BMJ open

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Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-001370
Article Type:	Research
Date Submitted by the Author:	24-Apr-2012
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Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Public health, Nutrition and metabolism, Paediatrics, Rheumatology, Genetics and genomics
Keywords:	EPIDEMIOLOGY, Paediatric clinical genetics & dysmorphology < GENETICS, NUTRITION & DIETETICS, Musculoskeletal disorders < ORTHOPAEDIC & TRAUMA SURGERY, PAIN MANAGEMENT

Do omega-3 or other fatty acids influence the development of 'growing pains'? A pre-birth cohort study

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Abbreviations: AA = arachidonic acid; ALA = alpha-linolenic acid; ALSPAC= Avon Longitudinal Study of Parents and Children; BMI = body mass index; DHA = decosahexaenoic acid; EPA = eicosapentaenoic acid; ETS = environmental tobacco smoke; FFQ = food frequency questionnaire; LA = linoleic acid.

Subject Heading: Childhood limb pains, omega-3 fatty acids; breast feeding; maternal diet; FADS genetic markers.

Competing Interests Statement: None

Contributorship statement:

JG, KN and PE had the original idea; KN and CS carried out the data analysis; JG wrote the first draft; all authors contributed to subsequent drafts.

Structured Abstract

Objectives: To assess whether the prevalence of growing pains varies with indicators of fatty acid exposure. Growing pains (limb pains of no obvious explanation) have been shown to be strongly linked to a family history of arthritis, and are thought to predict an increased risk of the development of arthritis in adulthood. Much has been made of the possibility of fatty acids, particularly the omega-3 fatty acids, playing a preventive role in the development of arthritis, but little research has been undertaken to determine whether such fatty acids might reduce the risk of growing pains. We aimed to assess whether the prevalence of growing pains varies with indicators of fatty acid exposures.

Design: Case-control study nested within a prospective longitudinal cohort comparing prenatal and postnatal diet, blood measures, and variants in FADS genes that influence the metabolism of fatty acids. Statistical analysis took account of factors such as gender, smoke exposure, maternal age and education, social class and parity.

Setting: Avon Longitudinal Study of Parents & Children (ALSPAC)

Participants: All children born between 1st April 1991 and 31st December 1992 (approximately 14,000) within the Avon area (only that part of Avon under the SWRHA). This project compared 1676 children who reported 'growing pains' at age 8 with 6155 with no such pain.

Primary outcome: Reported limb pains of no apparent origin

Results: There was no indication that the affected children had diets that differed in regard to omega-3 plasma levels of fatty acids, or the FADS genetic variants. We also assessed fetal and infant exposure but neither maternal prenatal blood levels nor maternal dietary intake, or duration of breast feeding showed any significant relationships even after adjustment for confounders.

Conclusions: Thus there is no evidence that omega-3 fatty acid status protects against the development of growing pains in childhood.

Article Summary

Article Focus: To assess whether prevalence of growing pains varies

- with indicators of fatty acid exposure through pre- and post-natal diet
- And with variation in FADS genes that influence the metaholism of fatty acids.

Key messages

- There was no evidence that omega-3 fatty acid status protects against the development of growing pains in childhood.

Strengths and Limitations

- The strength of the study lie in the fact that the data are collected prospectively; it is population based, with a large sample. It includes biological markers and genetic variants which allow independent assessment of fatty acid metabolism.
- Limitations concern the fact that the information on limb pains is collected from mothers, albeit using structured questions.

Data Sharing statement: ALSPAC is committed to share data with bona fide researchers.

Funding Statement: The UK Medical Research Council (MRC), the Wellcome Trust and the University of Bristol currently provide core support for ALSPAC. The assays of the maternal blood samples were carried out by Scotia at the instigation of the late David Horrobin, to whom we are extremely grateful. Funding for the genotyping and analysis of the genetic variants was partly undertaken with funding from the EU grant no.FP7-212652 and partly by the Waterloo Foundation. The assays of the child's blood were conducted and supported by the intramural research program of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) with funding from the National Oceanic and Atmospheric Administration (NOAA). Statistical analysis of the information was funded by the Arthritic Association.

Introduction

Growing pains are common in childhood, with about 56% of children reporting them as occurring frequently between the ages of 5 and 13.[1] Little attention has been paid though as to possible causes. Indeed there has been an assumption that frequent limb pain, like recurrent abdominal pain, has a mainly psychosomatic aetiology.[2] However an ultrasound study comparing the tibias of 39 children reporting growing pains with a control group showed significant physiological differences in both affected boys (P= 0.004) and girls (P <0.001).[3] This physical finding suggests that any association with malaise in the child is more likely to be a consequence rather than a precursor of the pain.

Growing pains in childhood are usually self-limiting. However, they may be an indicator of increased risk of arthritis in adulthood, since it has been shown that children with limb pains are more likely to have parents and/or grandparents with a history of arthritis compared to the general population.[1] Among small studies of selected groups of children, recurrent limb pains have been reported to be particularly prevalent in obese children,[4] and in those with some vitamin D deficiency.[5]

Adult arthritis has long been considered to be a condition that could be ameliorated or prevented by dietary manipulation, with suggestions of different components of the diet being important – particularly omega-3 fatty acids.[6] To our knowledge the only specific dietary factor that has been considered in regard to childhood limb pain concerns a study of 532 Greek children aged 4 – 12. A quarter of these children had experienced limb pains in the previous year.[7] The non-affected children were substantially more likely to have been breast fed for at least 40 days compared with the affected children (P<0.005). Unfortunately this study took no account of other factors that may have influenced the relationship, such as socio-economic background. However if breast feeding were on the causal pathway, the major differences between breast and artificial milk at the time, such as omega-3 fatty acid content, could be prime candidates for prevention.

While the biological aetiology of growing pains is unknown, data on adults indicate that omega-3 fatty acids may reduce pain in joints and muscle and may have a role in regulating bone growth: Goldberg and Katz [8] conducted a meta-analysis of 17 trials assessing reduction of pain in rheumatoid arthritis and reported significant reductions in patient reported joint pain intensity, morning stiffness, number of painful and/or tender joints and analgesic consumption. Omega-3 supplementation in adults without arthritis has been

shown to reduce perceived pain and increase the range of motion post exercise among untrained men in comparison to placebo.[9] A randomized trial found that in older adults omega-3 fatty acids stimulated muscle protein synthesis and were thought to be useful in preventing or treating acropenia.[10] Fatty acid patterns in serum of healthy 8 year olds have been associated with bone mineralisation.[11] Arachidonic acid and other omega-6 fatty acids influence bone resorption and accretion in a dose-dependent manner while omega-3 fats appear to stimulate bone accretion affecting both trabecular and cortical bone.[12] Cartilage is also influenced by fatty acid compositions: both linoleic acid and arachidonic acid can reduce collagen synthesis while eicosapentaenoic acid has a stimulating effect.[13]

In this study we examine the possibility of omega-3 fatty acids protecting against growing pains in childhood. On the basis of the Greek study and relationships between omega-3 and omega-6 status and muscle, bone and cartilage health, we hypothesise that beneficial effects may occur from exposure as a fetus, or ingestion in infancy as well as later in childhood. Increases in specific fatty acids may occur from genetic variants and /or dietary intake. In this study we are hypothesis free in regard to critical ages at exposure.

Material and Methods

The study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) was designed to determine the genetic and environmental factors that influence the health and development of the child.[14] The eligible sample comprised all pregnant mothers resident in a defined area [that part of the County of Avon that was within the South Western Health Authority Area] who had an expected date of delivery between 1st April 1991 and 31st December 1992. The study started in pregnancy and collected information in a variety of ways including questionnaires completed by the parents and, once old enough, by the child. Biological samples were collected from the mother during pregnancy and from the child at various ages.

Outcome measures

Information on limb pain was collected via self-completion questionnaires sent to the study child's chief carer at 4, 5, 6, 8, 11 and 13 years of age. Questions asked ‘does he/she often have aches and pains in his/her arms or legs’; responses were: ‘yes arms, yes legs, yes both, no not often’. If yes, parents were asked to describe what they thought was the cause

and whether any particular treatment(s) had helped. For this study we used the data collected at age 8 and combined all three positive responses and compared them with the group that replied 'no not often'. It should be noted, however, that relatively few children were reported as having pain in their arms only (<2% of the population), so pain in the limbs almost entirely comprised pain in the lower limbs.[1]

Measures of fatty acids

Measures of fatty acids used in this paper are as follows: (a) the maternal prenatal dietary intake of omega-3 from seafood in late pregnancy using the data from food frequency questionnaires analysed in Hibbeln et al;[15] (b) maternal prenatal red blood cell fatty acids as described in Newson;[16] (c) the duration of breast feeding as assessed from a questionnaire administered at 6 months, categorised as none, < 4 months, 4 months or more; (d) the child's omega-3 intake from seafood as identified from food frequency questionnaires administered at ages 3 and 7; (e) the relationship with genetic variants in the FADS genes which are strongly related to levels of plasma fatty acids (rs numbers 174548, 174556 and 174561 from the FADS 1 gene; rs nos 3834458 and 968567 from the intergenic region between FADS 1 and FADS 2; rs nos. 174570, 1535, 174574, 174575, 2727271, 174576, 174578, 174579, 174602,498793 and 526126 from the FADS 2 gene; rs 174448 and 174449 from the intergenic region between FADS 2 and FADS 3; and rs174455 from FADS 3);[17] and (f) the child's plasma ((blood samples were obtained from the children aged about 7 years at a special ALSPAC clinic). From these samples, plasma was obtained after centrifugal separation and frozen immediately. All samples were stored at -70°C, thawed once to obtain a 100 μL aliquot, shipped airfreight on dry ice to Rockville MD and thawed a second time for analysis. Transmethylation of lipids with acetyl chloride and methanol was performed using a simplified method based upon the Lepage and Roy procedure [18] using a high throughput automated method.[19] Internal calibration was conducted by adding internal standards to each assay. A second standard was used to quantify the exact amount of internal standard in every batch for ongoing assay of experimental variability. Freedom Evo Instrument 200 (TECAN Trading AG, Switzerland) was utilized for the automatic transmethylation and extraction of FAs employing the customized control and automation software (EVOware v2.0, SP1, Patch3). Gas chromatography 6890 Plus LAN system (Agilent Technologies, Inc., Santa Clara, CA) coupled with a fused-silica, narrow-bored DB-FFAP capillary column (Agilent 127-32H2, 15 m × 0.1 mm I.D. × 0.1 μm film thickness) which was used for chromatographic separation of the FAME as reported previously.[19] The

assay was linear in the range of 1-600 μg/mL plasma. The within and between day imprecision was 3.26±1.2% and 2.95±1.6% for fatty acid concentrations. Assays were undertaken in 2009-2010. In all, 23 FAs were measured including 12 polyunsaturates. [Cord plasma was measured 2008-9]

