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Can early intake of dietary omega-3 predict childhood externalizing behaviour?

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Short title: Dietary omega-3 and childhood externalising behaviour

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ABSTRACT

Aim: To determine whether maternal and child intake of dietary omega-3 fatty acids, together with the presence or absence of breastfeeding, predicted psychiatric diagnosis of externalising disorders in childhood.

Methods: Data concerning childhood externalising disorders was collected from 8242 children aged 7.9 years in a large British cohort. Intake of omega-3 fatty acids was measured for the study mother during pregnancy and for the child at three years. Duration of breastfeeding was examined to account for moderating effects. Adjustment was made for a variety of potential confounders.

Results: Maternal intake of omega-3 and breastfeeding predicted oppositional / conduct disorder and comorbid externalising disorder before adjustment for confounding factors. However, there was no association between intake of omega-3 by mother or child and any type of externalising disorder once socio-demographic factors were taken into account.

Conclusions: Any association between intake of omega-3 and childhood externalising disorders appears to be strongly confounded with sociodemographic factors. This is important to note given the current popularity of omega-3 as a possible treatment for behaviour problems related to inattention and impulsivity. Care must be taken that studies investigating this relationship account fully for factors associated with both behaviour and diet.

Keywords: ALSPAC, breastfeeding, externalising behaviour, longitudinal study, omega-3

Nutrition in infancy or early childhood is associated with childhood behaviours such as inattentiveness, hyperactivity, impulsivity and anti-social behaviour; these, in turn are associated with childhood externalising disorders including ADHD [1]. One group of nutrients associated with externalising disorders in children is that of essential fatty acids (EFAs). Results from two recent randomised control trials conclude that nutritional interventions using a combined fatty acid supplement reduce problems such as inattention, hyperactivity and impulsivity in middle childhood and that these effects can be maintained over time [2, 3] but other studies report no effects [4].

EFAs are essential for both optimal development [5] and the functioning of the central nervous system (CNS) [6]. During the last trimester of pregnancy and up to two years of age, they accumulate rapidly in the CNS of the infant, transferring from mother to child via the placenta and in breast-milk. EFAs must be obtained from the maternal diet from foods like oily fish as they cannot be synthesised by humans [7]. However early neurodevelopment may be compromised if maternal levels of EFAs are not supplemented by diet during pregnancy and lactation [5] or if infant levels are not maintained post-natally via breast or supplemented feeding and complementary foods [8].

The current paper focuses upon one type of EFA, the omega-3 series of long-chain polyunsaturated fatty acids (LC-PUFAs). LC-PUFAs are found primarily in cell membrane phospholipids, are important in the regulation of cell function and affect gene transcription [5]. They are necessary during periods of rapid growth (pre-natal development, early childhood and adolescence) and nutrient loss (pregnancy and lactation) [6]. Neonates with neurological dysfunction have lower indices of both LC-PUFAs and EFAs [8] and there is increased risk of suboptimal cognitive, social and motor skills in children at eight years of age as maternal intake of FAs during pregnancy declines [9]. On the other hand, increased CNS maturity is observed in infants who are breastfed [10] possibly because of the transfer of LC-PUFAs and other fatty acids in breast milk [11]. The aim of the present study was to determine whether maternal intake of omega-3 LC-PUFAs at 32 weeks gestation and by the child aged three years predicted diagnosis of externalizing disorders overall and specific sub-types of disorders in middle childhood. We also examined the moderating effects of breastfeeding in the relationship between diagnosis of externalising disorders and intake of omega-3 in pregnancy and childhood.

MATERIALS AND METHODS

Patients: The data analysed here were collected for the Avon Longitudinal Study of Parents and Children (ALSPAC: see www.bristol.ac.uk/alspac/), reported in detail elsewhere [12]. Within the former county of Avon, UK, 14,541 pregnant women expected to deliver between April 1991 and December 1992 enrolled into the

study. By the time children entered primary school (aged 5), around 11,500 families were still participating. Mothers consented to join the study at recruitment and were free to withdraw at any time; all aspects of the study conform to the ethical regulations of both the ALSPAC Law and Ethics Committee and local research ethics committees.

As with many longitudinal studies there was attrition from ALSPAC during the data collection period. When the study children were aged 7.9 years (SD 1.4 months) behavioural data was returned by parents for 8242 children (59%) and by teachers for 5115 study children (39%). Mothers who dropped out from the study (non-responders) were younger than those who remained ($p<.001$), were more likely to leave school earlier ($p<.001$), were more likely to live in rented accommodation ($p<.001$) and were more likely to still be smoking in pregnancy ($p<.001$). These families were also exposed to higher levels of family adversity during the pregnancy ($p<.001$). Babies born to non-responders were more likely to have been born prematurely ($p<.001$) and to weigh less than 2500g ($p<.001$). These babies were less likely to have been breastfed ($p<.001$). Non-responder mothers had lower levels of omega-3 in their diet than those who remained in the study ($p<.001$) but there was no difference in the omega-3 intake of the children at 3 years of age. (Actual data for this comparison are provided in a supplementary file).

