Omega-3&6 Fatty Acids and Off springs Eruption Date of Diabetic and Non-Diabetic Pregnant Rats

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Abstract: Omega-3&6 fatty acids and its derivatives are so essential to a child's development. A well-nourished nursing mother provides her infant with a perfect blend of essential fatty acids most frequently from flax seeds and their long-chained derivatives, assuring the body tissues a rich supply**.** Gestational diabetes can have severe adverse effects on fetal and neonatal developmental outcomes. Several factors affecting eruption dates ranging from root development, nutrition, rich tissue vascularity and hormonal influences have been implicated in this process. The present study provide the first evidence on the effect of flax seeds as a source of omega 3 &6 on offsprings' tooth development and eruption dates of diabetic and non-diabetic pregnant rats.

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Key wards: Flax seeds, omega-3, eruption date,diabetes, pregnancy.

1. Introduction

Development includes all changes that occur during life cycle of an organism. It requires process of growth, differentiation and morphogenesis.(1)On the other hand tooth development or odontogenesis is the complex process by which teeth form from embryonic cells, grow, and erupt into the oral cavity.(2)The process of dental development occurs over a significant amount of time in an individual's life. Primary teeth start to form between the sixth and eighth weeks, and permanent teeth begin to form later in the twentieth weeks in utero.(3) The basic developmental process is similar for all teeth but each tooth developed as an anatomically distinct unit. Tooth eruption appears to be a programmed, localized event whereby a given tooth erupts at its appointed time. (4)

People with fasting glucose levels from 110 to 125 mg/dl (6.1 to 6.9 mmol/l) are considered to have impaired fasting glucose.(5) Patients with plasma glucose at or above 140 mg/dL (7.8 mmol/L), but not over 200 mg/dL (11.1 mmol/L), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two prediabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus, as well as cardiovascular disease.(6)

Gestational diabetes mellitus (GDM) is defined as glucose intolerance of various degrees that is first detected during pregnancy. It resembles type 2 diabetes in several respects. Pregnancy has long been recognized as a state of relative insulin resistance, and those women who cannot meet the increased demands for insulin during pregnancy have been labeled as having gestational diabetes mellitus (GDM).(7) It occurs in about 2%–5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. (8) Known risk factors for GDM are Age (older than 25 years; the risk is even greater after age 35, Race (occurs more often in African Americans, Hispanics, American Indians, and Asian Americans) Overweight and obesity Personal medical history of gestational diabetes, prediabetes, or previously delivering a baby weighing more than 9 pounds and Family history of type 2 diabetes (in parents or siblings)(7,9). It has been known that diabetes that occurs during pregnancy can have severe adverse effects on fetal and neonatal outcomes.(10) The degree of glucose intolerance during pregnancy was related to the risk of developing diabetes after pregnancy.(11) Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia, congenital cardiac and central nervous system anomalies, skeletal muscle malformations; GDM increases the risk of premature delivery and preeclampsia. In severe cases, perinatal death may occur.(12) Nutrition intervention for women with gestational diabetes mellitus (GDM) has been recognized as the cornerstone of therapy at all stages.(13,14) Dietary intake is foundational to optimal pregnancy outcomes because nutritional quality and quantity have an impact on the overall growth and development of the fetus.(15) Because, medical nutrition therapy (MNT) is the primary therapy for 30–90% of women diagnosed with GDM, the challenge for MNT for GDM is to balance the needs of a healthy pregnancy with the need to control glucose level.(16,17) Physical activity is an obvious adjunct therapy to MNT for women with GDM. It improve blood glucose control, reduce insulin resistance, reduce cardiovascular risk factors, contribute to weight control, and improve well-being.(18) Diabetes prevention trials using exercise and weight reduction have shown a 56% decrease in the incidence of diabetes in a population of people with impaired glucose tolerance.(19,20) Therefore, it is reasonable to consider that regular exercise may prevent GDM. Women who participated in any physical activity before and during pregnancy experienced a 69% reduced risk of GDM.(21)The food plan should be individualized, culturally appropriate and provided by a qualified individual with experience in GDM management as a frontline strategy for controlling gestational diabetes.(22)