Possible confounders

The following factors were taken into account in multivariable analyses: highest maternal educational achievement, categorised in 3 groups: low (< O level), medium (O level), high (A level or more); socioeconomic group as measured by the highest social class categorisation based on the occupation of the parents as recorded in pregnancy; parity of the mother at the time of delivery of the study child, measured as the number of previous pregnancies resulting in a live or stillbirth: 0, 1, 2+ ; maternal smoking in mid-pregnancy: none, 1-9, 10+ cigarettes per day; passive smoke exposure in childhood at 3 years of age, as assessed by the length of time the child was in a room where others were smoking: not at all, low-moderate, high ; the child's body mass index (BMI) at age 7 used as a continuous variable.

Statistical analysis

Differences in the proportions of children reporting growing pains at 8 years by possible confounding factors were examined using chi-squared tests. Comparisons of mean fatty acid levels were performed using t-tests, comparing those with and without growing pains. Logistic regression was performed adjusting for those confounders that showed significant unadjusted associations. All fatty acids were analysed as a percentage of total fatty acids.

Results

Growing pains of children aged 8 were more likely to be reported by mothers with lower educational achievements, those of lower social class, lower parity, and those who smoked in pregnancy. Growing pains were also more likely in children exposed to environmental tobacco smoke (ETS) in childhood. There was no difference in prevalence of growing pains between the sexes, and the affected children had a similar mean BMI to those without such a history (Table 1). These results were similar to the results at other ages.

Table 1: Differences in potential confounding factors and fatty acid measures according to growing pains reported at 8.5 years

	No growing pains (78.6%)	Growing pains (21.4%)	P value
Gender			
Boy (51.0%)	3137 (78.6%)	855 (21.4%)	0.972
Girl (49.0%)	3018 (78.6%)	821 (21.4%)	
Maternal education			
< O level (23.0%)	1350 (77.3%)	397 (22.7%)	0.022
O level (35.5%)	2115 (78.5%)	579 (21.5%)	
>O level (41.5%)	2539 (80.5%)	616 (19.5%)	
Family social class			
I and II (29.4%)	1710 (80.4%)	417 (19.6%)	0.001
III NM (26.5%)	1545 (80.7%)	370 (19.3%)	
III M (26.9%)	1473 (75.7%)	473 (24.3%)	
IV and V (17.2%)	981 (79.4%)	254 (20.6%)	
Parity			
0 (46.3%)	2700 (77.0%)	805 (23.0%)	0.002
1 (35.9%)	2179 (80.0%)	545 (20.0%)	
2+ (17.8%)	1091 (80.9%)	258 (19.1%)	
Maternal smoking in pregnancy			
None (85.1%)	5189 (79.4%)	1344 (20.6%)	0.005
1-9 (7.3%)	421 (75.6%)	136 (24.4%)	
10+ (7.6%)	436 (74.8%)	147 (25.2%)	
Child ETS at age 3			
None (62.1%)	3592 (80.4%)	874 (19.6%)	<0.0001
Low- moderate (25.0%)	1378 (76.8%)	417 (23.2%)	
High (12.9%)	701 (75.8%)	224 (24.2%)	
Mean BMI (sd) at age 7	16.2 (2.0)	16.3 (2.1)	0.171

BMI = body mass index; ETS = Environmental tobacco smoke

In Table 2 unadjusted indicators of omega-3 and omega-6 fatty acid status are shown for the fetus and child. There were no unadjusted differences between the children with limb pain and those without in regard to the amount of omega-3 consumed as estimated from the mother's dietary intake, the duration of breast feeding, or the child's dietary intake at 3 or 6 years; additionally there were no differences in the plasma levels of key omega-3 and omega-6 fatty acids at 7 years, nor of the maternal prenatal red cell levels. No differences were seen in plasma levels of monosaturated or saturated fatty acids at any age.

Table 2: Unadjusted differences in measures of estimated dietary intake of omega-3 and mean blood levels according to growing pains at 8.5 years

	No growing pains	Growing pains	P-value
Maternal prenatal FFQ [mean (sd)]			
Total omega-3	0.15 (0.15)	0.16 (0.15)	0.614
DHA	0.07 (0.07)	0.07 (0.07)	0.593
EPA	0.05 (0.05)	0.05 (0.05)	0.654
Breastfeeding duration			
None (20.7%)	1172 (77.7%)	336 (22.3%)	0.193
Up to 3 months (31.2%)	1775 (78.2%)	495 (21.8%)	
> 3 months (48.2%)	2797 (79.7%)	712 (20.3%)	
Child FFQ at 3 years [mean (sd)]			
Total n3	0.06 (0.06)	0.06 (0.05)	0.443
DHA	0.02 (0.03)	0.03 (0.03)	0.449
EPA	0.02 (0.02)	0.02 (0.02)	0.439
Child FFQ at 6 years [mean (sd)]			
Total n3	0.08 (0.09)	0.09 (0.09)	0.173
DHA	0.03 (0.04)	0.03 (0.04)	0.217
EPA	0.02 (0.02)	0.02 (0.02)	0.233
Child plasma levels at 7 years[mean (sd)]			
AA (20:4n6)	6.45 (1.32)	6.36 (1.32)	0.087
DHA (22:6n3)	1.89 (0.52)	1.90 (0.53)	0.426
EPA (20:5n3)	0.64 (0.20)	0.64 (0.20)	0.797
LA (18:2n6)	30.6 (3.16)	30.7 (3.25)	0.511
ALA (18:3n3)	0.71 (0.28)	0.72 (0.27)	0.318
Maternal prenatal red cell levels [mean (sd)]			
AA (20:4n6)	6.16 (2.98)	6.11(2.97)	0.727
DHA (22:6n3)	2.32(1.36)	2.25(1.31)	0.215
EPA (20:5n3)	0.27 (0.18)	0.27 (0.17)	0.331
LA (18:2n6)	11.10 (2.70)	10.98 (2.82)	0.610
ALA (18:3n3)	0.14 (0.07)	0.14 (0.07)	0.676

FFQ = food frequency questionnaire; DHA = decosahexaenoic acid; EPA = eicosapentaenoic acid
AA – arachidonic acid; LA = linoleic acid; ALA = alpha-linolenic acid

Reversing the calculations, Table 3 demonstrates the odds ratios (ORs) for developing growing pains given the level of exposure to fatty acids, adjusting for the various factors in Table 1. Of the 20 factors tested only one showed some evidence of a statistically significant association – there was a slight increase in the risk of growing pains with each unit increase in the child's plasma omega-6 linoleic acid levels [OR = 1.02; 95% CI 1.00,1.05]. There was

no evidence of any linear relationships between growing pains and the number of alleles of any of the 17 genetic variants examined (data not shown).

Table 3: Unadjusted and adjusted odds ratios (95% confidence intervals) for growing pains reported at 8.5 years according to various fatty acid measures

	Unadjusted	Adjusted*
Breastfeeding duration		
None	1.00 Reference	1.00 Reference
Up to 3 months	0.97 (0.83, 1.14)	1.00 (0.82, 1.22)
> 3 months	0.89 (0.77, 1.03)	0.95 (0.78, 1.16)
Maternal FFQ in pregnancy		
Total omega-3	1.10 (0.76, 1.60)	1.26 (0.81, 1.97)
DHA	1.24 (0.56, 2.74)	1.69 (0.66, 4.34)
EPA	1.30 (0.42, 4.05)	1.94 (0.50, 7.52)
Child FFQ at 3 years		
Total omega-3	1.48 (0.54, 4.06)	1.85 (0.57, 6.01)
DHA	2.26 (0.27, 18.57)	4.35 (0.37, 50.78)
EPA	3.96 (0.12, 129.4)	9.73 (0.17, 568.9)
Child FFQ at 6 years		
Total omega-3	1.55 (0.83, 2.91)	1.62 (0.79, 3.36)
DHA	2.70 (0.56, 13.04)	3.12 (0.51, 19.22)
EPA	4.37 (0.39, 49.32)	5.07 (0.31, 83.48)
Child plasma levels at 7 years		
AA (20:4n6)	0.95 (0.90, 1.01)	0.97 (0.91, 1.03)
DHA (22:6n3)	1.06 (0.92, 1.22)	1.13 (0.97, 1.32)
EPA (20:5n3)	1.05 (0.73, 1.50)	1.15 (0.77, 1.70)
LA (18:2n6)	1.01 (0.99, 1.03)	1.02 (1.00, 1.05)
ALA (18:3n3)	1.14 (0.88, 1.47)	1.16 (0.87, 1.55)
Maternal prenatal red cell levels		
AA (20:4n6)	1.00 (0.97,1.02)	0.99(0.95,1.02)
DHA (22:6n3)	0.96 (0.90,1.02)	0.95 (0.88,1.03)
EPA (20:5n3)	0.78 (0.47,1.29)	0.63 (0.34,1.17)
LA (18:2n6)	0.99 (0.96,1.02)	0.99 (0.95,1.03)
ALA (18:3n3)	0.76 (0.21,2.75)	0.40 (0.09,1.83)

*Adjusted for gender, maternal education, family social class, parity, maternal smoking, childhood environmental tobacco smoke

AA = arachidoic acid; ALA = alpha-linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LA = linoleic acid; FFQ = food frequency questionnaire

We also explored gender differences in the associations. No differences were observed in fatty acid relationships between boys and girls or for the associations with the

FADS genetic variants assuming an additive genetic model. However when a recessive model for the minor allele was explored, 47% of the 34 analyses prior to age 7 years showed significant associations for girls compared to 2.9% in boys. A formal interaction test suggested many of these differences could have occurred by chance but nevertheless 6 of these differences (18%) were nominally significant. After 7 years, differences in the associations by gender persisted to a lesser extent but none exceeded the formal requirements for an interaction.