Diet Assessment: Information on mother's diet at 32 weeks gestation and child's diet at 3 years was collected using food-frequency questionnaires; details are provided elsewhere [13]. The transfer of EFAs during the final trimester of pregnancy is important for the infant's neurological development and although the CNS is no longer so demanding of omega-3 for development at 3 years of age, intake at this stage might be considered as a maintenance dose. Three questions on seafood consumption were used to estimate omega-3 intake per week for mothers and children and intake was categorised as either high or low [9, 14]. Mothers and children with high intake were used as the reference group in analyses. Infant-feeding data were taken from the 15-month questionnaire with the reference group being children who were breastfed for any period of time. Confounding factors associated with diet were: maternal age and education, family adversity during pregnancy (an aggregate measure of 18 variables measuring adverse family circumstances including maternal psychopathology, financial difficulties and standard of housing) [15] and whether the mother smoked cigarettes during pregnancy (never smoked, stopped smoking in pregnancy, still smoking in pregnancy).

Behavioural data: Information about child behaviour at 7.9 years was collected from parents and teachers using a questionnaire version of the Development and Well-Being Assessment (DAWBA) [16]. This is a validated measure consisting of structured and semi-structured questions. An experienced clinician (TF) combined all information about symptoms and their impact using a computerised heuristic to make DSM-IV diagnoses of

childhood psychiatric disorders [17]. Data from teacher questionnaires were available for 66% of children with parent data. Confounding factors associated with behaviour were gender, gestational age (<37 weeks or >=37 weeks) and birth weight (<2500g or >=2500g).

Statistical methods: All analyses were undertaken using STATA 9.0[18]. Diagnoses of externalizing disorders were categorised into groups: oppositional defiant disorder or conduct disorder without any type of comorbid disorder ("pure ODD/CD": N = 189), attention-deficit hyperactive disorder without any type of comorbid disorder ("pure ADHD": N = 103) or ADHD plus ODD/CD (comorbid disorder, N = 72). We focussed on "pure" disorders and ADHD plus ODD / CD as a separate group so that children with comorbid disorders were not double-counted. Descriptive statistics were calculated by diagnostic category (Table 1). *(Table 1 about here)*

Unadjusted independent logistic regressions were carried out to predict diagnosis using mother and child omega-3 FA consumption and whether or not the child was breast-fed, together with an interaction term for breast-feeding by maternal intake of omega-3 FAs. The interaction term allowed the identification of a) mothers who breastfed but did not eat oily fish during pregnancy and b) those who ate oily fish during pregnancy but did not breastfeed. Multivariate step-wise models were built adjusting for all confounding factors. To account for multiple analyses, a conservative value of p<.001 was used.

RESULTS

Dietary factors: Children with "pure ODD / CD" were less likely to have been breastfed (p<.001). However, the evidence for differences in omega-3 intake by either mother or child between children diagnosed with a disorder and those without was weak (p>=.001) (Table 1).

Externalising disorders: For every type of externalizing disorder, more boys were diagnosed than girls (p<.001). Mothers of children with externalizing disorders were younger than those with no disorder (p<.05) but there was no difference in their education (p=.68). The families of children with externalising disorders experienced more family adversity during pregnancy than those with no disorder (p<.001). With respect to specific disorders, children diagnosed with "pure ADHD" were more likely to have been born before 37 weeks than those without (p<.001). Mothers of children with "pure ODD / CD" and "ADHD plus ODD / CD were more likely to continue to smoke throughout pregnancy (p<.001).

Dietary factors and externalising disorders: Neither maternal nor child intake of omega-3 nor breastfeeding predicted "pure ADHD" in the unadjusted analyses (p>=.38; odds ratios and confidence intervals in Table 2). Formula-feeding (never breastfed) predicted "pure ODD/CD" in the unadjusted analyses (p<.001) but after adjustment for confounding factors, there was no evidence for formula feeding as a predictor (p>.40).

There was weak evidence that maternal omega-3 (p =.01), formula feeding (p=.02) and the interaction between the two (p=0.18) predicted the combination of ADHD plus ODD/CD in the unadjusted model but after adjustment for confounding factors these relationships disappeared (p>=.34).

