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Lipids, Nutrition and Development

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1. Introduction

Lipids are classified as simple, compound and derived based on the hydrolysis, which result in breaking the fatty acids off, leaving free fatty acids and a glycerol, using up three water molecules. Simple lipids are esters of fatty acids with various types of alcohol. They are distinguished into fats and oils. Compound lipids contain an inorganic or organic group in addition to fatty acids and glycerol. They include phospholipids, glicolipids and lipoproteins. Finally, derived lipids are obtained by hydrolysis of simple and complex lipids. These lipids contain glycerol and other alcohols. They correspond to steroid hormones, ketone bodies, hydrocarbons, fatty acids, fatty alcohols, mono and diglycerides, terpenes and carotenoids. Sometimes they are present as waste products of metabolism. Lipids also can be classified, depending on its solubility or function, as polar or apolar compounds and as structural or reserve substances, respectively (Basso, 2007).

The main source of body energy comes from the triglycerides. These compounds are esters formed by one molecule of glycerol and three molecules of fatty acids. Fatty acids are carboxylic acids that usually have in its structure an unbranched carbon chain and one carboxyl. According to the saturation of the carbon chain, they can be classified as saturated, monounsaturated and polyunsaturated fatty acids (Basso, 2007).

In general, all mammals are able to synthesize saturated and monounsaturated fatty acids, but this ability is limited to polyunsaturated fatty acids (PUFAs), without them the organisms could not function properly. For this reason, these compounds are considered "essential" fatty acids. Thus, these fatty acids must be supplied by the diet. Linoleic acid 18:2 (n-6), a member of the n-6 family of fatty acids, was identified as the first "essential" fatty acid, whereas α -linolenic acid, 18:3 (n-3) represents the other essential fatty acids and physiological synthesis of complex lipids (Yehuda et al., 1999). Linoleic acid [18:2 (n-6)] is usually found in large quantities in soybean, corn, canola and safflower oil while α – linolenic [18:3 (n-3)] is easily found in green leafy vegetables, linseed and marine fish oil (Takahashi, 2005).

The main source of dietary lipids is obtained through the intake of triglycerides which can be found as fats or oils. The concept of fat or oil is based on the consistency and on the fatty acid present in the triglyceride molecule. At room temperature, oils are liquid because are constituted of tiacylglycerols containing a high proportion of mono and/or polyunsaturated fatty acids. These come from the vegetable sources such as soybean, corn, sunflower, olive or canola oil or from animal source such as fish oil. On the other hand, fats are solid or pasty at room temperature and contain a large proportion of saturated fatty acids and/or unsaturated with trans double bonds. Fats can be from animal source such as butter, beef tallow or pig and from vegetable source such as cocoa butter and hydrogenated fats. Trans fats are produced naturally at the rumen or from an industrial process adding hydrogen to unsaturated fatty acids found in vegetable oils (partial or total hydrogenation) (Basso, 2007). After hydrogenation, vegetable oils may be converted from liquids to solids, resulting in margarines and shortenings which have excellent culinary properties even though have detrimental health effects. The partial hydrogenation increases the melt pointing of the fats at a room temperature and the degree of hydrogenation controls the final consistency of the manufactured products. These fats are regularly found in foods such as ice cream, chocolates, biscuits, cookies, cakes, mass and margarines (Basso, 2007; Kinsella et al., 1981). Changes in the structure of the lipids molecules have metabolic and nutritional repercussions (Kummerow, 2009), since, produce a significant loss of essential fatty acids (Martin et al., 2004). According to Martin et al. (2004), human nutrition has been moulded substantially along the modernization and industrialization process. Because the property of hydrogenated fats in increasing validity of food products and making them look like more crispy and less oily, food industry and "fast food" restaurants has been intensifying the use of trans fatty acids. On the other hand, since 1990's it has been a substantial interest of the scientif community in investigating the adverse effects over health caused by the long lasting intake of trans fatty acids.

