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Pregnancy Diet and Offspring Asthma risk over a 10-year period

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ABSTRACT:

Objective

The association of maternal pregnancy diet with offspring asthma risk have been reported. However, literature on longitudinal patterns of asthma risk relative to intra-uterine nutrient exposure is limited. We aimed to establish whether vegetable, oily fish and vitamin D intake during pregnancy influence childhood asthma risk over a 10 year period.

Design

Mother-child pairs (n=897) from the Lifeways prospective birth cohort, with data on nutrient intake during pregnancy and asthma status respectively, were eligible for inclusion in the analysis. Data on socio-economic and morbidity indicators over 10 years of follow-up on mothers and the index child were collected through self-administered questionnaires. Asthma status as diagnosed by the GP at any time-point over 10 years was related to maternal vegetable, oily fish and vitamin D intake during pregnancy, while adjusting for gestational age, socio-economic status, smoking at delivery, breast-feeding, season of birth and supplement use. Data were modelled with a Generalised Linear Mixed Model (GLMM); personal id was modelled as random effect with random intercepts and slopes over time for individuals.

Results

In the fully adjusted GLMM, higher daily average intake of oily fish was significantly protective of asthma risk (OR 0.13, 95% CI 0.02-0.86); vegetable intake was non-significant (OR 0.95, 95% CI 0.87-1.04). A higher daily vitamin D intake significantly reduced the odds of asthma (OR 0.93, 95% CI 0.89-0.97).

Conclusion

This analysis suggests higher daily average intake of oily fish and vitamin D in pregnancy to be protective of asthma risk in offspring at any time-point over a 10 year follow-up period.

STRENGTHS

- The prospective design with an a priori purpose of examining intergenerational transmission of risk.
- As there is a strong social gradient applied to diet in pregnancy, confounding by socio-demographic factors and lifestyle was a concern; we controlled comprehensively for various socio-demographic factors
- The GLMM enabled us to control for repeated measurements over time for participants

LIMITATIONS

- Limitations were differential follow-up, leading to varied numbers of missing data for certain variables, potentially introducing bias to follow-up data. Countering this, it is notable that the same associations were found separately at the three different time points.
- The authors recognise that asthma in childhood is a heterogeneous phenotype and that grouping of the 3 time-points could potentially mask different phenotypes; however our study was not powered to differentiate asthma on phenotype at various follow-up.
- Data on familial atopy or asthma were not available; it is possible that the suggested relationships between maternal diet and offspring asthma have been confounded by the presence or absence of familial predisposition.
- Data on foetal growth parameters were not available; the effect of foetal growth on childhood asthma could not be controlled for.

INTRODUCTION

Asthma is the most common chronic disease of childhood;(1-3) reports indicate a continuous and consistent increase in worldwide prevalence, especially in westernised societies. Prevalence rates in the United Kingdom and Ireland are among the highest in Europe.(4-11) According to the Centres for Disease Control and Prevention, between 2001 and 2010 asthma prevalence in children in the United States increased 1.4% each year.(12) This increase is most likely multi-factorial, with complex interactions of genetic-immunological-environmental factors leading to the phenotypic expression of disease.(5) Recently multiple studies have attempted to deconstruct this multifactorial relationship, focusing on the change

in dietary habits over recent decades.(13-19) The progressive trend in early presentation of allergic disease in childhood, with the implication of possible exposure in utero, has placed an emphasis on maternal pregnancy diet as a prominent factor in the development of offspring asthma.(20, 21) As allergen-specific immune responses are established in foetal life, maternal nutrient intake during pregnancy is pivotal; intake may potentially influence the development of both the innate and acquired immune responses, predisposing to atopy in later life.(22)

A growing body of persuasive epidemiological evidence suggests that deficiency of maternal vitamin D intake prenatally has an inverse relationship with atopic disease in childhood.(23-28) Observational studies on the association of maternal serum and/or infant cord blood 25-hydroxy-vitamin D_3 ($25(OH)D_3$) levels with atopic markers are conflicting. Some demonstrated similar inverse associations;(29-36) others demonstrated direct (37-39) and null (40-48) associations. U-shaped associations were suggested by both Rothers *et al* (49) and Maslova *et al*.(50) Most recently intervention studies exploring pregnancy vitamin D supplementation and asthma risk at 3 years of age suggested a protective effect (statistically non-significant). The authors suggested that longer follow-up of children is needed to determine the clinical importance of findings.(51, 52)

The Lifeways study has previously reported the association between pregnancy intake of oily fish and vegetables and General Practitioner (GP) diagnosed asthma in offspring at age 3 years; a higher daily mean intake suggested a significant protective effect.(53) Literature on pregnancy consumption of fish with subsequent atopic risk in offspring is mostly consistent with our findings, indicating a protective effect.(13, 54-60) Our current aim was twofold: firstly to build on the aforementioned Lifeways findings and test the hypothesis that a higher pregnancy intake of vegetables and oily fish might be protective of asthma risk at any stage over 10 years follow-up; secondly, as suggested in the literature, to explore the association of pregnancy vitamin D intake and offspring asthma risk within our cohort at any stage over 10 years of follow-up.

METHODS

Study design and sample selection

The Lifeways study was established 2001-2003 as a prospective birth cohort in the Republic of Ireland. The *a priori* purpose was to examine determinants of health status in children, including diet and lifestyle, and to establish patterns and links across generations. Recruitment, data collection and study instruments have previously been discussed in

detail.(61, 62) In brief, mothers were recruited at first ante-natal visit (14-16 weeks gestation) in one of two regional maternity hospitals in the more rural West (Galway) and the more industrialised East (Dublin). Of 1124 mothers recruited, 1082 gave birth resulting in 1096 live mother-child pairs. Analysis was limited to current live mother-child pairs, where data on the proband's asthma status were available for at least one time-point. Babies with congenital anomalies and delivery <34 weeks gestational age (63) were excluded from analysis. Due to attrition over time participants had differential patterns of follow-up through phases; 614 mother-child pairs were included in the year 3 analyses, 511 in year 5 and 432 in year 10 follow-up. The sample for this analysis of 897 mother-child pairs comprised respondents for whom at least one follow-up point of asthma health status was recorded. Ethical approval for all phases of the study was granted by the Human Research Ethics Committee, University College Dublin, Ireland.

Assessment of pregnancy diet

Data on maternal nutrient intake during pregnancy were captured by a semi-quantitave Food Frequency Questionnaire (FFQ) as part of a self-administered questionnaire to the mother at her first ante-natal visit. The FFQ was developed from the international version used in the European Prospective Investigation in Cancer studies by the National Nutritional Surveillance Centre and extensively validated for use in an Irish population by the National University of Ireland, Galway. (64) The main food groups regularly consumed in the Irish diet were included, and consisted of 149 food items.(53, 65) The questionnaire focused on maternal dietary intake since pregnant. Intake as a medium serving (detailed in the FFQ for relevant food items) was recorded on a 9-grade scale, with categories subsequently transformed to continuous daily portion averages for all 149 food items. To arrive at distinct food groups, the continuous intake of various food items were summed and reported as total servings per day (Supplement 1). The daily average intake of energy and nutrients was calculated by linking frequency selections from the FFQ with food equivalents in McCance and Widdowson's nutritional composition database, 6th edition,(66) using software developed specifically for the Lifeways database.(62) Vitamin D was reported in micrograms per day (µg/day); energy as kilocalories (kCal/day).

Assessment of outcome

Data on doctor diagnosed asthma in offspring were collected at 3 time-points: ages 3, 5 and 9 years. Various studies have used doctor diagnosed asthma/parental report of doctor diagnosed

asthma to ascertain diagnosis.(36, 51) Our questions on asthma were adapted from the validated International Study on Asthma and Allergies in Childhood (ISAAC) questionnaire.(67, 68) Diagnosis from age 3 and 9 follow-up were reported by the General Practitioner (GP), information on GP diagnosed asthma at age 5 was obtained from the mother. For the univariate analysis, asthma as a dichotomous outcome variable ('Yes' vs 'No') at each of the three time-points were analysed separately. For the multivariable analysis a composite, dichotomous outcome variable was created; it described doctor diagnosed asthma in the child at any of the 3 time-points over a period of 10-year follow-up, versus never asthma.(69)

Statistical analysis

Definite doctor diagnosed asthma in offspring at any time point over 10-year follow-up, versus never, was related to maternal pregnancy intake of oily fish, vegetables and vitamin D.

Uni-variate analysis and Covariates

Variables that could potentially confound the diagnosis of asthma were identified from the literature. To identify significance of these variables in our cohort the odds of an asthma diagnosis at the specific time-point of the 3 follow-up phases were tested against identified background variables using unadjusted binomial logistic regression and the independent t-test. Predictors with $p<0.1$ were identified as being significant for inclusion in the multivariable models and are discussed below.

Total Energy Intake (EI)

To control for variance in accuracy of maternal energy reporting, total energy intake (kCal) was adjusted for in all models. Nutrients were adjusted for total energy intake using the residual method.(70, 71) To account for mothers who potentially over- or under reported energy intake, the ratio of energy intake (EI) to basal metabolic rate in pregnancy (BMR_{preg}) was calculated to identify extreme outliers.(69, 72, 73)

Gestational age

Gestational age in weeks.

Socio-economic status

We used eligibility for the General Medical Scheme (GMS) as an indicator of socio-economic status. In the Republic of Ireland this is a robust means tested indicator.(74-76) As maternal

and offspring GMS eligibility is strongly correlated only the maternal predictor was used in the models.(69, 77)

Smoking

Smoking status at time of delivery; hospital delivery records.

Breastfeeding

Data from the self-administered maternal questionnaire; 'Was your Lifeways child ever breastfed?'

Season of birth

Seasons were comprised as follows: Summer (May, June, July), Autumn (Aug, Sept, Oct), Winter (Nov, Dec, Jan), Spring (Feb, March, April). This follows the grouping suggested by the Irish ROLO study with data on maternal serum 25(OH)D levels.(78) Summer and Autumn were collapsed to form 'Summer', with Spring and Winter collapsed to form 'Winter', making up the final dichotomous variable, 'Summer' vs 'Winter'.

Supplement use

Data on supplement use were generic: 'Have you taken any vitamins, minerals or food supplements?' 'Yes' or 'No' and did not allow for specification on supplement type or content quantification.

Multivariable analysis

The aforementioned association was analysed in the multivariable models. Vegetables, oily fish and vitamin D intake were analysed as continuous variables. Multivariable analysis was done using the full sample of 897 mother-child pairs in a Generalised Linear Mixed Model (GLMM). Covariates were entered into the GLMM sequentially as fixed factors based on significance at univariate level. Time was consistently included as a fixed factor. Personal id was modelled as a random effect with random intercepts and slopes over time for each individual. Predictor variables with $p<0.05$ were regarded as significant. The Log Pseudo-likelihood as a measure of model fit was used to compare the models. A best fit model for vegetable, oily fish and vitamin D intake each was selected by evaluating the log pseudo-likelihood; the lower the number the better the model fit.

The Statistical Package for the Social Sciences (SPSS) version 20 was used to conduct univariate analysis; multivariable analysis was done using Statistical Analysis Software (SAS) version 9.3.

RESULTS

Study subjects' characteristics and asthma prevalence

In the final sample, 66.9% of the mother-child pairs were resident in the Dublin area and 33.1% in the Galway area, proportionate to recruitment patterns. Mothers with a 3rd level education were marginally higher (51.9%) than those with None/Primary/Secondary school education (48.1%); 19.4% of mothers were smokers at the time of giving birth. The mean (SD) age of mothers at time of giving birth was 30.2 (5.9) years and the mean (SD) pre-pregnancy body mass index of mothers were 23.7 (4.0). The offspring sex distribution was about equal; 48.8% males and 51.2% females. The mean (SD) birth-weight was 3515.3 (568.6) grams, with a mean (SD) gestational age of 39.9 (1.9) weeks. Just under half (45.8%) of probands were the first born and 86.20% were delivered vaginally. Doctor diagnosed asthma in offspring at the 3 phases of follow-up respectively, increased from 10.90% at 3-years, to 14.33% at 5-years and 23.10% at 10-years follow-up. Maternal socio-economic and biological characteristics relative to food group intake are presented in Table 1. Maternal and child characteristics as related to asthma diagnosis are further presented in supplemental material (Table S1 and S2).

Univariate associations of background variables with childhood asthma from the cross-sectional data for the 3 follow-up phases respectively have previously been discussed in detail.(53, 68, 69)

Table 1 Maternal food group intake during pregnancy in relation to socio-economic factors (n=897)

	Food group					
	n[1]	Oily fish mean (SD)	p-value	n[1]	Vegetables mean (SD)	p-value
Education			<0.001[a]			<0.001[a]
None/Primary/Secondary school	416	0.05 (0.10)		420	2.61 (1.88)	
3rd level education	451	0.07 (0.13)		451	3.26 (2.16)	
Marital status (baseline)			0.01[a]			<0.001[a]
Lone	179	0.04 (0.09)		180	2.51 (2.06)	
Cohabiting	701	0.06 (0.12)		703	3.05 (2.03)	
GMS Eligibility (baseline)			<0.001[a]			<0.001[a]
No	750	0.06 (0.12)		754	3.02 (2.06)	
Yes	131	0.03 (0.07)		131	2.43 (1.91)	
Smoking status at delivery			0.07[a]			<0.001[a]
Non-smoker	693	0.06 (0.12)		695	3.04 (2.10)	
Smoker	167	0.04 (0.11)		169	2.39 (1.61)	
Region			0.04[a]			<0.001[a]
Galway	292	0.07 (0.13)		294	3.21 (2.17)	
Dublin	594	0.05 (0.10)		596	2.79 (1.96)	
Age group at delivery (y)			<0.001[b]			<0.001[b]
Under 18	16	0.02 (0.04)		16	2.52 (1.51)	
18 to 29	373	0.03 (0.07)		377	2.56 (1.92)	
30+	491	0.08 (0.14)		492	3.22 (2.09)	

[a]Independent samples t-test [b]ANOVA GMS=General Medical Scheme
[1]Numbers do not always add up to 897 because of varied numbers of missing data for some variables

Within-time representativeness of responders vs. non-responders that contributed to the final sample is presented in Table 2.

Table 2 Within-time representativeness of final sample (n=897)

Characteristics	Responders[a] n=614	Non-responders[a] n=283
Year 3 follow-up		
Maternal educational attainment[c]		
None/Primary/Secondary school, No. (%)	290 (48.6)	130 (47.1)
Some/Completed 3rd level education, No. (%)	307 (51.4)	146 (52.9)
Maternal GMS eligibility at baseline[c]		
Not eligible, No. (%)	512 (84.3)	244 (87.1)
Eligible, No.(%)	95 (15.7)	36 (12.9)
Region[c]		
West, No. (%)	217 (35.3)	80 (28.3)
East, No. (%)	397 (64.7)	203 (71.7)
Maternal age at birth of proband, mean (SD), y[c]	30.35 (5.9)	29.75 (5.9)
Maternal oily fish intake in pregnancy, mean (SD), servings/d[c]	0.06 (0.1)	0.05 (0.1)
Maternal Vegetable intake in pregnancy, mean (SD), servings/d[c]	2.95 (1.9)	2.88 (2.2)
Maternal Vitamin D intake in pregnancy, mean (SD), μg/d[c]	4.70 (4.8)	5.60 (10.2)
Year 5 follow-up	n=511	n=386
Maternal educational attainment[c]		
None/Primary/Secondary school, No. (%)	241 (48.7)	179 (47.4)
Some/Completed 3rd level education, No. (%)	254 (56.1)	199 (52.6)
Maternal GMS eligibility at baseline[c]		
Not eligible, No. (%)	429 (85.0)	327 (85.6)
Eligible, No. (%)	76 (15.0)	55 (14.4)
Region[c]		
West, No. (%)	165 (32.3)	132 (34.2)
East, No. (%)	346 (67.7)	254 (65.8)
Maternal age at birth of proband, mean (SD), y[c]	30.10 (5.8)	30.24 (5.9)
Maternal oily fish intake in pregnancy, mean (SD), servings/d[c]	0.06 (0.1)	0.06 (0.1)
Maternal Vegetable intake in pregnancy, mean (SD), servings/d[c]	2.93 (2.1)	2.94 (1.9)
Maternal Vitamin D intake in pregnancy, mean (SD), μg/d[c]	4.70 (6.1)	5.30 (7.9)
Year 10 follow-up	n=432	n=465
Maternal educational attainment[b]		
None/Primary/Secondary school, No. (%)	174 (40.7)	246 (55.2)
Some/Completed 3rd level education, No. (%)	253 (59.3)	200 (44.8)
Maternal GMS eligibility at baseline[b]		
Not eligible, No. (%)	385 (89.7)	371 (81.0)
Eligible, No. (%)	44 (10.3)	87 (19.0)
Region[b]		
West, No. (%)	166 (38.4)	131 (28.2)
East, No. (%)	266 (61.6)	334 (71.8)
Maternal age at birth of proband, mean (SD), y[b]	31.55 (5.2)	28.86 (6.1)
Maternal oily fish intake in pregnancy, mean (SD), servings/d[c]	0.06 (0.1)	0.06 (0.1)
Maternal Vegetable intake in pregnancy, mean (SD), servings/d[c]	3.11 (2.1)	2.76 (2.0)
Maternal Vitamin D intake in pregnancy, mean (SD), μg/d[c]	5.00 (7.5)	4.90 (6.4)

[a]Numbers do not always add up to total as varied numbers of missing data at certain variables

[b]p<0.001 [c]Non-significant

μg: micrograms SD:Standard Deviation d:day y:years

Pregnancy dietary intake

Reported intake of vegetables and oily fish were directly and significantly associated with the maternal social gradient (Table 1). Mean (SD) daily intake of oily fish and vegetables were 0.06 (0.12) and 2.93 (2.04) portions respectively. The Food Safety Authority of Ireland (FSAI) recommends that pregnant women should have one portion of oily fish weekly and 6-7 portions of vegetables daily.(79) The mean intake of Lifeways mothers did not reach this recommendation.

Vitamin D intake during pregnancy between those mothers with consistent follow-up, and those with attrition did not differ significantly. A social gradient for pregnancy vitamin D intake in the final sample was not observed. The distribution of mean vitamin D intake was investigated according to the EI/BMR_{preg} ratio; no marked difference in the distribution of vitamin D was observed.(69) Energy adjusted mean (SD) intake of vitamin D in mothers was 4.3 (4.1) µg/d. The FSAI recommends a daily allowance of 10µg/day vitamin D for Irish pregnant women.(80). (Table S3 and S4). At baseline 327 (36.9%) mothers from the final sample reported using supplements.

Pregnancy dietary associations with offspring asthma

Vegetable intake was negatively associated with offspring asthma, although not significantly so in the best fit, fully adjusted model (OR 0.95, 95% CI 0.87-1.04) (Model 6) (Table 3).

Oily fish intake was significantly associated with offspring asthma in the fully adjusted model (Model 6), with an increase in daily average serving of oily fish suggesting a protective effect at any time point over the 10 year follow-up period (OR 0.12, 95% CI 0.02-0.83) (Table 4). Further adjustment for season of birth, vitamin D intake and supplement use (Models 7-9) saw oily fish remaining protective, however the Log Pseudo-likelihood increased suggesting goodness of fit for the models decreased.

The fully adjusted, best fit model (Model 6), suggested vitamin D to have a significant inverse association (OR 0.93, 95% CI 0.87-0.97) with offspring asthma at any time-point of follow-up (Table 5). Further adjustment for season of birth, oily fish intake and supplement use (Models 7-9) suggested an ongoing, significant protective effect of vitamin D, however the Log Pseudo-likelihood increased suggesting goodness of fit for the models decreased.

Table 3 Generalised linear mixed model: asthma at any time-point vs. never (vegetable intake as main exposure of interest)

Independent variable		**OR (95% CI)**							
		Model 1 n=890	Model 2 n=883	Model 3 n=781	Model 4 n=777	Model 5 n=756	Model 6 n=682	Model 7 n=682	Model 8 n=677
Time	Year 3 follow-up	0.40 (0.29-0.57)	0.41 (0.29-0.57)	0.37 (0.26-0.54)	0.36 (0.25-0.53)	0.36 (0.25-0.53)	0.36 (0.24-0.54)	0.37 (0.54-0.54)	0.36 (0.24-0.53)
Ref: Year 10 follow-up	Year 5 follow-up	0.54 (0.39-0.76)	0.54 (0.39-0.76)	0.49 (0.34-0.71)	0.49 (0.34-0.71)	0.49 (0.32-0.68)	0.48 (0.33-0.71)	0.48 (0.33-0.71)	0.48 (0.33-0.71)
Vegetable intake (serving/d)		0.93 (0.86-0.99)	0.93 (0.86-0.99)	0.94 (0.87-1.02)	0.95 (0.87-1.03)	0.95 (0.86-1.03)	0.95 (0.87-1.04)	0.95 (0.87-1.04)	0.95 (0.87-1.04)
Total EI (kCal)			1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)
Gestational Age (weeks)				0.92 (0.85-0.99)	0.92 (0.85-0.99)	0.93 (0.86-1.00)	0.93 (0.85-1.00)	0.92 (0.85-1.00)	0.93 (0.85-1.00)
GMS Eligibility (baseline)	No				0.80 (0.51-1.25)	0.84 (0.53-1.34)	0.99 (0.59-1.67)	0.98 (0.58-1.63)	0.98 (0.58-1.64)
Ref: Yes									
Smoking at delivery	Non-smoker					1.07 (0.69-1.64)	1.23 (0.77-1.95)	1.24 (0.78-1.99)	1.24 (0.78-1.99)
Ref: Smoker									
Breastfeeding	No						1.25 (0.90-1.73)	1.27 (0.91-1.78)	1.28 (0.91-1.77)
Ref: Yes									
Season of birth	Summer							1.41 (0.99-1.99)	1.41 (0.99-1.99)
Ref: Winter									
Supplement use	No								1.71 (0.83-1.66)
Ref: Yes									
Log Pseudo-likelihood		7627.72	7587.68	6874.38	6841.02	6621.85	6077.47	6094.97	6052.34

EI=Energy Intake GMS=General Medical Scheme

Table 4 Generalised linear mixed model: asthma at any time-point vs. never (oily fish intake as main exposure of interest)

Independent variable		OR (95% CI)								
		Model 1 n=886	Model 2 n=879	Model 3 n=778	Model 4 n=774	Model 5 n=753	Model 6 n=678	Model 7 n=678	Model 8 n=753	Model 9 n=673
Time	Year 3 follow-up	0.41 (0.29-0.58)	0.42 (0.30-0.59)	0.38 (0.26-0.55)	0.37 (0.26-0.54)	0.37 (0.25-0.54)	0.37 (0.25-0.55)	0.37 (0.25-0.56)	0.36 (0.24-0.54)	0.35 (0.24-0.52)
Ref: Year 10 follow-up	Year 5 follow-up	0.54 (0.39-0.77)	0.55 (0.39-0.77)	0.49 (0.35-0.72)	0.50 (0.35-0.72)	0.47 (0.32-0.69)	0.49 (0.33-0.72)	0.49 (0.33-0.73)	0.48 (0.32-0.71)	0.48 (0.32-0.71)
Oily fish intake (serving/d)		0.11 (0.02-0.60)	0.11 (0.02-0.61)	0.15 (0.03-0.84)	0.16 (0.03-0.96)	0.15 (0.03-0.87)	0.12 (0.02-0.83)	0.13 (0.02-0.86)	0.15 (0.02-0.99)	0.16 (0.03-1.05)
Total EI (kCal)			1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)
Gestational Age (weeks)				0.92 (0.85-0.99)	0.92 (0.85-0.99)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.93 (0.86-1.01)
GMS Eligibility (baseline)	No				0.82 (0.53-1.28)	0.87 (0.54-1.39)	1.03 (0.62-1.73)	1.01 (0.60-1.69)	0.94 (0.56-1.58)	0.94 (0.56-1.59)
Ref: Yes										
Smoking at delivery	Non-smoker					1.06 (0.69-1.63)	1.23 (0.76-1.96)	1.2 (0.78-2.03)	1.26 (0.78-2.03)	1.26 (0.78-2.02)
Ref: Smoker										
Breastfeeding	No						1.22 (0.87-1.70)	1.23 (0.76-1.97)	1.25 (0.89-1.75)	1.26 (0.90-1.76)
Ref: Yes										
Season of birth	Summer							1.42 (1.00-2.02)	1.39 (0.89-1.99)	1.39 (0.89-1.98)
Ref: Winter										
[a]**Vitamin D** (μg/d)									0.93 (0.89-0.98)	0.93 (0.89-0.97)
Supplement use	No									1.19 (0.84-1.68)
Ref: Yes										
Log Pseudo-likelihood		7620.85	7579.16	6863.30	6828.52	6614.40	6069.88	6085.90	6138.38	6102.47

