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Review

The benefits of fish intake: Results concerning prenatal mercury exposure and child outcomes from the ALSPAC prebirth cohort

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ABSTRACT

Health advice to pregnant women concerning consumption of mercury-containing foods has resulted in anxiety, with subsequent avoidance of fish consumption during pregnancy. However, seafood contains many nutrients crucial for children's growth and development. Longitudinal studies in the Seychelles, where fish is a major component of the diet, have not demonstrated harmful cognitive effects in children with increasing maternal mercury levels. Is the same true in a more developed country (the UK) where fish is eaten less frequently? We review publications using data collected by the Avon Longitudinal Study of Parents and Children (ALSPAC) to address this topic. Total mercury levels were measured in maternal whole blood and umbilical cord tissue. Offspring were followed throughout childhood, especially their cognitive development. No adverse associations were noted. Significantly beneficial associations with prenatal mercury levels were shown for total and performance IQ, mathematical/scientific reasoning, and birthweight in fish-consuming vs non-fish consuming mothers. These beneficial findings are similar to those observed in the Seychelles where fish consumption is high and prenatal Hg levels are x10 higher than US levels. Government recommendations should be reviewed to emphasise the beneficial value of fish consumption during pregnancy.
Data availability: ALSPAC data access is through a system of managed open access. The steps below highlight how to apply for access to the data included in this paper and all other ALSPAC data.

1. Please read the ALSPAC access policy (http://www.bristol.ac.uk/media-library/sites/alspac/documents/researchers/data-access/ALSPAC_Access_Policy.pdf) which describes the process of accessing the data and biological samples in detail, and outlines the costs associated with doing so.

2. You may also find it useful to browse our fully searchable research proposals database (https://proposals.epi.bristol.ac.uk/), which lists all research projects that have been approved since April 2011.

3. Please submit your research proposal (https://proposals.epi.bristol.ac.uk/) for consideration by the ALSPAC Executive Committee using the online process. You will receive a response within 10 working days to advise you whether your proposal has been approved.

If you have any questions about accessing data, please email: alspac-data@bristol.ac.uk.

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1. Introduction

Although there is substantial evidence that fish intake in pregnancy is beneficial (Hibbeln et al., 2019; Spiller et al., 2019), there is a fear that pollutants such as mercury in fish may have detrimental effects. The most influential study concerning the possible dangers of eating seafood concerned a study from the Faroe Islands (Grandjean et al., 1997), where the level of methylmercury in maternal hair and cord blood was shown to be negatively associated with cognitive abilities of the offspring at age 7 years. Although it was clearly stated that the methylmercury levels were associated with consumption of pilot whale (a sea mammal, not a fish), the subsequent assumptions were that seafood in general was responsible for increased mercury levels in the mother, and thence the fetus, and that mercury levels in pregnancy should be minimised. In consequence, although advice to pregnant women was generally that fish was good, the accompanying caveat was to avoid fish with high levels of mercury. Psychologically, the latter was the message that women remembered, and the general reaction has been for women to reduce their intake of all seafood (Oken et al., 2003; Bloomingdale et al., 2010).

Although there are a number of studies that have considered the question, here we have considered two contrasting studies of population samples with a measure of prenatal mercury exposure and whose offspring were followed up at frequent intervals: they comprise one in the Seychelles (an archipelago of 115 islands in the Indian Ocean, off East Africa, where fish provides a major component of the diet), and for which summaries of the findings are available (Davidson et al., 2006, 2011; Shamlaye et al., 2020; van Wijngaarden et al., 2020), and one in the UK (the Avon Longitudinal Study of Parents and Children (ALSPAC)), an area in south-west England where fish are consumed far less frequently, and where no summary of the findings has been published heretofore.

With this background it was clear that further research was required to assess the true association between maternal fish intake in pregnancy, levels of mercury biomarkers and offspring outcomes, particularly measures of cognition.

2. Materials and methods using ALSPAC

2.1. The ALSPAC study

This pre-birth cohort study enrolled a geographical population of pregnant women in 1990–1992, and followed their pregnancies, and then their offspring throughout childhood, adolescence and into adulthood. Detailed information on the ALSPAC study aims and methods are available elsewhere (Golding et al., 2001; Boyd et al., 2013; Fraser et al., 2013; Northstone et al., 2019). Data sources included: (a) self-completion questionnaires completed by the mother and her partner initially and later by their offspring; (b) linkage to medical records; (c) assays of biological samples (including blood, urine, umbilical cord tissue, hair and toe-nails); physical examinations; (d) various measures of subsamples concerning the environment (e.g. measures of air pollutants and trace metals); and (e) linkage to geographic sources of pollution using GIS (Geographic Information Systems). (The study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool: http://www.bristol.ac.uk/alspac/researchers/our-data/).