Essential fatty acids (EFA's) are long-chain polyunsaturated fatty acids that are necessary for normal biological activities. They are an essential dietary nutrient, as are vitamins, minerals, and amino acids (protein). (23) The EFA's are omega 3 and omega 6 fatty acids which cannot by synthesized by human body, but instead must be consumed in their diet.(24,25) These fatty acids are essential to the formation of new tissues, which occurs at an elevated rate during pregnancy and fetal development.(26,27) Flaxseed is high in polyunsaturated fatty acids (73%), moderate in monounsaturated fatty acids (18%), and low in saturated fatty acids (9%). Flaxseed is a rich plant source of alpha-linolenic acid (ALA), an essential fatty acid in the human diet and the parent fatty acid of the omega-3 family.(31) ALA is converted to two main long-chain fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in a series of enzymatic reactions.(32) ALA has been shown to modulate eicosanoid synthesis,(28) its concentration in breast milk exceeds that of DHA, suggesting a particular requirement for ALA by infants.(29,30) Studies have shown that flax seeds may lower cholesterol levels, although with differing results in terms of gender. (33) Flax may also lessen the severity of diabetes by stabilizing blood-sugar levels.(34). EFAs also have antibacterial actions.(35).

2.Materials and Methods

A total of sixteen males & thirty two females' healthy albino rats of seven weeks old, with an average initial body weight 225±25 grams were used in the present study. The rats were allotted at random in which each every two females were housed with one male for nearly one week. When the females were recognized to be pregnant by palpation and by sharp weight gain, they were separated. The pregnant rats were individually caged in metal galvanized cages under the same managerial conditions in well ventilated block building. Fresh water was automatically available all the time by stainless steel nipples for each cage. A basal diet (control, BD) was formulated to cover all essential nutrients requirement for pregnant rats according to AIN-93G diet to meet the nutritional needs of developing and lactating rats.(35)A 10% FS diet (the experimental diet, FSD) was prepared by supplementing the basal diet with 10 % (w/w) ground flaxseed (IMTENAN heath shop for healthy and functional food; Organic Flax Seeds, rich source of omega 3 &6 Egypt) after adjustment for total calories, macronutrients, and fiber contributed by the added flaxseed.(36) The basal and experimental diets were nearly iso-nitrogenous, iso-caloric and contained similar levels of macro-elements. Throughout the experimental periods, the basal and the experimental diets were offered for the pregnant rats ad libitum in pelleted form to ensure that the pregnant females had taken all administrated supplements without selection. The experimental period were extended for seven weeks. The day of birth was designed as day one. Within 24 hours after parturition the offspring's were reduced to six, to make the stress upon lactating mothers more uniform and to provide a nearly equal opportunity to individual offspring. The same experimental diet for each group was continued throughout lactation period.

Weaning always take place after 21 days from delivery, hence all offspring in control and experimental litters were weaned at 21 days of age. Fresh diets were stored at 4°C and intake was monitored throughout the study period.

The composition and chemical analysis of the control diet is summarized in Table 1. A commercial vitamin and mineral premix was added for all diet.

Vitamins and mineral premix per kilogram contained: Vit. A 2,OOO,OOO Iμ, Vit. D3 150,000 lμ, Vit. K 0.33 mg, Vit. B1 0.33 g, Vit B2 1.0 g, Vit B6 0.33 g, Vit. B12 1.7 mg, Panthonicacid 3.33 g, Biotin 33 m, Folic acid 0.83 g, Choline chloride 200 mg, Zn 11.7 g, Mn 5.0 g, Fe 12.5 g, Mg 66.7 mg, Se 16.6 mg, Co 1.33 mg, Cu 0.5 g, I 16.6 mg and Antioxidant 10.0 g. Chemical composition of the control diet, faeces and urine were analyzed according to A.O.A.C. (1990).

Pregnant females were divided equally (eight in each group) as follows; Group I: Pregnant females fed with basal diet (BD).Group II: Pregnant females fed with basal diet enriched with flax seeds (FSD).Group III: Pregnant females with induced diabetes and fed with basal diet (BD).

Group IV: Pregnant females with induced diabetes and fed with basal diet enriched with flax seeds (FSD).

Table 1. The composition and chemical analysis of the control diet

Ingredients %	Experimental diets	
	Control(BD)	Flaxseed diet(FSD)
Egyptian berseem hay	9.0	9.0
Bean straw	17.5	14.5
Gluten	16.0	12.0
Flaxseed	-	10.0
Yellow corn	15.0	15.0
Wheat bran	18.0	15.0
Barley grain	15.0	15.0
Soybean meal (44%)	3.0	3.0
Molasses	3.0	3.0
Common salt	1.0	1.0
Limestone	1.0	1.0
Dicalcium phosphate	1.0	1.0
Vitamin & mineral premix*	0.2	0.2
Mineral mixture	0.3	0.3
Total	100	100
Chemical composition (%)		
Dry matter	90.46	90.86
OM basis (%):		
Organic matter	90.81	89.96
Crude protein	18.64	18.85
Ether extract	4.21	4.43
Crude fiber	11.42	11.31
Nitrogen free extract	56.54	55.37
Ash	9.19	10.04