Discussion

Growing pains may have deleterious effects on the child's quality of life. For example a German study of children aged 5 – 18 showed that 35% of those with limb pain had been absent from school in consequence, 36% had disturbed sleep and 55% were unable to pursue hobbies.[20] Consequently any ways in which the occurrence of these pains can be avoided is likely to have major benefits on the children's quality of life. Omega-3 fatty acids were hypothesised to be candidate substances that might ameliorate or prevent such pains. However we could find no convincing evidence here that fatty acids have such a part to play in reducing the risk of limb pain at 8 years of age.

In this study we have taken account of factors such as exposure to cigarette smoke, whether *in utero* or in childhood, socio-economic status and maternal education level, the child's gender and BMI. Neither the unadjusted markers of fatty acid exposure, nor those demonstrated after adjustment showed more statistically significant results than would be expected by chance. This applied to the duration of breast feeding, the child's dietary exposure as estimated from food frequency questionnaires of the mother during pregnancy and of the child at ages 3 and 6 years. In addition the levels of 5 different omega-3 and omega-6 fatty acids measured in the mothers' blood in pregnancy, and in the child's blood at age 7 years, were not associated with limb pain.

It is well documented that any dietary effects shown can be artefacts associated with socioeconomic and personality factors, and the same can be true for lack of associations. We therefore have used the Mendelian randomisation approach to test this further.[21] We considered 17 genetic variants from the FADS1, 2 and 3 genes and their intergenic markers. We have already shown that 14 of the minor alleles of these markers are strongly negatively associated with the blood levels of DHA, and all 17 are associated with the ratios of AA to LA (omega-6) and of EPA to ALA (omega-3).[17] Thus, if there were an association of

blood levels between fatty acids and growing pains, one would expect it to be demonstrated by an association with one or more of the genetic variants. No such relationships were found with the number of minor alleles. Some differences were observed in girls using a recessive model. These results might suggest that pre-pubertal girls homozygous for the minor allele tend to have lower levels of long chain fatty acids below a requirement to alleviate limb pains. Irrespective of its validity, the increasing (not decreasing) prevalence of limb pain with age in girls and the absence of any genetic associations in boys suggest a different aetiology for the majority of children.

The major strength of this study concerns the large sample size, having objective measures of fatty acid levels from the mother during pregnancy, and of the child at 7 years of age, availability of genetic markers related to the FADS1, 2 and 3 genes, and the information on the diet of mother prenatally and of the child at various time points. Here we used the duration of breast feeding, and the diet of the child at ages 3 and 7, the latter being the closest age at which we had collected dietary information to that of the child's report of growing pains used in this study. The data also benefits from the prospectively collected study design, thus avoiding the biases inherent in retrospective questioning.

A possible weakness concerns the fact that the data were provided by the parents. The child him/herself is the only person who would be able to accurately reveal such a history. However it is likely to only be parents of 8-year-olds from the most dysfunctional families that would be oblivious to their child's pain. Such families are likely to be poor at responding in general, and are unlikely to have made a detectable difference to the results reported here.

In conclusion, we could find no strong evidence to indicate that variations in fatty acids would prevent the occurrence of growing pains in children. That is not to say, however, that omega-3 fatty acids might not be beneficial as a treatment for such pains, just as they seem to be for rheumatoid arthritis.[6]

What is already known on this topic:

- 'Growing pains' are common complaints in childhood and adolescence.
- No large studies have been undertaken to determine whether diet, particularly omega-3 fatty acids, might have a preventive role.

What this study adds

- No evidence was found that components of the diet or blood levels of fatty acids either prenatally or childhood influence the development of growing pains.

- Minimal associations were found with the genetic variants in the FADS genes using a Mendelian randomisation approach.
- We conclude that dietary intake of omega-3 fatty acids is unlikely to prevent the development of growing pains in childhood.

Acknowledgements

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

Ethics

Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees.

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STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any pre-specified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	2
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	2-3
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3,5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	3,5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3, 5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-6*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	3,5-6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5,6-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-10
		(*b*) Describe any methods used to examine subgroups and interactions	7-10
		(*c*) Explain how missing data were addressed	N/A
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	N/A

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	3
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	3
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	N/A
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	3,5-6,8-10
		Cross-sectional study—Report numbers of outcome events or summary measures	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	7-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7,11-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	N/A
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ open

Do omega-3 or other fatty acids influence the development of 'growing pains'? A pre-birth cohort study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-001370.R1
Article Type:	Research
Date Submitted by the Author:	25-Jun-2012
Complete List of Authors:	Golding, Jean; University of Bristol, Centre for Child and Adolescent Health Northstone, Kate; University of Bristol, School of Social & Community Medicine Emmett, Pauline; University of Bristol, School of Social & Community Medicine Steer, Colin; University of Bristol, School of Social & Community Medicine Hibbeln, Joseph; National Institutes of Health (NIAAA), Section on Nutritional Neuroscience, LMBB
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Public health, Nutrition and metabolism, Paediatrics, Rheumatology, Genetics and genomics
Keywords:	EPIDEMIOLOGY, Paediatric clinical genetics & dysmorphology < GENETICS, NUTRITION & DIETETICS, Musculoskeletal disorders < ORTHOPAEDIC & TRAUMA SURGERY, PAIN MANAGEMENT

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Do omega-3 or other fatty acids influence the development of 'growing pains'? A pre-birth cohort study

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Abbreviations: AA = arachidonic acid; ALA = alpha-linolenic acid; ALSPAC= Avon Longitudinal Study of Parents and Children; BMI = body mass index; DHA = decosahexaenoic acid; EPA = eicosapentaenoic acid; ETS = environmental tobacco smoke; FFQ = food frequency questionnaire; LA = linoleic acid.

Subject Heading: Childhood limb pains, omega-3 fatty acids; breast feeding; maternal diet; FADS genetic markers.

Competing Interests Statement: None

Contributorship statement:

JG, KN and PE had the original idea; KN and CS carried out the data analysis; JG wrote the first draft; all authors contributed to subsequent drafts.

Structured Abstract

Objectives: To assess whether the prevalence of growing pains varies with indicators of fatty acid exposure. Growing pains (limb pains of no obvious explanation) have been shown to be strongly linked to a family history of arthritis, and are thought to predict an increased risk of the development of arthritis in adulthood. Much has been made of the possibility of fatty acids, particularly the omega-3 fatty acids, playing a preventive role in the development of arthritis, but little research has been undertaken to determine whether such fatty acids might reduce the risk of growing pains. We aimed to assess whether the prevalence of growing pains varies with indicators of fatty acid exposures.

Design: Case-control study nested within a prospective longitudinal cohort comparing prenatal and postnatal diet, blood measures, and variants in FADS genes that influence the metabolism of fatty acids. Statistical analysis took account of factors such as gender, smoke exposure, maternal age and education, social class and parity.

Setting: Avon Longitudinal Study of Parents & Children (ALSPAC)

Participants: All children born between 1st April 1991 and 31st December 1992 (approximately 14,000) within the Avon area (only that part of Avon under the SWRHA). This project compared 1676 children who reported 'growing pains' at age 8 with 6155 with no such pain.

Primary outcome: Reported limb pains of no apparent origin

Results: There was no indication that the affected children had diets that differed in regard to omega-3 plasma levels of fatty acids, or the FADS genetic variants. We also assessed fetal and infant exposure but neither maternal prenatal blood levels nor maternal dietary intake, or duration of breast feeding showed any significant relationships even after adjustment for confounders.

Conclusions: Thus there is no evidence that omega-3 fatty acid status protects against the development of growing pains in childhood.

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Article Summary

Article Focus: To assess whether prevalence of growing pains varies

- with indicators of fatty acid exposure through pre- and post-natal diet
- And with variation in FADS genes that influence the metabolism of fatty acids.

Key messages

- There was no evidence that omega-3 fatty acid status protects against the development of growing pains in childhood.

Strengths and Limitations

- The strength of the study lie in the fact that the data are collected prospectively; it is population based, with a large sample. It includes biological markers and genetic variants which allow independent assessment of fatty acid metabolism.
- Limitations concern the fact that the information on limb pains is collected from mothers, albeit using structured questions.

Data Sharing statement: ALSPAC is committed to share data with bona fide researchers.

