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Archives of Medical Research 44 (2013) 429–436

Archives of Medical Research

ORIGINAL ARTICLE

Protein Restriction in the Rat Negatively Impacts Long-chain Polyunsaturated Fatty Acid Composition and Mammary Gland Development at the End of Gestation

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Received for publication February 14, 2013; accepted August 14, 2013 (ARCMED–D–13–00098).

Background and Aims. Maternal nutrition during gestation is critical for mammary gland cell proliferation and differentiation and development of optimal delta-6 (Δ6D) and delta-5 (Δ5D) desaturase and elongase 2 and 5 (Elovl 2 and 5) activity for synthesis of the long chain polyunsaturated fatty acids (LC-PUFAs), arachidonic (AA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, important for normal fetal and neonatal brain development. We hypothesized that maternal low protein diet (LPD) impairs mammary gland preparation for lactation and PUFA synthesis. The aim of the study was to evaluate consequences of maternal LPD on mammary gland structure and development and expression of enzymes responsible for LC-PUFA production.

Methods. Pregnant rats were assigned to control or protein restricted, isocaloric diet (R). At 19 days gestation, mammary gland tissue was removed for histological analysis and lipid, AA, EPA and DHA determination by gas chromatography. Gene transcription was quantified by RT–PCR and protein by Western blot.

Results. In R mothers, mammary gland lobuloalveolar development was decreased and showed fat cell infiltration. Δ6D, Δ5D, and Elovl 5 mRNA were lower in R, whereas protein levels measured by Western blot were unchanged. This is the first report that detects mammary gland desaturase and elongase protein. Although Elovl 2 mRNA was not detectable by RT–PCR, Elovl 2 protein was not different between groups. AA and DHA were lower and EPA undetectable in the mammary gland of R mothers.

Conclusions. Maternal LPD decreased late gestation mammary gland lobuloalveolar development and LC–PUFAs. Protein restriction negatively impacts maternal mammary gland development prior to lactation.

Key Words: Mammary gland, Low protein, Pregnancy and LC–PUFAs.

Introduction

Appropriate maternal nutrition during gestation is critical for optimal fetal growth and organ development (1). In addition, an adequate maternal nutrient supply is necessary to support the changes that occur in maternal structure and function during pregnancy, especially development and

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0188-4409/$ - see front matter.
http://dx.doi.org/10.1016/j.arcmed.2013.08.002

function of the mammary gland for lactation. Normal mammary epithelial cell proliferation is essential to the production of a mature mammary gland capable of synthesizing the enzymes responsible for the formation of long-chain polyunsaturated fatty acid (LC–PUFAs) through the conversion of precursors such as linoleic acid (LA 18:2 n-6) to arachidonic (AA) and α-linolenic acid (LNA 18:3 n-3) to eicosapentaenoic (EPA) and docosahexaenoic (DHA) via delta-6 (Δ6D) and delta-5 (Δ5D) desaturases. Both enzymes introduce an unsaturated double bond into the fatty acid carbon backbone, whereas elongases 2 and 5 (Elovl 2 and 5) are responsible for elongation of the fatty acid chain (2,3). It is well established that the developing fetus and neonate lack the ability to synthesize adequate amounts of PUFAs (4,5), which therefore have to be provided to the fetus by the mother via the placenta and to the newborn via the mother's milk during lactation. LC–PUFAs are then incorporated into cell membranes throughout the developing nervous system and retina as essential elements of infant maturation (6,7), especially cognitive development (8).

There are many studies of the impact of the quantity and quality of fat intake during pregnancy and lactation and its effects on fetal and neonatal development. However, to our knowledge, there are no studies that have explored effects of low protein intake during gestation on mammary epithelial cell proliferation and lipid metabolism, particularly the enzymes involved in production of LC–PUFAs (Δ5D, Δ6D and Elovl 2 and Elovl 5). In a previous study we demonstrated that maternal body fat including liver lipids were lower in protein-restricted mothers. In addition, protein restriction reduces desaturase and elongase gene expression in the maternal liver as well as the concentration of AA and DHA (8). We therefore hypothesized that protein restriction during pregnancy would impair lobuloalveolar development of the mammary gland and decrease expression of the enzymes involved in LC–PUFAs formation. We investigated effects of a well-established model of maternal low protein dietary intake on mammary gland cell differentiation and histology, metabolic function and enzymes responsible for LC–PUFAs production in the pregnant rat at 19 days of gestation.

Materials and Methods

Care and Maintenance of Animals

All procedures were approved by the Animal Experimentation Ethics Committee of the Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán" (INNSZ). Details of maternal diet, breeding and management have been previously published in detail (9). Twenty one virgin female albino adult Wistar rats aged 15–17 weeks and weighing 220–260 g with regular cycles were fed Purina 5001 rodent diet and maintained under controlled lighting conditions (lights on from 07:00–19:00 h at 22/23°C). Female rats were mated overnight with proven male breeders and the day on which spermatozoa were present in a vaginal smear was designated as the day of conception (day 0). Female rats that did not have spermatozoa in a vaginal smear in 5 consecutive days were excluded. As soon as the female rats that were positive for spermatozoa in the vaginal smear were immediately transferred to an individual cage, they were randomly allocated to be fed either with 20% protein (C– control diet group, $n = 10$) or 10% protein isocaloric diet (R – restricted diet group, $n = 11$) (10). Rats were weighed daily and provided with free access to the experimental diet and water. Food was delivered in the form of large flat biscuits retained behind a grill through which the biscuits could be nibbled. The amount of food provided each day was weighed as well as the amount remaining after 24 h. Food intake was also measured in six female age-matched non pregnant rats. On day 19 of gestation (dG) (chosen as a day representative of late gestation but avoiding the rapid and accentuated hormonal and metabolic changes leading to parturition), food was removed from pregnant rats at 6:00 AM. Between 10:00 and 11:00 AM rats were rapidly euthanized by decapitation by experienced personnel trained in the use of the rodent guillotine (Thomas Scientific, USA). To ensure homogeneity of the study subjects, rats with litters of >12 or <8 pups were excluded from the experiment; three control and five restricted mothers were excluded because of these criteria. Trunk blood was collected into polyethylene tubes, allowed to clot at 4°C for 1 h, centrifuged at 1500 g for 15 min at 4°C and serum stored at −20°C until assayed. The right inguinal (4^{th}, 5^{th} and 6^{th} nipples/gland, counted from the cephalad end) mammary gland chain was excised. The 6^{th} was fixed in paraformaldehyde for morphometric analysis, whereas the 4^{th} and 5^{th} were immediately frozen at −75°C for later study by real-time RT–PCR and Western blot analysis. Mammary glands 4^{th}, 5^{th} and 6^{th} from the left inguinal mammary gland chain were collected for Folch analysis of fatty acids.

Morphometric Analysis

Mammary gland number six, including the nipple and skin, was dissected, sectioned longitudinally into two halves and immediately fixed by immersion in 4% paraformaldehyde in neutral phosphate saline buffer. After 24 h of fixation, tissue sections were dehydrated with ethanol at increasing concentrations from 75–95% and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin (H&E). Five random fields in each section from each rat were used to determine the percentage area occupied by each of the adipose and parenchymal tissues (acinar and ductal epithelium) at low power magnification (10x). Total acinar and acinar cell sizes (area of cytoplasm and nucleus) were evaluated at higher magnification (100x) in five