2.2. Measurements of exposure to mercury

2.2.1. Maternal whole blood mercury

Gestational age at collection of a blood sample collected specifically for the identification of levels of trace metals was known for 4472 of the 4484 pregnancies (99.7%) for which a blood measurement was made; it ranged from 1 to 42 weeks, with a median value of 11 weeks and interquartile range (IQR) of 9–13 weeks. In all, 93% of the samples were collected in the first half of pregnancy (< 18 weeks gestation).

Whole blood was assayed for mercury content by the laboratory of Robert Jones at CDC in Atlanta, Georgia, USA. The techniques used are described in detail elsewhere (Golding et al., 2013). In brief, clotted whole blood was digested to remove all clots before being analysed using inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS). The blood sample was heated in a microwave oven at a controlled temperature and time during which the organic matrix of the blood was digested thus removing the clots. ICP-DRC-MS internal standards (iridium and tellurium) were added at a constant concentration to all blanks, calibrators, and samples (at the time of 1:9 dilution of digestate) to facilitate correction for instrument noise and drift. The standard additions method of calibration was used to optimize the analytical sensitivity of the method for the whole blood samples. A recovery spike was included in each analytical run for calibration verification and as a blind quality control (QC) sample. Two levels of bench QC materials as well as in-house QC samples with control limits unknown to the analysts were used for daily quality control.

Of the 4484 samples, 4131 had accurate results for mercury. Three samples with values below the assay limit of detection (LOD) (0.24 μg/L) were assigned the LOD value divided by the square root of 2. (Because the distribution of mercury exposure was log-normally distributed (Fig. 1), a factor > 0.5 was deemed appropriate to reflect the likelihood that more of the results below the LOD would be closer to the LOD than zero.).

2.2.2. Mercury levels in umbilical cord tissue

Cord tissue samples were taken by the midwife at birth and frozen at − 20 °C. Samples were defrosted briefly to divide the sample into several 1-cm slices, and then stored again at − 20°.

These pieces of umbilical cord tissue were used to measure total mercury in three ways: (a) using wet weight in two different machines (the Sheffield assays), and (b) using dry weight in a different laboratory (the Danish assays). The latter measure was assumed by Julvez and co-authors (2013, 2019) to provide a measure of methylmercury.

The Sheffield assays: Total mercury concentration was analysed in the umbilical cord tissue using a convenience sample of children, as study funds allowed. Samples were analysed using cold vapor fluorescence spectrometry at Sheffield Hallam University using reference samples and standard laboratory practices to ensure validity and reliability (Winfield et al., 1994). Before analyses, the selected 1 cm cord tissue samples were washed with distilled water to remove cord blood, weighed, and digested by closed system microwave digestion using nitric acid and hydrogen peroxide, made to 10 mL solution. (Daniels et al., 2004). In phase 1 the majority of samples (n = 2005) were assayed for up to 13 elements, including magnesium, manganese, iron, copper and zinc, using inductively coupled plasma-optical emission spectrometry (ICP-OES) (separate analyses were carried out for elements present in relatively high concentration e.g. iron, and those present in low concentration e.g. cadmium). Selenium and mercury were assayed using atomic fluorescence techniques (hydride generation for selenium, cold vapour for mercury). However, the last 911 samples were assayed for these elements plus lead, using inductively coupled plasma mass spectrometry (ICP-MS) (phase 2), which was more sensitive than ICP-OES but had not been available for the earlier samples. Element concentrations were measured as ng 0.10 mL^{-1} digest and divided by the cord wet weight to give $Ng.g^{-1}$ of cord and hence parts per billion (ppb). Quality control procedures used bovine liver as a standard reference material to check that each assay method was producing consistent and reliable data over time. Nevertheless, the results for mercury using the second