1.1 Effects of early and prolonged intake of hydrogenated fat for health

Once considered a problem only in high-income countries, overweight and obesity are now dramatically present in high rates in low- and middle-income countries, particularly in urban areas. World Health Organization predicts that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese. According to World Health Organization/Food and Agriculture Organization (2003), obesity and overweight is caused by an energy imbalance between calories consumed and calories expended. Among several factors related to the present development of overweight and obesity, an increased intake of foods high in fat and sugar but low in vitamins, minerals and fiber is one of the worldwide problems of this century (WHO, 2003; Remig et al., 2010). Fats are important nutrients in diet and have wide chemical properties that drive diverse metabolic effects.

Although optimal dietary fat quantity has been keenly pursued over past decades, attention has recently centered on the value of dietary fat quality (Gillingham et al., 2011). Recent population studies have shown the important role of monounsaturated and polyunsaturated fats as key nutrients in preventing chronic diseases in modern societies. In addition, sufficient intakes of polyunsaturated fats during childhood are required for optimal growth and development (Carrillo-Fernandez et al., 2011). Consumption of a healthy diet, containing adequate rates of omega6:omega3 throughout the life is important to maintain cardiovascular and possibly also cognitive and immune health (WHO/FAO, 2003). In addition to PUFA, dietary monounsaturated also promote benefits to health in terms of blood lipid profile, blood pressure, insulin sensitivity and glycemic control. Due to existing and emerging research on health attributes of monounsaturated rich diets, and to the low prevalence of chronic disease in populations consuming monounsaturated rich Mediterranean diets, recommendations have been made to replace saturated fat acids with unsaturated fats (Gillingham et al., 2011).

Although several studies have been shown the importance for health in replacing saturated for unsaturated fats in diets, currently, food products consumed in a typical Western diet contain a significant proportion of fats that have been industrially altered. It is estimated that 2% to 8% of energy needs in the typical Western diet come from chemically modified lipid products (Craig-Schmidt, 2006). Trans fatty acids frequently present in partially hydrogenated vegetable oils have been consumed on large scale all over the world. In terms of consumption, trans fatty acids have long been used in food manufacturing for reasons that were described above. Increasing epidemiologic and biochemical evidence suggest that high consumption of trans fats is related to many metabolic alterations and also induce a significant risk factor for cardiovascular disturbs (de Oliveira et al, 2011). Recently, Mozaffarian et al. (2006) showed that a 2% absolute increase in energy intake from trans fat has been associated with a 23% increase in cardiovascular risk. In general, these cardiovascular disturbs are related to some of known mechanisms such as reduction of c-HDL concentration, increase of low density lipoprotein and triglycerides; disturbance in prostaglandin balance and they may also promote insulin resistance (Castro-Martínez et al., 2010). Since , the American Heart Association as well as the World Health Organization recommend limiting trans fats to <1% energy and many others health institutions in the United States of America all recommend limiting dietary trans-fat intake from industrial sources as much as possible.

The presence of an inflammatory process in the arteries seems to be another risk factor in heart disease, and studies show that hydrogenated trans fats increase the inflammation in the arteries and promote endothelial dysfunction (Sun et al., 2007; Lopez-Garcia et al., 2005). Endothelial cells are responsible for the regulation of local vascular tone by means of releasing relaxing and contracting factors synthesized mainly from arachidonic acid. Trans fat inhibits COX-2, an enzyme which converts arachidonic acid to prostacyclin that is needed to prevent blood clots in the coronary arteries (Kummerow, 2009).

In addition to the relation between high rates of trans fatty acids in diet and increased risk for developing cardiovascular disease, a detrimental relationship was found between trans fatty acids intake and depression risk. Rising secular trends in the incidence of depressive disorders have been paralleled by a dramatic change in the sources of fat in the Western diet. This change mainly consists in the replacement of polyunsaturated or monounsaturated fatty acids by saturated fats and trans-unsaturated fats (Pawels & Volterrani, 2008). These findings suggest that cardiovascular disease and depression may share some common nutritional determinants related to subtypes of fat intake (Sanchez-Villegas et al., 2011).