EI=Energy Intake [a]Energy Adjusted GMS=General Medical Scheme

Table 5 Generalised linear mixed model: asthma at any time-point vs. never (vitamin D intake as main exposure of interest)

Independent variable		OR (95% CI)								
		Model 1 n=890	Model 2 n=890	Model 3 n=787	Model 4 n=779	Model 5 n=758	Model 6 n=683	Model 7 n=681	Model 8 n=678	Model 9 n=677
Time	Year 3 follow-up	0.41 (0.29-0.57)	0.41 (0.29-0.57)	0.37 (0.25-0.53)	0.36 (0.25-0.52)	0.36 (0.24-0.52)	0.35 (0.23-0.52)	0.35 (0.24-0.52)	0.36 (0.24-0.54)	0.35 (0.24-0.52)
Ref: Year 10 follow-up	Year 5 follow-up	0.55 (0.39-0.77)	0.55 (0.39-0.77)	0.49 (0.34-0.71)	0.49 (0.34-0.71)	0.46 (0.32-0.67)	0.47 (0.32-0.69)	0.47 (0.32-0.70)	0.48 (0.32-0.71)	0.48 (0.32-0.71)
[a]**Vitamin D** (μg/d)		0.96 (0.93-0.99)	0.96 (0.93-0.99)	0.96 (0.92-0.99)	0.95 (0.92-0.99)	0.95 (0.92-0.99)	0.93 (0.87-0.97)	0.93 (0.89-0.97)	0.93 (0.89-0.98)	0.93 (0.89-0.97)
Total EI (kCal)			1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)
Gestational Age (weeks)				0.92 (0.85-0.99)	0.92 (0.85-0.99)	0.90 (0.86-1.01)	0.93 (0.86-1.00)	0.93 (0.85-1.00)	0.93 (0.86-1.01)	0.93 (0.86-1.01)
GMS Eligibility (baseline)	No				0.74 (0.47-1.16)	0.77 (0.48-1.24)	0.91 (0.55-1.52)	0.89 (0.53-1.49)	0.94 (0.56-1.58)	0.94 (0.56-1.59)
Ref: Yes										
Smoking at delivery	Non-smoker					1.04 (0.67-1.59)	1.25 (0.78-1.99)	1.24 (0.77-1.99)	1.26 (0.78-2.03)	1.26 (0.78-2.02)
Ref: Smoker										
Breastfeeding	No						1.32 (0.95-1.84)	1.33 (0.96-1.85)	1.25 (0.89-1.75)	1.26 (0.90-1.76)
Ref: Yes										
Season of birth	Summer							1.40 (0.99-1.98)	1.40 (0.98-1.99)	1.39 (0.98-1.98)
Ref: Winter										
Oily fish intake (serving/d)									0.15 (0.02-0.99)	0.16 (0.03-1.05)
Supplement use	No									1.19 (0.84-1.68)
Ref: Yes										
Log Pseudo-likelihood		7646.41	7666.29	6965.61	6894.82	6678.52	6151.79	6158.31	6138.38	6127.49

EI=Energy Intake [a]Energy Adjusted GMS=General Medical Scheme

DISCUSSION

Birth cohort studies with follow-up of 6 years or more on the association of maternal pregnancy diet and childhood asthma are limited.(81) This analysis suggests that higher vitamin D intake during pregnancy is significantly associated with reduced likelihood of asthma in offspring at any point during 10-years follow-up. A significant inverse association with oily fish consumption was also demonstrated. Although vegetable intake suggested an initial protective effect, significance was lost early in model progression. Literature on the association with vegetable intake is scarce and inconsistent, with both an increased risk (82) and no effect reported.(83) Literature on the association of oily fish intake in pregnancy with offspring atopic disease is consistent with findings from the current analysis.(55, 57, 82, 83) Analyses from all of these studies were limited to children in the age range 2-6 years; to our knowledge there are no studies with findings extending up to 10 years of age follow-up. A systematic review (84) concluded that there is little evidence to recommend supplementation/modification of diet to include fish oil for children or adults with established asthma. They found no evidence of improvement or increased risk relative to fish oil and established asthma. This is likely evidence that the window period for fish oil to have an effect on immune regulation is most relevant in foetal life, with limited potential for effect once immune responses are established.(83) The pro-inflammatory mediators leukotriene B4 (LTB_4) and tumour necrosis factor alpha (TNF-α) increase airway inflammation and hyper-responsiveness.(85, 86) The protective effect of oily fish could possibly be mediated via eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA). Competitive binding with cyclooxygenase (COX) and lipoxygenase (LOX) reduce LTB_4 and PGE_2 production.(55, 87-89)

Our findings suggest a significant inverse relationship of pregnancy vitamin D consumption with childhood asthma. Results are consistent with the main body of literature on the association of pregnancy vitamin D intake and offspring atopic disease.(23-28) Miyake *et al* (27) reported a protective effect of increased vitamin D consumption in pregnancy with wheeze and eczema in 16-24 month old offspring. A follow-up paper from the same cohort reported higher intake of maternal vitamin D suggested an increased risk of eczema in offspring, now 23-29 months old.(90) More recently a significant increase in the risk of asthma in 20-25 year old offspring of mothers who had high concentrations of serum 25(OH)D ante-natally was reported.(91) This suggests that the long-term effect of pre-natal vitamin D exposure differs from the initial, mostly protective, effect. Findings from vitamin D status studies are less consistent and at times not in agreement with intake studies. Lack of concordance in findings may be an indication that intake data is a surrogate for the intake of other important nutrients.(21)

The possible mechanism of action in the relationship of vitamin D and asthma could relate to T-helper 2 cell (Th2) differentiation. Literature is conflicting; one immunologic study reported that vitamin D supplementation (in vitro) promoted Th2 cell differentiation.(92) It was thought that the increased vitamin D supplementation in European and high latitude countries to prevent rickets led to the current high prevalence of asthma.(15, 93) Contrary to this, the proposal is that vitamin D inhibits Th2 differentiation, thus having a protective effect.(94)

Vitamin D intervention studies are under way;(95-97) results from a randomised controlled trial in the UK failed to find any association between prenatal vitamin D supplementation and wheezing in offspring 3 years of age.(98) An Aberdeen cohort demonstrated an *in vivo* anti-inflammatory effect of maternal serum $25(OH)D_3$ on interleukin-10 secretion from the airway epithelial cells of cultured neonatal nasal samples.(99) Dick *et al* (100) suggested that a specific exposure might act in a different manner in different population groups and demographic areas, as the genetic and epigenetic factors are likely to be differential. Inconsistencies in findings from the association between pregnancy nutrition and offspring asthma may furthermore relate to interactions between specific nutrients or additional interactions with the human microbiome and environment.(101)

Strengths of this study and analysis were the prospective design with an a priori purpose of examining intergenerational transmission of risk. As there is a strong social gradient applied to diet in pregnancy,(102) confounding by socio-demographic factors and lifestyle was a concern; we controlled comprehensively for various socio-demographic factors. The GLMM enabled us to control for repeated measurements over time for participants. Limitations were differential follow-up, leading to varied numbers of missing data for certain variables, potentially introducing bias to follow-up data. Countering this, it is notable that the same associations were found separately at the three different time points. The authors recognise that asthma in childhood is a heterogeneous phenotype and that grouping of the 3 time-points could potentially mask different phenotypes;(103) however our study was not powered to differentiate asthma on phenotype at various follow-up. Data on familial atopy or asthma were not available; it is possible that the suggested relationships between maternal diet and offspring asthma have been confounded by the presence or absence of familial predisposition. Data on foetal growth parameters were not available; the effect of foetal growth on childhood asthma could not be controlled for.(104) The fact that the associations found in this analysis were between maternal food intake and doctor diagnosed asthma raised the possibility of ascertainment bias; more health conscious mothers were more likely to follow good pregnancy diets, and were more likely to take their ill children to the doctor to receive a formal diagnosis. However, if this ascertainment bias were to be true, this analysis would have showed the opposite, i.e. that an increase in healthy eating was adversely associated with doctor diagnosed asthma.

CONCLUSION

This analysis suggests that higher intakes of oily fish and vitamin D in the maternal pregnancy diet have a protective effect on childhood asthma. This is consistent with the developmental origins of health and disease hypothesis, suggesting that certain exposures of the foetus in utero can affect the development of allergic diseases in childhood by modulating immune response (105). As there is no anticipated cure for asthma in the near future, adjustment of the environment as early as possible, e.g. in utero and in infancy, might provide the best way to achieve a reduction in the asthma burden.(100, 106)

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CONTRIBUTORS' STATEMENT: The Principal Investigator of the Lifeways Study, Professor Kelleher, conceptualised the design of the study and supervised the overall project, including data analysis and interpretation. Dr. Viljoen drafted the manuscript and undertook data collection, analyses and interpretation as part of her PhD. Dr. Murrin assisted with data collection, analyses and interpretation. Dr. Segurado provided statistical input relating to analyses and interpretation of data. Drs. O'Brien and Mehegan assisted with data management and data interpretation. All contributors reviewed and revised the manuscript and approved the final version as submitted.

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What is already known on this topic: The rising burden of childhood asthma worldwide has placed an emphasis on the potential for primary prevention and disease modification. The association of pregnancy nutrient intake (particularly oily fish, vegetables, vitamin D) and season of birth with offspring asthma/atopy has suggested disease originating in utero. Findings from observational studies are inconsistent; intervention studies suggest an inverse association of asthma with vitamin D.

What this study adds: Only one other cohort study investigating the association of maternal pregnancy diet and offspring asthma with follow-up beyond 6 years was identified. In children with persistent wheezing up to age 3 only a small percentage progress to asthma; in our study offspring was followed-up for 10 years. Mothers with higher oily fish and vitamin D intake had offspring with significant decreased odds of asthma at any time point over 10 years follow-up.

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Supplement 1: Food items included in 'Oily fish' and 'Vegetables'

Oily fish (fresh or canned):	Mackerel, Kippers, Tuna, Salmon, Sardines, Herring
Vegetables:	Carrots, Spinach, Broccoli, Spring greens & Cale, Brussel sprouts, Cabbage, Peas, Green beans, Runner beans, Parsnips & Turnips, Leeks, Onions, Garlic, Mushrooms, Sweet peppers, Bean sprouts, Green salad & Lettuce, Cucumber, Tomatoes, Sweetcorn, Beetroot, Coleslaw, Avocado, Watercress, Cauliflower, Celery, Marrow & Courgettes

Table S1 Simple mixed model association between childhood determinants and childhood asthma at any point (n=897)

	Asthma diagnosis True vs False 95% CI		
	OR	**Lower**	**Upper**
Time (n=897)			
Year 10 follow-up	1.00		
Year 5 follow-up	0.55	0.40	0.77
Year 3 follow-up	0.41	0.29	0.57
Birthweight[1] (n=885)	1.00	1.00	1.00
Gestational age[1] (n=793)	0.92	0.85	0.99
Gender[1] (n=893)			
Female	1.00		
Male	1.55	1.17	2.05
GMS Eligibility[1] (n=694)			
TRUE	1.00		
FALSE	0.45	0.29	0.69
Birth order[1] (n=882)			
Not firstborn	1.00		
Firstborn	1.14	0.86	1.51
Mode of delivery[1] (n=839)			
Ceasarean section	1.00		
Vaginal (spontaneous or assisted)	0.85	0.57	1.26
Region[1] (n=897)			
Dublin	1.00		
Galway	0.86	0.64	1.16

[1]Controlling for time as a fixed factor GMS=General Medical Scheme

OR=Odds Ratio SD=Standard Deviation CI=Confidence interval

Table S2 Simple mixed model association between maternal determinants and childhood asthma at any point (n=897)

	Asthma diagnosis True vs False 95% CI		
	OR	**Lower**	**Upper**
Time (n=897)			
Year 10 follow-up	1.00		
Year 5 follow-up	0.55	0.40	0.77
Year 3 follow-up	0.41	0.29	0.57
Education[1] (n=873)			
Some/completed 3rd level	1.00		
None/Primary/Secondary school	1.28	0.97	1.70
Marital status[1] (baseline) (n=885)			
Lone	1.00		
Cohabiting	0.66	0.47	0.94
Marital status[1] (any time-point) (n=557)			
Lone	1.00		
Cohabiting	1.21	0.57	2.57
Age at giving birth[1] (n=889)	0.98	0.96	1.01
BMI prior to falling pregnant[1]	0.98	0.94	1.02
GMS Eligibility[1] (baseline) (n=887)			
TRUE	1.00		
FALSE	0.73	0.5	1.07
GMS Eligibility[1] (any time point) (n=797)			
TRUE	1.00		
FALSE	0.72	0.51	1.03
Maternal Health[1] (baseline) (n=871)			
Excellent/Very good	1.00		
Poor/Fair/Good	0.82	0.59	1.13
Maternal Health[1] (any time point) (n=560)			
Excellent/Very good	1.00		
Poor/Fair/Good	1.23	0.79	1.9
Smoking status at delivery[1] (n=870)			
Smoker	1.00		
Non-smoker	0.84	0.59	1.2
Breastfeeding[1] (n=897)			
TRUE	1.00		
FALSE	1.21	0.85	1.73

[1]Controlling for time as a fixed factor GMS=General Medical Scheme

OR=Odds Ratio SD=Standard Deviation CI=Confidence interval

Table S3 Distribution of maternal nutirent intake during pregnancy in Normal-Reporters (EI/BMRpreg<1.35-2.39) relative to Irish Recommended Daily Allowances (n=453)

Nutrient[1]	$\bar{x}$ ± SD	25th Percentile	Median	75th Percentile	RDAs for Irish women* 19-64 yrs	Pregnancy**
Total fat (g/day)	129.0±110.4	51.8	100.4	175.3	-	-
MUFA (g/day)	41.1±35.9	16.8	31.7	53.9	-	-
PUFA (g/day)	20.3±20.3	7.0	13.5	25.8	-	-
Saturated Fatty Acids (g/day)	57.8±58.5	20.1	39.1	74.6	-	-
Cholesterol (mg/day)	402.1±330.9	182.3	312.9	522.2	-	-
Retinol (µg/day)	763.2±1004.2	290.6	515.7	890.4	-	-
Carotene (µg/day)	3594.9±2758.3	1356.7	3127.5	4581.2	-	-
Selenium (µg/day)	86.7±83.0	36.3	60.7	110.3	55.0	55.0
Zinc (mg/day)	14.7±11.7	6.6	12.2	18.5	15.0	20.0
Magnesium (mg/day)	545.5±557.9	191.1	362.7	660.9	255-65‡	290-300‡
Copper (mg/day)	2.0±3.4	0.6	1.2	2.1	1.1	1.1
Manganese (mg/day)	4.9±4.8	1.7	3.3	5.9	≤10.0‡	-
Calcium (mg/day)	1712.9±1467.4	614.6	1222.0	2429.9	800.0	1200.0
Vitamin C (mg/day)	248.1±246.5	90.3	157.9	316.0	60.0	80.0
Vitamin D (µg/day)	4.3±4.1	1.7	3.1	5.4	7.5	10.0
Vitamin E (mg/day)	13.6±18.7	4.9	7.9	14.2	8.0	10.0

[1]Calculated from food-frequency questionnaires in first trimester; Energy adjusted - residual method;

MUFA=Monounsaturated Fatty Acids PUFA=Polyunsaturated Acids EI=Energy Intake BMR=Basal Metabolic Rate preg=Pregnancy

g=gram mg=milligram µg=microgram

* Recommended Daily Allowance; Irish Food Safety Authority (1999) **From 20 weeks gestation ‡Estimated Average Requirement, European Food Safety Authority (2006)

‡Scientific Committee for Food of the European Union (SCF, 1993)

Table S4 Distribution of maternal food group intake during pregnancy relative to SLAN females (n=886)

	Lifeways Mothers 2001-2003			SLAN 2007 Females		Recommended intake*	
	Under 18 years (n=16)	18-29 years (n=377)	30-44 years (n=493)	18-29 years	30-44 years	19-64 yrs	Pregnancy
Food group[1] (total daily portions)	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	Portions	Portions
Oily fish	0.02 ± 0.04	0.03 ± 0.07	0.08 ± 0.14	-	-	1[b]	1[b]
Vegetables	2.5 ± 1.5	2.6 ± 1.9	3.2 ± 2.1	4.1 ± 2.9	4.5 ± 2.8	5[a]	6-7[a]
Fruit	2.2 ± 1.6	2.0 ± 2.5	2.2 1.6	2.8 ± 2.7	3.0 ± 2.8	5[a]	6-7[a]
Added Fat	2.2 ± 3.1	2.4 ± 2.4	2.6 ± 2.2	-	-	-	-

[1]Calculated from food-frequency questionnaires in first trimester *Food Safety Authority of Ireland SLAN=Survey of Lifestyle, Attitudes and Nutrition

[a]Daily portion [b]Weekly portion

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract – **PAGE 1 & 2**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found – **PAGE 2**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported – **PAGE 3 & 4**
Objectives	3	State specific objectives, including any prespecified hypotheses – **PAGE 4**
Methods		
Study design	4	Present key elements of study design early in the paper – **PAGE 4 & 5**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection – **PAGE 4, 5 & 6**
Participants	6	*Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up – **PAGE 5**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable – **PAGE 5, 6, 7 & 9**
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 – **PAGE 6 &7**
Bias	9	Describe any efforts to address potential sources of bias – **PAGE 16**
Study size	10	Explain how the study size was arrived at – DUE TO WORD COUNT RESTRICTION PLEASE SEE DETAIL OF REFERENCE 61, 62 & 69
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why – **PAGE 4, 5, 6 & 7**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding – **PAGE 4, 5, 6, 7, 12, 13 & 14**
		(*b*) Describe any methods used to examine subgroups and interactions – **PAGE 7, 12, 13 & 14**
		(*c*) Explain how missing data were addressed – **PAGE 10 & 16,** ADDITIONAL DETAIL IN REFERENCE 69
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed – **PAGE 10 &16**, ADDITIONAL DETAIL IN REFERENCE 69

Continued on next page

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 – **PAGE 10, 16**, ADDITIONAL DETAIL REFERENCE 69
		(b) Give reasons for non-participation at each stage - DUE TO WORD COUNT RESTRICTION PLEASE SEE DETAIL OF REFERENCE 61, 62 & 69
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders – **PAGE 8, 9, & 11**
		(b) Indicate number of participants with missing data for each variable of interest – **PAGE 10**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) – **PAGE 10**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time – **PAGE 10**, ADDITIONAL DETAIL REFERENCE 69
Main results	6	(*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 – **PAGE 12, 13, & 14**
		(*b*) Report category boundaries when continuous variables were categorized - DUE TO WORD COUNT RESTRICTION PLEASE SEE DETAIL OF REFERENCE 69
Discussion		
Key results	18	Summarise key results with reference to study objectives – **PAGE 11**
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 – **PAGE 16**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence – **PAGE 16**
Generalisability	21	Discuss the generalisability (external validity) of the study results – **PAGE 16**
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 – **PAGE 18**

*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.

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Pregnancy Diet and Offspring Asthma risk over a 10-year period: the Lifeways Cross Generation Cohort Study, Republic of Ireland

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Pregnancy Diet and Offspring Asthma risk over a 10-year period: the Lifeways Cross Generation Cohort Study, Republic of Ireland

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Key Words: asthma, childhood, DOHaD, pregnancy, vitamin D

Word count: 3110

ABSTRACT:

Objective

The association of maternal pregnancy diet with offspring asthma risk have been reported. However, literature on longitudinal patterns of asthma risk relative to intra-uterine nutrient exposure is limited. We aimed to establish whether vegetable, oily fish and vitamin D intake during pregnancy influence childhood asthma risk over a 10 year period in Irish Republic.

Design

Mother-child pairs (n=897) from the Lifeways prospective birth cohort, with data on nutrient intake during pregnancy and asthma status respectively, were eligible for inclusion in the analysis. Data on socio-economic and morbidity indicators over 10 years of follow-up on mothers and the index child were collected through self-administered questionnaires. Asthma status as diagnosed by the GP at any time-point over 10 years was related to maternal vegetable, oily fish and vitamin D intake during pregnancy, while adjusting for gestational age, socio-economic status, smoking at delivery, breast-feeding, season of birth and supplement use. Data were modelled with a Generalised Linear Mixed Model (GLMM); personal id was modelled as random effect with random intercepts and slopes over time for individuals.

Results

In the fully adjusted GLMM, higher daily average intake of oily fish was significantly protective of asthma risk (OR 0.13, 95% CI 0.02-0.86); vegetable intake was non-significant (OR 0.95, 95% CI 0.87-1.04). A higher daily vitamin D intake significantly reduced the odds of asthma (OR 0.93, 95% CI 0.89-0.97).

Conclusion

This analysis suggests higher daily average intake of oily fish and vitamin D in pregnancy to be protective of asthma risk in offspring at any time-point over a 10 year follow-up period.

STRENGTHS

- The prospective design with an a priori purpose of examining intergenerational transmission of risk.
- As there is a strong social gradient applied to diet in pregnancy, confounding by socio-demographic factors and lifestyle was a concern; we controlled comprehensively for various socio-demographic factors
- The GLMM enabled us to control for repeated measurements over time for participants

LIMITATIONS

- Limitations were differential follow-up, leading to varied numbers of missing data for certain variables, potentially introducing bias to follow-up data. Countering this, it is notable that the same associations were found separately at the three different time points.
- The authors recognise that asthma in childhood is a heterogeneous phenotype and that grouping of the 3 time-points could potentially mask different phenotypes; however our study was not powered to differentiate asthma on phenotype at various follow-up.
- Data on familial atopy or asthma were not available; it is possible that the suggested relationships between maternal diet and offspring asthma have been confounded by the presence or absence of familial predisposition.
- Data on foetal growth parameters were not available; the effect of foetal growth on childhood asthma could not be controlled for.
- GP'S criteria to diagnose asthma could be diverse, with potential for variability.

