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MATERNAL DIETARY SUPPLEMENTATION OF N-3 FATTY ACIDS: EFFECT ON Δ^6 -DESATURASE EXPRESSION AND ACTIVITY OF SUCKLING RABBITS

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ABSTRACT

Fish oil and linseed are concentrated sources of n-3 polyunsaturated fatty acid: fish oil contains a higher proportion of long chain polyunsaturated (LCP) whereas linseed contains their precursors (α -linolenic acid, ALA). The inclusion of LCP in the maternal diet can influence the fatty acid composition of offspring tissues and the postnatal expression of Δ^5 and Δ^6 -desaturase, thus improving the quality of animal products. The aim of this study was to check whether the maternal dietary supplementation of n-3 fatty acids (ALA or LCP) affected the gene expression and activity of Δ^6 -desaturase in the liver of suckling rabbits. Thirty New Zealand White rabbit does belonging to 3 experimental groups were studied: control, 10% of linseed (ALA) and 3% of fish oil (LCP). Two pups per does were killed at two different ages: 0 (T0) and 20 days (T20) and the liver was sampled for subsequent analysis. At birth day (T0) the enzyme activity and the gene expression of pups was lower in the linseed group compared to the others ($P < 0.05$). After 20 days of lactation the mRNA expression was upregulated in the offspring liver of ALA group, whereas the enzyme activity didn't show significant differences. Our study suggested that the main effect on liver mRNA expression and the activity in the rabbit pups was mainly due to the LCP supply in maternal milk. The epigenetic effect of maternal diet on rabbit pups was not clear probably related to the relatively low dietary LCP amount administered.

Key words: Rabbit, Δ^6 -desaturase, mRNA expression, Long-chain polyunsaturated fatty acids, Liver metabolism

INTRODUCTION

The current human consumption of eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) fatty acids in many western countries is only 25% of the recommended daily intake (Givens and Gibbs, 2008). Fish is the most concentrated source of long-chain polyunsaturated fatty acid n-3 (LCP) but the current technique of fishing and aquaculture are becoming more and more unsustainable. Further, changing the food habits of humans is a very difficult goal and the best way to enhance the intake of LCP is to increase the levels of LCP in a wide range of terrestrial foods. The main strategy to enlarge the amount of EPA and DHA in animal products is the inclusion of fish oils or linseed (α -linolenic acid, ALA) in the diet (Toral et al., 2013, Wang et al., 2012). The latter approach is more sustainable but requires an efficient conversion of ALA into LCP; namely a high Δ^6 -desaturase (Δ^6) and Δ^5 -desaturase (Δ^5) efficiency. The inclusion of LCP in the maternal diet can influence the fatty acid composition of offspring tissues (Lauridsen and Jensen, 2007) and the postnatal expression of Δ^5 and Δ^6 (Neuringer et al., 1988; Xiang et al., 2006, De Quelen et al., 2013). Accordingly, it's a way for programming the EPA and DHA contents in the animal foodstuffs. To our knowledge, no studies have been performed on the effects of dietary LCP supplementation during pregnancy on Δ^6 metabolism of rabbit progeny. The objective of this study is to determine whether the maternal dietary supplementation of n-3 fatty acids (linseed or fish oil) affects the liver Δ^6 gene expression (FADS2) and desaturating ability of suckling rabbits.

MATERIALS AND METHODS

Animals and experimental design

A total of 30 New Zealand White rabbit does belonging to the three experimental groups were studied. Rabbits were housed in cages of the Research Centre of the University of Perugia (IT). Each group consisted of 10 rabbits with different diet (Table 1): Control, fed *ad libitum* with the standard diet; Fish oil, fed the standard diet containing 3% of fish oil (Nordos®); Linseed, fed the standard diet added with 10% of extruded linseed.

Two pups per does were killed at two different ages: 0 (T0) and 20 days (T20). The liver was quickly excised with sterile scissors (about 1 g imbibed in 5 volumes of RNAlater - Sigma, Milan, Italy). After 1 day at +4°C RNAlater was removed and samples placed -80°C until analysis. The rest was stored at -20 °C for subsequent enzyme activity analysis.