It seems that the exposure to trans fatty acids in utero has negative consequences early in life. How much the in utero environment dictates birth weight and the programming of long-term obesity related disorders is still unclear, especially when compared with that of early neonatal growth rate. The placental transfer of trans fatty acids is still contradictory, both in human and animals (Haggarty, 2010). However, experimental studies have demonstrated that placenta is not completely impermeable to these compounds, since a number of trans fatty acids cross this barrier and accumulates in the liver and in the total body lipids of the fetus. However, despite that the myelinogenesis process is not finished in fetus, the amount of trans isomers transferred to fetal brain was negligible in all studies. At least in animals, this finding suggests the brain might be protected from the trans fatty acids accumulation, but no data have yet been reported for human newborns (Haggarty, 2010).

1.2 The role of lipids on the development of nervous system

Among the various organic systems, the nervous system plays the main role in controlling several physiological processes. During development, this system presents a rapid growth spurt period or a vulnerable period which corresponds to the highest rate of cellular migration and differentiation, neurogenesis, synaptogenesis, myelinization and maturation of neurotransmitter pathways. Depending on the animal species, this critical period of intense neural development occurs at different time points early in life (Dobbing, 1968). In human, for example, it occurs during the last trimester of gestation until the second year of life, while in rats it corresponds the lactation period. During this period, the nutrition is one of the essential environmental factors to a normal development because it provides nutrients without them the neurodevelopment would be impaired (Walker, 2005).

The classical studies about malnutrition show that nutritional deficiency in macro or micronutrients has deleterious effects on the brain (Winick & Rosso, 1969). In rats, malnutrition induces functional and developmental failures, as well as reduction in brain size (Morgane et al., 1992;1993). In children, malnutrition showed influence both short and long term problems of cognition and behavior (Grantham-McGregor & Baker-Henningham, 2005; Benton, 2008).

Essential fatty acids are important constituents of structural lipids in nervous membranes cell and signaling molecules, as such, are involved in many brain functions. Around 30–40% of the total phospholipids in these structures are docosahexaenoic acid molecules (Young et al., 2000), which appear to be specifically concentrated in membranes surrounding synapses (Carlson, 2001). Changes in the quantity and quality of the dietary fatty acids are often associated with developmental and functional alterations in the nervous system.

At the cellular level, an α -linolenic deficient diet can induce less complex patterns of dendritic branching (Wainwright, 2002), smaller neurite growth in hippocampal neurons (Calderon & Kim 2004) and reduced neuronal soma size in some brain regions (Ahmad et al., 2002). Modification in the fatty acid composition of rat brain cell membranes of neurons, astrocytes, oligodendrocytes, and of subcellular fractions, such as myelin and synaptosomes, are also induced by a diet with reduced levels of n-3 fatty acids (Bourre et al., 1984). It has been shown that essential fatty acids imbalance as well as specific fatty acid deficiencies in the maternal diet can affect the neuromotor development of pups, including the ability to respond to environmental stimulation (Lamptey & Walker, 1976; Wainwright, 2002; Anselmo et al., 2006).

Fats and oils as they exist in nature must be processed before they are suitable for human consumption (González et al., 2007). On the other hand, only a few studies have described the effects of the ingestion of trans fatty acids early in life on the rat brain development. In this study we investigated the replacement of soybean oil in the diet by partially hydrogenated vegetable oil, rich in trans fatty acids, from the beginning of gestation through lactation on reflex ontogeny.

2. Methods

2.1 Animals and diets

Female pregnant *Wistar* rats weighing 200-250 g were obtained from the colony of Department of Nutrition of the Federal University of Pernambuco. Twenty-four hours after the birth of the entire mothers' nestling, born on the same day, were contained in a large group and randomly each litter was culled to six males and two female pups. The litters

were also randomly assigned to isocaloric diets containing as lipid source 7% soybean oil (control group-C; n=32) or 7% of hydrogenated vegetal fat (experimental group-E; n=39), since gestation until weaning (21 days old). Both diets (table 1) were formulated based on recommendations of the American Institute of Nutrition-AIN-93 (Reeves et al., 1993). The animals were kept on a 12:12-h light –dark photoperiod at 24o C temperature during the whole period. The animals were maintained according to recommendations from the National Institute of Health (USA) and approved by the Ethics Committee for Use Animal, Federal University of Pernambuco (CEUA, protocol.23076.020339/2010-24). After delivery, from P1 until 21d, the pups were weighted at 1, 7, 14 and 21days of age. Indicators of somatic maturation and reflex ontogeny were studied daily from P1 to P21 between 07:00 am and 09:00 am. Daily it was observed the occurrence of the reflex responses, being considered the consolidation day to be the first one, of a series of three consecutive days where the reply was verified. For each reflex it was established a maximum observation time of 10s according to the experimental model established by Smart & Dobbing (1971).