INTRODUCTION

Asthma is the most common chronic disease of childhood;(1, 2) reports indicate a continuous and consistent increase in worldwide prevalence, especially in westernised societies. Prevalence rates in the United Kingdom and Ireland are among the highest in Europe. (3, 4) According to the Centres for Disease Control and Prevention, between 2001 and 2010 asthma prevalence in children in the United States increased 1.4% each year.(5) This increase is most likely multi-factorial, with complex interactions of genetic-immunological-environmental

factors leading to the phenotypic expression of disease.(6) Recently multiple studies have attempted to deconstruct this multifactorial relationship, focusing on the change in dietary habits over recent decades.(7-13) The progressive trend in early presentation of allergic disease in childhood, with the implication of possible exposure in utero, has placed an emphasis on maternal pregnancy diet as a prominent factor in the development of offspring asthma.(14, 15) As allergen-specific immune responses are established in foetal life, maternal nutrient intake during pregnancy is pivotal; intake may potentially influence the development of both the innate and acquired immune responses, predisposing to atopy in later life.(16)

A growing body of persuasive epidemiological evidence suggests that deficiency of maternal vitamin D intake prenatally has an inverse relationship with atopic disease in childhood.(17-22) Observational studies on the association of maternal serum and/or infant cord blood 25-hydroxy-vitamin D_3 ($25(OH)D_3$) levels with atopic markers are conflicting. Some demonstrated similar inverse associations;(23-30) others demonstrated direct (31-33) and null (34-42) associations. U-shaped associations were suggested by both Rothers *et al* (43) and Maslova *et al*.(44) Most recently intervention studies exploring pregnancy vitamin D supplementation and asthma risk at 3 years of age suggested a protective effect (statistically non-significant). The authors suggested that longer follow-up of children is needed to determine the clinical importance of findings.(45, 46)

The Lifeways study has previously reported the association between pregnancy intake of oily fish and vegetables and General Practitioner (GP) diagnosed asthma in offspring at age 3 years; a higher daily mean intake suggested a significant protective effect.(47) Literature on pregnancy consumption of fish with subsequent atopic risk in offspring is mostly consistent with our findings, indicating a protective effect.(7, 48-54) Our current aim was twofold: firstly to build on the aforementioned Lifeways findings and test the hypothesis that a higher pregnancy intake of vegetables and oily fish might be protective of asthma risk at any stage over 10 years follow-up; secondly, as suggested in the literature, to explore the association of pregnancy vitamin D intake and offspring asthma risk within our cohort at any stage over 10 years of follow-up.

METHODS

Study design and sample selection

The Lifeways study was established 2001-2003 as a prospective birth cohort in the Republic of Ireland. The *a priori* purpose was to examine determinants of health status in children, including diet and lifestyle, and to establish patterns and links across generations. Recruitment, data collection and study instruments have previously been discussed in detail.(55) In brief, mothers were recruited at first ante-natal visit (14-16 weeks gestation) in one of two regional maternity hospitals in the more rural West (Galway) and the more industrialised East (Dublin). Of 1124 mothers recruited, 1082 gave birth resulting in 1096 live mother-child pairs. Analysis was limited to current live mother-child pairs, where data on the proband's asthma status were available for at least one time-point. Babies with congenital anomalies and delivery <34 weeks gestational age (56) were excluded from analysis. Due to attrition over time participants had differential patterns of follow-up through phases; 614 mother-child pairs were included in the year 3 analyses, 511 in year 5 and 432 in year 10 follow-up. The sample for this analysis of 897 mother-child pairs comprised respondents for whom at least one follow-up point of asthma health status was recorded (Figure S1). Ethical approval for all phases of the study was granted by the Human Research Ethics Committee, University College Dublin, Ireland. Written informed consent was obtained from all adult participants at each follow-up phase of the study. Parental consent was obtained for child subjects at each follow-up phase; additional assent was obtained from all child subjects at the year 10 follow-up phase.

Assessment of pregnancy diet

Data on maternal nutrient intake during pregnancy were captured by a semi-quantitave Food Frequency Questionnaire (FFQ) as part of a self-administered questionnaire to the mother at her first ante-natal visit. The FFQ was developed from the international version used in the European Prospective Investigation in Cancer studies by the National Nutritional Surveillance Centre and extensively validated for use in an Irish population by the National University of Ireland, Galway. (57) The main food groups regularly consumed in the Irish diet were included, and consisted of 149 food items.(47, 58) The questionnaire focused on maternal dietary intake since pregnant. Intake as a medium serving (detailed in the FFQ for relevant food items) was recorded on a 9-grade scale, with categories subsequently transformed to continuous daily portion averages for all 149 food items. To arrive at distinct food groups, the

continuous intake of various food items were summed and reported as total portions per day (Supplement; Table S1). The daily average intake of energy and nutrients was calculated by linking frequency selections from the FFQ with food equivalents in McCance and Widdowson's nutritional composition database, 6th edition,(59) using software developed specifically for the Lifeways database.(60) Vitamin D was reported in micrograms per day (μg/day); energy as kilocalories (kCal/day).

Assessment of outcome

Data on doctor diagnosed asthma in offspring were collected at 3 time-points: ages 3, 5 and 9 years. Various studies have used doctor diagnosed asthma/parental report of doctor diagnosed asthma to ascertain diagnosis.(30, 45) Questions to ascertain asthma diagnosis were adapted from the validated International Study on Asthma and Allergies in Childhood (ISAAC) questionnaire.(61, 62) Diagnosis from age 3 and 9 follow-up were reported by the General Practitioner (GP), information on GP diagnosed asthma at age 5 was obtained from the mother. For the univariate analysis, asthma as a dichotomous outcome variable ('Yes' vs 'No') at each of the three time-points were analysed separately. For the multivariable analysis a composite, dichotomous outcome variable was created; it described doctor diagnosed asthma in the child at any of the 3 time-points over a period of 10-year follow-up, versus never asthma.(63)

Statistical analysis

Definite doctor diagnosed asthma in offspring at any time point over 10-year follow-up, versus never, was related to maternal pregnancy intake of oily fish, vegetables and vitamin D.

Uni-variate analysis and Covariates

Variables that could potentially confound the diagnosis of asthma were identified from the literature, and screened in our cohort for association with an asthma diagnosis at the specific time-point using unadjusted binomial logistic regression or the independent samples t-test. Predictors with $p<0.1$ were identified as being potential confounders and selected for inclusion in the multivariable models and are discussed below.

Total Energy Intake (EI)

To control for variance in accuracy of maternal energy reporting, total energy intake (kCal) was adjusted for in all models. Nutrients were adjusted for total energy intake using the residual method.(64, 65) To account for mothers who potentially over- or under reported energy intake, the ratio of energy intake (EI) to basal metabolic rate in pregnancy (BMR_{preg}) was calculated to identify extreme outliers.(63, 66, 67) BMR_{preg} was calculated using the Schofield equations.(67)

Gestational age

Gestational age in weeks.

Socio-economic status

We used eligibility for the General Medical Scheme (GMS) as an indicator of socio-economic status. The Republic of Ireland has a two-tiered healthcare system, where certain individuals are eligible to different levels of free healthcare which are dependent on their income and certain medical conditions. GMS is thus a robust means tested indicator.(68-70) As maternal and offspring GMS eligibility are strongly correlated only the maternal predictor was used in the models.(63, 71)

Smoking

Smoking status at time of delivery; hospital delivery records.

Breastfeeding

Data from the self-administered maternal questionnaire; 'Was your Lifeways child ever breastfed?'

Season of birth

Seasons were comprised as follows: Summer (May, June, July), Autumn (Aug, Sept, Oct), Winter (Nov, Dec, Jan), Spring (Feb, March, April). This follows the grouping suggested by the Irish ROLO study with data on maternal serum 25(OH)D levels.(72) Summer and Autumn were collapsed to form 'Summer', with Spring and Winter collapsed to form 'Winter', making up the final dichotomous variable, 'Summer' vs 'Winter'.

Supplement use

Data on supplement use were generic: 'Have you taken any vitamins, minerals or food supplements?' 'Yes' or 'No' and did not allow for specification on supplement type or content quantification.

Multivariable analysis

The associations with the three aforementioned dietary intakes were analysed in multivariable models to assess their independent association with asthma, and verify the extent of confounding in a stepwise manner. Vegetables, oily fish and vitamin D intakes were analysed as continuous predictor variables. Multivariable analysis was done using the full sample of 897 mother-child pairs in a Generalised Linear Mixed Model (GLMM). Covariates were entered into the GLMM sequentially as fixed factors based on their univariate p-value. Time was consistently included as a fixed factor. Personal id was modelled as a random effect with random intercepts and slopes over time for each individual. Models using asthma as a time-varying outcome failed to converge, therefore a composite variable for asthma at any time point was used as the dependent variable. Predictor variables with $p<0.05$ were regarded as significant. The Log Pseudo-likelihood as a measure of model fit was used to compare the models. A best fit model for vegetables, oily fish and vitamin D intake each was selected by evaluating the log pseudo-likelihood; the lower the number the better the model fit.

The Statistical Package for the Social Sciences (SPSS) version 20 was used to conduct univariate analysis; multivariable analysis was done using Statistical Analysis Software (SAS) version 9.3.

RESULTS

Study subjects' characteristics and asthma prevalence

In the final sample, 66.9% of the mother-child pairs were resident in the Dublin area and 33.1% in the Galway area, proportionate to recruitment patterns. Mothers with a 3rd level education were marginally higher (51.9%) than those with None/Primary/Secondary school education (48.1%); 19.4% of mothers were smokers at the time of giving birth. The mean (SD) age of mothers at time of giving birth was 30.2 (5.9) years and the mean (SD) pre-pregnancy body mass index of mothers were 23.7 (4.0). The offspring sex distribution was about equal; 48.8% males and 51.2% females. The mean (SD) birth-weight was 3515.3 (568.6) grams, with a mean (SD) gestational age of 39.9 (1.9) weeks. Just under half (45.8%) of probands were the first born and 86.20% were delivered vaginally. Doctor diagnosed asthma in offspring at the 3 phases of follow-up respectively, increased from 10.90% at 3-years, to 14.33% at 5-years and 23.10% at 10-years follow-up. In general the literature suggests a downward trend in prevalence with increasing age. There are however, cohorts that noted an increase of prevalence with age.(73-75) Maternal socio-economic and biological characteristics relative to food group intake are presented in Table 1. Maternal and child

characteristics as related to asthma diagnosis are further presented in supplemental material (Table S2 and S3).

Univariate associations of background variables with childhood asthma from the cross-sectional data for the 3 follow-up phases respectively have previously been discussed in detail.(47, 62, 63)

Table 1 Maternal food group intake during pregnancy in relation to socio-economic factors (n=897)

		Food group				
	n[1]	**Oily fish** (portion/day) mean (SD)	**p-value**	n[1]	**Vegetables** (portion/day) mean (SD)	**p-value**
Education			<0.001[a]			<0.001[a]
None/Primary/Secondary school	416	0.05 (0.10)		420	2.61 (1.88)	
3rd level education	451	0.07 (0.13)		451	3.26 (2.16)	
Marital status (baseline)			0.01[a]			<0.001[a]
Lone	179	0.04 (0.09)		180	2.51 (2.06)	
Cohabiting	701	0.06 (0.12)		703	3.05 (2.03)	
GMS Eligibility (baseline)			<0.001[a]			<0.001[a]
No	750	0.06 (0.12)		754	3.02 (2.06)	
Yes	131	0.03 (0.07)		131	2.43 (1.91)	
Smoking status at delivery			0.07[a]			<0.001[a]
Non-smoker	693	0.06 (0.12)		695	3.04 (2.10)	
Smoker	167	0.04 (0.11)		169	2.39 (1.61)	
Region			0.04[a]			<0.001[a]
Galway	292	0.07 (0.13)		294	3.21 (2.17)	
Dublin	594	0.05 (0.10)		596	2.79 (1.96)	
Age group at delivery (y)			<0.001[b]			<0.001[b]
Under 18	16	0.02 (0.04)		16	2.52 (1.51)	
18 to 29	373	0.03 (0.07)		377	2.56 (1.92)	
30+	491	0.08 (0.14)		492	3.22 (2.09)	

[a]Independent samples t-test [b]ANOVA GMS=General Medical Scheme

[1]Numbers do not always add up to 897 because of varied numbers of missing data for some variables

Within-time representativeness of responders vs. non-responders that contributed to the final sample is presented in Table 2.

Table 2 Within-time representativeness of final sample (n=897)

Characteristics	Responders[a] n=614	Non-responders[a] n=283
Year 3 follow-up		
Maternal educational attainment[c]		
None/Primary/Secondary school, No. (%)	290 (48.6)	130 (47.1)
Some/Completed 3rd level education, No. (%)	307 (51.4)	146 (52.9)
Maternal GMS eligibility at baseline[c]		
Not eligible, No. (%)	512 (84.3)	244 (87.1)
Eligible, No.(%)	95 (15.7)	36 (12.9)
Region[c]		
West, No. (%)	217 (35.3)	80 (28.3)
East, No. (%)	397 (64.7)	203 (71.7)
Maternal age at birth of proband, mean (SD), y[c]	30.35 (5.9)	29.75 (5.9)
Maternal oily fish intake in pregnancy, mean (SD), servings/d[c]	0.06 (0.1)	0.05 (0.1)
Maternal Vegetable intake in pregnancy, mean (SD), servings/d[c]	2.95 (1.9)	2.88 (2.2)
Maternal Vitamin D intake in pregnancy, mean (SD), µg/d[c]	4.70 (4.8)	5.60 (10.2)
Year 5 follow-up	n=511	n=386
Maternal educational attainment[c]		
None/Primary/Secondary school, No. (%)	241 (48.7)	179 (47.4)
Some/Completed 3rd level education, No. (%)	254 (56.1)	199 (52.6)
Maternal GMS eligibility at baseline[c]		
Not eligible, No. (%)	429 (85.0)	327 (85.6)
Eligible, No. (%)	76 (15.0)	55 (14.4)
Region[c]		
West, No. (%)	165 (32.3)	132 (34.2)
East, No. (%)	346 (67.7)	254 (65.8)
Maternal age at birth of proband, mean (SD), y[c]	30.10 (5.8)	30.24 (5.9)
Maternal oily fish intake in pregnancy, mean (SD), servings/d[c]	0.06 (0.1)	0.06 (0.1)
Maternal Vegetable intake in pregnancy, mean (SD), servings/d[c]	2.93 (2.1)	2.94 (1.9)
Maternal Vitamin D intake in pregnancy, mean (SD), µg/d[c]	4.70 (6.1)	5.30 (7.9)
Year 10 follow-up	n=432	n=465
Maternal educational attainment[b]		
None/Primary/Secondary school, No. (%)	174 (40.7)	246 (55.2)
Some/Completed 3rd level education, No. (%)	253 (59.3)	200 (44.8)
Maternal GMS eligibility at baseline[b]		
Not eligible, No. (%)	385 (89.7)	371 (81.0)
Eligible, No. (%)	44 (10.3)	87 (19.0)
Region[b]		
West, No. (%)	166 (38.4)	131 (28.2)
East, No. (%)	266 (61.6)	334 (71.8)
Maternal age at birth of proband, mean (SD), y[b]	31.55 (5.2)	28.86 (6.1)
Maternal oily fish intake in pregnancy, mean (SD), servings/d[c]	0.06 (0.1)	0.06 (0.1)
Maternal Vegetable intake in pregnancy, mean (SD), servings/d[c]	3.11 (2.1)	2.76 (2.0)
Maternal Vitamin D intake in pregnancy, mean (SD), µg/d[c]	5.00 (7.5)	4.90 (6.4)

[a]Numbers do not always add up to total as varied numbers of missing data at certain variables
[b]p<0.001 [c]Non-significant
µg: micrograms SD:Standard Deviation d:day y:years

Pregnancy dietary intake

Reported intake of vegetables and oily fish were directly and significantly associated with the maternal social gradient (Table 1). Mean (SD) daily intake of oily fish and vegetables were 0.06 (0.12) and 2.93 (2.04) portions per day respectively. The Food Safety Authority of Ireland (FSAI) recommends that pregnant women should have one portion of oily fish weekly (i.e. 0.1 portions/day) and 6-7 portions of vegetables daily.(76) The mean intake of Lifeways mothers did not reach this recommendation. Data captured by the FFQ detailed intake as a medium portion (90-100 gram of fish; a piece of fish about the size of a woman's palm).

Vitamin D intake during pregnancy between those mothers with consistent follow-up, and those with attrition did not differ significantly. A social gradient for pregnancy vitamin D intake in the final sample was not observed. The distribution of mean vitamin D intake was investigated according to the EI/BMR_{preg} ratio; no marked difference in the distribution of vitamin D was observed.(63) Energy adjusted mean (SD) intake of vitamin D in mothers was 4.3 (4.1) µg/d. The FSAI recommends a daily allowance of 10µg/day vitamin D for Irish pregnant women.(77). (Table S4 and S5). At baseline 327 (36.9%) mothers from the final sample reported using supplements.

Pregnancy dietary associations with offspring asthma

Vegetable intake was negatively associated with offspring asthma, although not significantly so in the best fit, fully adjusted model (OR 0.95, 95% CI 0.87-1.04) (Model 6) (Table 3).

Oily fish intake was significantly and inversely associated with offspring asthma in the fully adjusted model (Model 6), with an increase in daily average serving of oily fish suggesting a protective effect at any time point over the 10 year follow-up period (OR 0.12, 95% CI 0.02-0.83) (Table 4). Further adjustment for season of birth, vitamin D intake and supplement use (Models 7-9) saw oily fish remaining protective, however the Log Pseudo-likelihood increased suggesting goodness of fit for the models decreased.

The fully adjusted, best fit model (Model 9), suggested vitamin D to have a significant inverse association (OR 0.93, 95% CI 0.87-0.97) with offspring asthma at any time-point of follow-up (Table 5). Further adjustment for season of birth, oily fish intake and supplement use (Models 7-9) suggested an ongoing, significant protective effect of vitamin D, however the Log Pseudo-likelihood increased suggesting goodness of fit for the models decreased.

Table 3 Generalised linear mixed model: asthma at any time-point vs. never (vegetable intake as main exposure of interest)

Independent variable		OR (95% CI)							
		Model 1 n=890	Model 2 n=883	Model 3 n=781	Model 4 n=777	Model 5 n=756	Model 6 n=682	Model 7 n=682	Model 8 n=677
Time	Year 3 follow-up	0.40 (0.29-0.57)	0.41 (0.29-0.57)	0.37 (0.26-0.54)	0.36 (0.25-0.53)	0.36 (0.25-0.53)	0.36 (0.24-0.54)	0.37 (0.54-0.54)	0.36 (0.24-0.53)
Ref: Year 10 follow-up	Year 5 follow-up	0.54 (0.39-0.76)	0.54 (0.39-0.76)	0.49 (0.34-0.71)	0.49 (0.34-0.71)	0.49 (0.32-0.68)	0.48 (0.33-0.71)	0.48 (0.33-0.71)	0.48 (0.33-0.71)
Vegetable intake (serving/d)		0.93 (0.86-0.99)	0.93 (0.86-0.99)	0.94 (0.87-1.02)	0.95 (0.87-1.03)	0.95 (0.86-1.03)	0.95 (0.87-1.04)	0.95 (0.87-1.04)	0.95 (0.87-1.04)
Total EI (kCal)			1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)
Gestational Age (weeks)				0.92 (0.85-0.99)	0.92 (0.85-0.99)	0.93 (0.86-1.00)	0.93 (0.85-1.00)	0.92 (0.85-1.00)	0.93 (0.85-1.00)
GMS Eligibility (baseline)	No				0.80 (0.51-1.25)	0.84 (0.53-1.34)	0.99 (0.59-1.67)	0.98 (0.58-1.63)	0.98 (0.58-1.64)
Ref: Yes									
Smoking at delivery	Non-smoker					1.07 (0.69-1.64)	1.23 (0.77-1.99)	1.24 (0.78-1.99)	1.24 (0.78-1.99)
Ref: Smoker									
Breastfeeding	No						1.35 (0.90-1.76)	1.27 (0.91-1.78)	1.28 (0.91-1.77)
Ref: Yes									
Season of birth	Summer							1.41 (0.99-1.99)	1.41 (0.99-1.99)
Ref: Winter									
Supplement use	No								1.71 (0.83-1.66)
Ref: Yes									
Log Pseudo-likelihood		7627.72	7587.68	6874.38	6841.02	6621.85	6077.47	6094.97	6052.34

EI=Energy Intake GMS=General Medical Scheme

Table 4 Generalised linear mixed model: asthma at any time-point vs. never (oily fish intake as main exposure of interest)

Independent variable		OR (95% CI)								
		Model 1 n=886	Model 2 n=879	Model 3 n=778	Model 4 n=774	Model 5 n=753	Model 6 n=678	Model 7 n=678	Model 8 n=753	Model 9 n=673
Time	Year 3 follow-up	0.41 (0.29-0.58)	0.42 (0.30-0.59)	0.38 (0.26-0.55)	0.37 (0.26-0.54)	0.37 (0.25-0.54)	0.37 (0.25-0.55)	0.37 (0.25-0.56)	0.36 (0.24-0.54)	0.35 (0.24-0.52)
Ref: Year 10 follow-up	Year 5 follow-up	0.54 (0.39-0.77)	0.55 (0.39-0.77)	0.49 (0.35-0.72)	0.50 (0.35-0.72)	0.47 (0.32-0.69)	0.49 (0.33-0.72)	0.49 (0.33-0.73)	0.48 (0.32-0.71)	0.48 (0.32-0.71)
Oily fish intake (serving/d)		0.11 (0.02-0.60)	0.11 (0.02-0.61)	0.15 (0.03-0.84)	0.16 (0.03-0.96)	0.15 (0.03-0.87)	0.12 (0.02-0.83)	0.13 (0.02-0.86)	0.15 (0.02-0.99)	0.16 (0.03-1.05)
Total EI (kCal)			1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)
Gestational Age (weeks)				0.92 (0.85-0.99)	0.92 (0.85-0.99)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.93 (0.86-1.01)
GMS Eligibility (baseline)	No				0.82 (0.53-1.28)	0.87 (0.54-1.39)	1.03 (0.62-1.73)	1.01 (0.60-1.69)	0.94 (0.56-1.58)	0.94 (0.56-1.59)
Ref: Yes										
Smoking at delivery	Non-smoker					1.06 (0.69-1.63)	1.23 (0.76-1.96)	1.2 (0.78-2.03)	1.26 (0.78-2.03)	1.26 (0.78-2.02)
Ref: Smoker										
Breastfeeding	No						1.22 (0.87-1.70)	1.23 (0.76-1.97)	1.25 (0.89-1.75)	1.26 (0.90-1.76)
Ref: Yes										
Season of birth	Summer							1.42 (1.00-2.02)	1.39 (0.89-1.99)	1.39 (0.89-1.98)
Ref: Winter										
[a]**Vitamin D** (µg/d)									0.93 (0.89-0.98)	0.93 (0.89-0.97)
Supplement use	No									1.19 (0.84-1.68)
Ref: Yes										
Log Pseudo-likelihood		7620.85	7579.16	6863.30	6828.52	6614.40	6069.88	6085.90	6138.38	6102.47

EI=Energy Intake [a]Energy Adjusted GMS=General Medical Scheme

Table 5 Generalised linear mixed model: asthma at any time-point vs. never (vitamin D intake as main exposure of interest)

Independent variable		OR (95% CI)								
		Model 1 n=890	Model 2 n=890	Model 3 n=787	Model 4 n=779	Model 5 n=758	Model 6 n=683	Model 7 n=683	Model 8 n=678	Model 9 n=677
Time	Year 3 follow-up	0.41 (0.29-0.57)	0.41 (0.29-0.57)	0.37 (0.25-0.53)	0.36 (0.25-0.52)	0.36 (0.24-0.52)	0.35 (0.23-0.52)	0.35 (0.24-0.52)	0.36 (0.24-0.54)	0.35 (0.24-0.52)
Ref: Year 10 follow-up	Year 5 follow-up	0.55 (0.39-0.77)	0.55 (0.39-0.77)	0.49 (0.34-0.71)	0.49 (0.34-0.71)	0.46 (0.32-0.67)	0.47 (0.32-0.69)	0.47 (0.32-0.70)	0.48 (0.32-0.71)	0.48 (0.32-0.71)
[a]**Vitamin D** (µg/d)		0.96 (0.93-0.99)	0.96 (0.93-0.99)	0.96 (0.92-0.99)	0.95 (0.92-0.99)	0.95 (0.92-0.99)	0.93 (0.87-0.97)	0.93 (0.89-0.97)	0.93 (0.89-0.98)	0.93 (0.89-0.97)
Total EI (kCal)			1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00)
Gestational Age (weeks)				0.92 (0.85-0.99)	0.92 (0.85-0.99)	0.90 (0.86-1.01)	0.93 (0.86-1.00)	0.93 (0.85-1.00)	0.93 (0.86-1.01)	0.93 (0.86-1.01)
GMS Eligibility (baseline)	No				0.74 (0.47-1.16)	0.77 (0.48-1.24)	0.91 (0.55-1.52)	0.89 (0.53-1.49)	0.94 (0.56-1.58)	0.94 (0.56-1.59)
Ref: Yes										
Smoking at delivery	Non-smoker					1.04 (0.67-1.59)	1.25 (0.78-1.99)	1.24 (0.77-1.99)	1.26 (0.78-2.03)	1.26 (0.78-2.02)
Ref: Smoker										
Breastfeeding	No						1.32 (0.95-1.84)	1.33 (0.96-1.85)	1.25 (0.89-1.75)	1.26 (0.90-1.76)
Ref: Yes										
Season of birth	Summer							1.40 (0.99-1.98)	1.40 (0.98-.99)	1.39 (0.98-.98)
Ref: Winter										
Oily fish intake (serving/d)									0.15 (0.02-0.99)	0.16 (0.03-1.05)
Supplement use	No									1.19 (0.84-.68)
Ref: Yes										
Log Pseudo-likelihood		7646.41	7666.29	6965.61	6894.82	6678.52	6151.79	6158.30	6138.38	6127.49

