospholipid fatty acid composition

Fatty acids (%)	Controls	Ethanol	Pair-fed
C16:0	34.40 ± 0.79	36.83 ± 1.40	$41.31 \pm 1.16^{*\dagger}$
C18:0	17.96 ± 0.66	$20.83 \pm 0.89^{*}$	$15.91 \pm 0.63^{\dagger}$
C18:1 *n*-9	10.83 ± 0.39	11.11 ± 0.28	11.73 ± 0.36
C18:2 *n*-6	1.01 ± 0.06	$0.75 \pm 0.06^{*}$	0.87 ± 0.05
C18:3 *n*-3	0.28 ± 0.02	$0.20 \pm 0.05^{*}$	$0.15 \pm 0.01^{*\dagger}$
C20:3 *n*-9 (Mead)	2.36 ± 0.02	$3.06 \pm 0.17^{*}$	$2.42 \pm 0.09^{\dagger}$
C20:4 *n*-6 (AA)	8.54 ± 0.32	5.99 ± 0.85	7.23 ± 0.53
C20:5 *n*-3 (EPA)	2.17 ± 0.20	$1.35 \pm 0.14^{*}$	$1.66 \pm 0.02^{*}$
C22:6 *n*-3 (DHA)	7.20 ± 0.73	$3.16 \pm 0.39^{*}$	$4.86 \pm 0.42^{*\dagger}$

The results are means ± SEM of 6–8 samples.Statistical comparisons versus controls are shown by * ($p < 0.05$), whereas those between pair-fed and ethanol groups † ($p < 0.05$).

mothers as compared to pups from either control or pair-fed dams. The proportion of C18:3 *n*-3 and C22:6 *n*-3 fatty acids was lower in brain phospholipids of pups from ethanol and pair-fed rats compared to controls, whereas C22:6 in ethanol pups had lower values than pair-fed ones. Besides, the proportion of C16:0 was higher in pups from pair-fed rats than in either ethanol or controls, while C18:0 was higher in ethanol than in controls and pair-feds. Pups from ethanol and pair-fed mothers showed also lower proportion of C20:5 *n*-3 compared to controls, whereas only pups from ethanol mothers presented lower proportion of C18:2 *n*-6 than controls (Table IV).

DISCUSSION

To our knowledge this is the first report of fatty acid profile in milk from rats receiving ethanol (20%) in drinking water during lactation. Present findings show that milk produced by ethanol treated dams have a different fatty acid pattern than either control rats or pair-fed rats, indicating that differences are not caused by the decreased food intake of ethanol treated rats but are a direct consequence of ethanol action. Average amount of food intake, ethanol consumption, blood ethanol concentrations and weight gain of lactating rats given ethanol found in the present study were similar to our previous findings (Tavares do Carmo, *et al.*, 1996; Tavares do Carmo *et al.*, 1999). From previous studies we know that although milk production is greatly impaired in ethanol treated lactating rats, it has an enhanced triglyceride content (Vilaró *et al.*, 1987), and this change may be the result of both an enhanced mammary gland uptake of circulating fatty acid triglycerides caused by an augmented lipoprotein lipase activity (Vilaró *et al.*, 1987) and enhanced mammary gland lipogenesis (Tavares do Carmo, *et al.*, 1996). The fatty acid profile in milk of ethanol-treated rats in this study may well be also a consequence of both effects. The proportion of medium-chain fatty acids appeared either higher (C8:0 and C10:0) or similar (C12:0 and C14:0) in ethanol-treated rats as compared to pair-fed rats, which together with the enhanced triglyceride concentration present in milk of ethanol treated rats (Vilaró *et al.*, 1987) would indicate a specific abundance of these lipogenic products in the mammary gland of ethanol treated rats. Since it is known that triglycerides containing medium-chain fatty acids are more easily hydrolyzed and absorbed during suckling than long-chain fatty acids (Neville and Picciano, 1997; Genzel-Boroviczény *et al.*, 1997), preservation of those fatty acids in milk of ethanol-treated lactating mothers may well be a metabolic adaptation addressed to guarantee offspring survival, despite their decreased availability of sufficient amount of milk.

Both saturated and unsatured long-chain fatty acids (> 16 C) enter the mammary alveolar cell from the plasma, being supplied from circulating lipids, arising from either dietary sources or from lipid stores (Williamson and DaCosta, 1993). Furthermore, in rats and presumably humans, desaturation and elongation of oleic and other monounsaturated fatty acids takes place in the liver (Neville and Picciano, 1997), and desaturation and elongation of the essential fatty acids, linoleic and linolenic acids, produce the long-chain polyunsatured fatty acids (LC-PUFA) present in milk (Bandyopadhyay *et al.*,

1995). Thus, proportional increments in C18:1, C16:1 and C18:0, and the decrease in C22:6 *n*-3 in milk found here in the ethanol treated rats are probably the result of ethanol action on the metabolism of fatty acids.