Ingredients	Control diet (g/100g)	Experimental diet (g/100g)
Casein	20.0	20.0
Cellulose	5.0	5.0
Corn starch	52.95	53.49
Sucrose	10.0	10.0
Soyabean oil	7.0	-
Hydrogenated vegetable fat	_	6.46
Vitamin mix ¹	1.0	1.0
Mineral mix ²	3.5	3.5
L-Cystine	0.3	0.3
Choline Bitartrate	0.25	0.25

Table 1. Composition of the diets. Vitamin mixture (Rhoster Ind.Com. LTDA. SP. Brazil) containing (m%): folic acid (20); niacin (300); biotin (2); calcium pantothenate 160; pyridoxine 70; riboflavin 60; thiamine chloride 60; vitamin B_{12} 0.25; vitamin K_1 7.5. Additionally containing (UI%): vitamin A 40.000; vitamin D₃ 10.000; vitamin E 750. 2 Mineral mixture (Rhoster Ind. Com. LTDA. SP. Brazil) containing (m%): CaHP0₄ (38); K_2 HP0₄ (24); CaCO₃ (18.1); NaF (0.1); NaCl(7.0); MgO (2.0); MgS0₄ 7H₂0 (9.0); FeS0₄ 7H₂0 (0.7); ZnS0₄ H₂0 (0.5); MnSO⁺ H₂0 (0.5); CuS0₄ 5H₂0 (0.1); Al₂ (S0₄)₃ K_2 S0₄ 24H₂0 (0.02); Na₂SeO₃ 5H₂0 (0.001); KCI (0.008).

2.2 Indicators of somatic maturation

The following indicators of somatic maturation, as illustrated in figure 1, were analyzed in order to study whether the replacement of soybean oil by vegetable hydrogenated fat in the diet influenced the development of physical features of the rat pups.

2.3 Indicators of reflex ontogeny

The following indicators of the reflex ontogeny investigated at the present study are described and illustrated below (figure 2):

Palmar Grasp (PG)- This reflex is present at birth and consists of a dorso flexion of the digits ('grasping') in response to the stimulation of the hand-palm with a small metallic stick. The

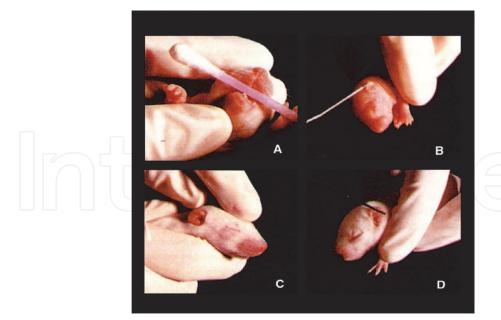


Fig. 1. Indicators of somatic maturation. A) Eruption of the Upper Incisors (EUI) and Eruption of the Lower Incisors (ELI); B) Ear Unfolding (EU); C) Eye Opening (EO); D) Auditory Conduit Opening (ACO).



Fig. 2. The figure represents the indicators of reflex ontogeny observed during lactation period.

expected response is the disappearance of the palmar grasping response, as the organism matures.

Righting (R)- The newborn is placed on its back on a flat surface and the expected mature response is to turn over on the ventral surface, resting in the normal position with the four feet on the ground.

Vibrissa Placing (VP)- The pup is held by the tail, with the head facing the edge of a table and the vibrissae just touching the vertical surface of the table. The expected response is to lift the head and to extend the fore legs in direction of the table.

Cliff Avoidance (*CA*) - The newborn is placed on the edge of a 'cliff' (for instance, on the edge of a table), with the forepaws and face just over the edge. The expected response is to move away from the cliff, to avoid dropping.

Negative Geotaxis (NG)- The pup is placed on an inclined ramp (45° slope) with its head pointing to the ground. The expected mature response is to turn around and crawl up the slope.