Funding Statement: The UK Medical Research Council (MRC), the Wellcome Trust and the University of Bristol currently provide core support for ALSPAC. The assays of the maternal blood samples were carried out by Scotia at the instigation of the late David Horrobin, to whom we are extremely grateful. Funding for the genotyping and analysis of the genetic variants was partly undertaken with funding from the EU grant no.FP7-212652 and partly by the Waterloo Foundation. The assays of the child's blood were conducted and supported by the intramural research program of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) with funding from the National Oceanic and Atmospheric Administration (NOAA). Statistical analysis of the information was funded by the Arthritic Association.

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Introduction

Growing pains are common in childhood, with about 56% of children reporting them as occurring frequently between the ages of 5 and 13.[1] Little attention has been paid though as to possible causes. Indeed there has been an assumption that frequent limb pain, like recurrent abdominal pain, has a mainly psychosomatic aetiology.[2] However an ultrasound study comparing the tibias of 39 children reporting growing pains with a control group showed significant physiological differences in both affected boys (P= 0.004) and girls (P <0.001).[3] This physical finding suggests that any association with malaise in the child is more likely to be a consequence rather than a precursor of the pain.

Growing pains in childhood are usually self-limiting. However, they may be an indicator of increased risk of arthritis in adulthood, since it has been shown that children with limb pains are more likely to have parents and/or grandparents with a history of arthritis compared to the general population.[1] Among small studies of selected groups of children, recurrent limb pains have been reported to be particularly prevalent in obese children,[4] and in those with some vitamin D deficiency.[5], though this was notnot found in a small study in New Mexico[6a]. Other possibilities have been summarised by Evans and Scutter.[7]

Adult arthritis has long been considered to be a condition that could be ameliorated or prevented by dietary manipulation, with suggestions of different components of the diet being important – particularly omega-3 fatty acids.[86] To our knowledge the only specific dietary factor that has been considered in regard to childhood limb pain concerns a study of 532 Greek children aged 4 – 12. A quarter of these children had experienced limb pains in the previous year.[97] The non-affected children were substantially more likely to have been breast fed for at least 40 days compared with the affected children (P<0.005). Unfortunately this study took no account of other factors that may have influenced the relationship, such as socio-economic background. However if breast feeding were on the causal pathway, the major differences between breast and artificial milk at the time, such as omega-3 fatty acid content, could be prime candidates for prevention.

While the biological aetiology of growing pains is unknown, data on adults indicate that omega-3 fatty acids may reduce pain in joints and muscle and may have a role in regulating bone growth: Goldberg and Katz [810] conducted a meta-analysis of 17 trials assessing reduction of pain in rheumatoid arthritis and reported significant reductions in patient reported joint pain intensity, morning stiffness, number of painful and/or tender joints

and analgesic consumption. Omega-3 supplementation in adults without arthritis has been shown to reduce perceived pain and increase the range of motion post exercise among untrained men in comparison to placebo.[119] A randomized trial found that in older adults omega-3 fatty acids stimulated muscle protein synthesis and were thought to be useful in preventing or treating acropenia.[120] Fatty acid patterns in serum of healthy 8 year olds have been associated with bone mineralisation.[131] Arachidonic acid and other omega-6 fatty acids influence bone resorption and accretion in a dose-dependent manner while omega-3 fats appear to stimulate bone accretion affecting both trabecular and cortical bone.[142] Cartilage is also influenced by fatty acid compositions: both linoleic acid and arachidonic acid can reduce collagen synthesis while eicosapentaenoic acid has a stimulating effect.[153]

In this study we examine the possibility of omega-3 fatty acids protecting against growing pains in childhood. On the basis of the Greek study and relationships between omega-3 and omega-6 status and muscle, bone and cartilage health, we hypothesise that beneficial effects may occur from exposure as a fetus, or ingestion in infancy as well as later in childhood. Increases in specific fatty acids may occur from genetic variants and /or dietary intake. In this study we are hypothesis free in regard to critical ages at exposure.

Material and Methods

The study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) was designed to determine the genetic and environmental factors that influence the health and development of the child.[164] The eligible sample comprised all pregnant mothers resident in a defined area [that part of the County of Avon that was within the South Western Health Authority Area] who had an expected date of delivery between 1st April 1991 and 31st December 1992. The study started in pregnancy and collected information in a variety of ways including questionnaires completed by the parents and, once old enough, by the child. Biological samples were collected from the mother during pregnancy and from the child at various ages.

Outcome measures

Information on limb pain was collected via self-completion questionnaires sent to the study child's chief carer at 4, 5, 6, 8, 11 and 13 years of age. Questions asked 'does he/she

often have aches and pains in his/her arms or legs'; responses were: 'yes arms, yes legs, yes both, no not often'. If yes, parents were asked to describe what they thought was the cause and whether any particular treatment(s) had helped. There was concurrent validity to these questions by virtue of the causes and treatments described being similar to those found in small studies in the literature, as well as by the expected correlations with family history of arthritis.[1] We also demonstrated test-retest reliability by comparing results over time, and showing that limb pains were usually reported on more than one consecutive occasion.

For this study we used the data collected at age 8 and combined all three positive responses and compared them with the group that replied 'no not often'. The children for whom a known physical cause was given were excluded. (The actual descriptions of causes given are described in the Supplementary Table to ref [1].) It should be noted, ~~however,~~ that relatively few children were reported as having pain in their arms only (<2% of the population), so pain in the limbs almost entirely comprised pain in the lower limbs.[1]

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Measures of fatty acids

Measures of fatty acids used in this paper are as follows: (a) the maternal prenatal dietary intake of omega-3 from seafood in late pregnancy using the data from food frequency questionnaires analysed in Hibbeln et al;[17~~5~~] (b) maternal prenatal red blood cell fatty acids as described in Newson;[18~~6~~] (c) the duration of breast feeding as assessed from a questionnaire administered at 6 months, categorised as none, < 4 months, 4 months or more; (d) the child's omega-3 intake from seafood as identified from food frequency questionnaires administered at ages 3 and 7; (e) the relationship with genetic variants in the FADS genes which are strongly related to levels of plasma fatty acids (rs numbers 174548, 174556 and 174561 from the FADS 1 gene; rs nos 3834458 and 968567 from the intergenic region between FADS 1 and FADS 2; rs nos. 174570, 1535, 174574, 174575, 2727271, 174576, 174578, 174579, 174602,498793 and 526126 from the FADS 2 gene; rs 174448 and 174449 from the intergenic region between FADS 2 and FADS 3; and rs174455 from FADS 3);[19-~~7~~] and (f) the child's plasma ((blood samples were obtained from the children aged about 7 years at a special ALSPAC clinic). From these samples, plasma was obtained after centrifugal separation and frozen immediately. All samples were stored at -70°C, thawed once to obtain a 100 µL aliquot, shipped airfreight on dry ice to Rockville MD and thawed a second time for analysis. Transmethylation of lipids with acetyl chloride and methanol was

performed using a simplified method based upon the Lepage and Roy procedure [2018] using a high throughput automated method.[2119] Internal calibration was conducted by adding internal standards to each assay. A second standard was used to quantify the exact amount of internal standard in every batch for ongoing assay of experimental variability. Freedom Evo Instrument 200 (TECAN Trading AG, Switzerland) was utilized for the automatic transmethylation and extraction of FAs employing the customized control and automation software (EVOware v2.0, SP1, Patch3). Gas chromatography 6890 Plus LAN system (Agilent Technologies, Inc., Santa Clara, CA) coupled with a fused-silica, narrow-bored DB-FFAP capillary column (Agilent 127-32H2, 15 m × 0.1 mm I.D. × 0.1 µm film thickness) which was used for chromatographic separation of the FAME as reported previously.[2119] The assay was linear in the range of 1-600 µg/mL plasma. The within and between day imprecision was 3.26±1.2% and 2.95±1.6% for fatty acid concentrations. Assays were undertaken in 2009-2010. In all, 23 FAs were measured including 12 polyunsaturates. [Cord plasma was measured 2008-9]

Possible confounders

The following factors were taken into account in multivariable analyses: highest maternal educational achievement, categorised in 3 groups: low (< O level), medium (O level), high (A level or more); socioeconomic group as measured by the highest social class categorisation based on the occupation of the parents as recorded in pregnancy; parity of the mother at the time of delivery of the study child, measured as the number of previous pregnancies resulting in a live or stillbirth: 0, 1, 2+ ; maternal smoking in mid-pregnancy: none, 1-9, 10+ cigarettes per day; passive smoke exposure in childhood at 3 years of age, as assessed by the length of time the child was in a room where others were smoking: not at all, low-moderate, high ; the child's body mass index (BMI) at age 7 used as a continuous variable.

Statistical analysis

Differences in the proportions of children reporting growing pains at 8 years by possible confounding factors were examined using chi-squared tests. Comparisons of mean fatty acid levels were performed using t-tests, comparing those with and without growing pains. Logistic regression was performed adjusting for those confounders that showed

significant unadjusted associations. All fatty acids were analysed as a percentage of total fatty acids.

Results

Growing pains of children aged 8 were more likely to be reported by mothers with lower educational achievements, those of lower social class, lower parity, and those who smoked in pregnancy. Growing pains were also more likely in children exposed to environmental tobacco smoke (ETS) in childhood. There was no difference in prevalence of growing pains between the sexes, and the affected children had a similar mean BMI to those without such a history (Table 1). These results were similar to the results at other ages.