Induction of diabetes:

For group I and II, the rats were injected with vehicle (citrate buffer) to simulate the influence of injection stress or buffer-induced effects on the animals.(37)For groups III and IV, the rats were fasted overnight and diabetes were induced by a single intravenous injection of Streptozotocin 60 mg/kg body weight in 0.1 M citrate buffer.(38) Streptozotocin (STZ) was freshly prepared immediately before injection, and it was kept in cold store and refrigerator temperature (2-8°C) away from light. If it is not used fresh, Streptozotocin (STZ) solution can exhibit reduced ability to induce diabetes.(39)

Measurement of Blood Glucose Level

Blood glucose level of the animals was measured 48 hours after the administration of Streptozotocin then every two weeks throughout the time of the experiment. Blood sample were obtained from the tail vein of the animals and their blood glucose level were determined in mg/dl using a digital glucometer (Accu-chek. Advantage. Roche Diagnostic. Germany).(40)

Recording the Dates of Eruption

In vivo examinations were conducted periodically for each pup to determine the dates of eruption of mandibular first molar teeth. At the 14 days of age the offspring were examined daily for the molar eruption. Data collected were then subjected to statistical analysis for variance.

3.Results

The data was collected and entered into the personal computer. Statistical analysis was done using Statistical Package for Social Sciences (SPSS/ version 20) software. The statistical test used as follow; (1) Mean and standard deviation of each category. (2) Mann Whitney test was used for comparison between unpaired signed ranks test. The 5% was chosen as the cut off level of significance.

Eruption time of lower first molar

Table (2), showed, the eruption times of the lower first molar in different studied groups, the eruption time in control group was ranged from 13-17 days with a mean of 16.08±0.90, while in control with omega was ranged from 13-15 days with a mean of 14.0±0.85, in diabetic group the eruption days was ranged from 16-18 days with a mean of 17.67±0.78, finally in diabetic group with omega was ranged from 15-18 days with a mean of 16.71±1.06, on comparing the four groups it was found that there was a significant difference between the four studied groups.

Table (2): The eruption times of the lower first molar in different studied groups.

	13th day		14th day		15th day		16th day		17th day		18th day	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control(BD)	1	8.3	0	0.0	4	33.3	3	25.0	4	33.3	0	0.0
Control(FSD)	4	33.3	4	33.3	4	33.3	0	0.0	0	0.0	0	0.0
Diabetes(BD)	0	0.0	0	0.0	0	0.0	2	16.7	6	50.0	4	33.3
Diabetes(FSD)	0	0.0	0	0.0	5	41.7	2	16.7	4	33.3	1	8.3

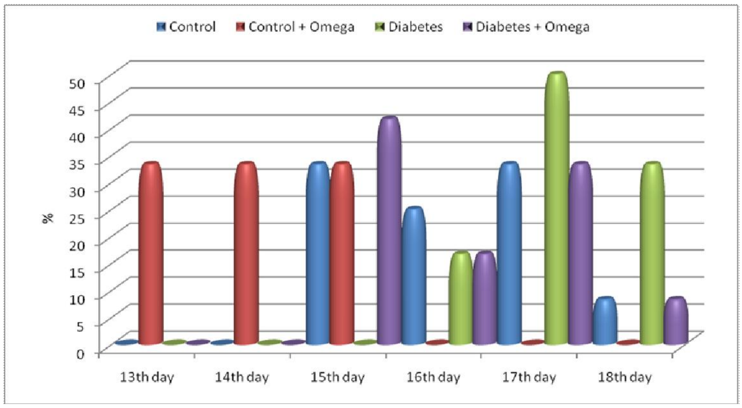

Fig. (1): The eruption times of the lower first molar in different studied groups.

Table (3): Statistical analysis of the eruption time of the different studied groups.