Free-Fall Righting (FFR)- The pup is held with the back downwards 35cm above a cotton pad and dropped. The expected response is to turn in mid-air to land on its four paws.

Auditory Startle Response (ASR)- The newborn is exposed suddenly to a loud, sharp noise. The expected response is a prompt extension of the head and the limbs, followed by withdrawal of the limbs and a crouching posture.

2.4 Statistical analysis

The body weight was analyzed by Student's t-test. Results are presented as means±standard error of the mean (SEM). Differences were significant when p< 0.05. Results of the somatic maturation and reflex ontogeny were evaluated by Mann-Whitney test. Results are expressed as median \pm interquartile. Statistical significance was set at p < 0.05.

3. Results

3.1 Body weight

The figure 3 shows that pups fed an experimental diet did not exhibited significant differences in the body weight at the 1st, 7th, 14th and 21th day, during the lactation period when compared to the controls. (C = 6.7 g ± 0.12; 16.3 g ± 0.49; 29.6 g ± 1.18; 46.4 g ±1.52; E = 6.6 g ± 0.11; 16.5 g ± 0.22; 30.3 g ± 0.48; 47 g ± 0.86).

3.2 Indicators of somatic maturation

The effect of the experimental diet on somatic development is shown in Figure 4, where the results are expressed in median (min. – max.) and compared with the control group. There was no difference between the number of days for ear unfolding, eye opening and the eruptions of superior and inferior incisors. However, the opening of the external auditory canal was significantly delayed in the experimental group as compared to the control (C: 12-1; E: 14-2.5; p<0.05).

3.3 Indicators of reflex ontogeny

As can be seen in Figure 5, the development of the early reflexes which appear in the first postnatal week such as righting, cliff avoidance and vibrissa placing did not differ between control and experimental pups. In the second postnatal week, the negative geotaxis was the only reflex which was delayed in the experimental group when compared to the control (C: 11-3; E: 9-1.5).

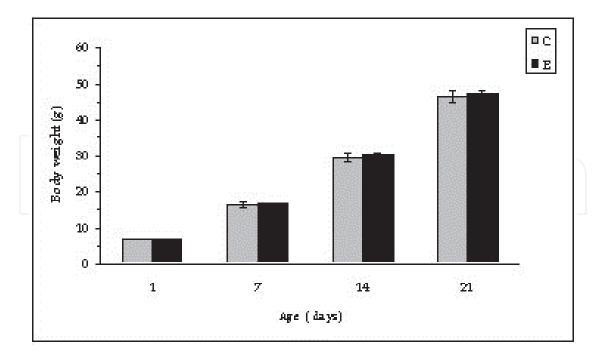


Fig. 3. Body weight in control (C) and experimental (E) offsprings from the 1^{st} , 7^{th} , 14^{th} and the 21^{st} day. Each bar represents the mean ± SEM. C = control group fed a diet containing 7% of soybean oil (n=32); E = experimental group fed a diet containing 7% of hydrogenated vegetal fat (n=39).

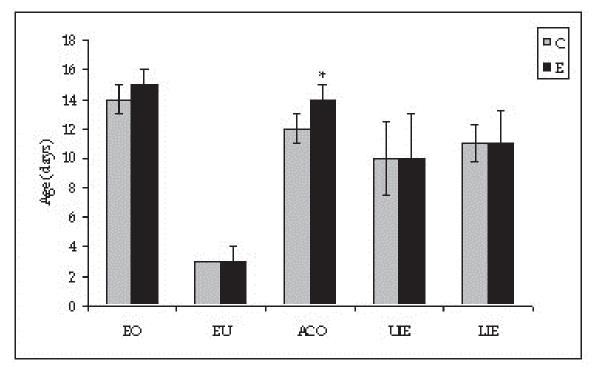


Fig. 4. Somatic maturation indicators of suckling rats fed the control diet (C; n=32) and the experimental diet (E; n=39). Each bar represents the median \pm interquartile. EO = Eye Opening; EU = Ear Unfolding; ACO = Auditory Conduit Opening; UIE = Upper Incisors Eruption; LIE = Low Incisors Eruption. * p< 0.05 vs C group (Mann-Whitney Test).