Table 1: Differences in potential confounding factors and fatty acid measures according to growing pains reported at 8.5 years

	No growing pains (78.6%)	Growing pains (21.4%)	P value
Gender			
Boy (51.0%)	3137 (78.6%)	855 (21.4%)	0.972
Girl (49.0%)	3018 (78.6%)	821 (21.4%)	
Maternal education			
< O level (23.0%)	1350 (77.3%)	397 (22.7%)	0.022
O level (35.5%)	2115 (78.5%)	579 (21.5%)	
>O level (41.5%)	2539 (80.5%)	616 (19.5%)	
Family social class			
I and II (29.4%)	1710 (80.4%)	417 (19.6%)	0.001
III NM (26.5%)	1545 (80.7%)	370 (19.3%)	
III M (26.9%)	1473 (75.7%)	473 (24.3%)	
IV and V (17.2%)	981 (79.4%)	254 (20.6%)	
Parity			
0 (46.3%)	2700 (77.0%)	805 (23.0%)	0.002
1 (35.9%)	2179 (80.0%)	545 (20.0%)	
2+ (17.8%)	1091 (80.9%)	258 (19.1%)	
Maternal smoking in pregnancy			
None (85.1%)	5189 (79.4%)	1344 (20.6%)	0.005
1-9 (7.3%)	421 (75.6%)	136 (24.4%)	
10+ (7.6%)	436 (74.8%)	147 (25.2%)	
Child ETS at age 3			
None (62.1%)	3592 (80.4%)	874 (19.6%)	<0.0001
Low- moderate (25.0%)	1378 (76.8%)	417 (23.2%)	
High (12.9%)	701 (75.8%)	224 (24.2%)	
Mean BMI (sd) at age 7	16.2 (2.0)	16.3 (2.1)	0.171

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	No growing pains (78.6%)	Growing pains (21.4%)	P value

BMI = body mass index; ETS = Environmental tobacco smoke

In Table 2 unadjusted indicators of omega-3 and omega-6 fatty acid status are shown for the fetus and child. There were no unadjusted differences between the children with limb pain and those without in regard to the amount of omega-3 consumed as estimated from the mother's dietary intake, the duration of breast feeding, or the child's dietary intake at 3 or 6 years; additionally there were no differences in the plasma levels of key omega-3 and omega-6 fatty acids at 7 years, nor of the maternal prenatal red cell levels. No differences were seen in plasma levels of monosaturated or saturated fatty acids at any age.

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Table 2: Unadjusted differences in measures of estimated dietary intake of omega-3 and mean blood levels according to growing pains at 8.5 years

	No growing pains	Growing pains	P-value
Maternal prenatal FFQ [mean (sd)]			
Total omega-3			
DHA	0.15 (0.15)	0.16 (0.15)	0.614
EPA	0.07 (0.07)	0.07 (0.07)	0.593
	0.05 (0.05)	0.05 (0.05)	0.654
Breastfeeding duration			
None (20.7%)	1172 (77.7%)	336 (22.3%)	0.193
Up to 3 months (31.2%)	1775 (78.2%)	495 (21.8%)	
> 3 months (48.2%)	2797 (79.7%)	712 (20.3%)	
Child FFQ at 3 years [mean (sd)]			
Total n3	0.06 (0.06)	0.06 (0.05)	0.443
DHA	0.02 (0.03)	0.03 (0.03)	0.449
EPA	0.02 (0.02)	0.02 (0.02)	0.439
Child FFQ at 6 years [mean (sd)]			
Total n3	0.08 (0.09)	0.09 (0.09)	0.173
DHA	0.03 (0.04)	0.03 (0.04)	0.217
EPA	0.02 (0.02)	0.02 (0.02)	0.233
Child plasma levels at 7 years[mean (sd)]			
AA (20:4n6)	6.45 (1.32)	6.36 (1.32)	0.087
DHA (22:6n3)	1.89 (0.52)	1.90 (0.53)	0.426
EPA (20:5n3)	0.64 (0.20)	0.64 (0.20)	0.797
LA (18:2n6)	30.6 (3.16)	30.7 (3.25)	0.511
ALA (18:3n3)	0.71 (0.28)	0.72 (0.27)	0.318
Maternal prenatal red cell levels [mean (sd)]			
AA (20:4n6)	6.16 (2.98)	6.11(2.97)	0.727
DHA (22:6n3)	2.32(1.36)	2.25(1.31)	0.215
EPA (20:5n3)	0.27 (0.18)	0.27 (0.17)	0.331
LA (18:2n6)	11.10 (2.70)	10.98 (2.82)	0.610
ALA (18:3n3)	0.14 (0.07)	0.14 (0.07)	0.676

FFQ = food frequency questionnaire; DHA = decosahexaenoic acid; EPA = eicosapentaenoic acid
AA – arachidonic acid; LA = linoleic acid; ALA = alpha-linolenic acid

Reversing the calculations, Table 3 demonstrates the odds ratios (ORs) for developing growing pains given the level of exposure to fatty acids, adjusting for the various factors in Table 1. Of the 20 factors tested only one showed some evidence of a statistically significant association – there was a slight increase in the risk of growing pains with each unit increase in the child's plasma omega-6 linoleic acid levels [OR = 1.02; 95% CI 1.00,1.05]. There was

no evidence of any linear relationships between growing pains and the number of alleles of any of the 17 genetic variants examined (data not shown).

Table 3: Unadjusted and adjusted odds ratios (95% confidence intervals) for growing pains reported at 8.5 years according to various fatty acid measures

	Unadjusted	Adjusted*
Breastfeeding duration		
None	1.00 Reference	1.00 Reference
Up to 3 months	0.97 (0.83, 1.14)	1.00 (0.82, 1.22)
> 3 months	0.89 (0.77, 1.03)	0.95 (0.78, 1.16)
Maternal FFQ in pregnancy		
Total omega-3	1.10 (0.76, 1.60)	1.26 (0.81, 1.97)
DHA	1.24 (0.56, 2.74)	1.69 (0.66, 4.34)
EPA	1.30 (0.42, 4.05)	1.94 (0.50, 7.52)
Child FFQ at 3 years		
Total omega-3	1.48 (0.54, 4.06)	1.85 (0.57, 6.01)
DHA	2.26 (0.27, 18.57)	4.35 (0.37, 50.78)
EPA	3.96 (0.12, 129.4)	9.73 (0.17, 568.9)
Child FFQ at 6 years		
Total omega-3	1.55 (0.83, 2.91)	1.62 (0.79, 3.36)
DHA	2.70 (0.56, 13.04)	3.12 (0.51, 19.22)
EPA	4.37 (0.39, 49.32)	5.07 (0.31, 83.48)
Child plasma levels at 7 years		
AA (20:4n6)	0.95 (0.90, 1.01)	0.97 (0.91, 1.03)
DHA (22:6n3)	1.06 (0.92, 1.22)	1.13 (0.97, 1.32)
EPA (20:5n3)	1.05 (0.73, 1.50)	1.15 (0.77, 1.70)
LA (18:2n6)	1.01 (0.99, 1.03)	1.02 (1.00, 1.05)
ALA (18:3n3)	1.14 (0.88, 1.47)	1.16 (0.87, 1.55)
Maternal prenatal red cell levels		
AA (20:4n6)	1.00 (0.97,1.02)	0.99(0.95,1.02)
DHA (22:6n3)	0.96 (0.90,1.02)	0.95 (0.88,1.03)
EPA (20:5n3)	0.78 (0.47,1.29)	0.63 (0.34,1.17)
LA (18:2n6)	0.99 (0.96,1.02)	0.99 (0.95,1.03)
ALA (18:3n3)	0.76 (0.21,2.75)	0.40 (0.09,1.83)

*Adjusted for gender, maternal education, family social class, parity, maternal smoking, childhood environmental tobacco smoke

AA = arachidoic acid; ALA = alpha-linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LA = linoleic acid; FFQ = food frequency questionnaire

We also explored gender differences in the associations. No differences were observed in fatty acid relationships between boys and girls or for the associations with the

FADS genetic variants assuming an additive genetic model. However when a recessive model for the minor allele was explored, 47% of the 34 analyses prior to age 7 years showed significant associations for girls compared to 2.9% in boys. A formal interaction test suggested many of these differences could have occurred by chance but nevertheless 6 of these differences (18%) were nominally significant. After 7 years, differences in the associations by gender persisted to a lesser extent but none exceeded the formal requirements for an interaction.

Discussion

Growing pains may have deleterious effects on the child's quality of life. For example a German study of children aged 5 – 18 showed that 35% of those with limb pain had been absent from school in consequence, 36% had disturbed sleep and 55% were unable to pursue hobbies.[220] Consequently any ways in which the occurrence of these pains can be avoided is likely to have major benefits on the children's quality of life. Omega-3 fatty acids were hypothesised to be candidate substances that might ameliorate or prevent such pains. However we could find no convincing evidence here that fatty acids have such a part to play in reducing the risk of limb pain at 8 years of age.

In this study we have taken account of factors such as exposure to cigarette smoke, whether *in utero* or in childhood, socio-economic status and maternal education level, the child's gender and BMI. Neither the unadjusted markers of fatty acid exposure, nor those demonstrated after adjustment showed more statistically significant results than would be expected by chance. This applied to the duration of breast feeding, the child's dietary exposure as estimated from food frequency questionnaires of the mother during pregnancy and of the child at ages 3 and 6 years. In addition the levels of 5 different omega-3 and omega-6 fatty acids measured in the mothers' blood in pregnancy, and in the child's blood at age 7 years, were not associated with limb pain.