	Control (BD)	**Control (FSD)**	**Diabetes (BD)**	**Diabetes (FSD)**
Min.	13	13	16	15
Max	17	15	18	18
Mean± S.D.	16.08±0.90	14.00±0.85	17.67±0.78	16.71±1.06
*P*1		0.001*	0.022*	0.042*
*P*2			0.001*	0.003*
*P*3				0.017*

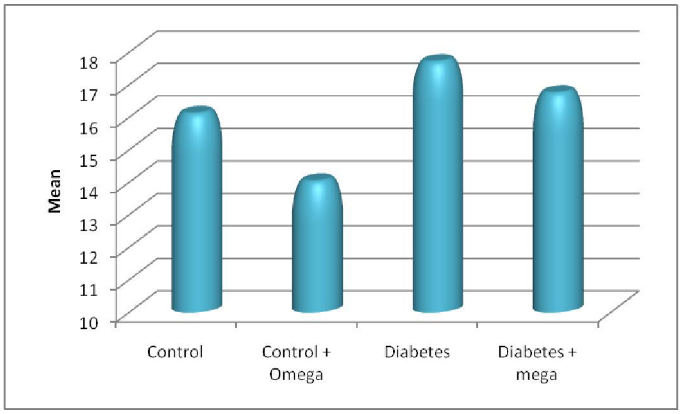

Fig. (2): Statistical analysis of the eruption time of the different studied groups

4.Discussion

Dental eruption is the gradual movement of a tooth from its formative position in the osseous crypt through alveolar bone and into functional occlusion in the oral cavity. Several factors ranging from molecular signaling to osteoclastic activity, root development, nutrition, and hormonal influences have been implicated.(32)It has been suggested that an eruptive force is derived from the deposition of alveolar bone and the vascularity of the tissues surrounding the tooth have an impelling force which carries the tooth to its functional position.(34)Disturbances in dental development (ie, timing or sequence of eruption) may contribute to a chain of complications such as malocclusion, crowding, impaired oral hygiene, periodontal disease, and associated dental and orthodontic treatment needs.(41)Timing in development process is important.(42)

Nutrition plays a major role in maternal and child health. Poor maternal nutritional status has been related to adverse birth outcomes; however, the association between maternal nutrition and birth outcome is complex and is influenced by many biologic, socioeconomic, and demographic factors, which vary widely in different populations. Understanding the relation between maternal nutrition and birth outcomes may provide a basis for developing nutritional interventions that will improve birth outcomes and long-term quality of life and reduce mortality, morbidity, and health-care costs.(23)Since, the early 1990s, there has been increasing interest, in omega3&6, mainly because of the evidence that supplementation in early pregnancy can reduce the incidence of congenital abnormalities. Omega-3 fatty acids are essential dietary nutrients and one of their important roles is providing the fatty acid with 22 carbons and 6 double bonds known as docosahexaenoic acid (DHA) for nervous tissue growth and function.(43)

The diet and body stores of essential polyunsaturated fatty acids in pregnant women need to meet the polyunsaturated fatty acid requirements of both mother and fetus, because the developing fetus depends upon maternal fatty acids and polyunsaturated fatty acids for its supply. The omega-6 and omega-3 fatty acid status of mothers has been found to decline during pregnancy, and while normalization occurs after delivery, it appears to take more than 6 months.(44)

The present study provide the first evidence on the effect of flax seedsas a source of omega 3 &6 on tooth development and eruption dates.

The data obtained from clinical examination of the offspring'sjaws have suggested that the flax seeds may target, specifically or preferentially, different tissue compartment. Evidence presented over the past 20 years has shown that long-chain polyunsaturated fatty acids (LCPUFAs), especially the n-3 fatty acids such as eicospentaenoic acid (EPA) and docosahexaenoic acid (DHA) are beneficial for bone health.(45)

Skeletal biologistsview the process of tooth eruption as a valuable model tostudy bone remodeling, since the emergence of a tooth into the oral cavity involves both coupled and uncoupled bone turnover events.What has emerged is the realization that interactions among osteoblasts, osteoclasts, and dental follicle involve a complex interplay of regulatory genes that encode various transcription factors, proto-oncogenes, and soluble factors.(46)Recent investigations indicate that the type and amount of polyunsaturated fatty acids (PUFA) influence bone formation in animal models and osteoblastic cell functions in culture. (47)In growing rats, a diet supplemented with omega-3 PUFA results in greater bone formation rates and moderates ex vivo prostaglandin E2 production in bone organ cultures. The actions of omega-3 fatty acids on bone formation appear to be linked to altering osteoblast functions.Where omega-3 PUFA modulated COX-2 protein expression, reduced prostaglandin E2 production, and increased alkaline phosphatase activity.(48)In our study supplementing FSD had the greater effect on the offsprings' eruption time of the mandibular first molar in both control and diabetic groups. Eruption dates is mostly affected by nutritional factors during pregnancy and the statistical analysis of our results of eruption rates, revealed that flax seeds as a source of omega-3&6supplementation affects positively this rate.

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