EI=Energy Intake [a]Energy Adjusted GMS=General Medical Scheme

DISCUSSION

Birth cohort studies with follow-up of 6 years or more on the association of maternal pregnancy diet and childhood asthma are limited.(78) This analysis suggests that higher vitamin D intake during pregnancy is significantly associated with reduced likelihood of asthma in offspring at any point during 10-years follow-up. A significant inverse association with oily fish consumption was also demonstrated. Although vegetable intake suggested an initial protective effect, significance was lost early in model progression. Literature on the association with vegetable intake is scarce and inconsistent, with both an increased risk (79) and no effect reported.(80) Literature on the association of oily fish intake in pregnancy with offspring atopic disease is consistent with findings from the current analysis.(49, 51, 79, 80) Analyses from all of these studies were limited to children in the age range 2-6 years; to our knowledge there are no studies with findings extending up to 10 years of age follow-up. A systematic review (81) concluded that there is little evidence to recommend supplementation/modification of diet to include fish oil for children or adults with established asthma. They found no evidence of improvement or increased risk relative to fish oil and established asthma. This is likely evidence that the window period for fish oil to have an effect on immune regulation is most relevant in foetal life, with limited potential for effect once immune responses are established.(80) The pro-inflammatory mediators leukotriene B4 (LTB_4) and tumour necrosis factor alpha (TNF-α) increase airway inflammation and hyper-responsiveness.(82, 83) The protective effect of oily fish could possibly be mediated via eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA). Competitive binding with cyclooxygenase (COX) and lipoxygenase (LOX) reduce LTB_4 and PGE_2 production.(49, 84-86)

Our findings suggest a significant inverse relationship of pregnancy vitamin D consumption with childhood asthma. Results are consistent with the main body of literature on the association of pregnancy vitamin D intake and offspring atopic disease.(17-22) Miyake *et al* (21) reported a protective effect of increased vitamin D consumption in pregnancy with wheeze and eczema in 16-24 month old offspring. A follow-up paper from the same cohort reported higher intake of maternal vitamin D suggested an increased risk of eczema in offspring, now 23-29 months old.(87) More recently a significant increase in the risk of asthma in 20-25 year old offspring of mothers who had high concentrations of serum 25(OH)D ante-natally was reported.(88) This suggests that the long-term effect of pre-natal vitamin D exposure differs from the initial, mostly protective, effect. Findings from vitamin D status studies are less consistent and at times not in agreement with intake studies. Lack of concordance in findings may be an indication that intake data is a surrogate for the intake of other important nutrients.(15)

The possible mechanism of action in the relationship of vitamin D and asthma could relate to T-helper 2 cell (Th2) differentiation. Literature is conflicting; one immunologic study reported that vitamin D supplementation (in vitro) promoted Th2 cell differentiation.(89) It was thought that the increased vitamin D supplementation in European and high latitude countries to prevent rickets led to the current high prevalence of asthma.(9, 90) Contrary to this, the proposal is that vitamin D inhibits Th2 differentiation, thus having a protective effect.(91)

Vitamin D intervention studies are under way; results from a randomised controlled trial in the UK failed to find any association between prenatal vitamin D supplementation and wheezing in offspring 3 years of age.(92) Litonjua *et al* (45) found a lower incidence (non-significant) of recurrent wheeze and asthma in 3 year old offspring of mothers who received higher vitamin D supplementation in pregnancy. An Aberdeen cohort demonstrated an *in vivo* anti-inflammatory effect of maternal serum $25(OH)D_3$ on interleukin-10 secretion from the airway epithelial cells of cultured neonatal nasal samples.(93) Dick *et al* (94) suggested that a specific exposure might act in a different manner in different population groups and demographic areas, as the genetic and epigenetic factors are likely to be differential. Inconsistencies in findings from the association between pregnancy nutrition and offspring asthma may furthermore relate to interactions between specific nutrients or additional interactions with the human microbiome and environment.(95)

Strengths of this study and analysis were the prospective design with an a priori purpose of examining intergenerational transmission of risk. As there is a strong social gradient applied to diet in pregnancy,(96) confounding by socio-demographic factors and lifestyle was a concern; we controlled comprehensively for various socio-demographic factors. The GLMM enabled us to control for repeated measurements over time for participants. Limitations were differential follow-up, leading to varied numbers of missing data for certain variables, potentially introducing bias to follow-up data. Countering this, it is notable that the same associations were found separately at the three different time points. The authors recognise that asthma in childhood is a heterogeneous phenotype and that grouping of the 3 time-points could potentially mask different phenotypes;(97, 98) however our study was not powered to differentiate asthma on phenotype at various follow-up. Data on familial atopy or asthma were not available; it is possible that the suggested relationships between maternal diet and offspring asthma have been confounded by the presence or absence of familial predisposition. Data on foetal growth parameters were not available; the effect of foetal growth on childhood asthma could not be controlled for.(99) The fact that the associations found in this analysis were between maternal food intake and doctor diagnosed asthma raised the possibility of ascertainment bias; more health conscious mothers were more likely to follow good pregnancy diets, and were more likely to take their ill children to the doctor to receive a formal diagnosis.

However, if this ascertainment bias were to be true, this analysis would have showed the opposite, i.e. that an increase in healthy eating was adversely associated with doctor diagnosed asthma.

CONCLUSION

This analysis suggests that higher intakes of oily fish and vitamin D in the maternal pregnancy diet have a protective effect on childhood asthma. This is consistent with the developmental origins of health and disease hypothesis, suggesting that certain exposures of the foetus in utero can affect the development of allergic diseases in childhood by modulating immune response (100). As there is no anticipated cure for asthma in the near future, adjustment of the environment as early as possible, e.g. in utero and in infancy, might provide the best way to achieve a reduction in the asthma burden.(94, 101)

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CONTRIBUTORS' STATEMENT: The Principal Investigator of the Lifeways Study, Professor Kelleher, conceptualised the design of the study and supervised the overall project, including data analysis and interpretation. Dr. Viljoen drafted the manuscript and undertook data collection, analyses and interpretation as part of her PhD. Dr. Murrin assisted with data collection, analyses and interpretation. Dr. Segurado provided statistical input relating to analyses and interpretation of data. Drs. O'Brien and Mehegan assisted with data management and data interpretation. All contributors reviewed and revised the manuscript and approved the final version as submitted.

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DATA SHARING: Additional unpublished data from the Lifeways study relating to cardiovascular risk/mortality and healthcare utilization are available. Data can be accessed by collaborators or peers via written request to the principal investigator Professor CC Kelleher (cecily.kelleher@ucd.ie) and the study data manager: Dr John Mehegan (john.mehegan@ucd.ie).

What is already known on this topic: The rising burden of childhood asthma worldwide has placed an emphasis on the potential for primary prevention and disease modification. The association of pregnancy nutrient intake (particularly oily fish, vegetables, vitamin D) and season of birth with offspring asthma/atopy has suggested disease originating in utero. Findings from observational studies are inconsistent; intervention studies suggest an inverse association of asthma with vitamin D.

What this study adds: Only one other cohort study investigating the association of maternal pregnancy diet and offspring asthma with follow-up beyond 6 years was identified. In children with persistent wheezing up to age 3 only a small percentage progress to asthma; in our study offspring was followed-up for 10 years. Mothers with higher oily fish and vitamin D intake had offspring with significant decreased odds of asthma at any time point over 10 years follow-up.

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Supplement

Table S1 Food items included in 'Oily fish' and 'Vegetables'

Oily fish (fresh or canned):	Mackerel, Kippers/Herring, Tuna, Salmon, Sardines
Vegetables:	Carrots, Spinach, Broccoli, Spring greens & Cale, Brussel sprouts, Cabbage, Peas, Green beans, Runner beans, Parsnips & Turnips, Leeks, Onions, Garlic, Mushrooms, Sweet peppers, Bean sprouts, Green salad & Lettuce, Cucumber, Tomatoes, Sweetcorn, Beetroot, Coleslaw, Avocado, Watercress, Cauliflower, Celery, Marrow & Courgettes

Table S2 Simple mixed model association between childhood determinants and childhood asthma at any point (n=897)

		Asthma diagnosis True vs False 95% CI	
	OR	**Lower**	**Upper**
Time (n=897)			
Year 10 follow-up	1.00		
Year 5 follow-up	0.55	0.40	0.77
Year 3 follow-up	0.41	0.29	0.57
Birthweight[1] (n=885)	1.00	1.00	1.00
Gestational age[1] (n=793)	0.92	0.85	0.99
Gender[1] (n=893)			
Female	1.00		
Male	1.55	1.17	2.05
GMS Eligibility[1] (n=694)			
TRUE	1.00		
FALSE	0.45	0.29	0.69
Birth order[1] (n=882)			
Not firstborn	1.00		
Firstborn	1.14	0.86	1.51
Mode of delivery[1] (n=839)			
Ceasarean section	1.00		
Vaginal (spontaneous or assisted)	0.85	0.57	1.26
Region[1] (n=897)			
Dublin	1.00		
Galway	0.86	0.64	1.16

[1]Controlling for time as a fixed factor

OR=Odds Ratio SD=Standard Deviation CI=Confidence interval

Table S3 Simple mixed model association between maternal determinants and childhood asthma at any point (n=897)

		Asthma diagnosis True vs False 95% CI	
	OR	**Lower**	**Upper**
Time (n=897)			
Year 10 follow-up	1.00		
Year 5 follow-up	0.55	0.40	0.77
Year 3 follow-up	0.41	0.29	0.57
Education[1] (n=873)			
Some/completed 3rd level	1.00		
None/Primary/Secondary school	1.28	0.97	1.70
Marital status[1] (baseline) (n=885)			
Lone	1.00		
Cohabiting	0.66	0.47	0.94
Marital status[1] (any time-point) (n=557)			
Lone	1.00		
Cohabiting	1.21	0.57	2.57
Age at birth of proband[1] (n=889)	0.98	0.96	1.01
BMI prior to falling pregnant[1] (n=750)	0.98	0.94	1.02
GMS Eligibility[1] (baseline) (n=887)			
TRUE	1.00		
FALSE	0.73	0.5	1.07
GMS Eligibility[1] (any time point) (n=797)			
TRUE	1.00		
FALSE	0.72	0.51	1.03
Maternal Health[1] (baseline) (n=871)			
Excellent/Very good	1.00		
Poor/Fair/Good	0.82	0.59	1.13
Maternal Health[1] (any time point) (n=560)			
Excellent/Very good	1.00		
Poor/Fair/Good	1.23	0.79	1.9
Smoking status at delivery[1] (n=870)			
Smoker	1.00		
Non-smoker	0.84	0.59	1.2
Breastfeeding[1] (n=897)			
TRUE	1.00		
FALSE	1.21	0.85	1.73

[1]Controlling for time as a fixed factor GMS=General Medical Scheme
OR=Odds Ratio SD=Standard Deviation CI=Confidence Interval

Table S4 Distribution of maternal nutrient intake during pregnancy in Normal-Reporters (EI/BMRpreg<1.35-2.39) relative to Irish Recommended Daily Allowances (n=453)

		25th		75th	RDAs for Irish women*	
Nutrient[1]	**$\bar{x}$ ± SD**	**Percentile**	**Median**	**Percentile**	**19-64 yrs**	**Pregnancy****
Total fat (g/day)	129.0±110.4	51.8	100.4	175.3	-	-
MUFA (g/day)	41.1±35.9	16.8	31.7	53.9	-	-
PUFA (g/day)	20.3±20.3	7.0	13.5	25.8	-	-
Saturated Fatty Acids (g/day)	57.8±58.5	20.1	39.1	74.6	-	-
Cholesterol (mg/day)	402.1±330.9	182.3	312.9	522.2	-	-
Retinol (µg/day)	763.2±1004.2	290.6	515.7	890.4	-	-
Carotene (µg/day)	3594.9±2758.3	1356.7	3127.5	4581.2	-	-
Selenium (µg/day)	86.7±83.0	36.3	60.7	110.3	55.0	55.0
Zinc (mg/day)	14.7±11.7	6.6	12.2	18.5	15.0	20.0
Magnesium (mg/day)	545.5±557.9	191.1	362.7	660.9	255-65‡	290-300‡
Copper (mg/day)	2.0±3.4	0.6	1.2	2.1	1.1	1.1
Manganese (mg/day)	4.9±4.8	1.7	3.3	5.9	≤10.0‡	-
Calcium (mg/day)	1712.9±1467.4	614.6	1222.0	2429.9	800.0	1200.0
Vitamin C (mg/day)	248.1±246.5	90.3	157.9	316.0	60.0	80.0
Vitamin D (µg/day)	4.3±4.1	1.7	3.1	5.4	7.5	10.0
Vitamin E (mg/day)	13.6±18.7	4.9	7.9	14.2	8.0	10.0

[1]Calculated from food-frequency questionnaires in first trimester; Energy adjusted - residual method;

MUFA=Monounsaturated Fatty Acids PUFA=Polyunsaturated Acids EI=Energy Intake BMR=Basal Metabolic Rate preg=Pregnancy

g=gram mg=milligram µg=microgram

* Recommended Daily Allowance; Irish Food Safety Authority (1999) **From 20 weeks gestation ‡Estimated Average Requirement, European Food Safety Authority (2006)

‡Scientific Committee for Food of the European Union (SCF, 1993)

Table S5 Distribution of maternal food group intake during pregnancy relative to SLAN females (n=886)

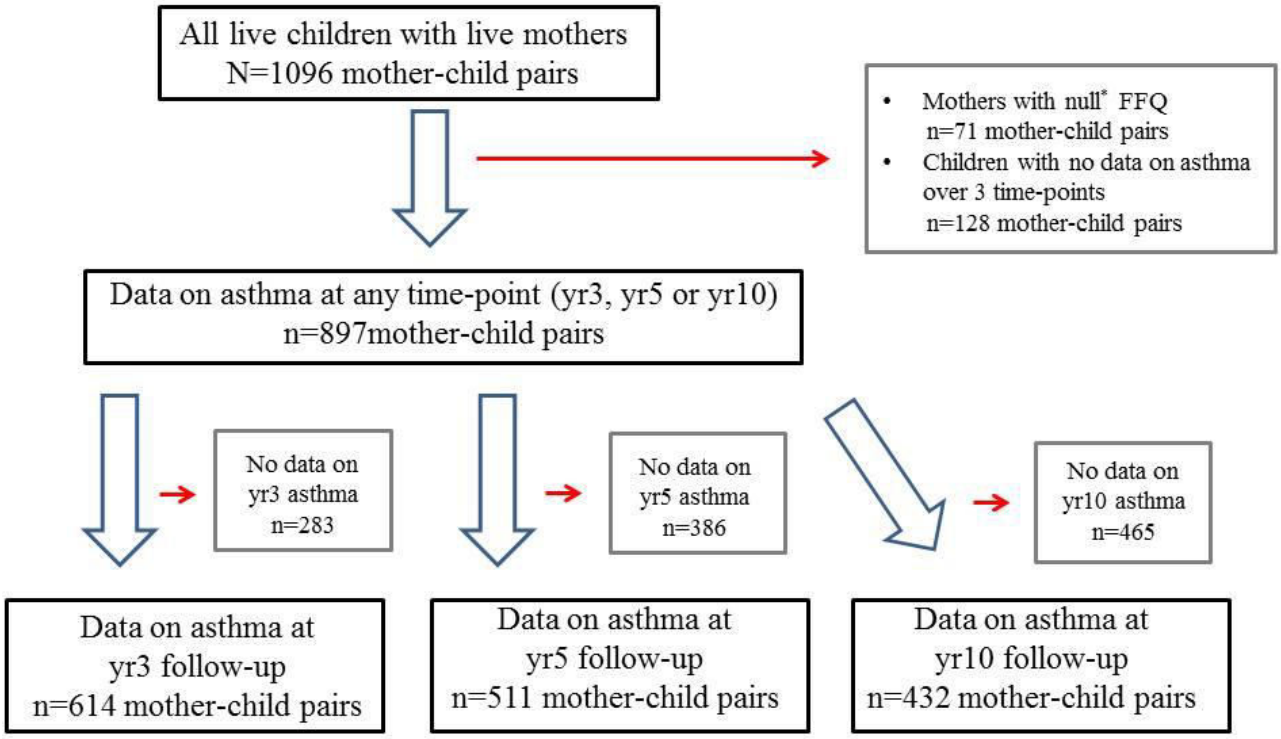
(n=886)

	Lifeways Mothers 2001-2003			SLAN 2007 Females		Recommended intake*	
	Under 18 years (n=16)	**18-29 years (n=377)**	**30-44 years (n=493)**	**18-29 years**	**30-44 years**	**19-64 yrs**	**Pregnancy**
Food group[1] (total daily portions)	**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**Portions**	**Portions**
Oily fish	0.02 ± 0.04	0.03 ± 0.07	0.08 ± 0.14	-	-	1[b]	1[b]
Vegetables	2.5 ± 1.5	2.6 ± 1.9	3.2 ± 2.1	4.1 ± 2.9	4.5 ± 2.8	5[a]	6-7[a]
Fruit	2.2 ± 1.6	2.0 ± 2.5	2.2 1.6	2.8 ± 2.7	3.0 ± 2.8	5[a]	6-7[a]
Added Fat	2.2 ± 3.1	2.4 ± 2.4	2.6 ± 2.2	-	-	-	-

[1]Calculated from food-frequency questionnaires in first trimester [*]Food Safety Authority of Ireland SLAN=Survey of Lifestyle, Attitudes and Nutrition

[a]Daily portion [b]Weekly portion

Figure S1 Numbers through analysis



FFQ – Food Frequency Questionnaire
*More than 10 rows missing from FFQ

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract – **PAGE 1 & 2**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found – **PAGE 2**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported – **PAGE 3 & 4**
Objectives	3	State specific objectives, including any prespecified hypotheses – **PAGE 4**
Methods		
Study design	4	Present key elements of study design early in the paper – **PAGE 4 & 5**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection – **PAGE 4, 5 & 6**
Participants	6	*Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up – **PAGE 5**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable – **PAGE 5, 6 & 7**
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 – **PAGE 6 &7**
Bias	9	Describe any efforts to address potential sources of bias – **PAGE 17**
Study size	10	Explain how the study size was arrived at – DUE TO WORD COUNT RESTRICTION PLEASE SEE DETAIL OF REFERENCE 60, 62 & 63
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why – **PAGE 5, 6, 7 & 8**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding – **PAGE 5, 6, 7, 13, 14 & 15**
		(*b*) Describe any methods used to examine subgroups and interactions – **PAGE 8, 13, 14 & 15**
		(*c*) Explain how missing data were addressed – **PAGE 11 & 17,** ADDITIONAL DETAIL IN REFERENCE 63
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed – **PAGE 11 &17**, ADDITIONAL DETAIL IN REFERENCE 63

Continued on next page

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 – **PAGE 11, 17**, ADDITIONAL DETAIL REFERENCE 63
		(b) Give reasons for non-participation at each stage - DUE TO WORD COUNT RESTRICTION PLEASE SEE DETAIL OF REFERENCE 60, 62 & 63
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders – **PAGE 8, 9, 10, 11 & 12**
		(b) Indicate number of participants with missing data for each variable of interest – **PAGE 11**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) – **PAGE 11**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time – **PAGE 11**, ADDITIONAL DETAIL REFERENCE 63
Main results	6	(*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 – **PAGE 13, 14, & 15**
		(*b*) Report category boundaries when continuous variables were categorized - DUE TO WORD COUNT RESTRICTION PLEASE SEE DETAIL OF REFERENCE 63
Discussion		
Key results	18	Summarise key results with reference to study objectives – **PAGE 12**
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 – **PAGE 17**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence – **PAGE 17**
Generalisability	21	Discuss the generalisability (external validity) of the study results – **PAGE 17**
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 – **PAGE 19**

*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.

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Pregnancy Diet and Offspring Asthma risk over a 10-year period: the Lifeways Cross Generation Cohort Study, Republic of Ireland

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Pregnancy Diet and Offspring Asthma risk over a 10-year period: the Lifeways Cross Generation Cohort Study, Republic of Ireland

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Key Words: asthma, childhood, DOHaD, pregnancy, vitamin D

Word count: 3110

ABSTRACT:

Objective

The association of maternal pregnancy diet with offspring asthma risk have been reported. However, literature on longitudinal patterns of asthma risk relative to intra-uterine nutrient exposure is limited. We aimed to establish whether vegetable, oily fish and vitamin D intake during pregnancy are associated with childhood asthma risk over a 10 year period in the Irish Republic.

Design

Mother-child pairs (n=897) from the Lifeways prospective birth cohort, with data on nutrient intake during pregnancy and asthma status respectively, were eligible for inclusion in the analysis. Data on socio-economic and morbidity indicators over 10 years of follow-up on mothers and the index child were collected through self-administered questionnaires. Asthma status as diagnosed by the GP at any time-point over 10 years was related to maternal vegetable, oily fish and vitamin D intake during pregnancy, while adjusting for gestational age, socio-economic status, smoking at delivery, breast-feeding, season of birth and supplement use. Data were modelled with a Generalised Linear Mixed Model (GLMM) with correlated residuals over time for individuals.

Results

In the fully adjusted GLMM, higher daily average intake of oily fish (OR 0.23 per serving/day, 95% CI 0.04-1.41) and of vegetables (OR 0.96 per serving/day, 95% CI 0.88-1.05) indicated a protective direction of effect, but the confidence limits overlapped 1. A higher daily vitamin D intake significantly reduced the odds of asthma (OR 0.93 per μg/day, 95% CI 0.89-0.98).

Conclusion

This analysis suggests higher daily average intake of vitamin D in pregnancy may be protective of asthma risk in offspring over the first 10 years of life.

STRENGTHS

- The prospective design with an a priori purpose of examining intergenerational transmission of risk.
- As there is a strong social gradient applied to diet in pregnancy, confounding by socio-demographic factors and lifestyle was a concern; we controlled for a comprehensive range of socio-demographic factors.
- The GLMM enabled us to take advantage of repeated measurements over time for participants.

LIMITATIONS

- Limitations were differential follow-up, leading to varied numbers of missing data for certain variables, potentially introducing bias to follow-up data. Countering this, it is notable that the same associations were found separately at the three different time points.
- The authors recognise that asthma in childhood is a heterogeneous phenotype and that grouping of the 3 time-points could potentially mask different phenotypes; however our study was not powered to differentiate asthma on phenotype at various follow-up.
- Data on familial atopy or asthma were not available; it is possible that the suggested relationships between maternal diet and offspring asthma have been confounded by the presence or absence of familial predisposition.
- Data on foetal growth parameters were not available; the effect of foetal growth on childhood asthma could not be controlled for.
- GPs criteria to diagnose asthma could be diverse, with potential for variability.