It is well documented that any dietary effects shown can be artefacts associated with socioeconomic and personality factors, and the same can be true for lack of associations. We therefore have used the Mendelian randomisation approach to test this further.[21] We considered 17 genetic variants from the FADS1, 2 and 3 genes and their intergenic markers. We have already shown that 14 of the minor alleles of these markers are strongly negatively associated with the blood levels of DHA, and all 17 are associated with the ratios of AA to LA (omega-6) and of EPA to ALA (omega-3).[179] Thus, if there were an association of

blood levels between fatty acids and growing pains, one would expect it to be demonstrated by an association with one or more of the genetic variants. No such relationships were found with the number of minor alleles. Some differences were observed in girls using a recessive model. These results might suggest that pre-pubertal girls homozygous for the minor allele tend to have lower levels of long chain fatty acids below a requirement to alleviate limb pains. Irrespective of its validity, the increasing (not decreasing) prevalence of limb pain with age in girls and the absence of any genetic associations in boys suggest a different aetiology for the majority of children.

The major strength of this study concerns the large sample size, having objective measures of fatty acid levels from the mother during pregnancy, and of the child at 7 years of age, availability of genetic markers related to the FADS1, 2 and 3 genes, and the information on the diet of mother prenatally and of the child at various time points. Here we used the duration of breast feeding, and the diet of the child at ages 3 and 7, the latter being the closest age at which we had collected dietary information to that of the child's report of growing pains used in this study. The data also benefits from the prospectively collected study design, thus avoiding the biases inherent in retrospective questioning.

A possible weakness concerns the fact that the data were provided by the parents. The child him/herself is the only person who would be able to accurately reveal such a history. However it is likely to only be parents of 8-year-olds from the most dysfunctional families that would be oblivious to their child's pain. Such families are likely to be poor at responding in general, and are unlikely to have made a detectable difference to the results reported here.

In conclusion, we could find no strong evidence to indicate that variations in fatty acids would prevent the occurrence of growing pains in children. That is not to say, however, that omega-3 fatty acids might not be beneficial as a treatment for such pains, just as they seem to be for rheumatoid arthritis.[8~~6~~]

What is already known on this topic:

- 'Growing pains' are common complaints in childhood and adolescence.
- No large studies have been undertaken to determine whether diet, particularly omega-3 fatty acids, might have a preventive role.

What this study adds

- No evidence was found that components of the diet or blood levels of fatty acids either prenatally or childhood influence the development of growing pains.

- Minimal associations were found with the genetic variants in the FADS genes using a Mendelian randomisation approach.
- We conclude that dietary intake of omega-3 fatty acids is unlikely to prevent the development of growing pains in childhood.

Acknowledgements

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

Ethics

Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees.

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STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any pre-specified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	2
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	2-3
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3,5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	3,5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3, 5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-6*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	3,5-6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5,6-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-10
		(*b*) Describe any methods used to examine subgroups and interactions	7-10
		(*c*) Explain how missing data were addressed	N/A
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	N/A

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	3
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	3
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	N/A
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	3,5-6,8-10
		Cross-sectional study—Report numbers of outcome events or summary measures	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	7-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7,11-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	N/A
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ open

Do omega-3 or other fatty acids influence the development of 'growing pains'? A pre-birth cohort study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-001370.R2
Article Type:	Research
Date Submitted by the Author:	23-Jul-2012
Complete List of Authors:	Golding, Jean; University of Bristol, Centre for Child and Adolescent Health Northstone, Kate; University of Bristol, School of Social & Community Medicine Emmett, Pauline; University of Bristol, School of Social & Community Medicine Steer, Colin; University of Bristol, School of Social & Community Medicine Hibbeln, Joseph; National Institutes of Health (NIAAA), Section on Nutritional Neuroscience, LMBB
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Public health, Nutrition and metabolism, Paediatrics, Rheumatology, Genetics and genomics
Keywords:	EPIDEMIOLOGY, Paediatric clinical genetics & dysmorphology < GENETICS, NUTRITION & DIETETICS, Musculoskeletal disorders < ORTHOPAEDIC & TRAUMA SURGERY, PAIN MANAGEMENT

Do omega-3 or other fatty acids influence the development of 'growing pains'? A pre-birth cohort study

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Abbreviations: AA = arachidonic acid; ALA = alpha-linolenic acid; ALSPAC= Avon Longitudinal Study of Parents and Children; BMI = body mass index; DHA = decosahexaenoic acid; EPA = eicosapentaenoic acid; ETS = environmental tobacco smoke; FFQ = food frequency questionnaire; LA = linoleic acid.

Subject Heading: Childhood limb pains, omega-3 fatty acids; breast feeding; maternal diet; FADS genetic markers.

Competing Interests Statement: None

Contributorship statement:

JG, KN and PE had the original idea; KN and CS carried out the data analysis; JG wrote the first draft; all authors contributed to subsequent drafts.

Structured Abstract

Objectives: To assess whether the prevalence of growing pains varies with indicators of fatty acid exposure. Growing pains (limb pains of no obvious explanation) have been shown to be strongly linked to a family history of arthritis, and are thought to predict an increased risk of the development of arthritis in adulthood. Much has been made of the possibility of fatty acids, particularly the omega-3 fatty acids, playing a preventive role in the development of arthritis, but little research has been undertaken to determine whether such fatty acids might reduce the risk of growing pains. We aimed to assess whether the prevalence of growing pains varies with indicators of fatty acid exposures.

Design: Case-control study nested within a prospective longitudinal cohort comparing prenatal and postnatal diet, blood measures, and variants in FADS genes that influence the metabolism of fatty acids. Statistical analysis took account of factors such as gender, smoke exposure, maternal age and education, social class and parity.

Setting: Avon Longitudinal Study of Parents & Children (ALSPAC)

Participants: All children born between 1st April 1991 and 31st December 1992 (approximately 14,000) within the Avon area (only that part of Avon under the SWRHA). This project compared 1676 children who reported 'growing pains' at age 8 with 6155 with no such pain.

Primary outcome: Reported limb pains of no apparent origin

Results: There was no indication that the affected children had diets that differed in regard to omega-3 plasma levels of fatty acids, or the FADS genetic variants. We also assessed fetal and infant exposure but neither maternal prenatal blood levels nor maternal dietary intake, or duration of breast feeding showed any significant relationships even after adjustment for confounders.

Conclusions: Thus there is no evidence that omega-3 fatty acid status protects against the development of growing pains in childhood.

Article Summary

Article Focus: To assess whether prevalence of growing pains varies

- with indicators of fatty acid exposure through pre- and post-natal diet
- And with variation in FADS genes that influence the metabolism of fatty acids.

Key messages

- There was no evidence that omega-3 fatty acid status protects against the development of growing pains in childhood.

Strengths and Limitations

- The strength of the study lie in the fact that the data are collected prospectively; it is population based, with a large sample. It includes biological markers and genetic variants which allow independent assessment of fatty acid metabolism.
- Limitations concern the fact that the information on limb pains is collected from mothers, albeit using structured questions.

Data Sharing statement: ALSPAC is committed to share data with bona fide researchers.

Funding Statement: The UK Medical Research Council (MRC), the Wellcome Trust and the University of Bristol currently provide core support for ALSPAC. The assays of the maternal blood samples were carried out by Scotia at the instigation of the late David Horrobin, to whom we are extremely grateful. Funding for the genotyping and analysis of the genetic variants was partly undertaken with funding from the EU grant no.FP7-212652 and partly by the Waterloo Foundation. The assays of the child's blood were conducted and supported by the intramural research program of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) with funding from the National Oceanic and Atmospheric Administration (NOAA). Statistical analysis of the information was funded by the Arthritic Association.

Introduction

Growing pains are common in childhood, with about 56% of children reporting them as occurring frequently between the ages of 5 and 13.[1] Little attention has been paid though as to possible causes. Indeed there has been an assumption that frequent limb pain, like recurrent abdominal pain, has a mainly psychosomatic aetiology.[2] However an ultrasound study comparing the tibias of 39 children reporting growing pains with a control group showed significant physiological differences in both affected boys (P= 0.004) and girls (P <0.001).[3] This physical finding suggests that any association with malaise in the child is more likely to be a consequence rather than a precursor of the pain.

Growing pains in childhood are usually self-limiting. However, they may be an indicator of increased risk of arthritis in adulthood, since it has been shown that children with limb pains are more likely to have parents and/or grandparents with a history of arthritis compared to the general population.[1] Among small studies of selected groups of children, recurrent limb pains have been reported to be particularly prevalent in obese children,[4] and in those with some vitamin D deficiency.[5], though this was not found in a small study in New Mexico[6]. Other possibilities have been summarised by Evans and Scutter.[7]

Adult arthritis has long been considered to be a condition that could be ameliorated or prevented by dietary manipulation, with suggestions of different components of the diet being important – particularly omega-3 fatty acids.[8] To our knowledge the only specific dietary factor that has been considered in regard to childhood limb pain concerns a study of 532 Greek children aged 4 – 12. A quarter of these children had experienced limb pains in the previous year.[9] The non-affected children were substantially more likely to have been breast fed for at least 40 days compared with the affected children (P<0.005). Unfortunately this study took no account of other factors that may have influenced the relationship, such as socio-economic background. However if breast feeding were on the causal pathway, the major differences between breast and artificial milk at the time, such as omega-3 fatty acid content, could be prime candidates for prevention.