DISCUSSION

This study aimed to investigate the ability of maternal intake of omega-3 during pregnancy and child intake of omega-3 at 3 years to predict externalising disorders in middle childhood. Children with externalising disorders were more likely to be boys and to be born into families where there was strong evidence of relatively high levels of family adversity during pregnancy. Children diagnosed with ADHD were more likely to have been born before 37 weeks.

Conclusions from previous research regarding the effects of omega-3 on externalising disorders have been inconsistent: some studies report positive effects of omega-3 [2, 3] while other work has been less conclusive [5]. Our results showed no overall benefit of omega-3 intake by either mother or child on the diagnosis of externalising disorders after adjustment. There were no associations between omega-3 intake and diagnosis of "pure ADHD" in this British cohort, consistent with some [4] but not other research [19]. Children who were breastfed had a lower rate of "pure ODD/CD" than children who were formula-fed before adjustment [20]. Similarly, when maternal intake of omega-3 was low during pregnancy and the baby was subsequently formula-fed, there was an increased risk of ADHD plus ODD/CD in unadjusted analyses. However, the predictive ability of these variables disappeared after adjustment, perhaps because the unadjusted relationships were a function of residual confounding eg breast-feeding may be easier to manage for mothers who, for example, are not depressed or have more time to spend supporting and supervising their children. Alternatively, we may have over-adjusted in some circumstances and the unadjusted relationships may be accurate eg omega-3 intake may be reduced in families exposed to adversity and reduced intake during pregnancy may be associated with an increased risk of oppositional and / or conduct disorders in childhood. In order to examine this hypothesis, the effects of omega-3 on such disorders should be further investigated. We are unable to rule out the possibility that other processes (not measured here) may influence the risk of externalising disorders in childhood [21] eg inefficient metabolism as opposed to a dietary deficiency of omega-3 [4] or the ratio of different fatty acids to one another [11] or variation in the nutritional content of breast-milk [22]. Further research is needed to improve our understanding of the influence of absolute levels of nutrients in diet on externalizing behaviours, particularly ADHD.

While the strengths of the study are large sample size and observer-rated psychiatric diagnoses, there are limitations. Only 59% of the original cohort provided DAWBA data and fewer than 5% of this group met the

criteria for diagnosis of an externalising disorder. Given the lack of teacher data on 34% of the sample, we will have misclassified some children as having no disorder [17], reducing our chances of detecting statistical associations. Other work on this cohort [23] has suggested that a lower proportion of families remaining in the study have children with externalising disorders compared to those who dropped out but bias will only occur if the association between omega-3 and externalising symptoms varies between responders and non-responders and we have no reason to believe that this is the case. In statistical simulations this type of selection bias has been shown to attenuate rather than alter the relationship between predictors [24].

We were restricted to using dietary intake of omega-3 FAs and breast-feeding practice as proxies for physiological measures of omega-3. This is problematic regarding the transfer of omega-3 via breast-milk given that the omega-3 content of breast-milk can vary ten-fold [22]. Similarly, other factors in human milk may affect neurodevelopment so studies such as this, comparing breast- and formula-fed infants, cannot completely resolve the role of EFAs in development. Also, the dichotomisation of each of these variables may have resulted in a loss of statistical power to detect differences between groups but given that the outcome of interest was diagnosis rather than symptom counts, we believe that categorisation allows the possibility of capturing non-linear effects in the relationship with externalising disorders.

In conclusion, neither maternal nor child intake of omega-3 nor breastfeeding predicted any type of externalising disorder after adjusting for social factors associated with diet and behaviour Our results are important to consider in light of the current tendency to promote omega-3 as a possible treatment for ADHD-related problems [2]. If studies do not adjust sufficiently for confounding factors such as family adversity and pre-term delivery then they may misrepresent the relationship between externalising disorders and omega-3 [25]. Stronger evidence is required from randomised control trials to clarify the role of omega-3 alone and in conjunction with other LC-PUFAs in childhood externalising disorders.

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ABBREVIATIONS

EFA, essential fatty acid; CNS, central nervous system; LC-PUFA, long-chain polyunsaturated fatty acid; FA, fatty acid; IQ, intelligence quotient; ALSPAC, Avon Longitudinal Study of Parents and Children; UK, United Kingdom; DAWBA, Development and Well-Being Assessment; ODD/CD, oppositional development disorder / conduct disorder; ADHD, attention-deficit hyperactive disorder; OR, odds ratio; CI, confidence interval; SES, socio-economic status; FAI, family adversity index; n-3, omega-3

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Table 1: Descriptive data for responders according to DSM-IV diagnosis (SD)[a]