Fatty acid composition of milk lipids seems to depend on the maternal energy and lipid composition of the diet (Del Prado *et al.*, 1999). In the present study the proportion of *n*-3 fatty acids in milk was found much lower than *n*-6 fatty acids, which may be a consequence of the abundance of *n*-6 fatty acids in maternal diet, which is known to suppress the metabolism and incorporation of *n*-3 fatty acids into milk fat (Huang *et al.*, 1992). Besides, ethanol is known to inhibit both Δ6-desaturase and Δ5-desaturase activities (Nervi *et al.*, 1980), which are responsible for the conversion of dietary linoleic acid (18:2 *n*-6) and alpha-linolenic (18:3 *n*-3) to their respective metabolites, arachidonic acid (20:4 *n*-6) and docosahexaenoic acid (22:6 *n*-3). Thus, these effects may be responsible for the decrease in the C22:6 *n*-3 fatty acid found here in milk of the ethanol treated lactating rats.

Analysis of fatty acid composition in brain phospholipids of pups showed that the ethanol treated lactating rats had a consistent decrease in the proportion of polyunsaturated fatty acids. Essential fatty acid (EFA) deficiency is characterized by a decrease in (*n*-6) and (*n*-3) fatty acids and by an accumulation of (*n*-9) fatty acids (Lefkowith, *et al.*, 1985). In particular, EFA deficiency leads to an accumulation of mead acid (20:3, *n*-9), a fatty acid which is normally not present in tissues (Leichsenring *et al.*, 1992). The ratio of 20:3 *n*-9 to C20:4 *n*-6 is used to define the deficiency state: a ratio over 0.4 is the biochemical criterion of EFA deficiency (Lefkowith *et al.*, 1985). In the present study the ratio in ethanol treated pup's brain averaged 0.51 whereas it was lower than 0.4 in those from control or pair-fed dams, thus demonstrating EFA deficiency in the formers.

In summary, this investigation presents evidence of the deleterious effects of dietary ethanol intake during lactation, modifying milk fatty acid composition and pups brain fatty acid composition, with an EFA deficiency, as well as greatly impairing their growth rate, and that such effects are not caused by the undernutrition produced by the ethanol. Those effects on brain fatty acid composition occur during a sensitive brain developmental period, and may contribute to the permanent effects known to occur in offsprings of high ethanol intake mothers.

References

Bandyopadhyay, G.H., Lee, L-Y., Guzman, R.C. and Nandi, S. (1995) Effect of reproductive states on lipid mobilization and linoleic acid metabolism in mammary glands. *Lipids* **30**, 155–62.

Del Prado, M., Villalpando, S., Gordillo, J. and Hernández-Montes, H. (1999) A high dietary lipid intake during pregnancy and lactation enhances mammary gland lipid uptake and lipoprotein lipase activity in rats. *J. Nutr.* **129**, 1574–1578.

Dutta-Roy, A.K. (1994) Insulin mediated processes in platelets, monocytes, macrophages and erytrocytes: Effects of essential fatty acid metabolism. *Prostaglandins Leukotrienes and essential fatty Acids* **51**, 385–399.

Genzel-Boroviczény, Wahle, J. and Koletzko, B. (1997) Fatty acid composition of human milk during the 1st month after term and preterm delivery. *European Journal of Pediatric* **156**, 142–147.

Girard, J., Ferré, P., Pégorier, J.P. and Duée, P.H. (1992) Adaptation of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. *Physiological Reviews* **72**, 507–562.

Goldstein, D. and Chin, J.H. (1981) Interaction of ethanol with biological membranes. *Federation Proc.* **40**, 2073–2076.

Huang, Y.S., Wainwright, P.E., Redden, P.R., Mills, D.E., Bulman-Fleming, B. and Horrobin, D.F. (1992) Effect of maternal dietary fats with variable (*n*-3)/(*n*-6) ratios on tissue fatty aid composition in suckling mice. *Lipid* **27**, 104–110.

Innis, S.M. (1986) Essential fatty acids in growth and development. *Progress in Lipid Research* **30**, 39–103.

Jones, P.J.H., Leitchter, J. and Lee, M. (1981) Placental Blood Flow in rats fed alcohol before and during gestation. *Life Sciences* **29**, 1153–1159.

Kates, M. (1972) Techniques of lipidology: isolation, analysis and identification of lipids. In: Work, T.S., Work, E. (Eds), Laboratory in Biochemistry and Molecular Biology. Elsevier, North-Holland/Amsterdam, vol 3, pp. 553.