While the biological aetiology of growing pains is unknown, data on adults indicate that omega-3 fatty acids may reduce pain in joints and muscle and may have a role in regulating bone growth: Goldberg and Katz [10] conducted a meta-analysis of 17 trials assessing reduction of pain in rheumatoid arthritis and reported significant reductions in patient reported joint pain intensity, morning stiffness, number of painful and/or tender joints

and analgesic consumption. Omega-3 supplementation in adults without arthritis has been shown to reduce perceived pain and increase the range of motion post exercise among untrained men in comparison to placebo.[11] A randomized trial found that in older adults omega-3 fatty acids stimulated muscle protein synthesis and were thought to be useful in preventing or treating acropenia.[12] Fatty acid patterns in serum of healthy 8 year olds have been associated with bone mineralisation.[13] Arachidonic acid and other omega-6 fatty acids influence bone resorption and accretion in a dose-dependent manner while omega-3 fats appear to stimulate bone accretion affecting both trabecular and cortical bone.[14] Cartilage is also influenced by fatty acid compositions: both linoleic acid and arachidonic acid can reduce collagen synthesis while eicosapentaenoic acid has a stimulating effect.[15]

In this study we examine the possibility of omega-3 fatty acids protecting against growing pains in childhood. On the basis of the Greek study and relationships between omega-3 and omega-6 status and muscle, bone and cartilage health, we hypothesise that beneficial effects may occur from exposure as a fetus, or ingestion in infancy as well as later in childhood. Increases in specific fatty acids may occur from genetic variants and /or dietary intake. In this study we are hypothesis free in regard to critical ages at exposure.

Material and Methods

The study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) was designed to determine the genetic and environmental factors that influence the health and development of the child.[16] The eligible sample comprised all pregnant mothers resident in a defined area [that part of the County of Avon that was within the South Western Health Authority Area] who had an expected date of delivery between 1st April 1991 and 31st December 1992. The study started in pregnancy and collected information in a variety of ways including questionnaires completed by the parents and, once old enough, by the child. Biological samples were collected from the mother during pregnancy and from the child at various ages.

Outcome measures

Information on limb pain was collected via self-completion questionnaires sent to the study child's chief carer at 4, 5, 6, 8, 11 and 13 years of age. Questions asked ‘does he/she often have aches and pains in his/her arms or legs’; responses were: ‘yes arms, yes legs, yes

both, no not often'. If yes, parents were asked to describe what they thought was the cause and whether any particular treatment(s) had helped. There was concurrent validity to these questions by virtue of the causes and treatments described being similar to those found in small studies in the literature, as well as by the expected correlations with family history of arthritis (P,0.0001 for each parent).[1] We also demonstrated test-retest reliability by comparing results over time; of 4491 children for whom responses were obtained on 6 time points, 43% repeatedly said 'no' to the question, and 35% responded positively on more than one occasion [1].. For reasons of space we did not ask supplementary questions that would have allowed some of Peterson's criteria [17] to be used [e.g. bilateral nature, occurring exclusively in the late afternoon or evening]. However the major criteria of exclusion of physical causes was included in this project.

For this study we used the data collected at age 8 and combined all three positive responses and compared them with the group that replied 'no not often'. The children for whom a known physical cause was given were excluded. (The actual descriptions of causes given are described in the Supplementary Table to ref [1].) It should be noted, that relatively few children were reported as having pain in their arms only (<2% of the population), so pain in the limbs almost entirely comprised pain in the lower limbs.[1]

Measures of fatty acids

Measures of fatty acids used in this paper are as follows: (a) the maternal prenatal dietary intake of omega-3 from seafood in late pregnancy using the data from food frequency questionnaires analysed in Hibbeln et al;[18] (b) maternal prenatal red blood cell fatty acids as described in Newson;[19] (c) the duration of breast feeding as assessed from a questionnaire administered at 6 months, categorised as none, < 4 months, 4 months or more; (d) the child's omega-3 intake from seafood as identified from food frequency questionnaires administered at ages 3 and 7; (e) the relationship with genetic variants in the FADS genes which are strongly related to levels of plasma fatty acids (rs numbers 174548, 174556 and 174561 from the FADS 1 gene; rs nos 3834458 and 968567 from the intergenic region between FADS 1 and FADS 2; rs nos. 174570, 1535, 174574, 174575, 2727271, 174576, 174578, 174579, 174602,498793 and 526126 from the FADS 2 gene; rs 174448 and 174449 from the intergenic region between FADS 2 and FADS 3; and rs174455 from FADS 3);[20] and (f) the child's plasma ((blood samples were obtained from the children aged about 7 years at a special ALSPAC clinic). From these samples, plasma was obtained after

centrifugal separation and frozen immediately. All samples were stored at -70°C, thawed once to obtain a 100 µL aliquot, shipped airfreight on dry ice to Rockville MD and thawed a second time for analysis. Transmethylation of lipids with acetyl chloride and methanol was performed using a simplified method based upon the Lepage and Roy procedure [21] using a high throughput automated method.[22] Internal calibration was conducted by adding internal standards to each assay. A second standard was used to quantify the exact amount of internal standard in every batch for ongoing assay of experimental variability. Freedom Evo Instrument 200 (TECAN Trading AG, Switzerland) was utilized for the automatic transmethylation and extraction of FAs employing the customized control and automation software (EVOware v2.0, SP1, Patch3). Gas chromatography 6890 Plus LAN system (Agilent Technologies, Inc., Santa Clara, CA) coupled with a fused-silica, narrow-bored DB-FFAP capillary column (Agilent 127-32H2, 15 m × 0.1 mm I.D. × 0.1 µm film thickness) which was used for chromatographic separation of the FAME as reported previously.[22] The assay was linear in the range of 1-600 µg/mL plasma. The within and between day imprecision was 3.26±1.2% and 2.95±1.6% for fatty acid concentrations. Assays were undertaken in 2009-2010. In all, 23 FAs were measured including 12 polyunsaturates. [Cord plasma was measured 2008-9]

Possible confounders

The following factors were taken into account in multivariable analyses: highest maternal educational achievement, categorised in 3 groups: low (< O level), medium (O level), high (A level or more); socioeconomic group as measured by the highest social class categorisation based on the occupation of the parents as recorded in pregnancy; parity of the mother at the time of delivery of the study child, measured as the number of previous pregnancies resulting in a live or stillbirth: 0, 1, 2+ ; maternal smoking in mid-pregnancy: none, 1-9, 10+ cigarettes per day; passive smoke exposure in childhood at 3 years of age, as assessed by the length of time the child was in a room where others were smoking: not at all, low-moderate, high ; the child's body mass index (BMI) at age 7 used as a continuous variable.

Statistical analysis

Differences in the proportions of children reporting growing pains at 8 years by possible confounding factors were examined using chi-squared tests. Comparisons of mean

fatty acid levels were performed using t-tests, comparing those with and without growing pains. Logistic regression was performed adjusting for those confounders that showed significant unadjusted associations. All fatty acids were analysed as a percentage of total fatty acids.

Results

Growing pains of children aged 8 were more likely to be reported by mothers with lower educational achievements, those of lower social class, lower parity, and those who smoked in pregnancy. Growing pains were also more likely in children exposed to environmental tobacco smoke (ETS) in childhood. There was no difference in prevalence of growing pains between the sexes, and the affected children had a similar mean BMI to those without such a history (Table 1). These results were similar to the results at other ages.

Table 1: Differences in potential confounding factors and fatty acid measures according to growing pains reported at 8.5 years

	No growing pains (78.6%)	Growing pains (21.4%)	P value
Gender			
Boy (51.0%)	3137 (78.6%)	855 (21.4%)	0.972
Girl (49.0%)	3018 (78.6%)	821 (21.4%)	
Maternal education			
< O level (23.0%)	1350 (77.3%)	397 (22.7%)	0.022
O level (35.5%)	2115 (78.5%)	579 (21.5%)	
>O level (41.5%)	2539 (80.5%)	616 (19.5%)	
Family social class			
I and II (29.4%)	1710 (80.4%)	417 (19.6%)	0.001
III NM (26.5%)	1545 (80.7%)	370 (19.3%)	
III M (26.9%)	1473 (75.7%)	473 (24.3%)	
IV and V (17.2%)	981 (79.4%)	254 (20.6%)	
Parity			
0 (46.3%)	2700 (77.0%)	805 (23.0%)	0.002
1 (35.9%)	2179 (80.0%)	545 (20.0%)	
2+ (17.8%)	1091 (80.9%)	258 (19.1%)	
Maternal smoking in pregnancy			
None (85.1%)	5189 (79.4%)	1344 (20.6%)	0.005
1-9 (7.3%)	421 (75.6%)	136 (24.4%)	
10+ (7.6%)	436 (74.8%)	147 (25.2%)	
Child ETS at age 3			
None (62.1%)	3592 (80.4%)	874 (19.6%)	<0.0001

	No growing pains (78.6%)	**Growing pains (21.4%)**	**P value**
Low- moderate (25.0%) High (12.9%)	1378 (76.8%) 701 (75.8%)	417 (23.2%) 224 (24.2%)	
Mean BMI (sd) at age 7	16.2 (2.0)	16.3 (2.1)	0.171

BMI = body mass index; ETS = Environmental tobacco smoke

In Table 2 unadjusted indicators of omega-3 and omega-6 fatty acid status are shown for the fetus and child. There were no unadjusted differences between the children with limb pain and those without in regard to the amount of omega-3 consumed as estimated from the mother's dietary intake, the duration of breast feeding, or the child's dietary intake at 3 or 6 years; additionally there were no differences in the plasma levels of key omega-3 and omega-6 fatty acids at 7 years, nor of the maternal prenatal red cell levels. No differences were seen in plasma levels of monosaturated or saturated fatty acids at any age.