INTRODUCTION

Asthma is the most common chronic disease of childhood;(1, 2) reports indicate a continuous and consistent increase in worldwide prevalence, especially in westernised societies. Prevalence rates in the United Kingdom and Ireland are among the highest in Europe. (3, 4) According to the Centres for Disease Control and Prevention, between 2001 and 2010 asthma prevalence in children in the United States increased 1.4% each year.(5) This increase is most likely multi-factorial, with complex interactions of genetic-immunological-environmental factors leading to the phenotypic expression of disease.(6) Recently multiple studies have attempted to deconstruct this multifactorial relationship, focusing on the change in dietary habits over recent decades.(7-13) The progressive trend in early presentation of allergic disease in childhood, with the implication of possible exposure in utero, has placed an emphasis on maternal pregnancy diet as a prominent factor in the development of offspring asthma.(14, 15) As allergen-specific immune responses are established in foetal life, maternal nutrient intake during pregnancy is pivotal; intake may potentially influence the development of both the innate and acquired immune responses, predisposing to atopy in later life.(16)

A growing body of persuasive epidemiological evidence suggests that deficiency of maternal vitamin D intake prenatally has an inverse relationship with atopic disease in childhood.(17-22) Observational studies on the association of maternal serum and/or infant cord blood 25-hydroxy-vitamin D_3 ($25(OH)D_3$) levels with atopic markers are conflicting. Some demonstrated similar inverse associations;(23-30) others demonstrated direct (31-33) and null (34-42) associations. U-shaped associations were suggested by both Rothers *et al* (43) and Maslova *et al*.(44) Most recently intervention studies exploring pregnancy vitamin D supplementation and asthma risk at 3 years of age suggested a protective effect (statistically non-significant). The authors suggested that longer follow-up of children is needed to determine the clinical importance of findings.(45, 46)

The Lifeways study has previously reported the association between pregnancy intake of oily fish and vegetables and General Practitioner (GP) diagnosed asthma in offspring at age 3 years; a higher daily mean intake suggested a significant protective effect.(47) Literature on pregnancy consumption of fish with subsequent atopic risk in offspring is mostly consistent with our findings, indicating a protective effect.(7, 48-54) Our current aim was twofold: firstly to build on the aforementioned Lifeways findings and test the hypothesis that a higher pregnancy intake of vegetables and oily fish might be inversely associated with asthma risk at any stage over 10 years follow-up; secondly, as suggested in the literature, to explore the

association of pregnancy vitamin D intake and offspring asthma risk within our cohort at any stage over 10 years of follow-up.

METHODS

Study design and sample selection

The Lifeways study was established 2001-2003 as a prospective birth cohort in the Republic of Ireland. The *a priori* purpose was to examine determinants of health status in children, including diet and lifestyle, and to establish patterns and links across generations. Recruitment, data collection and study instruments have previously been discussed in detail.(55) In brief, mothers were recruited at first ante-natal visit (14-16 weeks gestation) in one of two regional maternity hospitals in the more rural West (Galway) and the more industrialised East (Dublin). Of 1124 mothers recruited, 1082 gave birth resulting in 1096 live mother-child pairs. Analysis was limited to current live mother-child pairs, where data on the proband's asthma status were available for at least one time-point. Babies with congenital anomalies and delivery <34 weeks gestational age (56) were excluded from analysis. Due to attrition over time participants had differential patterns of follow-up through phases; 614 mother-child pairs were included in the year 3 analyses, 511 in year 5 and 432 in year 10 follow-up. The sample for this analysis of 897 mother-child pairs comprised respondents for whom at least one follow-up point of asthma health status was recorded (Figure 1). Ethical approval for all phases of the study was granted by the Human Research Ethics Committee, University College Dublin, Ireland. Written informed consent was obtained from all adult participants at each follow-up phase of the study. Parental consent was obtained for child subjects at each follow-up phase; additional assent was obtained from all child subjects at the year 10 follow-up phase.

Assessment of pregnancy diet

Data on maternal nutrient intake during pregnancy were captured by a semi-quantitative Food Frequency Questionnaire (FFQ) as part of a self-administered questionnaire to the mother at her first ante-natal visit. The FFQ was developed from the international version used in the European Prospective Investigation in Cancer studies by the National Nutritional Surveillance Centre and extensively validated for use in an Irish population by the National University of Ireland, Galway.(57) The main food groups regularly consumed in the Irish diet were included, and consisted of 149 food items.(47, 58) The questionnaire focused on maternal dietary intake since pregnant. Intake as a medium serving (detailed in the FFQ for relevant food items) was recorded on a 9-grade scale, with categories subsequently transformed to continuous daily portion averages for all 149 food items. To arrive at distinct food groups, the continuous intake of various food items were summed and reported as total portions per day (Supplement; Table S1). The daily average intake of energy and nutrients i.e. ingested portions/day of food containing vitamin D, was calculated by linking frequency selections from the FFQ with food equivalents in McCance and Widdowson's nutritional composition database, 6th edition,(59) using software developed specifically for the Lifeways database.(60) An estimation of the intake of Vitamin D in micrograms per day (μg/day) and energy in kilocalories (kCal/day) were made.

Assessment of outcome

Data on doctor diagnosed asthma in offspring were collected at 3 time-points: ages 3, 5 and 9 years. Various studies have used doctor diagnosed asthma/parental report of doctor diagnosed asthma to ascertain diagnosis.(30, 45) Questions to ascertain asthma diagnosis were adapted from the validated International Study on Asthma and Allergies in Childhood (ISAAC) questionnaire.(61, 62) Diagnosis from age 3 and 9 follow-up were reported by the General Practitioner (GP), information on GP diagnosed asthma at age 5 was obtained from the mother. For the univariate analysis, asthma as a dichotomous outcome variable ('Yes' vs 'No') at each of the three time-points were analysed separately. For the multivariable analysis the effect of dietary intakes during pregnancy on doctor diagnosed asthma in the child at the 3 time-points over a period of 10-year follow-up was examined controlling for different prevalence at each age, but assuming protective factors would have the same effect at each age.(63)

Statistical analysis

Definite doctor diagnosed asthma in offspring at any time point over 10-year follow-up, was related to maternal pregnancy intake of oily fish, vegetables, expressed in servings per day, and vitamin D expressed in μg per day.

Univariate analysis and Covariates

Variables that could potentially confound the diagnosis of asthma were identified from the literature, and screened in our cohort for association with an asthma diagnosis at the specific time-point using unadjusted binomial logistic regression or the independent samples t-test. Predictors with $p<0.1$ were identified as being potential confounders and selected for inclusion in the multivariable models, and are discussed below.

Total Energy Intake (EI)

To control for variance in accuracy of maternal energy reporting, total energy intake (kCal) was adjusted for in all models. Nutrients were adjusted for total energy intake using the residual method.(64, 65) To account for mothers who potentially over- or under reported energy intake, the ratio of energy intake (EI) to basal metabolic rate in the first trimester of pregnancy (BMR_{preg}) was calculated to identify extreme outliers.(63, 66, 67) BMR_{preg} was calculated using the pre-pregnancy BMI and Schofield equations.(67) Mothers were asked to report their pre-pregnancy weight at consultation during the first ante-natal visit.

Gestational age

Gestational age in weeks.

Socio-economic status

We used eligibility for the General Medical Scheme (GMS) as an indicator of socio-economic status. The Republic of Ireland has a two-tiered healthcare system, where certain individuals are eligible for different levels of free healthcare dependent on their income and certain medical conditions. GMS is thus a robust means tested indicator.(68-70) As maternal and offspring GMS eligibility are strongly correlated only the maternal predictor was used in the models.(63, 71)

Smoking

Smoking status at time of delivery; hospital delivery records.

Breastfeeding

Data from the self-administered maternal questionnaire; 'Was your Lifeways child ever breastfed?'

Season of birth

Seasons were comprised as follows: Summer (May, June, July), Autumn (Aug, Sept, Oct), Winter (Nov, Dec, Jan), Spring (Feb, March, April). This follows the grouping suggested by the Irish ROLO study with data on maternal serum 25(OH)D levels.(72) Summer and Autumn were collapsed to form 'Summer', with Spring and Winter collapsed to form 'Winter', making up the final dichotomous variable, 'Summer' vs 'Winter'.

Supplement use

Data on supplement use were generic: 'Have you taken any vitamins, minerals or food supplements?' 'Yes' or 'No' and did not allow for specification on supplement type or content quantification.

Multivariable analysis

The associations with the three aforementioned dietary intakes were analysed in multivariable models to assess their independent association with asthma, and verify the extent of confounding in a stepwise manner. Vegetables, oily fish and vitamin D intakes were analysed as continuous predictor variables. Multivariable analysis was run using the full sample of 897 mother-child pairs in a Generalised Linear Mixed Model (GLMM) with repeated measures of asthma marginally modelled over time, estimating an unstructured variance-covariance matrix to allow unequal variances and residual correlations of asthma diagnosis over time. Covariates were entered into the GLMM sequentially as fixed factors based on their univariate association with asthma, up to the cut-off of p=0.1. Time was consistently included as a fixed factor. Predictor variables were regarded as significant if the 95% confidence intervals for the odds ratios did not include 1. The Statistical Package for the Social Sciences (SPSS) version 20 was used to conduct univariate analysis; multivariable analysis was run with Statistical Analysis Software (SAS) version 9.3.

RESULTS

Study subjects' characteristics and asthma prevalence

In the final sample, 66.9% of the mother-child pairs were resident in the Dublin area and 33.1% in the Galway area, proportionate to recruitment patterns. Mothers with a 3rd level education were marginally higher (51.9%) than those with None/Primary/Secondary school education (48.1%); 19.4% of mothers were smokers at the time of giving birth. The mean (SD) age of mothers at time of giving birth was 30.2 (5.9) years and the mean (SD) pre-pregnancy body mass index of mothers were 23.7 (4.0). The offspring sex distribution was about equal; 48.8% males and 51.2% females. The mean (SD) birth-weight was 3515.3 (568.6) grams, with a mean (SD) gestational age of 39.9 (1.9) weeks. Just under half (45.8%) of probands were the first born and 86.20% were delivered vaginally. Doctor diagnosed asthma in offspring at the 3 phases of follow-up respectively, increased from 10.90% at 3-years, to 14.33% at 5-years and 23.10% at 10-years follow-up. In general the literature suggests a downward trend in prevalence with increasing age. There are however, cohorts that noted an increase of prevalence with age.(73-75) Maternal socio-economic and biological characteristics relative to food group intake are presented in Table 1. Maternal and child characteristics as related to asthma diagnosis are further presented in supplemental material (Table S2 and S3).

Univariate associations of background variables with childhood asthma from the cross-sectional data for the 3 follow-up phases respectively have previously been discussed in detail.(47, 62, 63)

Table 1 Maternal food group intake during pregnancy in relation to socio-economic factors (n=897)

		Food group				
	n[1]	Oily fish (portion/day) mean (SD)	p-value	n[1]	Vegetables (portion/day) mean (SD)	p-value
Education			<0.001[a]			<0.001[a]
None/Primary/Secondary school	416	0.05 (0.10)		420	2.61 (1.88)	
3rd level education	451	0.07 (0.13)		451	3.26 (2.16)	
Marital status (baseline)			0.01[a]			<0.001[a]
Lone	179	0.04 (0.09)		180	2.51 (2.06)	
Cohabiting	701	0.06 (0.12)		703	3.05 (2.03)	
GMS Eligibility (baseline)			<0.001[a]			<0.001[a]
No	750	0.06 (0.12)		754	3.02 (2.06)	
Yes	131	0.03 (0.07)		131	2.43 (1.91)	
Smoking status at delivery			0.07[a]			<0.001[a]
Non-smoker	693	0.06 (0.12)		695	3.04 (2.10)	
Smoker	167	0.04 (0.11)		169	2.39 (1.61)	
Region			0.04[a]			<0.001[a]
Galway	292	0.07 (0.13)		294	3.21 (2.17)	
Dublin	594	0.05 (0.10)		596	2.79 (1.96)	
Age group at delivery (y)			<0.001[b]			<0.001[b]
Under 18	16	0.02 (0.04)		16	2.52 (1.51)	
18 to 29	373	0.03 (0.07)		377	2.56 (1.92)	
30+	491	0.08 (0.14)		492	3.22 (2.09)	

[a]Independent samples t-test [b]ANOVA GMS=General Medical Scheme

[1]Numbers do not always add up to 897 because of varied numbers of missing data for some variables

Within-time representativeness of responders vs. non-responders that contributed to the final sample is presented in Table 2.

Table 2 Within-time representativeness of final sample (n=897)

Characteristics	Responders[a] n=614	Non-responders[a] n=283
Year 3 follow-up		
Maternal educational attainment[c]		
None/Primary/Secondary school, No. (%)	290 (48.6)	130 (47.1)
Some/Completed 3rd level education, No. (%)	307 (51.4)	146 (52.9)
Maternal GMS eligibility at baseline[c]		
Not eligible, No. (%)	512 (84.3)	244 (87.1)
Eligible, No.(%)	95 (15.7)	36 (12.9)
Region[c]		
West, No. (%)	217 (35.3)	80 (28.3)
East, No. (%)	397 (64.7)	203 (71.7)
Maternal age at birth of proband, mean (SD), y[c]	30.35 (5.9)	29.75 (5.9)
Maternal oily fish intake in pregnancy, mean (SD), servings/d[c]	0.06 (0.1)	0.05 (0.1)
Maternal Vegetable intake in pregnancy, mean (SD), servings/d[c]	2.95 (1.9)	2.88 (2.2)
Maternal Vitamin D intake in pregnancy, mean (SD), µg/d[c]	4.70 (4.8)	5.60 (10.2)
Year 5 follow-up	n=511	n=386
Maternal educational attainment[c]		
None/Primary/Secondary school, No. (%)	241 (48.7)	179 (47.4)
Some/Completed 3rd level education, No. (%)	254 (56.1)	199 (52.6)
Maternal GMS eligibility at baseline[c]		
Not eligible, No. (%)	429 (85.0)	327 (85.6)
Eligible, No. (%)	76 (15.0)	55 (14.4)
Region[c]		
West, No. (%)	165 (32.3)	132 (34.2)
East, No. (%)	346 (67.7)	254 (65.8)
Maternal age at birth of proband, mean (SD), y[c]	30.10 (5.8)	30.24 (5.9)
Maternal oily fish intake in pregnancy, mean (SD), servings/d[c]	0.06 (0.1)	0.06 (0.1)
Maternal Vegetable intake in pregnancy, mean (SD), servings/d[c]	2.93 (2.1)	2.94 (1.9)
Maternal Vitamin D intake in pregnancy, mean (SD), µg/d[c]	4.70 (6.1)	5.30 (7.9)
Year 10 follow-up	n=432	n=465
Maternal educational attainment[b]		
None/Primary/Secondary school, No. (%)	174 (40.7)	246 (55.2)
Some/Completed 3rd level education, No. (%)	253 (59.3)	200 (44.8)
Maternal GMS eligibility at baseline[b]		
Not eligible, No. (%)	385 (89.7)	371 (81.0)
Eligible, No. (%)	44 (10.3)	87 (19.0)
Region[b]		
West, No. (%)	166 (38.4)	131 (28.2)
East, No. (%)	266 (61.6)	334 (71.8)
Maternal age at birth of proband, mean (SD), y[b]	31.55 (5.2)	28.86 (6.1)
Maternal oily fish intake in pregnancy, mean (SD), servings/d[c]	0.06 (0.1)	0.06 (0.1)
Maternal Vegetable intake in pregnancy, mean (SD), servings/d[c]	3.11 (2.1)	2.76 (2.0)
Maternal Vitamin D intake in pregnancy, mean (SD), µg/d[c]	5.00 (7.5)	4.90 (6.4)

[a]Numbers do not always add up to total as varied numbers of missing data at certain variables
[b]p<0.001 [c]Non-significant
µg: micrograms SD:Standard Deviation d:day y:years

Pregnancy dietary intake

Reported intake of vegetables and oily fish were directly and significantly associated with the maternal social gradient (Table 1). Mean (SD) daily intake of oily fish and vegetables were 0.06 (0.12) and 2.93 (2.04) portions per day respectively. The Food Safety Authority of Ireland (FSAI) recommends that pregnant women should have one portion of oily fish weekly (i.e. 0.1 portions/day) and 6-7 portions of vegetables daily.(76) The mean intake of Lifeways mothers did not reach this recommendation. Data captured by the FFQ detailed intake as a medium portion (90-100 gram of fish; a piece of fish about the size of a woman's palm).

Vitamin D intake during pregnancy between those mothers with consistent follow-up, and those with attrition did not differ significantly. A social gradient for pregnancy vitamin D intake in the final sample was not observed. The distribution of mean vitamin D intake was investigated according to the EI/BMR_{preg} ratio; no marked difference in the distribution of vitamin D was observed.(63) Energy adjusted mean (SD) intake of vitamin D in mothers was 4.3 (4.1) µg/d. The FSAI recommends a daily allowance of 10µg/day vitamin D for Irish pregnant women.(77). (Table S4 and S5). At baseline 327 (36.9%) mothers from the final sample reported using supplements.

Pregnancy dietary associations with offspring asthma

Vegetable intake was negatively associated with offspring asthma, although not significantly so in the fully adjusted model (OR 0.96 per serving/day, 95% CI 0.88-1.05) (Model 8) (Table 3).

Oily fish intake was significantly and inversely associated with offspring asthma in the first two models, with an increase in daily average serving of oily fish suggesting a protective effect at any time point over the 10 year follow-up period (model 2 OR 0.17 per serving/day, 95% CI 0.03-0.87) (Table 4). Change in the odds ratios across models suggested that gestational age may have been a confounder (model 2 versus 3), and all models up to the fully adjusted (OR 0.23 per serving/day, 95% CI 0.04-1.41) were not significant, despite a substantial protective direction to the odds ratio.

All models suggested vitamin D had a significant inverse association, including the fully adjusted analysis (model 9 OR 0.93 per µg/day, 95% CI 0.89-0.98) with offspring asthma at any time-point of follow-up (Table 5).. Adjustment for breastfeeding in particular increased the odds ratio for vitamin D (model 5 versus 6), in part due to the loss of 85 women with no data on breastfeeding habits.

Table 3 Generalised linear mixed model: asthma at any time-point vs. never (vegetable intake as main exposure of interest)

Independent variable		**OR (95% CI)**							
		Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8
		n=890	n=883	n=781	n=777	n=756	n=682	n=682	n=677
Time	Year 5 follow-up	1.31 (0.92-1.86)	1.30 (0.92-1.84)	1.28 (0.87-1.90)	1.31 (0.88-1.94)	1.25 (0.84-1.86)	1.27 (0.83-1.92)	1.27 (0.83-1.93)	1.29 (0.84-1.96)
Ref: Year 3 follow-up	Year 10 follow-up	2.59 (1.97-3.41)	2.56 (1.94-3.37)	2.81 (2.09-3.78)	2.86 (2.11-3.86)	2.88 (2.13-3.90)	2.81 (2.07-3.83)	2.80 (2.06-3.82)	2.84 (2.08-3.88)
Vegetable intake (serving/d)		0.94 (0.87-1.01)	0.94 (0.87-1.01)	0.94 (0.86-1.03)	0.95 (0.88-1.03)	0.96 (0.88-1.04)	0.96 (0.88-1.05)	0.96 (0.88-1.05)	0.96 (0.88-1.05)
Total EI (1,000 kCal)			0.96 (0.85-1.08)	0.97 (0.86-1.09)	0.97 (0.86-1.09)	0.97 (0.86-1.09)	0.96 (0.84-1.09)	0.95 (0.83-1.09)	0.95 (0.83-1.09)
Gestational Age (weeks)				0.92 (0.85-0.99)	0.92 (0.85-0.99)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.93 (0.85-1.01)	0.93 (0.86-1.01)
GMS Eligibility (baseline)	Yes				1.20 (0.76-1.91)	1.13 (0.69-1.84)	0.93 (0.54-1.59)	0.94 (0.55-1.34)	0.95 (0.55-1.63)
Ref: No									
Smoking at delivery	Smoker					0.96 (0.62-1.49)	0.84 (0.52-1.34)	0.83 (0.52-1.34)	0.83 (0.52-1.34)
Ref: Non-smoker									
Breastfeeding	No						1.27 (0.90-1.79)	1.28 (0.91-1.81)	1.28 (0.91-1.81)
Ref: Yes									
Season of birth	Summer							1.36 (0.95-1.94)	1.35 (0.95-1.94)
Ref: Winter									
Supplement use	No								1.17 (0.82-1.66)
Ref: Yes									

EI=Energy Intake GMS=General Medical Scheme

Table 4 Generalised linear mixed model: asthma at any time-point vs. never (oily fish intake as main exposure of interest)

Independent variable		OR (95% CI)								
		Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9
		n=886	n=879	n=778	n=774	n=753	n=678	n=678	n=678	n=673
Time	Year 5 follow-up	1.31 (0.92-1.86)	2.52 (1.91-3.33)	1.29 (0.87-1.92)	1.32 (0.89-1.96)	1.25 (0.84-1.87)	1.27 (0.84-1.94)	1.27 (0.83-1.95)	1.29 (0.84-1.96)	1.31 (0.86-2.00)
Ref: Year 3 follow-up	Year 10 follow-up	2.56 (1.94-3.38)	1.30 (0.92-1.85)	2.78 (2.06-3.75)	2.83 (2.09-3.83)	2.85 (2.10-3.88)	2.78 (2.03-3.80)	2.77 (2.02-3.78)	2.85 (2.08-3.91)	2.91 (2.11-4.00)
Oily fish intake (serving/d)		0.16 (0.03-0.85)	0.17 (0.03-0.87)	0.22 (0.04-1.16)	0.24 (0.05-1.29)	0.21 (0.04-1.20)	0.19 (0.03-1.20)	0.20 (0.03-1.23)	0.22 (0.04-1.34)	0.23 (0.04-1.41)
Total EI (1,000 kCal)			0.97 (0.86-1.08)	0.98 (0.87-1.10)	0.97 (0.87-1.09)	0.97 (0.87-1.09)	0.96 (0.85-1.09)	0.96 (0.84-1.10)	0.96 (0.84-1.10)	0.96 (0.84-1.10)
Gestational Age (weeks)				0.92 (0.85-0.99)	0.92 (0.85-0.99)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.94 (0.86-1.02)	0.94 (0.87-1.02)
GMS Eligibility (baseline)	Yes				1.18 (0.75-1.87)	1.10 (0.68-1.79)	0.90 (0.53-1.55)	0.93 (0.54-1.59)	0.98 (0.57-1.68)	0.98 (0.57-1.68)
Ref: No										
Smoking at delivery	Smoker					0.97 (0.63-1.50)	0.84 (0.53-1.35)	0.84 (0.52-1.35)	0.82 (0.51-1.32)	0.82 (0.51-1.33)
Ref: Non-smoker										
Breastfeeding	No						1.24 (0.88-1.75)	1.25 (0.89-1.77)	1.26 (0.89-1.78)	1.27 (0.90-1.79)
Ref: No										
Season of birth	Summer							1.37 (0.96-1.96)	1.34 (0.94-1.92)	1.34 (0.93-1.91)
Ref: Winter										
[a]**Vitamin D** (µg/d)									0.94 (0.89-0.98)	0.93 (0.89-0.98)
Supplement use	No									1.20 (0.84-1.71)
Ref: Yes										

EI=Energy Intake [a]Energy Adjusted GMS=General Medical Scheme

Table 5 Generalised linear mixed model: asthma at any time-point vs. never (vitamin D intake as main exposure of interest)