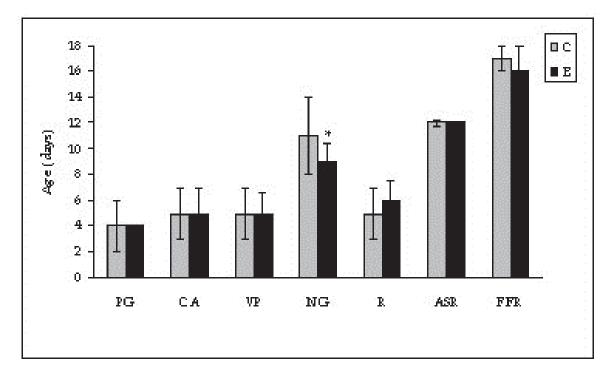


Fig. 5. Reflex ontogeny. Each bar represents the median + inertquartile. C = control group (n=32); E = experimental group (n=39). PG = Palmar Grasp; CA = Cliff Avoidance; VP = Vibrissa Placing; NG = Negative Geotaxis; R = Righting; ASR = Auditory Startle Response; FFR = Free-fall Righting. * p< 0.05 vs C group (Mann-Whitney Test).

4. Discussion

The currently hydrogenation of vegetable oils produces trans fatty acids which can be found in most manufactured products. The high consumption of these foods is related with the increase of health problems. When consumed, trans fat acids can be found in plasma and in the maternal milk (Carlson et al., 1997) and its concentration varies with daily mother intake. Circulating trans fat acids are also carried and incorporated into tissues such as the brain, liver, adipose tissue and spleen and its levels on these tissues depend on the amount ingested (Larqué et al., 2001).

In this study, we observed that offsprings fed a hydrogenated fat based diet did not exhibited differences in body weight from P0 until P21 when compared to the controls. One could explain this finding by the fact of the experimental drawing, herein used, kept the same proportion of lipids in the both diets, only replacing soybean oil for hydrogenated vegetal fat in the experimental diet. Although this dietary treatment has been offered during a period of an intense growth and body development, it did not compromise the body weight gain of the pups over the lactation period. These data are similar to those found in a previous study of our laboratory when rats fed a diet containing 5% coconut-oil or soybeanoil during pregnancy and lactation did not show significant changes in body weight gain from P0 to P21 when compared to the control group (Borba et al., 2010). Santillán et al., (2010) did not find differences in the body weight of mice fed a commercial diet enriched with soybean or sunflower oil diet over gestation and lactation when compared with those fed a commercial diet. On the other hand, mice known to be prone to obesity and insulin resistance when consumed a high fat diet during pregnancy and lactation exhibited a growth delay at the first week of life, but accelerated the growth in the subsequent two weeks (Kavanagh, et al., 2010). It is known that the fat content variation in human milk is clearly the result of different dietary, metabolic and physiologic controls (German, 2011). In humans, the fatty acid composition in maternal diet and in breastmilk during lactation may not affect the infant body composition in the early postpartum period but may be a factor in the development of childhood overweight later in life (Anderson et al., 2010).

The somatic maturation and reflex development from the 2nd over the 21st postnatal days of life are good indicators to understand how environmental factors can influence the functional maturity of the brain development (Smart & Dobbing, 1971; Gramsbergen, 1998).

Among the somatic parameters herein investigated, the opening of the external auditory canal was delayed in the experimental group when compared to the control group. However, we did not observe this effect on the auditory startle response. These results suggest that the consumption of trans fat during the critical period of development did not influence the function of the auditory system since the hearing sensation was preserved. It has been known since the 19th century that hearing may occur through bone conduction; however the way how this physiologic pathway works is not completely understood. Some factors can contribute to the bone conduction, such as: sound radiated into the external ear canal, middle ear ossicle inertia, inertia of the cochlear fluids, compression of the cochlear fluid seems the most important. The efficiency of the bone conduction is largely dependent on the skull bone where the skull acts as a rigid body at low frequencies and incorporates different types of wave transmission at higher frequencies (Stenfelt & Goode, 2005).