	N (%)	Maternal age	Maternal education % O levels or less[±]	FAI[§] in pregnancy	% Still smoking in pregnancy	Child gender % male	Maternal n3[¤] (32 weeks gestation) % Low intake <340g / week	% < 37 weeks gestation	% <2500g birth-weight	Whether breastfed % Never	Child n3[¤] (3 years) % Low intake <280mg / week
No disorder	7654 (92.9)	29.0 (4.6)	58.3	1.1 (1.4)	15.2	50.1	78.3	4.8	4.5	22.2	49.6
Any externalizing disorder	364 (4.4)	28.2 (5.1)*	61.5	1.8 (1.9)***	24.2 ***	75.5 ***	82.2 *	7.2	5.7	32.2***	50.5
Pure ODD/CD	189 (2.3)	28.3 (5.0)*	61.4	1.9 (1.9)***	29.4***	68.6***	82.3 **	4.3	4.8	33.9***	57.1**
Pure ADHD	103 (1.3)	29.0 (5.1)	56.1	1.5 (1.8)***	14.4	81.6***	76.1	13.6***	8.7 *	26.1	48.9
ADHD plus ODD/CD	72 (0.9)	27.4 (5.2)*	72.3	2.1 (2.0)***	29.4**	88.6***	88.9 *	5.7	5.7	34.8*	47.5

[a] the total number of children in this table with externalising disorders only is 8018; 224 children from the complete sample of 8242 were diagnosed with an internalising disorder but these were excluded from all analyses.

[±] O levels: educational qualification taken at 16 years of age [§] Family Adversity Index [¤] n3 = omega-3 fatty acids

* p<.05 ** p<.01 *** p<.001 (strength of evidence for difference from those with no disorder)

Table 2: Odds ratios and 95% CI for omega-3 and breastfeeding factors in the prediction of DSM-IV externalizing behaviors

DSM-IV diagnosis		Reference group	Unadjusted			Adjusted §		
			OR	95% CI	p	OR	95% CI	p
ODD/CD	Maternal n3	None	**1.73**	**1.03 – 2.90**	**0.04**	0.98	0.64 – 1.49	.92
N = 189	Child n3	None	1.62	0.92 – 2.85	0.09	1.39	0.99 – 1.96	.06
	Never breastfed		**1.83**	**1.30 – 2.56**	**<.001**	1.16	0.57 – 2.36	.68
	Maternal omega-3 * breastfed	None*never	1.20	0.40 – 3.61	0.74	1.42	0.63 – 3.23	.40
ADHD	Maternal n3	None	1.17	0.60 – 2.28	0.65	0.89	0.53 – 1.49	.66
N = 103	Child n3	None	1.31	0.62 – 2.79	0.48	0.77	0.49 – 1.21	.25
	Never breastfed		1.24	0.77 – 1.98	0.38	0.83	0.32 – 2.17	.70
	Maternal omega-3 * breastfed	None*never	0.60	0.11 – 3.32	0.56	1.40	0.45 – 4.40	.56
ADHD plus ODD/CD	Maternal n3	None	**3.56**	**1.41 – 8.95**	**0.01**	1.41	0.67 – 2.98	.37
N = 72	Child n3	None	1.02	0.36 – 2.89	0.98	0.92	0.52 – 1.63	.78
	Never breastfed		**1.87**	**1.13 – 3.12**	**0.02**	1.76	0.55 – 5.59	.34
	Maternal omega-3 * breastfed	None*never	**2.06**	**1.13 – 3.73**	**.018**	0.99	0.26 – 3.79	.99

§ Adjustment factors: Maternal age and education level, family adversity (including maternal psychopathology, financial difficulties and standard of housing during pregnancy), gestational age, birthweight, breastfeeding and smoking

Table 1: Descriptive data for responders vs. non-responders

	Responders (N = 8242)	Non-responders (N =3098)	p
Mean maternal age (SD)	29.0 (4.6)	26.7 (5.1)	<.001
Maternal education: O levels or less[a]	58%	76%	<.001
Home owner	82%	61%	<.001
Mean family adversity score (SD)	1.1 (1.4)	1.6 (1.8)	<.001
Still smoking in pregnancy	16%	29%	<.001
Gestational age <37 weeks	5%	15%	<.001
Birth weight <2500g	5%	8.4%	<.001
Never breastfed	23%	35%	<.001
Mean maternal intake (g) of omega-3 (SD)	1.4 (0.5)	1.3 (0.5)	<.001
Mean child intake (g) of omega-3 (SD)	0.51 (0.50)	0.51 (0.50)	.082
Gender of study child	51%	52%	.17

[a] In England and Wales, O levels are a national qualification taken at 16 years of age