Independent variable		OR (95% CI)								
		Model 1 n=890	Model 2 n=890	Model 3 n=787	Model 4 n=779	Model 5 n=758	Model 6 n=683	Model 7 n=683	Model 8 n=678	Model 9 n=673
Time	Year 5 follow-up	1.33 (0.94-1.89)	1.33 (0.93-1.89)	1.29 (0.87-1.92)	1.32 (0.89-1.97)	1.26 (0.84-1.89)	1.28 (0.84-1.95)	1.28 (0.84-1.95)	1.29 (0.84-1.96)	1.31 (0.86-2.00)
Ref: Year 3 follow-up	Year 10 follow-up	2.57 (1.96-3.38)	2.57 (1.95-3.38)	2.84 (2.11-3.82)	2.89 (2.14-3.90)	2.92 (2.16-3.95)	2.89 (2.12-3.95)	2.88 (2.11-3.92)	2.85 (2.08-3.91)	2.91 (2.11-4.00)
[a]**Vitamin D** (µg/d)		0.96 (0.93-0.99)	0.96 (0.93-0.99)	0.96 (0.92-0.99)	0.96 (0.92-0.99)	0.96 (0.92-0.99)	0.93 (0.89-0.98)	0.93 (0.89-0.98)	0.94 (0.89-0.98)	0.93 (0.89-0.98)
Total EI (1,000 kCal)			0.95 (0.84-1.08)	0.97 (0.86-1.10)	0.97 (0.86-1.09)	0.97 (0.86-1.10)	0.96 (0.84-1.10)	0.95 (0.83-1.10)	0.96 (0.84-1.10)	0.96 (0.84-1.10)
Gestational Age (weeks)				0.92 (0.85-0.99)	0.92 (0.85-0.99)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.94 (0.86-1.02)	0.94 (0.87-1.02)
GMS Eligibility (baseline)	Yes				1.29 (0.81-2.04)	1.22 (0.75-1.98)	1.00 (0.59-1.71)	1.02 (0.60-1.75)	0.98 (0.57-1.68)	0.98 (0.57-1.68)
Ref: No										
Smoking at delivery	Smoker					0.98 (0.64-1.52)	0.83 (0.52-1.33)	0.83 (0.52-1.33)	0.82 (0.51-1.34)	0.82 (0.51-1.33)
Ref: Non-smoker										
Breastfeeding	No						1.32 (0.94-1.85)	1.33 (0.95-1.87)	1.26 (0.89-1.78)	1.27 (0.90-1.79)
Ref: Yes										
Season of birth	Summer							1.34 (0.94-1.91)	1.34 (0.94-1.92)	1.34 (0.93-1.91)
Ref: Winter										
Oily fish intake (serving/d)									0.22 (0.04-1.34)	0.23 (0.04-1.41)
Supplement use	No									1.20 (0.84-1.71)
Ref: Yes										

EI=Energy Intake [a]Energy Adjusted GMS=General Medical Scheme

DISCUSSION

Birth cohort studies with follow-up of 6 years or more on the association of maternal pregnancy diet and childhood asthma are limited.(78) This analysis suggests that higher vitamin D intake during pregnancy is significantly associated with reduced likelihood of asthma in offspring at any point during 10-years follow-up. An interesting inverse association with oily fish consumption was also demonstrated initially suggesting a protective effect, but with significance lost early in model progression. Vegetable intake, however, showed a very small protective effect not statistically distinguishable from zero at a 5% level. Literature on the association with vegetable intake is scarce and inconsistent, with both an increased risk (79) and no effect reported.(80) Literature on the association of oily fish intake in pregnancy with offspring atopic disease is consistent with findings from the current analysis.(49, 51, 79, 80) Analyses from all of these studies were limited to children in the age range 2-6 years; to our knowledge there are no studies with findings extending up to 10 years of age follow-up. A systematic review (81) concluded that there is little evidence to recommend supplementation/modification of diet to include fish oil for children or adults with established asthma. They found no evidence of improvement or increased risk relative to fish oil and established asthma. This is likely evidence that the window period for fish oil to have an effect on immune regulation is most relevant in foetal life, with limited potential for effect once immune responses are established.(80) The pro-inflammatory mediators leukotriene B4 (LTB_4) and tumour necrosis factor alpha (TNF-α) increase airway inflammation and hyper-responsiveness.(82, 83) A protective effect of oily fish could possibly be mediated via eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA). Competitive binding with cyclooxygenase (COX) and lipoxygenase (LOX) reduce LTB_4 and PGE_2 production.(49, 84-86)

Our findings suggest a significant inverse relationship of pregnancy vitamin D consumption with childhood asthma. Results are consistent with the main body of literature on the association of pregnancy vitamin D intake and offspring atopic disease.(17-22) Miyake *et al* (21) reported a protective effect of increased vitamin D consumption in pregnancy with wheeze and eczema in 16-24 month old offspring. A follow-up paper from the same cohort reported higher intake of maternal vitamin D suggested an increased risk of eczema in offspring, now 23-29 months old.(87) More recently a significant increase in the risk of asthma in 20-25 year old offspring of mothers who had high concentrations of serum 25(OH)D ante-natally was reported.(88) This suggests that the long-term effect of pre-natal vitamin D exposure differs from the initial, mostly protective, effect. Findings from vitamin D status studies are less consistent and at times not in agreement with intake studies. Lack of concordance in findings may be an indication that intake data is a surrogate for the

intake of other important nutrients.(15)

The possible mechanism of action in the relationship of vitamin D and asthma could relate to T-helper 2 cell (Th2) differentiation. Literature is conflicting; one immunologic study reported that vitamin D supplementation (in vitro) promoted Th2 cell differentiation.(89) It was thought that the increased vitamin D supplementation in European and high latitude countries to prevent rickets led to the current high prevalence of asthma.(9, 90) Contrary to this, the proposal is that vitamin D inhibits Th2 differentiation, thus having a protective effect.(91)

Vitamin D intervention studies are under way; results from a randomised controlled trial in the UK failed to find any association between prenatal vitamin D supplementation and wheezing in offspring 3 years of age.(92) Litonjua *et al* (45) found a lower incidence (non-significant) of recurrent wheeze and asthma in 3 year old offspring of mothers who received higher vitamin D supplementation in pregnancy. An Aberdeen cohort demonstrated an *in vivo* anti-inflammatory effect of maternal serum $25(OH)D_3$ on interleukin-10 secretion from the airway epithelial cells of cultured neonatal nasal samples.(93) Dick *et al* (94) suggested that a specific exposure might act in a different manner in different population groups and demographic areas, as the genetic and epigenetic factors are likely to be differential. Inconsistencies in findings from the association between pregnancy nutrition and offspring asthma may furthermore relate to interactions between specific nutrients or additional interactions with the human microbiome and environment.(95)

Strengths of this study and analysis were the prospective design with an *a priori* purpose of examining intergenerational transmission of risk. As there is a strong social gradient applied to diet in pregnancy,(96) confounding by socio-demographic factors and lifestyle was a concern; we controlled for a comprehensive range of socio-demographic factors. The GLMM enabled us to control for repeated measurements over time for participants. Limitations were differential follow-up, leading to varied numbers of missing data for certain variables, potentially introducing bias to follow-up data. Countering this, it is notable that the same associations were found separately at the three different time points.(47, 63) The authors recognise that asthma in childhood is a heterogeneous phenotype and that grouping of the 3 time-points could potentially mask different phenotypes;(97, 98) however our study was not powered to differentiate asthma on phenotype at various follow-up. Data on familial atopy or asthma were not available; it is possible that the suggested relationships between maternal diet and offspring asthma have been confounded by the presence or absence of familial predisposition. Data on foetal growth parameters were not available; the effect of foetal growth on childhood asthma could not be controlled for.(99) The calculation of vitamin D (μg/d) and energy (kCal/day) intake was a surrogated calculation obtained from an estimated daily intake (portion/day) of food groups/nutrients.(59) As no data on serum-

25(OH)D_3were collected, it was not possible to correlate vitamin D intake with that of serum levels .The fact that the associations found in this analysis were between maternal food intake and doctor diagnosed asthma raised the possibility of ascertainment bias; more health conscious mothers were more likely to follow good pregnancy diets, and were more likely to take their ill children to the doctor to receive a formal diagnosis.

However, we propose that an ascertainment bias would be more likely to demonstrate the opposite finding, i.e. that an increase in healthy eating was adversely associated with doctor diagnosed asthma. A more comprehensive adjustment (for maternal age, education, and region of residence) did not substantially alter the conclusions and estimates and we feel we have adjusted as thoroughly as possible for potential socioeconomic factors and their influence on study retention.

CONCLUSION

This analysis suggests that higher intakes of oily fish and vitamin D in the maternal pregnancy diet have a protective effect on childhood asthma. This is consistent with the developmental origins of health and disease hypothesis, suggesting that certain exposures of the foetus in utero can affect the development of allergic diseases in childhood by modulating immune response (100). As there is no anticipated cure for asthma in the near future, adjustment of the environment as early as possible, e.g. in utero and in infancy, might provide the best way to achieve a reduction in the asthma burden.(94, 101)

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CONTRIBUTORS' STATEMENT: The Principal Investigator of the Lifeways Study, Professor Kelleher, conceptualised the design of the study and supervised the overall project, including data analysis and interpretation. Dr. Viljoen drafted the manuscript and undertook data collection, analyses and interpretation as part of her PhD. Dr. Murrin assisted with data collection, analyses and interpretation. Dr. Segurado provided statistical input relating to analyses and interpretation of data. Drs. O'Brien and Mehegan assisted with data management and data interpretation. All contributors reviewed and revised the manuscript and approved the final version as submitted.

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What is already known on this topic: The rising burden of childhood asthma worldwide has placed an emphasis on the potential for primary prevention and disease modification. The association of pregnancy nutrient intake (particularly oily fish, vegetables, vitamin D) and season of birth with offspring asthma/atopy has suggested disease originating in utero. Findings from observational studies are inconsistent; intervention studies suggest an inverse association of asthma with vitamin D.

What this study adds: Only one other cohort study investigating the association of maternal pregnancy diet and offspring asthma with follow-up beyond 6 years was identified. In children with persistent wheezing up to age 3 only a small percentage progress to asthma; in our study offspring was followed-up for 10 years. Mothers with higher oily fish and vitamin D intake had offspring with significant decreased odds of asthma at any time point over 10 years follow-up.

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Figure 1 Numbers through analysis. N 1096 mothers had a pregnancy which resulted in a live birth at cohort initiation. Mothers with a null FFQ and missing asthma data saw n 897 mother-child pairs with asthma data at any time-point of follow-up included in the final multivariable analysis. Attrition over time led to n 614 mother-child pairs at year 3 follow-up, n 511 at year 5 follow-up and n 432 at year 10 follow-up.

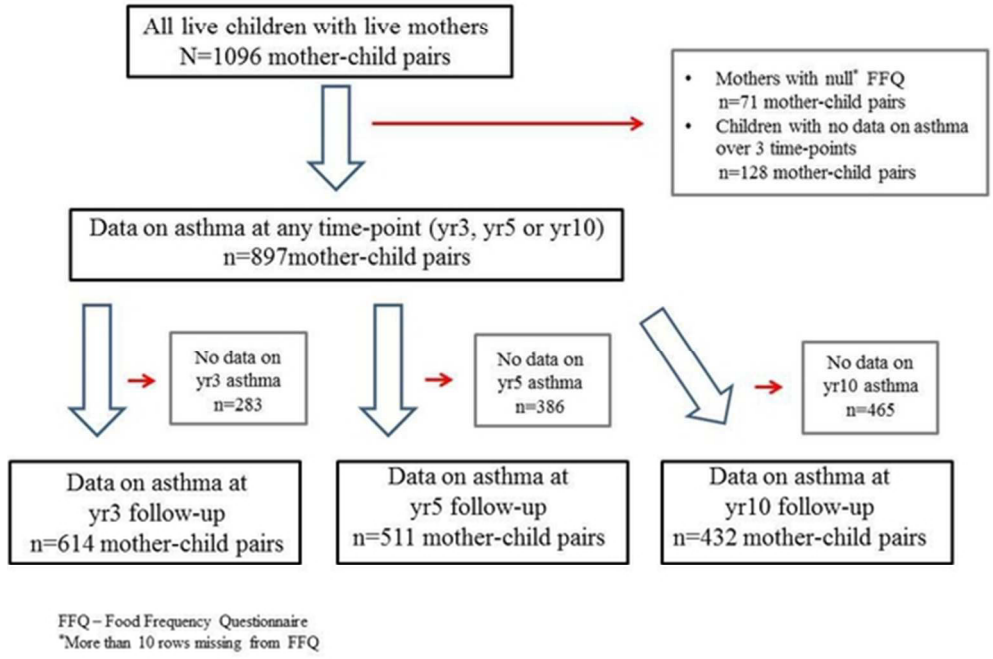

Figure 1 Numbers through analysis. N 1096 mothers had a pregnancy which resulted in a live birth at cohort initiation. Mothers with a null FFQ and missing asthma data saw n 897 mother-child pairs with asthma data at any time-point of follow-up included in the final multivariable analysis. Attrition over time led to n 614 mother-child pairs at year 3 follow-up, n 511 at year 5 follow-up and n 432 at year 10 follow-up.

52x39mm (300 x 300 DPI)

Supplement

Table S1 Food items included in 'Oily fish' and 'Vegetables'

Oily fish (fresh or canned):	Mackerel, Kippers/Herring, Tuna, Salmon, Sardines
Vegetables:	Carrots, Spinach, Broccoli, Spring greens & Cale, Brussel sprouts, Cabbage, Peas, Green beans, Runner beans, Parsnips & Turnips, Leeks, Onions, Garlic, Mushrooms, Sweet peppers, Bean sprouts, Green salad & Lettuce, Cucumber, Tomatoes, Sweetcorn, Beetroot, Coleslaw, Avocado, Watercress, Cauliflower, Celery, Marrow & Courgettes

Table S2 Simple mixed model association between childhood determinants and childhood asthma at any point (n=897)

		Asthma diagnosis True vs False 95% CI	
	OR	**Lower**	**Upper**
Time (n=897)			
Year 10 follow-up	1.00		
Year 5 follow-up	0.55	0.40	0.77
Year 3 follow-up	0.41	0.29	0.57
Birthweight[1] (n=885)	1.00	1.00	1.00
Gestational age[1] (n=793)	0.92	0.85	0.99
Gender[1] (n=893)			
Female	1.00		
Male	1.55	1.17	2.05
GMS Eligibility[1] (n=694)			
TRUE	1.00		
FALSE	0.45	0.29	0.69
Birth order[1] (n=882)			
Not firstborn	1.00		
Firstborn	1.14	0.86	1.51
Mode of delivery[1] (n=839)			
Ceasarean section	1.00		
Vaginal (spontaneous or assisted)	0.85	0.57	1.26
Region[1] (n=897)			
Dublin	1.00		
Galway	0.86	0.64	1.16

[1]Controlling for time as a fixed factor

OR=Odds Ratio SD=Standard Deviation CI=Confidence interval

Table S3 Simple mixed model association between maternal determinants and childhood asthma at any point (n=897)

		Asthma diagnosis True vs False 95% CI	
	OR	**Lower**	**Upper**
Time (n=897)			
Year 10 follow-up	1.00		
Year 5 follow-up	0.55	0.40	0.77
Year 3 follow-up	0.41	0.29	0.57
Education[1] (n=873)			
Some/completed 3rd level	1.00		
None/Primary/Secondary school	1.28	0.97	1.70
Marital status[1] (baseline) (n=885)			
Lone	1.00		
Cohabiting	0.66	0.47	0.94
Marital status[1] (any time-point) (n=557)			
Lone	1.00		
Cohabiting	1.21	0.57	2.57
Age at birth of proband[1] (n=889)	0.98	0.96	1.01
BMI prior to falling pregnant[1] (n=750)	0.98	0.94	1.02
GMS Eligibility[1] (baseline) (n=887)			
TRUE	1.00		
FALSE	0.73	0.5	1.07
GMS Eligibility[1] (any time point) (n=797)			
TRUE	1.00		
FALSE	0.72	0.51	1.03
Maternal Health[1] (baseline) (n=871)			
Excellent/Very good	1.00		
Poor/Fair/Good	0.82	0.59	1.13
Maternal Health[1] (any time point) (n=560)			
Excellent/Very good	1.00		
Poor/Fair/Good	1.23	0.79	1.9
Smoking status at delivery[1] (n=870)			
Smoker	1.00		
Non-smoker	0.84	0.59	1.2
Breastfeeding[1] (n=897)			
TRUE	1.00		
FALSE	1.21	0.85	1.73

[1]Controlling for time as a fixed factor GMS=General Medical Scheme
OR=Odds Ratio SD=Standard Deviation CI=Confidence Interval

Table S4 Distribution of maternal nutrient intake during pregnancy in Normal-Reporters (EI/BMRpreg<1.35-2.39) relative to Irish Recommended Daily Allowances (n=453)

Nutrient[1]	$\bar{x}$ ± SD	25th Percentile	Median	75th Percentile	RDAs for Irish women* 19-64 yrs	Pregnancy**
Total fat (g/day)	129.0±110.4	51.8	100.4	175.3	-	-
MUFA (g/day)	41.1±35.9	16.8	31.7	53.9	-	-
PUFA (g/day)	20.3±20.3	7.0	13.5	25.8	-	-
Saturated Fatty Acids (g/day)	57.8±58.5	20.1	39.1	74.6	-	-
Cholesterol (mg/day)	402.1±330.9	182.3	312.9	522.2	-	-
Retinol (µg/day)	763.2±1004.2	290.6	515.7	890.4	-	-
Carotene (µg/day)	3594.9±2758.3	1356.7	3127.5	4581.2	-	-
Selenium (µg/day)	86.7±83.0	36.3	60.7	110.3	55.0	55.0
Zinc (mg/day)	14.7±11.7	6.6	12.2	18.5	15.0	20.0
Magnesium (mg/day)	545.5±557.9	191.1	362.7	660.9	255-65‡	290-300‡
Copper (mg/day)	2.0±3.4	0.6	1.2	2.1	1.1	1.1
Manganese (mg/day)	4.9±4.8	1.7	3.3	5.9	≤10.0‡	-
Calcium (mg/day)	1712.9±1467.4	614.6	1222.0	2429.9	800.0	1200.0
Vitamin C (mg/day)	248.1±246.5	90.3	157.9	316.0	60.0	80.0
Vitamin D (µg/day)	4.3±4.1	1.7	3.1	5.4	7.5	10.0
Vitamin E (mg/day)	13.6±18.7	4.9	7.9	14.2	8.0	10.0

[1]Calculated from food-frequency questionnaires in first trimester; Energy adjusted - residual method;

MUFA=Monounsaturated Fatty Acids PUFA=Polyunsaturated Acids EI=Energy Intake BMR=Basal Metabolic Rate preg=Pregnancy

g=gram mg=milligram µg=microgram

* Recommended Daily Allowance; Irish Food Safety Authority (1999) **From 20 weeks gestation ‡Estimated Average Requirement, European Food Safety Authority (2006)

‡Scientific Committee for Food of the European Union (SCF, 1993)

Table S5 Distribution of maternal food group intake during pregnancy relative to SLAN females (n=886)

(n=886)

	Lifeways Mothers 2001-2003			SLAN 2007 Females		Recommended intake*	
	Under 18 years (n=16)	**18-29 years (n=377)**	**30-44 years (n=493)**	**18-29 years**	**30-44 years**	**19-64 yrs**	**Pregnancy**
Food group[1] (total daily portions)	**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**Portions**	**Portions**
Oily fish	0.02 ± 0.04	0.03 ± 0.07	0.08 ± 0.14	-	-	1[b]	1[b]
Vegetables	2.5 ± 1.5	2.6 ± 1.9	3.2 ± 2.1	4.1 ± 2.9	4.5 ± 2.8	5[a]	6-7[a]
Fruit	2.2 ± 1.6	2.0 ± 2.5	2.2 1.6	2.8 ± 2.7	3.0 ± 2.8	5[a]	6-7[a]
Added Fat	2.2 ± 3.1	2.4 ± 2.4	2.6 ± 2.2	-	-	-	-

[1]Calculated from food-frequency questionnaires in first trimester [*]Food Safety Authority of Ireland SLAN=Survey of Lifestyle, Attitudes and Nutrition

[a]Daily portion [b]Weekly portion

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract – **PAGE 1 & 2**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found – **PAGE 2**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported – **PAGE 3 & 4**
Objectives	3	State specific objectives, including any prespecified hypotheses – **PAGE 4**
Methods		
Study design	4	Present key elements of study design early in the paper – **PAGE 4 & 5**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection – **PAGE 4, 5 & 6**
Participants	6	*Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up – **PAGE 5**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable – **PAGE 5, 6 & 7**
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 – **PAGE 6 &7**
Bias	9	Describe any efforts to address potential sources of bias – **PAGE 17**
Study size	10	Explain how the study size was arrived at – DUE TO WORD COUNT RESTRICTION PLEASE SEE DETAIL OF REFERENCE 60, 62 & 63
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why – **PAGE 5, 6, 7 & 8**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding – **PAGE 5, 6, 7, 13, 14 & 15**
		(*b*) Describe any methods used to examine subgroups and interactions – **PAGE 8, 13, 14 & 15**
		(*c*) Explain how missing data were addressed – **PAGE 11 & 17,** ADDITIONAL DETAIL IN REFERENCE 63
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed – **PAGE 11 &17**, ADDITIONAL DETAIL IN REFERENCE 63

Continued on next page

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 – **PAGE 11, 17**, ADDITIONAL DETAIL REFERENCE 63
		(b) Give reasons for non-participation at each stage - DUE TO WORD COUNT RESTRICTION PLEASE SEE DETAIL OF REFERENCE 60, 62 & 63
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders – **PAGE 8, 9, 10, 11 & 12**
		(b) Indicate number of participants with missing data for each variable of interest – **PAGE 11**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) – **PAGE 11**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time – **PAGE 11**, ADDITIONAL DETAIL REFERENCE 63
Main results	6	(*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 – **PAGE 13, 14, & 15**
		(*b*) Report category boundaries when continuous variables were categorized - DUE TO WORD COUNT RESTRICTION PLEASE SEE DETAIL OF REFERENCE 63
Discussion		
Key results	18	Summarise key results with reference to study objectives – **PAGE 12**
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 – **PAGE 17**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence – **PAGE 17**
Generalisability	21	Discuss the generalisability (external validity) of the study results – **PAGE 17**
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 – **PAGE 19**

*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.

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Pregnancy Diet and Offspring Asthma risk over a 10-year period: the Lifeways Cross Generation Cohort Study, Republic of Ireland

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Pregnancy Diet and Offspring Asthma risk over a 10-year period: the Lifeways Cross Generation Cohort Study, Republic of Ireland

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Key Words: asthma, childhood, DOHaD, pregnancy, vitamin D

Word count: 3110

ABSTRACT:

Objective

The association of maternal pregnancy diet with offspring asthma risk have been reported. However, literature on longitudinal patterns of asthma risk relative to intra-uterine nutrient exposure is limited. We aimed to establish whether vegetable, oily fish and vitamin D intake during pregnancy are associated with childhood asthma risk over a 10 year period in the Irish Republic.

Design

Mother-child pairs (n=897) from the Lifeways prospective birth cohort, with data on nutrient intake during pregnancy and asthma status respectively, were eligible for inclusion in the analysis. Data on socio-economic and morbidity indicators over 10 years of follow-up on mothers and the index child were collected through self-administered questionnaires. Asthma status as diagnosed by the GP at any time-point over 10 years was related to maternal vegetable, oily fish and vitamin D intake during pregnancy, while adjusting for gestational age, socio-economic status, smoking at delivery, breast-feeding, season of birth and supplement use. Data were modelled with a marginal model with correlated residuals over time within individuals.

Results

In the fully adjusted model, asthma was inversely associated with higher daily average intake of oily fish (OR 0.23 per serving/day, 95% CI 0.04-1.41) and of vegetables (OR 0.96 per serving/day, 95% CI 0.88-1.05), but the confidence limits overlapped 1. A higher daily vitamin D intake was associated with reduced odds of asthma (OR 0.93 per μg/day, 95% CI 0.89-0.98).

Conclusion

This analysis suggests higher daily average intake of vitamin D in pregnancy is associated with asthma risk in offspring over the first 10 years of life.