Regarding the others somatic maturation indicators no differences were observed between the group fed a trans fat based diet and the group fed a soybean oil based diet. When the reflex ontogeny was analyzed, we observed that there was a significant delayed in the negative geotaxis of the pups fed an experimental diet. These data suggested that there was a negative effect possibly induced by the lack of any essential fatty acid or by the trans fat *per se* on the development of the motor and the cerebellar system. The negative geotaxis reflex is stimulated by the abnormal position of the head and the body which are under control of the vestibular and postural systems (Adams et. al, 1985). This reflex requires a sequence of organized motor events (Ramirez & Spear, 2010) but this only occurs if the motor system is maturated. In rats, spinal cord descending projections develop relatively early. Projections from vestibulospinal and reticulospinal origin reach the cervical levels of the spinal cord at 13rd or 14rd embrionary day. Around the same period, motoneurons in the ventral horn of thoracic and lumbosacral spinal cord segments start developing and two days thereafter, their axons invade the muscle mass of the caudal limb bud (Altman & Bayer, 1984; 1997; Gramsbergen, 1993).

The development of cerebellum occurs in the postnatal period, reaching its peak of development at the end of the first week (Smart & Dobbing, 1971). This period results of a number of events including: neuronal and glial proliferation, outgrowth of axons and dendrites, establishment of synaptical contacts, as well as myelination (Altman & Bayer, 1997). This late development makes the cerebellum a structure particularly vulnerable to insufficient supply of nutrients or to side and possible beneficial effects of pharmacological treatments (Gramsbergen, 2003). It has been shown that a restriction of daily food intake to dams delays the motor development and behavior associated with a disturbed cerebellar development of the offspring (Gramsbergen, 2003). On the other hand, Collucia et al. (2009) showed that omega-3 supplementation during gestation and lactation improved motor coordination in juvenile-adults rats.

One of the problems with the process of hydrogenation is the fact that it possibly produces a loss of essential fatty acids of the original vegetable oils. Hill et al. (1982) showed that rats fed a diet containing as a lipid source partially hydrogenated soybean oil showed a reduction of essential fatty acids levels in the liver and heart. It is evident that partially hydrogenated fats have excellent culinary properties, but from a nutritional point of view, the consumption of trans fatty acids represents a loss of essential fatty acids intake that may have a hazardous impact on health. This study is the first evidence that the consumption of hydrogenated vegetable fat during the critical period of development may compromise some parameters of the reflex and somatic development of rat pups.

5. Conclusion

Although the maternal intake of a diet containing trans fatty acid in replacement of soybean oil have not changed the body weight in the early postpartum period of the pups, it influenced negatively both somatic and reflex development. Recently, there is an increase in the level of interest in fatty acids and lipids. This interest is not limited to brain biochemistry, but also to the effects of levels and ratios of fatty acids on physiological and behavioral aspects. For these reasons, more research is warranted regarding the influence of maternal dietary on the fatty acid composition of the breast milk and their effects on body composition, the development of overweight and behavior changes later in life of rat pups.

6. Acknowledgements

This publication was made possible in part by support from the Universidade Federal de Pernambuco, Pró-Reitoria para Assuntos de Pesquisa e Pós-Graduação (PROPESQ). The authors gratefully acknowledge the invaluable assistance of Dr. Edeones França for the animal care.

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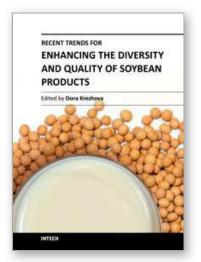
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Recent Trends for Enhancing the Diversity and Quality of Soybean Products

Edited by Prof. Dora Krezhova

ISBN 978-953-307-533-4 Hard cover, 536 pages **Publisher** InTech **Published online** 28, October, 2011 **Published in print edition** October, 2011

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Juliana M.C. Borba, Maria Surama P. da Silva and Ana Paula Rocha de Melo (2011). Lipids, Nutrition and Development, Recent Trends for Enhancing the Diversity and Quality of Soybean Products, Prof. Dora Krezhova (Ed.), ISBN: 978-953-307-533-4, InTech, Available from: http://www.intechopen.com/books/recent-trends-for-enhancing-the-diversity-and-quality-of-soybean-products/lipids-nutrition-and-development



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