Table 2: Unadjusted differences in measures of estimated dietary intake of omega-3 and mean blood levels according to growing pains at 8.5 years

	No growing pains	Growing pains	P-value
Maternal prenatal FFQ [mean (sd)]			
Total omega-3	0.15 (0.15)	0.16 (0.15)	0.614
DHA	0.07 (0.07)	0.07 (0.07)	0.593
EPA	0.05 (0.05)	0.05 (0.05)	0.654
Breastfeeding duration			
None (20.7%)	1172 (77.7%)	336 (22.3%)	0.193
Up to 3 months (31.2%)	1775 (78.2%)	495 (21.8%)	
> 3 months (48.2%)	2797 (79.7%)	712 (20.3%)	
Child FFQ at 3 years [mean (sd)]			
Total n3	0.06 (0.06)	0.06 (0.05)	0.443
DHA	0.02 (0.03)	0.03 (0.03)	0.449
EPA	0.02 (0.02)	0.02 (0.02)	0.439
Child FFQ at 6 years [mean (sd)]			
Total n3	0.08 (0.09)	0.09 (0.09)	0.173
DHA	0.03 (0.04)	0.03 (0.04)	0.217
EPA	0.02 (0.02)	0.02 (0.02)	0.233
Child plasma levels at 7 years[mean (sd)]			
AA (20:4n6)	6.45 (1.32)	6.36 (1.32)	0.087
DHA (22:6n3)	1.89 (0.52)	1.90 (0.53)	0.426
EPA (20:5n3)	0.64 (0.20)	0.64 (0.20)	0.797
LA (18:2n6)	30.6 (3.16)	30.7 (3.25)	0.511
ALA (18:3n3)	0.71 (0.28)	0.72 (0.27)	0.318
Maternal prenatal red cell levels [mean (sd)]			
AA (20:4n6)	6.16 (2.98)	6.11(2.97)	0.727
DHA (22:6n3)	2.32(1.36)	2.25(1.31)	0.215
EPA (20:5n3)	0.27 (0.18)	0.27 (0.17)	0.331
LA (18:2n6)	11.10 (2.70)	10.98 (2.82)	0.610
ALA (18:3n3)	0.14 (0.07)	0.14 (0.07)	0.676

FFQ = food frequency questionnaire; DHA = decosahexaenoic acid; EPA = eicosapentaenoic acid
AA – arachidonic acid; LA = linoleic acid; ALA = alpha-linolenic acid

Reversing the calculations, Table 3 demonstrates the odds ratios (ORs) for developing growing pains given the level of exposure to fatty acids, adjusting for the various factors in Table 1. Of the 20 factors tested only one showed some evidence of a statistically significant association – there was a slight increase in the risk of growing pains with each unit increase in the child's plasma omega-6 linoleic acid levels [OR = 1.02; 95% CI 1.00,1.05]. There was

no evidence of any linear relationships between growing pains and the number of alleles of any of the 17 genetic variants examined (data not shown).

Table 3: Unadjusted and adjusted odds ratios (95% confidence intervals) for growing pains reported at 8.5 years according to various fatty acid measures

	Unadjusted	Adjusted*
Breastfeeding duration		
None	1.00 Reference	1.00 Reference
Up to 3 months	0.97 (0.83, 1.14)	1.00 (0.82, 1.22)
> 3 months	0.89 (0.77, 1.03)	0.95 (0.78, 1.16)
Maternal FFQ in pregnancy		
Total omega-3	1.10 (0.76, 1.60)	1.26 (0.81, 1.97)
DHA	1.24 (0.56, 2.74)	1.69 (0.66, 4.34)
EPA	1.30 (0.42, 4.05)	1.94 (0.50, 7.52)
Child FFQ at 3 years		
Total omega-3	1.48 (0.54, 4.06)	1.85 (0.57, 6.01)
DHA	2.26 (0.27, 18.57)	4.35 (0.37, 50.78)
EPA	3.96 (0.12, 129.4)	9.73 (0.17, 568.9)
Child FFQ at 6 years		
Total omega-3	1.55 (0.83, 2.91)	1.62 (0.79, 3.36)
DHA	2.70 (0.56, 13.04)	3.12 (0.51, 19.22)
EPA	4.37 (0.39, 49.32)	5.07 (0.31, 83.48)
Child plasma levels at 7 years		
AA (20:4n6)	0.95 (0.90, 1.01)	0.97 (0.91, 1.03)
DHA (22:6n3)	1.06 (0.92, 1.22)	1.13 (0.97, 1.32)
EPA (20:5n3)	1.05 (0.73, 1.50)	1.15 (0.77, 1.70)
LA (18:2n6)	1.01 (0.99, 1.03)	1.02 (1.00, 1.05)
ALA (18:3n3)	1.14 (0.88, 1.47)	1.16 (0.87, 1.55)
Maternal prenatal red cell levels		
AA (20:4n6)	1.00 (0.97,1.02)	0.99(0.95,1.02)
DHA (22:6n3)	0.96 (0.90,1.02)	0.95 (0.88,1.03)
EPA (20:5n3)	0.78 (0.47,1.29)	0.63 (0.34,1.17)
LA (18:2n6)	0.99 (0.96,1.02)	0.99 (0.95,1.03)
ALA (18:3n3)	0.76 (0.21,2.75)	0.40 (0.09,1.83)

*Adjusted for gender, maternal education, family social class, parity, maternal smoking, childhood environmental tobacco smoke

AA = arachidoic acid; ALA = alpha-linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LA = linoleic acid; FFQ = food frequency questionnaire

We also explored gender differences in the associations. No differences were observed in fatty acid relationships between boys and girls or for the associations with the

FADS genetic variants assuming an additive genetic model. However when a recessive model for the minor allele was explored, 47% of the 34 analyses prior to age 7 years showed significant associations for girls compared to 2.9% in boys. A formal interaction test suggested many of these differences could have occurred by chance but nevertheless 6 of these differences (18%) were nominally significant. After 7 years, differences in the associations by gender persisted to a lesser extent but none exceeded the formal requirements for an interaction.

Discussion

Growing pains may have deleterious effects on the child's quality of life. For example a German study of children aged 5 – 18 showed that 35% of those with limb pain had been absent from school in consequence, 36% had disturbed sleep and 55% were unable to pursue hobbies.[23] Consequently any ways in which the occurrence of these pains can be avoided is likely to have major benefits on the children's quality of life. Omega-3 fatty acids were hypothesised to be candidate substances that might ameliorate or prevent such pains. However we could find no convincing evidence here that fatty acids have such a part to play in reducing the risk of limb pain at 8 years of age.

In this study we have taken account of factors such as exposure to cigarette smoke, whether *in utero* or in childhood, socio-economic status and maternal education level, the child's gender and BMI. Neither the unadjusted markers of fatty acid exposure, nor those demonstrated after adjustment showed more statistically significant results than would be expected by chance. This applied to the duration of breast feeding, the child's dietary exposure as estimated from food frequency questionnaires of the mother during pregnancy and of the child at ages 3 and 6 years. In addition the levels of 5 different omega-3 and omega-6 fatty acids measured in the mothers' blood in pregnancy, and in the child's blood at age 7 years, were not associated with limb pain.

It is well documented that any dietary effects shown can be artefacts associated with socioeconomic and personality factors, and the same can be true for lack of associations. We therefore have used the Mendelian randomisation approach to test this further.[24] We considered 17 genetic variants from the FADS1, 2 and 3 genes and their intergenic markers. We have already shown that 14 of the minor alleles of these markers are strongly negatively associated with the blood levels of DHA, and all 17 are associated with the ratios of AA to LA (omega-6) and of EPA to ALA (omega-3).[20] Thus, if there were an association of

blood levels between fatty acids and growing pains, one would expect it to be demonstrated by an association with one or more of the genetic variants. No such relationships were found with the number of minor alleles. Some differences were observed in girls using a recessive model. These results might suggest that pre-pubertal girls homozygous for the minor allele tend to have lower levels of long chain fatty acids below a requirement to alleviate limb pains. Irrespective of its validity, the increasing (not decreasing) prevalence of limb pain with age in girls and the absence of any genetic associations in boys suggest a different aetiology for the majority of children.

The major strength of this study concerns the large sample size, having objective measures of fatty acid levels from the mother during pregnancy, and of the child at 7 years of age, availability of genetic markers related to the FADS1, 2 and 3 genes, and the information on the diet of mother prenatally and of the child at various time points. Here we used the duration of breast feeding, and the diet of the child at ages 3 and 7, the latter being the closest age at which we had collected dietary information to that of the child's report of growing pains used in this study. The data also benefits from the prospectively collected study design, thus avoiding the biases inherent in retrospective questioning.

A possible weakness concerns the fact that the data were provided by the parents using questions that did not allow the complete categorisation of 'growing pains' using the Peterson criteria [17]. The child him/herself is the only person who would be able to accurately reveal such a history. However it is likely to only be parents of 8-year-olds from the most dysfunctional families that would be oblivious to their child's pain. Such families are likely to be poor at responding in general, and are unlikely to have made a detectable difference to the results reported here.

In conclusion, we could find no strong evidence to indicate that variations in fatty acids would prevent the occurrence of growing pains in children. That is not to say, however, that omega-3 fatty acids might not be beneficial as a treatment for such pains, just as they seem to be for rheumatoid arthritis.[8]

What is already known on this topic:

- 'Growing pains' are common complaints in childhood and adolescence.
- No large studies have been undertaken to determine whether diet, particularly omega-3 fatty acids, might have a preventive role.

What this study adds

- No evidence was found that components of the diet or blood levels of fatty acids either prenatally or childhood influence the development of growing pains.
- Minimal associations were found with the genetic variants in the FADS genes using a Mendelian randomisation approach.
- We conclude that dietary intake of omega-3 fatty acids is unlikely to prevent the development of growing pains in childhood.

Acknowledgements

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

Ethics

Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees.

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STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any pre-specified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	2
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	2-3
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3,5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	3,5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3, 5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-6*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	3,5-6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5,6-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-10
		(*b*) Describe any methods used to examine subgroups and interactions	7-10
		(*c*) Explain how missing data were addressed	N/A
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	N/A

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	3
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	3
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	N/A
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	3,5-6,8-10
		Cross-sectional study—Report numbers of outcome events or summary measures	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	7-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7,11-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	N/A
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.