STRENGTHS

- The prospective design with an a priori purpose of examining intergenerational transmission of risk.
- As there is a strong social gradient applied to diet in pregnancy, confounding by socio-demographic factors and lifestyle was a concern; we controlled for a comprehensive range of socio-demographic factors.
- The marginal model enabled us to take advantage of repeated measurements over time for participants.

LIMITATIONS

- Limitations were differential follow-up, leading to varied numbers of missing data for certain variables, potentially introducing bias to follow-up data. Countering this, it is notable that the same associations were found separately at the three different time points.
- The authors recognise that asthma in childhood is a heterogeneous phenotype and that grouping of the 3 time-points could potentially mask different phenotypes; however our study was not powered to differentiate asthma on phenotype at various follow-up.
- Data on familial atopy or asthma were not available; it is possible that the suggested relationships between maternal diet and offspring asthma have been confounded by the presence or absence of familial predisposition.
- Data on foetal growth parameters were not available; the effect of foetal growth on childhood asthma could not be controlled for.
- GPs criteria to diagnose asthma could be diverse, with potential for variability.

INTRODUCTION

Asthma is the most common chronic disease of childhood;(1, 2) reports indicate a continuous and consistent increase in worldwide prevalence, especially in westernised societies. Prevalence rates in the United Kingdom and Ireland are among the highest in Europe. (3, 4) According to the Centres for Disease Control and Prevention, between 2001 and 2010 asthma prevalence in children in the United States increased 1.4% each year.(5) This increase is most likely multi-factorial, with complex interactions of genetic-immunological-environmental factors leading to the phenotypic expression of disease.(6) Recently multiple studies have attempted to deconstruct this multifactorial relationship, focusing on the change in dietary habits over recent decades.(7-13) The progressive trend in early presentation of allergic disease in childhood, with the implication of possible exposure in utero, has placed an emphasis on maternal pregnancy diet as a prominent factor in the development of offspring asthma.(14, 15) As allergen-specific immune responses are established in foetal life, maternal nutrient intake during pregnancy is pivotal; intake may potentially influence the development of both the innate and acquired immune responses, predisposing to atopy in later life.(16)

A growing body of persuasive epidemiological evidence suggests that deficiency of maternal vitamin D intake prenatally has an inverse relationship with atopic disease in childhood.(17-22) Observational studies on the association of maternal serum and/or infant cord blood 25-hydroxy-vitamin D_3 ($25(OH)D_3$) levels with atopic markers are conflicting. Some demonstrated similar inverse associations;(23-30) others demonstrated direct (31-33) and null (34-42) associations. U-shaped associations were suggested by both Rothers *et al* (43) and Maslova *et al*.(44) Most recently intervention studies exploring pregnancy vitamin D supplementation and asthma risk at 3 years of age also suggested an inverse association (statistically non-significant). The authors suggested that longer follow-up of children is needed to determine the clinical importance of findings.(45, 46)

The Lifeways study has previously reported the association between pregnancy intake of oily fish and vegetables and General Practitioner (GP) diagnosed asthma in offspring at age 3 years; a higher daily mean intake suggested a significant inverse effect.(47) Literature on pregnancy consumption of fish with subsequent atopic risk in offspring is mostly consistent with our findings, indicating an inverse effect.(7, 48-54) Our current aim was twofold: firstly to build on the aforementioned Lifeways findings and test the hypothesis that a higher pregnancy intake of vegetables and oily fish might be inversely associated with asthma risk at any stage over 10 years follow-up; secondly, as suggested in the literature, to explore the

association of pregnancy vitamin D intake and offspring asthma risk within our cohort at any stage over 10 years of follow-up.

METHODS

Study design and sample selection

The Lifeways study was established 2001-2003 as a prospective birth cohort in the Republic of Ireland. The *a priori* purpose was to examine determinants of health status in children, including diet and lifestyle, and to establish patterns and links across generations. Recruitment, data collection and study instruments have previously been discussed in detail.(55) In brief, mothers were recruited at first ante-natal visit (14-16 weeks gestation) in one of two regional maternity hospitals in the more rural West (Galway) and the more industrialised East (Dublin). Of 1124 mothers recruited, 1082 gave birth resulting in 1096 live mother-child pairs. Analysis was limited to current live mother-child pairs, where data on the proband's asthma status were available for at least one time-point. Babies with congenital anomalies and delivery <34 weeks gestational age (56) were excluded from analysis. Due to attrition over time participants had differential patterns of follow-up through phases; 614 mother-child pairs were included in the year 3 analyses, 511 in year 5 and 432 in year 10 follow-up. The sample for this analysis of 897 mother-child pairs comprised respondents for whom at least one follow-up point of asthma health status was recorded (Figure 1). Ethical approval for all phases of the study was granted by the Human Research Ethics Committee, University College Dublin, Ireland. Written informed consent was obtained from all adult participants at each follow-up phase of the study. Parental consent was obtained for child subjects at each follow-up phase; additional assent was obtained from all child subjects at the year 10 follow-up phase.

Assessment of pregnancy diet

Data on maternal nutrient intake during pregnancy were captured by a semi-quantitative Food Frequency Questionnaire (FFQ) as part of a self-administered questionnaire to the mother at her first ante-natal visit. The FFQ was developed from the international version used in the European Prospective Investigation in Cancer studies by the National Nutritional Surveillance Centre and extensively validated for use in an Irish population by the National University of Ireland, Galway.(57) The main food groups regularly consumed in the Irish diet were included, and consisted of 149 food items.(47, 58) The questionnaire focused on maternal dietary intake since pregnant. Intake as a medium serving (detailed in the FFQ for relevant food items) was recorded on a 9-grade scale, with categories subsequently transformed to continuous daily portion averages for all 149 food items. To arrive at distinct food groups, the continuous intake of various food items were summed and reported as total portions per day (Supplement; Table S1). The daily average intake of energy and nutrients i.e. ingested portions/day of food containing vitamin D, was calculated by linking frequency selections from the FFQ with food equivalents in McCance and Widdowson's nutritional composition database, 6th edition,(59) using software developed specifically for the Lifeways database.(60) An estimation of the intake of Vitamin D in micrograms per day (μg/day) and energy in kilocalories (kCal/day) were made.

Assessment of outcome

Data on doctor diagnosed asthma in offspring were collected at 3 time-points: ages 3, 5 and 9 years. Various studies have used doctor diagnosed asthma/parental report of doctor diagnosed asthma to ascertain diagnosis.(30, 45) Questions to ascertain asthma diagnosis were adapted from the validated International Study on Asthma and Allergies in Childhood (ISAAC) questionnaire.(61, 62) Diagnosis from age 3 and 9 follow-up were reported by the General Practitioner (GP), information on GP diagnosed asthma at age 5 was obtained from the mother. For the univariate analysis, asthma as a dichotomous outcome variable ('Yes' vs 'No') at each of the three time-points were analysed separately. For the multivariable analysis the effect of dietary intakes during pregnancy on doctor diagnosed asthma in the child at the 3 time-points over a period of 10-year follow-up was examined controlling for different prevalence at each age, but assuming protective factors would have the same effect at each age.(63)

Statistical analysis

Definite doctor diagnosed asthma in offspring at any time point over 10-year follow-up, was related to maternal pregnancy intake of oily fish, vegetables, expressed in servings per day, and vitamin D expressed in μg per day.

Univariate analysis and Covariates

Variables that could potentially confound the diagnosis of asthma were identified from the literature, and screened in our cohort for association with an asthma diagnosis at the specific time-point using unadjusted binomial logistic regression or the independent samples t-test. Predictors with $p<0.1$ were identified as being potential confounders and selected for inclusion in the multivariable models, and are discussed below.

Total Energy Intake (EI)

To control for variance in accuracy of maternal energy reporting, total energy intake (kCal) was adjusted for in all models. Nutrients were adjusted for total energy intake using the residual method.(64, 65) To account for mothers who potentially over- or under reported energy intake, the ratio of energy intake (EI) to basal metabolic rate in the first trimester of pregnancy (BMR_{preg}) was calculated to identify extreme outliers.(63, 66, 67) BMR_{preg} was calculated using the pre-pregnancy BMI and Schofield equations.(67) Mothers were asked to report their pre-pregnancy weight at consultation during the first ante-natal visit.

Gestational age

Gestational age in weeks.

Socio-economic status

We used eligibility for the General Medical Scheme (GMS) as an indicator of socio-economic status. The Republic of Ireland has a two-tiered healthcare system, where certain individuals are eligible for different levels of free healthcare dependent on their income and certain medical conditions. GMS is thus a robust means tested indicator.(68-70) As maternal and offspring GMS eligibility are strongly correlated only the maternal predictor was used in the models.(63, 71)

Smoking

Smoking status at time of delivery; hospital delivery records.

Breastfeeding

Data from the self-administered maternal questionnaire; 'Was your Lifeways child ever breastfed?'

Season of birth

Seasons were comprised as follows: Summer (May, June, July), Autumn (Aug, Sept, Oct), Winter (Nov, Dec, Jan), Spring (Feb, March, April). This follows the grouping suggested by the Irish ROLO study with data on maternal serum 25(OH)D levels.(72) Summer and Autumn were collapsed to form 'Summer', with Spring and Winter collapsed to form 'Winter', making up the final dichotomous variable, 'Summer' vs 'Winter'.

Supplement use

Data on supplement use were generic: 'Have you taken any vitamins, minerals or food supplements?' 'Yes' or 'No' and did not allow for specification on supplement type or content quantification.

Multivariable analysis

The associations with the three aforementioned dietary intakes were analysed in multivariable models to assess their independent association with asthma, and verify the extent of confounding in a stepwise manner. Vegetables, oily fish and vitamin D intakes were analysed as continuous predictor variables. Multivariable analysis was run using the full sample of 897 mother-child pairs in a marginal model (or Generalised Estimating Equation model) with repeated measures of asthma over time with an unstructured variance-covariance matrix to allow unequal variances and residual correlations. Covariates were entered into the model sequentially as fixed factors based on their univariate association with asthma, up to the cut-off of p=0.1. Time was consistently included as a fixed factor. Predictor variables were regarded as significant if the 95% confidence intervals for the odds ratios did not include 1. The Statistical Package for the Social Sciences (SPSS) version 20 was used to conduct univariate analysis; multivariable analysis was run with Statistical Analysis Software (SAS) version 9.3.

Sensitivity Analysis

Considering that non-response (attrition or sparse point-wise) bias may be introduced by residual socio-economic or educational factors which correlate with diet and with asthma diagnosis, we applied a sensitivity analysis to explore how robust the observed associations were.(73). We assuming no, or a smaller effect in the full sample, and that non-response arises

in a subgroup with elevated asthma prevalence and decreased quality of dietary intake, and noted under which conditions false associations exist in the responders.

RESULTS

Study subjects' characteristics and asthma prevalence

In the final sample, 66.9% of the mother-child pairs were resident in the Dublin area and 33.1% in the Galway area, proportionate to recruitment patterns. Mothers with a 3rd level education were marginally higher (51.9%) than those with None/Primary/Secondary school education (48.1%); 19.4% of mothers were smokers at the time of giving birth. The mean (SD) age of mothers at time of giving birth was 30.2 (5.9) years and the mean (SD) pre-pregnancy body mass index of mothers were 23.7 (4.0). The offspring sex distribution was about equal; 48.8% males and 51.2% females. The mean (SD) birth-weight was 3515.3 (568.6) grams, with a mean (SD) gestational age of 39.9 (1.9) weeks. Just under half (45.8%) of probands were the first born and 86.20% were delivered vaginally. Doctor diagnosed asthma in offspring at the 3 phases of follow-up respectively, increased from 10.90% at 3-years, to 14.33% at 5-years and 23.10% at 10-years follow-up. In general the literature suggests a downward trend in prevalence with increasing age. There are however, cohorts that noted an increase of prevalence with age.(74-76) Maternal socio-economic and biological characteristics relative to food group intake are presented in Table 1. Maternal and child characteristics as related to asthma diagnosis are further presented in supplemental material (Table S2 and S3).

Univariate associations of background variables with childhood asthma from the cross-sectional data for the 3 follow-up phases respectively have previously been discussed in detail.(47, 62, 63)

Table 1 Maternal food group intake during pregnancy in relation to socio-economic factors (n=897)

		Food group				
	n[1]	Oily fish (portion/day) mean (SD)	p-value	n[1]	Vegetables (portion/day) mean (SD)	p-value
Education			<0.001[a]			<0.001[a]
None/Primary/Secondary school	416	0.05 (0.10)		420	2.61 (1.88)	
3rd level education	451	0.07 (0.13)		451	3.26 (2.16)	
Marital status (baseline)			0.01[a]			<0.001[a]
Lone	179	0.04 (0.09)		180	2.51 (2.06)	
Cohabiting	701	0.06 (0.12)		703	3.05 (2.03)	
GMS Eligibility (baseline)			<0.001[a]			<0.001[a]
No	750	0.06 (0.12)		754	3.02 (2.06)	
Yes	131	0.03 (0.07)		131	2.43 (1.91)	
Smoking status at delivery			0.07[a]			<0.001[a]
Non-smoker	693	0.06 (0.12)		695	3.04 (2.10)	
Smoker	167	0.04 (0.11)		169	2.39 (1.61)	
Region			0.04[a]			<0.001[a]
Galway	292	0.07 (0.13)		294	3.21 (2.17)	
Dublin	594	0.05 (0.10)		596	2.79 (1.96)	
Age group at delivery (y)			<0.001[b]			<0.001[b]
Under 18	16	0.02 (0.04)		16	2.52 (1.51)	
18 to 29	373	0.03 (0.07)		377	2.56 (1.92)	
30+	491	0.08 (0.14)		492	3.22 (2.09)	

[a]Independent samples t-test [b]ANOVA GMS=General Medical Scheme

[1]Numbers do not always add up to 897 because of varied numbers of missing data for some variables

Within-time representativeness of responders vs. non-responders that contributed to the final sample is presented in Table 2.

Table 2 Within-time representativeness of final sample (n=897)

Characteristics	Responders[a] n=614	Non-responders[a] n=283
Year 3 follow-up		
Maternal educational attainment[c]		
None/Primary/Secondary school, No. (%)	290 (48.6)	130 (47.1)
Some/Completed 3rd level education, No. (%)	307 (51.4)	146 (52.9)
Maternal GMS eligibility at baseline[c]		
Not eligible, No. (%)	512 (84.3)	244 (87.1)
Eligible, No.(%)	95 (15.7)	36 (12.9)
Region[c]		
West, No. (%)	217 (35.3)	80 (28.3)
East, No. (%)	397 (64.7)	203 (71.7)
Maternal age at birth of proband, mean (SD), y[c]	30.35 (5.9)	29.75 (5.9)
Maternal oily fish intake in pregnancy, mean (SD), servings/d[c]	0.06 (0.1)	0.05 (0.1)
Maternal Vegetable intake in pregnancy, mean (SD), servings/d[c]	2.95 (1.9)	2.88 (2.2)
Maternal Vitamin D intake in pregnancy, mean (SD), µg/d[c]	4.70 (4.8)	5.60 (10.2)
Year 5 follow-up	n=511	n=386
Maternal educational attainment[c]		
None/Primary/Secondary school, No. (%)	241 (48.7)	179 (47.4)
Some/Completed 3rd level education, No. (%)	254 (51.3)	199 (52.6)
Maternal GMS eligibility at baseline[c]		
Not eligible, No. (%)	429 (85.0)	327 (85.6)
Eligible, No. (%)	76 (15.0)	55 (14.4)
Region[c]		
West, No. (%)	165 (32.3)	132 (34.2)
East, No. (%)	346 (67.7)	254 (65.8)
Maternal age at birth of proband, mean (SD), y[c]	30.10 (5.8)	30.24 (5.9)
Maternal oily fish intake in pregnancy, mean (SD), servings/d[c]	0.06 (0.1)	0.06 (0.1)
Maternal Vegetable intake in pregnancy, mean (SD), servings/d[c]	2.93 (2.1)	2.94 (1.9)
Maternal Vitamin D intake in pregnancy, mean (SD), µg/d[c]	4.70 (6.1)	5.30 (7.9)
Year 10 follow-up	n=432	n=465
Maternal educational attainment[b]		
None/Primary/Secondary school, No. (%)	174 (40.7)	246 (55.2)
Some/Completed 3rd level education, No. (%)	253 (59.3)	200 (44.8)
Maternal GMS eligibility at baseline[b]		
Not eligible, No. (%)	385 (89.7)	371 (81.0)
Eligible, No. (%)	44 (10.3)	87 (19.0)
Region[b]		
West, No. (%)	166 (38.4)	131 (28.2)
East, No. (%)	266 (61.6)	334 (71.8)
Maternal age at birth of proband, mean (SD), y[b]	31.55 (5.2)	28.86 (6.1)
Maternal oily fish intake in pregnancy, mean (SD), servings/d[c]	0.06 (0.1)	0.06 (0.1)
Maternal Vegetable intake in pregnancy, mean (SD), servings/d[c]	3.11 (2.1)	2.76 (2.0)
Maternal Vitamin D intake in pregnancy, mean (SD), µg/d[c]	5.00 (7.5)	4.90 (6.4)

[a]Numbers do not always add up to total as varied numbers of missing data at certain variables
[b]p<0.001 [c]Non-significant
µg: micrograms SD:Standard Deviation d:day y:years

Pregnancy dietary intake

Reported intake of vegetables and oily fish were directly and significantly associated with the maternal social gradient (Table 1). Mean (SD) daily intake of oily fish and vegetables were 0.06 (0.12) and 2.93 (2.04) portions per day respectively. The Food Safety Authority of Ireland (FSAI) recommends that pregnant women should have one portion of oily fish weekly (i.e. 0.1 portions/day) and 6-7 portions of vegetables daily.(77) The mean intake of Lifeways mothers did not reach this recommendation. Data captured by the FFQ detailed intake as a medium portion (90-100 gram of fish; a piece of fish about the size of a woman's palm).

Vitamin D intake during pregnancy between those mothers with consistent follow-up, and those with attrition did not differ significantly. A social gradient for pregnancy vitamin D intake in the final sample was not observed. The distribution of mean vitamin D intake was investigated according to the EI/BMR_{preg} ratio; no marked difference in the distribution of vitamin D was observed.(63) Energy adjusted mean (SD) intake of vitamin D in mothers was 4.3 (4.1) µg/d. The FSAI recommends a daily allowance of 10µg/day vitamin D for Irish pregnant women.(78). (Table S4 and S5). At baseline 327 (36.9%) mothers from the final sample reported using supplements.

Pregnancy dietary associations with offspring asthma

Vegetable intake was negatively associated with offspring asthma, although not significantly so in the fully adjusted model (OR 0.96 per serving/day, 95% CI 0.88-1.05) (Model 8) (Table 3).

Oily fish intake was inversely associated with offspring asthma in the first two models, with an increase in daily average serving of oily fish demonstrating lower odds at any time point over the 10 year follow-up period (model 2 OR 0.17 per serving/day, 95% CI 0.03-0.87) (Table 4). Change in the odds ratios across models suggested that gestational age may have been a confounder (model 2 versus 3), and all models up to the fully adjusted (OR 0.23 per serving/day, 95% CI 0.04-1.41) were not significant, despite a substantial magnitude of the odds ratio.

All models suggested vitamin D had a significant inverse association, including the fully adjusted analysis (model 9 OR 0.93 per µg/day, 95% CI 0.89-0.98) with offspring asthma at any time-point of follow-up (Table 5). Adjustment for breastfeeding in particular increased the odds ratio for vitamin D (model 5 versus 6), in part due to the loss of 85 women with no data on breastfeeding habits.

Results of a sensitivity analysis on the Vitamin D association, indicated that if the true association was null, a false association of this magnitude could be generated by high missing data in a subgroup with higher asthma prevalence (by approximately 5%) and lower vitamin D intake (by 10% at the median), but only if the non-responders had an association in the opposite direction (high vitamin D associated with increased asthma risk). An inflation of a true but small effect could also occur in scenarios where non-responders have lower vitamin D intake, similar or *lower* asthma rates, and a weaker association between intake and asthma, than responders. The hypothetical scenarios consistent with our expectations of missing data patterns suggest our observed odds ratio of 0.93 may be attenuated relative to the true effect.

Table 3 Marginal model: asthma at any time-point (vegetable intake as main exposure of interest)

Independent variable		OR (95% CI)							
		Model 1 n=890	Model 2 n=883	Model 3 n=781	Model 4 n=777	Model 5 n=756	Model 6 n=682	Model 7 n=682	Model 8 n=677
Time	Year 5 follow-up	1.31 (0.92-1.86)	1.30 (0.92-1.84)	1.28 (0.87-1.90)	1.31 (0.88-1.94)	1.25 (0.84-1.86)	1.27 (0.83-1.92)	1.27 (0.83-1.93)	1.29 (0.84-1.96)
Ref: Year 3 follow-up	Year 10 follow-up	2.59 (1.97-3.41)	2.56 (1.94-3.37)	2.81 (2.09-3.78)	2.86 (2.11-3.86)	2.88 (2.13-3.90)	2.81 (2.07-3.83)	2.80 (2.06-3.82)	2.84 (2.08-3.88)
Vegetable intake (serving/d)		0.94 (0.87-1.01)	0.94 (0.87-1.01)	0.94 (0.86-1.03)	0.95 (0.88-1.03)	0.96 (0.88-1.04)	0.96 (0.88-1.05)	0.96 (0.88-1.05)	0.96 (0.88-1.05)
Total EI (1,000 kCal)			0.96 (0.85-1.08)	0.97 (0.86-1.09)	0.97 (0.86-1.09)	0.97 (0.86-1.09)	0.96 (0.84-1.09)	0.95 (0.83-1.09)	0.95 (0.83-1.09)
Gestational Age (weeks)				0.92 (0.85-0.99)	0.92 (0.85-0.99)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.93 (0.85-1.01)	0.93 (0.86-1.01)
GMS Eligibility (baseline)	Yes				1.20 (0.76-1.91)	1.13 (0.69-1.84)	0.93 (0.54-1.59)	0.94 (0.55-1.34)	0.95 (0.55-1.63)
Ref: No									
Smoking at delivery	Smoker					0.96 (0.62-1.49)	0.84 (0.52-1.34)	0.83 (0.52-1.34)	0.83 (0.52-1.34)
Ref: Non-smoker									
Breastfeeding	No						1.27 (0.90-1.79)	1.28 (0.91-1.81)	1.28 (0.91-1.81)
Ref: Yes									
Season of birth	Summer							1.36 (0.95-1.94)	1.35 (0.95-1.94)
Ref: Winter									
Supplement use	No								1.17 (0.82-1.66)
Ref: Yes									

EI=Energy Intake GMS=General Medical Scheme

Table 4 Marginal model: asthma at any time-point (oily fish intake as main exposure of interest)