Chemical Analyses

Total RNA was extracted from rabbit liver using Midiprep System (Promega, Italy -PureYield™ RNA). RNA was reverse transcribed into cDNA and an aliquot was amplified with GoTaq Polymerase (Promega) to perform PCR (Thermal Cycler, Mycycler, Biorad). The primer was (FADS2 sense + antisense) designed on the basis of *Oryctolagus cuniculus FADS2* gene sequence (Genebank database XM_002721017.1). The PCR product was then cloned (pGEMT-Easy cloning vector system, Promega, Italy) and sequenced in both directions (One Step TaqMan® PCR). Data were collected with StepOne Sequence Detector Program. Microsomes were isolated from 2 g of fresh rabbit liver by standard methods. The Δ^6 -desaturase activity was estimated by measuring the amount of [1-¹⁴C]18:4n-3 produced from [1-¹⁴C]18:3n-3 (Perkin Elmer) in a buffer A (4mM ATP, 0.1mM CoA, 1.25mM NADH, 0.5mM nicotinamide, 5mM MgCl₂, 62.5mM NaF, 1.5mM GSH and 35 of unlabeled fatty acid nmol substrate). For each sample, about 3nmol [1-¹⁴C]18:3 n-3 were blended with 30nmol of unlabeled fatty acid. The distribution of radioactivity between the substrate and the product of Δ^6 -desaturase activity was determined by thin-layer chromatography (10% w/w AgNO₃ silica gel plates, SiliCycle, Canada). Methyl esters of 18:3n-3 and 18:4n-3 were spotted near the labelled FAME to identify reaction product. Plates were developed in hexane/diethyl ether (2/3, v/v). The spots were identified under UV light with 0.2% (w/v) of 2',7'-dichlorofluorescein in ethanol. Radioactive spots were identified by Istant Imager (Packard) and scraped off directly into the scintillation vials and counted for radioactivity with 4mL of scintillation flour by using a scintillation analyzer (Tri-carb Packard, model 1600 CA).

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (STATA 2015, proc ANOVA) and Bonferroni test was used to analyse the significance of differences (P<0.05).

RESULTS AND DISCUSSION

Fish oil and linseed are good sources of polyunsaturated fatty acids (PUFA) of n-3 series. Fish oil is particularly high in LCP (3.60%), while linseed is high in ALA (30.56%) (Table 2), which is the precursor for the biosynthesis of LCP (Cleland et al., 2003).

Many studies have shown that prenatal nutrition can alters the epigenetic regulation and the PUFA content of cell membranes in the offspring (Burdge et al., 2010). However, Hoile et al. (2013) have demonstrated that the modulation of fatty acids metabolism in rodent newborns depends on LCP series and the amount (from 3 to 21% w/w) of dietary fat eaten by pregnant animals. In our study, at birth day (T0) the liver activity (Figure 2)

Table 1: Chemical composition (%) and nutritional value of diets

	Diets		
	Control	Fish oil	Linseed
Crude protein	16.91	16.11	16.32
Ether extract	4.93	5.03	4.72
Crude fiber	15.40	15.44	15.58
Ash	8.21	8.59	8.78
NDF	31.37	30.91	31.34
ADF	18.04	18.00	18.16
ADL	4.98	5.03	5.09
Cellulose	13.06	12.91	13.18
Hemicelluloses	13.33	12.97	13.07
Estimated digestible Energy**(MJ)	**	2.50	2.38

** According to Maertens et al., 1988

and the gene expression (Figure 1) of pups was lower in the linseed group compared to the others probably due to the higher affinity of Δ^6 for LA than ALA (Portolesi et al., 2007).

Table 2. Main fatty acid composition (% of total FAME) of diets (n=3)

Fatty acids	Diets			
	Control	Fish oil	Linseed	Pooled SE
SFA	19.76b	18.42a	18.54a	0.32
MUFA	18.46b	16.55a	17.14a	0.86
C18:2n-6	38.93b	32.08a	33.27a	1.26
C18:3n-6	0.01	0.02	0.01	0.02
C20:3n-6	0.14	0.04	0.05	0.02
C20:4n-6	0.19	0.11	0.09	0.08
PUFA n-6	39.27b	32.25a	33.42a	1.30
C 18:3n-3	22.19a	29.18b	30.56b	1.46
C20:5n-3	0.18A	1.01B	0.17A	0.12
C21:5n-3	0.01	0.09	0.01	0.08
C22:5n-3	0.02A	0.34B	0.09A	0.03
C22:6n-3	0.11A	2.16B	0.07A	0.21
LCPn-3	0.32A	3.60B	0.34A	0.28
PUFA n-3	22.51A	32.78B	30.90B	1.92
n-3/n-6	0.57a	1.02b	0.92b	0.32

Means in the same row with different letters differ significantly ($P < 0.05$ or $P < 0.01$ small or block letters, respectively).

LCP: long chain polyunsaturated fatty acids ($\geq 20C$).