Lee, M. and Leichter, J. (1980) Effect of litter size on the physical growth and maturation on the offspring of rat given alcohol during gestation. *Growth* **44**, 327–335.

Lefkowith, J.B., Flippo, V., Sprecher, H. and Needleman, P. (1985) Paradoxical conservation of cardiac and renal arachidonate content in essential fatty acid deficiency. *Journal of Biological Chemistry* **260**, 15735–15744.

Leichsenring, M., Ahmed, H.M., Laryea, M.D., Welchering, T., El Amin, E., El Karib, A.O. and Bremer, H.J. (1992) Polyunsaturated and essential fatty acids in malnourished children. *Nutrition Research* **12**, 595–603.

Lepage, G. and Roy, C.C. (1986) Direct transesterification of all classes of lipid in on-step reaction. *Journal of Lipid Research* **27**, 114–120.

López-Tejero, D., Ferrer, I., Llobera, M. and Herrera, E. (1986) Effects of prenatal ethanol exposure on physical growth, sensory reflex maturation and brain development in the rat. *Neuropath Appl. Neurobio.* **12**, 251–260.

Ludeña, M.C., Mena, M.A., Salinas, M. and Herrera, E. (1983) Effects of alcohol ingestion in the pregnant rat on daily food intake, offspring growth and matabolic parameters. *Gen Pharmac.* **14**, 327–332.

Marín, M.C., De Tomás, M.E., Serres, C. and Mercuri, O. (1995) Protein-Energy malnutrition during gestation and lactation in rats affects growth rats, brain development and essential fatty acid metabolism. *Journal of Nutrition* **125**, 1017–1024.

Martinez, M. (1992) Tissue levels of polyunsaturated fatty acids during early development. *Journal of Pediatrics* **120**, S129–S138.

Morrison, W.R. and Smith, L.M. (1964) Preparation of fatty acid methyl esters and di-methyl acetals from lipids with boron trifluoride-methanol. *Journal of Lipid Research* **5**, 600–608.

Nervi, A.M., Peluffo, R.O., Brenner, R.R. and Leiken, A.I. (1980) Effect of ethanol administration on fatty acid desaturation. *Lipids* **15**, 263–268.

Neville, M.C., Picciano, M.F. (1997) Regulation of milk lipid secretion and composition. *Annual Review of Nutrition* **17**, 159–84.

Reitz, R.C. (1993) Dietary fatty acids and alcohol: effects on cellular membranes. *Alcohol Alcohol* **28**, 59–71.

Suárez, A. Ramírez, M.C., Faus, M.J. and Gil, A. (1996) Dietary long-chain polyunsaturated fatty acids influence tissue fatty acid composition in rats at weaning. *Journal of Nutrition* **126**, 887–897.

Tavares do Carmo, M.G., Oller Do Nascimento, C.M., Martin, A. and Herrera, E. (1996) Effects of ethanol intake on lipid metabolism in the lactating rat. *Alcohol* **13**(5), 443–448.

Tavares do Carmo, M.G., Oller do Nascimento, C.M., Martin, A. and Herrera, E. (1999) Ethanol intake during lactation in rats impairs milk production and suckling pups growth and metabolism *Alcohol* **18**(1), 71–76.

Tavares do Carmo, M.G. and Nascimento Curi, C.M. (1990) Effect of ethanol intake during lactation on the metabolism of dams and on pup development. *Brazilian Journal of Medical and Biological Research* **23**, 1161–1163.

Testar, X., López, D., Llobera, M. and Herrera, E. (1986) Ethanol administration in the drinking fluid to pregnant rats as a model for the fetal alcohol syndrome. *Pharmacol Biochem Behav* **24**, 625–630.

Vilaró, S., Viñas, O., Remesar, X. and Herrera, E. (1987) Effects of chronic ethanol consumption on lactational performance in rat: Mammary gland and milk composition and pups' growth and metabolism. *Pharmacology Biochemistry & Behavior* **27**(2), 333–339.

Wang, D.L. and Reitz, R.C. (1983) Ethanol ingestion and polyunsaturated fatty acids: effects on the acyl-coA desaturases. *Alcoholism: Clinical and Experimental Research* **7**, 220–226.

Williamson, D.H. and DaCosta, M.T. (1993) The regulation of lipid metabolism in lactation. In: Medina, J.M., Quero, J., (Eds), Physiological Basis of Perinatal Care. *Salamanca, Spain: Ergon*, 63.

Zheng, Z., Barkai, A.J. and Hungund, B.L. (1996) Effects of ethanol on the incorporation of free fatty acids into cerebral membrane phospholipids. *Neurochem. Int.* **28**, 551–555.