Independent variable		OR (95% CI)								
		Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9
		n=886	n=879	n=778	n=774	n=753	n=678	n=678	n=678	n=673
Time	Year 5 follow-up	1.31 (0.92-1.86)	2.52 (1.91-3.33)	1.29 (0.87-1.92)	1.32 (0.89-1.96)	1.25 (0.84-1.87)	1.27 (0.84-1.94)	1.27 (0.83-1.95)	1.29 (0.84-1.96)	1.31 (0.86-2.00)
Ref: Year 3 follow-up	Year 10 follow-up	2.56 (1.94-3.38)	1.30 (0.92-1.85)	2.78 (2.06-3.75)	2.83 (2.09-3.83)	2.85 (2.10-3.88)	2.78 (2.03-3.80)	2.77 (2.02-3.78)	2.85 (2.08-3.91)	2.91 (2.11-4.00)
Oily fish intake (serving/d)		0.16 (0.03-0.85)	0.17 (0.03-0.87)	0.22 (0.04-1.16)	0.24 (0.05-1.29)	0.21 (0.04-1.20)	0.19 (0.03-1.20)	0.20 (0.03-1.23)	0.22 (0.04-1.34)	0.23 (0.04-1.41)
Total EI (1,000 kCal)			0.97 (0.86-1.08)	0.98 (0.87-1.10)	0.97 (0.87-1.09)	0.97 (0.87-1.09)	0.96 (0.85-1.09)	0.96 (0.84-1.10)	0.96 (0.84-1.10)	0.96 (0.84-1.10)
Gestational Age (weeks)				0.92 (0.85-0.99)	0.92 (0.85-0.99)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.94 (0.86-1.02)	0.94 (0.87-1.02)
GMS Eligibility (baseline)	Yes				1.18 (0.75-1.87)	1.10 (0.68-1.79)	0.90 (0.53-1.55)	0.93 (0.54-1.59)	0.98 (0.57-1.68)	0.98 (0.57-1.68)
Ref: No										
Smoking at delivery	Smoker					0.97 (0.63-1.50)	0.84 (0.53-1.35)	0.84 (0.52-1.35)	0.82 (0.51-1.32)	0.82 (0.51-1.33)
Ref: Non-smoker										
Breastfeeding	No						1.24 (0.88-1.75)	1.25 (0.89-1.77)	1.26 (0.89-1.78)	1.27 (0.90-1.79)
Ref: No										
Season of birth	Summer							1.37 (0.96-1.96)	1.34 (0.94-1.92)	1.34 (0.93-1.91)
Ref: Winter										
[a]**Vitamin D** (µg/d)									0.94 (0.89-0.98)	0.93 (0.89-0.98)
Supplement use	No									1.20 (0.84-1.71)
Ref: Yes										

EI=Energy Intake [a]Energy Adjusted GMS=General Medical Scheme

Table 5 Marginal model: asthma at any time-point (vitamin D intake as main exposure of interest)

Independent variable		OR (95% CI)								
		Model 1 n=890	Model 2 n=890	Model 3 n=787	Model 4 n=779	Model 5 n=758	Model 6 n=683	Model 7 n=683	Model 8 n=678	Model 9 n=673
Time	Year 5 follow-up	1.33 (0.94-1.89)	1.33 (0.93-1.89)	1.29 (0.87-1.92)	1.32 (0.89-1.97)	1.26 (0.84-1.89)	1.28 (0.84-1.95)	1.28 (0.84-1.95)	1.29 (0.84-1.96)	1.31 (0.86-2.00)
Ref: Year 3 follow-up	Year 10 follow-up	2.57 (1.96-3.38)	2.57 (1.95-3.38)	2.84 (2.11-3.82)	2.89 (2.14-3.90)	2.92 (2.16-3.95)	2.89 (2.12-3.95)	2.88 (2.11-3.92)	2.85 (2.08-3.91)	2.91 (2.11-4.00)
[a]**Vitamin D** (µg/d)		0.96 (0.93-0.99)	0.96 (0.93-0.99)	0.96 (0.92-0.99)	0.96 (0.92-0.99)	0.96 (0.92-0.99)	0.93 (0.89-0.98)	0.93 (0.89-0.98)	0.94 (0.89-0.98)	0.93 (0.89-0.98)
Total EI (1,000 kCal)			0.95 (0.84-1.08)	0.97 (0.86-1.10)	0.97 (0.86-1.09)	0.97 (0.86-1.10)	0.96 (0.84-1.10)	0.95 (0.83-1.10)	0.96 (0.84-1.10)	0.96 (0.84-1.10)
Gestational Age (weeks)				0.92 (0.85-0.99)	0.92 (0.85-0.99)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.93 (0.86-1.01)	0.94 (0.86-1.02)	0.94 (0.87-1.02)
GMS Eligibility (baseline)	Yes				1.29 (0.81-2.04)	1.22 (0.75-1.98)	1.00 (0.59-1.71)	1.02 (0.60-1.75)	0.98 (0.57-1.68)	0.98 (0.57-1.68)
Ref: No										
Smoking at delivery	Smoker					0.98 (0.64-1.52)	0.83 (0.52-1.33)	0.83 (0.52-1.33)	0.82 (0.51-1.34)	0.82 (0.51-1.33)
Ref: Non-smoker										
Breastfeeding	No						1.32 (0.94-1.85)	1.33 (0.95-1.87)	1.26 (0.89-1.78)	1.27 (0.90-1.79)
Ref: Yes										
Season of birth	Summer							1.34 (0.94-1.91)	1.34 (0.94-1.92)	1.34 (0.93-1.91)
Ref: Winter										
Oily fish intake (serving/d)									0.22 (0.04-1.34)	0.23 (0.04-1.41)
Supplement use	No									1.20 (0.84-1.71)
Ref: Yes										

EI=Energy Intake [a]Energy Adjusted GMS=General Medical Scheme

DISCUSSION

Birth cohort studies with follow-up of 6 years or more on the association of maternal pregnancy diet and childhood asthma are limited.(79) This analysis suggests that higher vitamin D intake during pregnancy is associated with reduced likelihood of asthma in offspring at any point during 10-years follow-up. An interesting inverse association with oily fish consumption was also demonstrated initially, but with significance lost early in model progression. Vegetable intake, however, showed a very small inverse association not statistically distinguishable from zero at a 5% level. Literature on the association with vegetable intake is scarce and inconsistent, with both an increased risk (80) and no effect reported.(81) Literature on the association of oily fish intake in pregnancy with offspring atopic disease is consistent with findings from the current analysis.(49, 51, 80, 81) Analyses from all of these studies were limited to children in the age range 2-6 years; to our knowledge there are no studies with findings extending up to 10 years of age follow-up. A systematic review (82) concluded that there is little evidence to recommend supplementation/modification of diet to include fish oil for children or adults with established asthma. They found no evidence of improvement or increased risk relative to fish oil and established asthma. This is likely evidence that the window period for fish oil to have an effect on immune regulation is most relevant in foetal life, with limited potential for effect once immune responses are established.(81) The pro-inflammatory mediators leukotriene B4 (LTB_4) and tumour necrosis factor alpha (TNF-α) increase airway inflammation and hyper-responsiveness.(83, 84) A protective effect of oily fish could possibly be mediated via eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA). Competitive binding with cyclooxygenase (COX) and lipoxygenase (LOX) reduce LTB_4 and PGE_2 production.(49, 85-87)

Our findings suggest a significant inverse association of pregnancy vitamin D consumption with childhood asthma, which in sensitivity analyses appears robust to bias arising from a reasonable range of missing data patterns. Results are consistent with the main body of literature on the association of pregnancy vitamin D intake and offspring atopic disease.(17-22) Miyake *et al* (21) reported that increased vitamin D consumption in pregnancy was associated with less wheeze and eczema in 16-24 month old offspring. A follow-up paper from the same cohort reported higher intake of maternal vitamin D with an increased risk of eczema in offspring, now 23-29 months old.(88) More recently a significant increase in the risk of asthma in 20-25 year old offspring of mothers who had high concentrations of serum 25(OH)D ante-natally was reported.(89) We speculate that the long-term effect of pre-natal vitamin D exposure differs from the initial, mostly protective, effect. Findings from vitamin D status studies are less consistent and at times not in agreement with intake studies. Lack of concordance in findings may be an indication that intake data is a surrogate for the

intake of other important nutrients.(15) The possible mechanism of action in the relationship of vitamin D and asthma could relate to T-helper 2 cell (Th2) differentiation. Literature is conflicting; one immunologic study reported that vitamin D supplementation (in vitro) promoted Th2 cell differentiation.(90) It has been proposed that the increased vitamin D supplementation in European and high latitude countries to prevent rickets led to the current high prevalence of asthma.(9, 91) Contrary to this, the proposal is that vitamin D inhibits Th2 differentiation, thus having a protective effect.(92) Vitamin D intervention studies are under way; results from a randomised controlled trial in the UK failed to find any association between prenatal vitamin D supplementation and wheezing in offspring 3 years of age.(93) Litonjua *et al* (45) found a lower incidence (non-significant) of recurrent wheeze and asthma in 3 year old offspring of mothers who received higher vitamin D supplementation in pregnancy. An Aberdeen cohort demonstrated an *in vivo* anti-inflammatory effect of maternal serum 25(OH)D_3 on interleukin-10 secretion from the airway epithelial cells of cultured neonatal nasal samples.(94) Dick *et al* (95) suggested that a specific exposure might act in a different manner in different population groups and demographic areas, as the genetic and epigenetic factors are likely to be differential. Inconsistencies in findings from the association between pregnancy nutrition and offspring asthma may furthermore relate to interactions between specific nutrients or additional interactions with the human microbiome and environment.(96) Strengths of this study and analysis were the prospective design with an *a priori* purpose of examining intergenerational transmission of risk. As there is a strong social gradient applied to diet in pregnancy,(97) confounding by socio-demographic factors and lifestyle was a concern; we controlled for a comprehensive range of socio-demographic factors. The marginal model enabled us to control for repeated measurements over time for participants. Limitations were differential follow-up, leading to varied numbers of missing data for certain variables, potentially introducing bias to follow-up data. Although a sensitivity analysis was encouraging, it remains possible that unknown, unobserved factors driving non-response have generated bias in an unexpected direction, and there is a dearth of literature to guide exploration of such effects. The fact that the associations found in this analysis were between maternal food intake and doctor diagnosed asthma raised the possibility of ascertainment bias; more health conscious mothers were more likely to follow good pregnancy diets, and were more likely to take their ill children to the doctor to receive a formal diagnosis. However, we propose that an ascertainment bias would be more likely to demonstrate the opposite finding, i.e. that an increase in healthy eating was adversely associated with doctor diagnosed asthma.

Countering this, it is notable that the same associations were found separately at the three different time points.(47, 63) A more comprehensive adjustment (for maternal age, education, and region of residence) also did not substantially alter the conclusions and estimates and we feel we have adjusted as thoroughly as possible for potential socioeconomic factors and their influence on bias.

The authors recognise that asthma in childhood is a heterogeneous phenotype and that grouping of the 3 time-points could potentially mask different phenotypes;(98, 99) however our study was not powered to differentiate asthma on phenotype at various follow-up. Data on familial atopy or asthma were not available; it is possible that the suggested relationships between maternal diet and offspring asthma have been confounded by the presence or absence of familial predisposition. Data on foetal growth parameters were not available; the effect of foetal growth on childhood asthma could not be controlled for.(100) The calculation of vitamin D (μg/d) and energy (kCal/day) intake was a surrogated calculation obtained from an estimated daily intake (portion/day) of food groups/nutrients.(59) As no data on serum-25(OH)D_3were collected, it was not possible to correlate vitamin D intake with that of serum levels.

CONCLUSION

This analysis suggests that higher intakes of oily fish and vitamin D in the maternal pregnancy diet are inversely associated with childhood asthma. This is consistent with the developmental origins of health and disease hypothesis, suggesting that certain exposures of the foetus in utero can affect the development of allergic diseases in childhood by modulating immune response (101). As there is no anticipated cure for asthma in the near future, adjustment of the environment as early as possible, e.g. in utero and in infancy, might provide the best way to achieve a reduction in the asthma burden.(95, 102)

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What is already known on this topic: The rising burden of childhood asthma worldwide has placed an emphasis on the potential for primary prevention and disease modification. The association of pregnancy nutrient intake (particularly oily fish, vegetables, vitamin D) and season of birth with offspring asthma/atopy has suggested disease originating in utero. Findings from observational studies are inconsistent; intervention studies suggest an inverse association of asthma with vitamin D.

What this study adds: Only one other cohort study investigating the association of maternal pregnancy diet and offspring asthma with follow-up beyond 6 years was identified. In children with persistent wheezing up to age 3 only a small percentage progress to asthma; in our study offspring was followed-up for 10 years. Mothers with higher oily fish and vitamin D intake had offspring with decreased odds of asthma at any time point over 10 years follow-up.

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Figure 1 Numbers through analysis. N 1096 mothers had a pregnancy which resulted in a live birth at cohort initiation. Mothers with a null FFQ and missing asthma data saw n 897 mother-child pairs with asthma data at any time-point of follow-up included in the final multivariable analysis. Attrition over time led to n 614 mother-child pairs at year 3 follow-up, n 511 at year 5 follow-up and n 432 at year 10 follow-up.

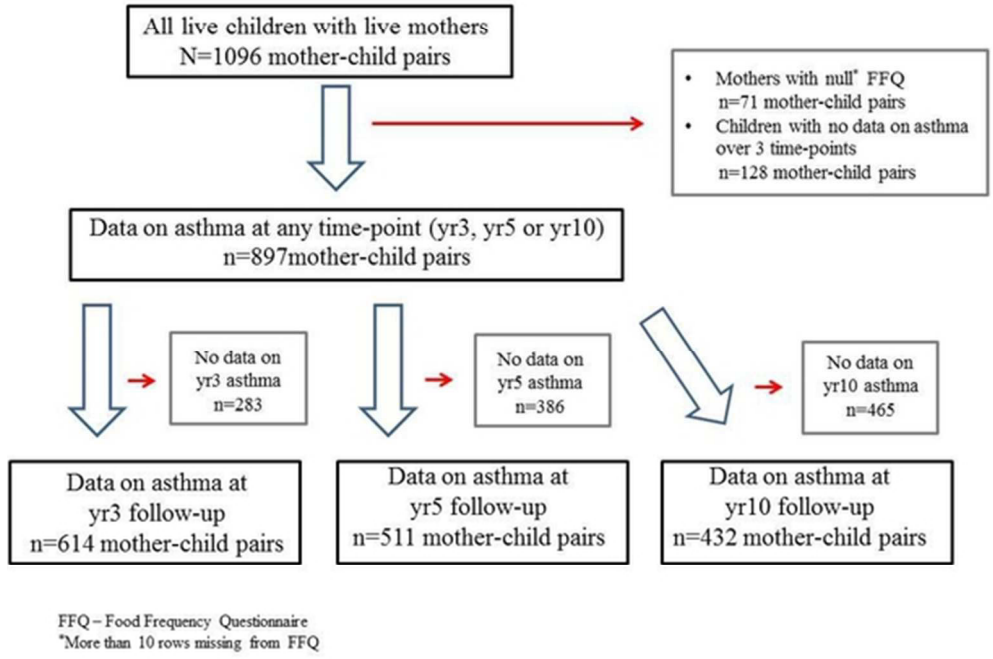

Figure 1 Numbers through analysis. N 1096 mothers had a pregnancy which resulted in a live birth at cohort initiation. Mothers with a null FFQ and missing asthma data saw n 897 mother-child pairs with asthma data at any time-point of follow-up included in the final multivariable analysis. Attrition over time led to n 614 mother-child pairs at year 3 follow-up, n 511 at year 5 follow-up and n 432 at year 10 follow-up.

52x39mm (300 x 300 DPI)

Supplement

Table S1 Food items included in 'Oily fish' and 'Vegetables'

Oily fish (fresh or canned):	Mackerel, Kippers/Herring, Tuna, Salmon, Sardines
Vegetables:	Carrots, Spinach, Broccoli, Spring greens & Cale, Brussel sprouts, Cabbage, Peas, Green beans, Runner beans, Parsnips & Turnips, Leeks, Onions, Garlic, Mushrooms, Sweet peppers, Bean sprouts, Green salad & Lettuce, Cucumber, Tomatoes, Sweetcorn, Beetroot, Coleslaw, Avocado, Watercress, Cauliflower, Celery, Marrow & Courgettes

Table S2 Simple mixed model association between childhood determinants and childhood asthma at any point (n=897)

		Asthma diagnosis True vs False 95% CI	
	OR	**Lower**	**Upper**
Time (n=897)			
Year 10 follow-up	1.00		
Year 5 follow-up	0.55	0.40	0.77
Year 3 follow-up	0.41	0.29	0.57
Birthweight[1] (n=885)	1.00	1.00	1.00
Gestational age[1] (n=793)	0.92	0.85	0.99
Gender[1] (n=893)			
Female	1.00		
Male	1.55	1.17	2.05
GMS Eligibility[1] (n=694)			
TRUE	1.00		
FALSE	0.45	0.29	0.69
Birth order[1] (n=882)			
Not firstborn	1.00		
Firstborn	1.14	0.86	1.51
Mode of delivery[1] (n=839)			
Ceasarean section	1.00		
Vaginal (spontaneous or assisted)	0.85	0.57	1.26
Region[1] (n=897)			
Dublin	1.00		
Galway	0.86	0.64	1.16

[1]Controlling for time as a fixed factor

OR=Odds Ratio SD=Standard Deviation CI=Confidence interval

Table S3 Simple mixed model association between maternal determinants and childhood asthma at any point (n=897)

		Asthma diagnosis True vs False 95% CI	
	OR	**Lower**	**Upper**
Time (n=897)			
Year 10 follow-up	1.00		
Year 5 follow-up	0.55	0.40	0.77
Year 3 follow-up	0.41	0.29	0.57
Education[1] (n=873)			
Some/completed 3rd level	1.00		
None/Primary/Secondary school	1.28	0.97	1.70
Marital status[1] (baseline) (n=885)			
Lone	1.00		
Cohabiting	0.66	0.47	0.94
Marital status[1] (any time-point) (n=557)			
Lone	1.00		
Cohabiting	1.21	0.57	2.57
Age at birth of proband[1] (n=889)	0.98	0.96	1.01
BMI prior to falling pregnant[1] (n=750)	0.98	0.94	1.02
GMS Eligibility[1] (baseline) (n=887)			
TRUE	1.00		
FALSE	0.73	0.5	1.07
GMS Eligibility[1] (any time point) (n=797)			
TRUE	1.00		
FALSE	0.72	0.51	1.03
Maternal Health[1] (baseline) (n=871)			
Excellent/Very good	1.00		
Poor/Fair/Good	0.82	0.59	1.13
Maternal Health[1] (any time point) (n=560)			
Excellent/Very good	1.00		
Poor/Fair/Good	1.23	0.79	1.9
Smoking status at delivery[1] (n=870)			
Smoker	1.00		
Non-smoker	0.84	0.59	1.2
Breastfeeding[1] (n=897)			
TRUE	1.00		
FALSE	1.21	0.85	1.73

[1]Controlling for time as a fixed factor GMS=General Medical Scheme
OR=Odds Ratio SD=Standard Deviation CI=Confidence Interval

Table S4 Distribution of maternal nutrient intake during pregnancy in Normal-Reporters (EI/BMRpreg<1.35-2.39) relative to Irish Recommended Daily Allowances (n=453)

Nutrient[1]	$\bar{x}$ ± SD	25th Percentile	Median	75th Percentile	RDAs for Irish women* 19-64 yrs	Pregnancy**
Total fat (g/day)	129.0±110.4	51.8	100.4	175.3	-	-
MUFA (g/day)	41.1±35.9	16.8	31.7	53.9	-	-
PUFA (g/day)	20.3±20.3	7.0	13.5	25.8	-	-
Saturated Fatty Acids (g/day)	57.8±58.5	20.1	39.1	74.6	-	-
Cholesterol (mg/day)	402.1±330.9	182.3	312.9	522.2	-	-
Retinol (µg/day)	763.2±1004.2	290.6	515.7	890.4	-	-
Carotene (µg/day)	3594.9±2758.3	1356.7	3127.5	4581.2	-	-
Selenium (µg/day)	86.7±83.0	36.3	60.7	110.3	55.0	55.0
Zinc (mg/day)	14.7±11.7	6.6	12.2	18.5	15.0	20.0
Magnesium (mg/day)	545.5±557.9	191.1	362.7	660.9	255-65‡	290-300‡
Copper (mg/day)	2.0±3.4	0.6	1.2	2.1	1.1	1.1
Manganese (mg/day)	4.9±4.8	1.7	3.3	5.9	≤10.0‡	-
Calcium (mg/day)	1712.9±1467.4	614.6	1222.0	2429.9	800.0	1200.0
Vitamin C (mg/day)	248.1±246.5	90.3	157.9	316.0	60.0	80.0
Vitamin D (µg/day)	4.3±4.1	1.7	3.1	5.4	7.5	10.0
Vitamin E (mg/day)	13.6±18.7	4.9	7.9	14.2	8.0	10.0

[1]Calculated from food-frequency questionnaires in first trimester; Energy adjusted - residual method;

MUFA=Monounsaturated Fatty Acids PUFA=Polyunsaturated Acids EI=Energy Intake BMR=Basal Metabolic Rate preg=Pregnancy

g=gram mg=milligram µg=microgram

* Recommended Daily Allowance; Irish Food Safety Authority (1999) **From 20 weeks gestation ‡Estimated Average Requirement, European Food Safety Authority (2006)

‡Scientific Committee for Food of the European Union (SCF, 1993)

Table S5 Distribution of maternal food group intake during pregnancy relative to SLAN females (n=886)

(n=886)

	Lifeways Mothers 2001-2003			SLAN 2007 Females		Recommended intake*	
	Under 18 years (n=16)	**18-29 years (n=377)**	**30-44 years (n=493)**	**18-29 years**	**30-44 years**	**19-64 yrs**	**Pregnancy**
Food group[1] (total daily portions)	**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**Portions**	**Portions**
Oily fish	0.02 ± 0.04	0.03 ± 0.07	0.08 ± 0.14	-	-	1[b]	1[b]
Vegetables	2.5 ± 1.5	2.6 ± 1.9	3.2 ± 2.1	4.1 ± 2.9	4.5 ± 2.8	5[a]	6-7[a]
Fruit	2.2 ± 1.6	2.0 ± 2.5	2.2 1.6	2.8 ± 2.7	3.0 ± 2.8	5[a]	6-7[a]
Added Fat	2.2 ± 3.1	2.4 ± 2.4	2.6 ± 2.2	-	-	-	-

[1]Calculated from food-frequency questionnaires in first trimester [*]Food Safety Authority of Ireland SLAN=Survey of Lifestyle, Attitudes and Nutrition

[a]Daily portion [b]Weekly portion

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract – **PAGE 1 & 2**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found – **PAGE 2**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported – **PAGE 3 & 4**
Objectives	3	State specific objectives, including any prespecified hypotheses – **PAGE 4**
Methods		
Study design	4	Present key elements of study design early in the paper – **PAGE 4 & 5**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection – **PAGE 4, 5 & 6**
Participants	6	*Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up – **PAGE 5**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable – **PAGE 5, 6 & 7**
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 – **PAGE 6 &7**
Bias	9	Describe any efforts to address potential sources of bias – **PAGE 17**
Study size	10	Explain how the study size was arrived at – DUE TO WORD COUNT RESTRICTION PLEASE SEE DETAIL OF REFERENCE 60, 62 & 63
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why – **PAGE 5, 6, 7 & 8**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding – **PAGE 5, 6, 7, 13, 14 & 15**
		(*b*) Describe any methods used to examine subgroups and interactions – **PAGE 8, 13, 14 & 15**
		(*c*) Explain how missing data were addressed – **PAGE 11 & 17,** ADDITIONAL DETAIL IN REFERENCE 63
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed – **PAGE 11 &17**, ADDITIONAL DETAIL IN REFERENCE 63

Continued on next page

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 – **PAGE 11, 17**, ADDITIONAL DETAIL REFERENCE 63
		(b) Give reasons for non-participation at each stage - DUE TO WORD COUNT RESTRICTION PLEASE SEE DETAIL OF REFERENCE 60, 62 & 63
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders – **PAGE 8, 9, 10, 11 & 12**
		(b) Indicate number of participants with missing data for each variable of interest – **PAGE 11**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) – **PAGE 11**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time – **PAGE 11**, ADDITIONAL DETAIL REFERENCE 63
Main results	6	(*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 – **PAGE 13, 14, & 15**
		(*b*) Report category boundaries when continuous variables were categorized - DUE TO WORD COUNT RESTRICTION PLEASE SEE DETAIL OF REFERENCE 63
Discussion		
Key results	18	Summarise key results with reference to study objectives – **PAGE 12**
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 – **PAGE 17**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence – **PAGE 17**
Generalisability	21	Discuss the generalisability (external validity) of the study results – **PAGE 17**
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 – **PAGE 19**

*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.