Many studies have shown that prenatal nutrition can alter the epigenetic regulation and the PUFA content of cell membranes in the offspring (Burdge et al., 2010). However, Hoile et al. (2013) have demonstrated that the modulation of fatty acid metabolism in rodent newborns depends on LCP series and the amount (from 3 to 21% w/w) of dietary fat eaten by pregnant animals. In our study, at birth day (T0) the liver activity (Figure 2) and the gene expression (Figure 1) of pups was lower in the linseed group compared to the others probably due to the higher affinity of Δ^6 for LA than ALA (Portolesi et al., 2007).

This hypothesis is further sustained by assessing the effect of PUFA n-3 supplementation in suckling rabbits (Figure 1, 2; T20). Indeed, in spite the maternal milk provided a high proportion of ALA (linseed) or LCP (fish oil) to suckling rabbits (data not shown), their Δ^6 enzyme activity was not significantly different.

FADS2 mRNA expression at T20, unlike of enzyme activity, was upregulated in the linseed group (Figure 1). There is emerging evidence which suggests a not direct correlation between mRNA expression and protein translation rate, since many other post-transcriptional mechanisms are involved (Schwanhäusser et al. 2011). The effect of fish oil on FADS2 expression is in agreement with the data of literature on other species. Matsuzaka et al., (2002) has showed a repression of lipogenic enzyme-encoding genes by LCP, and, most recently, Hoile et al. (2013) has demonstrated a negatively correlation with the promoter methylation rate of CpGs sites in FADS2 gene. Dietary-induced changes in the fatty-acid metabolism could be the result of activation/inhibition of Δ^6 enzyme expression, operated by n-3 fatty acids, through the regulation of transcription factors (Deckelbaum et al., 2006).

However, it remains unclear, why in newborn rabbits of mothers fed fish oil (Figure 2, T0), the enzyme activity was almost 3 times higher than linseed group (145.53 vs 54.87 pmol in 30min/mg protein, $P < 0.05$). With respect to the dietary manipulation during gestation, some authors suggested that only small amount of the dietary fatty acids can be transferred to the foetus and then modulate the liver lipid metabolism (Ramsay et al., 1991). The total lipid percentage of rabbit diets was relatively low in all the diets (Table 1), and probably the PUFA amount provided was not sufficient to modulate the Δ^6 activity and expression of offspring through epigenetic regulation of FADS2.

CONCLUSIONS

The main finding of our study is that the dietary supplementation of n-3 fatty acids (precursor/products) on prenatal period affected the fatty acid metabolism of offspring mainly during lactation. The dietary supplementations influenced the pups' mRNA expression but not the Δ^6 enzyme activity, where the effect of the competition by LA (preferential substrate) was higher. The epigenetic effect of rabbit maternal dietary was unclear. Further study was necessary to elucidate the amount of LCP necessary to produce the modulation of lipid metabolism in rabbit offspring.

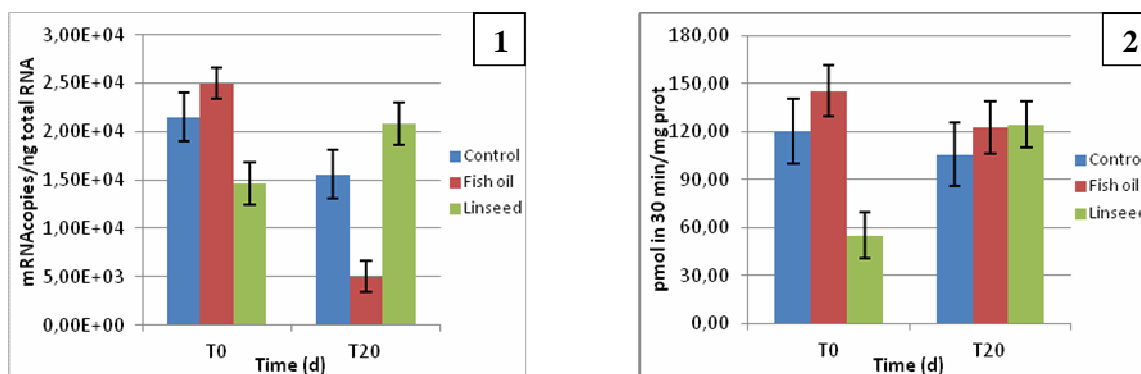


Figure 1. and 2. Δ^6 -desaturase expression (1; mRNA copies / ng of total RNA) and enzyme activity (2; pmol in 30 min / mg protein) in suckling rabbits (mean \pm sd; n=20/group) of different ages and with different diets.

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