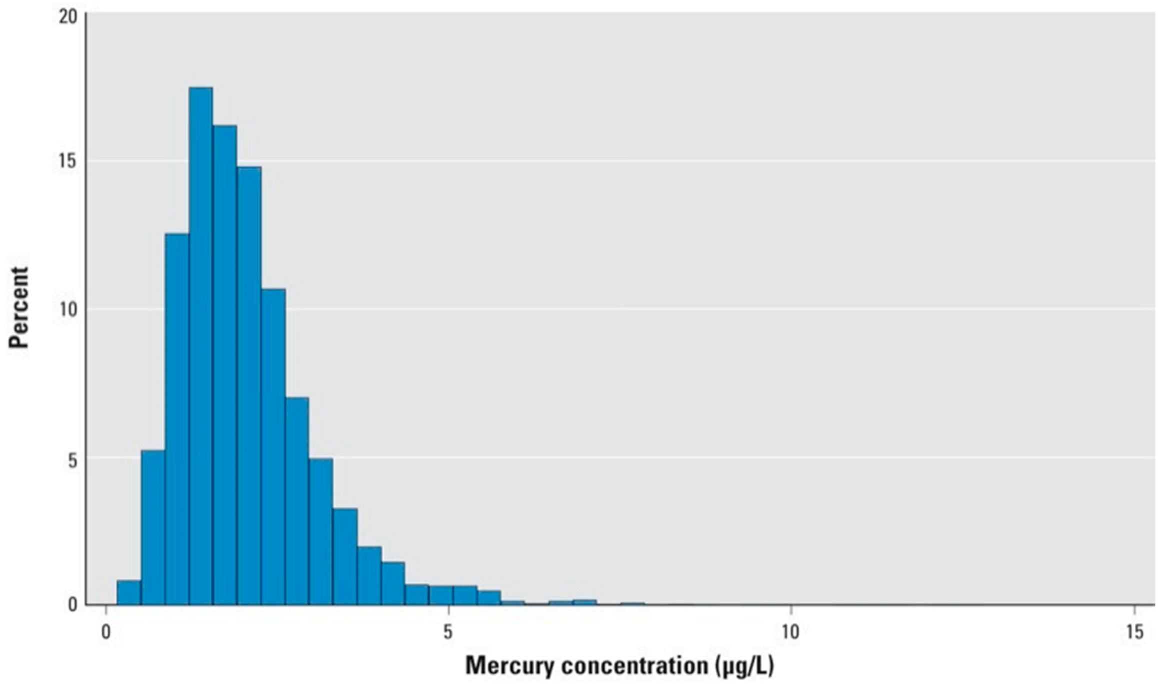

Fig. 1. The distribution of whole blood total mercury measured in 4134 women during pregnancy (from Golding et al., 2013).

method showed markedly different results from the first method. Shaheen and colleagues made attempts to control for this in their epidemiological analyses by dividing each measure by the geometric mean for all measures obtained using that particular machine (Shaheen et al., 2004).

The Danish analyses: A further sub-sample of ALSPAC umbilical cords (n = 1311) was selected to measure mercury concentrations using a different methodology. The samples were selected from everyone who had available both Genome-Wide Association Study (GWAS) data at that time (3233 persons) and a cord sample of suitable size. After freeze-drying the cord tissue samples, mercury was determined in duplicate using a Direct Mercury Analyzer (DMA-80, Milestone, Inc., CT) at the University of Southern Denmark. A specimen of about 0.5 g was weighed into a quartz boat. The sample boat was then placed in the auto-sampler and inserted into the quartz decomposition tube. Once the sample was completely decomposed, mercury trapped on a gold filter was rapidly released by heating the amalgamator. Released mercury was measured by atomic absorption spectroscopy at 253.7 nm as a function of mercury concentration. Samples were analysed by using a matrix-matched calibration (solid samples) curve created with various weights of certified reference material DOLT-3 (dogfish liver tissue certified reference material for trace metals, National Research Council, Institute of Environmental Chemistry, Ottawa, Canada) containing 3.37 ppm mercury. As calibration verification standards National Institute of Standard and Technology (NIST SRM) 1566b (Oyster tissue) was used. The detection limit for this method is 5 ng/g. In 14 cord tissue samples run in triplicate, the average coefficient of variation was 14.5% at average concentrations between 10.7 ng/g and 164 ng/g (both dry weight) The mercury concentration in the umbilical cord was taken to be a measure of prenatal methylmercury exposure (Julvez et al., 2013). A second set of 1045 ALSPAC umbilical cord samples were assayed subsequently using a similar methodology to the previous set (Julvez et al., 2019).

2.3. *Maternal exposures associated with Hg*

2.3.1. *Diet during pregnancy*

Importantly for the study of prenatal mercury and offspring outcomes, detailed estimates of maternal diet were obtained for over 11,000 pregnant women using a food frequency questionnaire (FFQ) during the last trimester of pregnancy. The mean age of the women was 27.9 years, self-reported pre-pregnancy weight was 61.6 kg, height 1.64 cm and BMI 22.9 kg/m^2 (Table 1).

The FFQ included three questions on seafood: the frequencies with which she ate (i) white fish, (ii) oily fish and (iii) shellfish. These details, together with information on standard portion sizes for women of child-bearing age in Britain, allowed estimates of total fish intake to be calculated (Emmett et al., 2015). Further dietary information estimated in this way ensured that levels of total energy intake could be calculated, giving a mean of 7.70 (S.D. 2.03) and median of 7.55 MJ/day (Rogers and Emmett, 1998). The variation in the amount of each type of seafood consumed is shown in Table 2. This shows that white fish were eaten more frequently than oily fish, and that shellfish were eaten less often. In all, 18% of the pregnant women were eating three or more portions of seafood per week, which was in excess of the UK national recommendations at that time (Golding et al., 2014). For the rest of this paper,

Table 1
The basic information concerning the women who answered the diet questions during pregnancy (from Rogers and Emmett, 1998).

Characteristics	Mean (S.D)	Range	N
Age at delivery (years)	27.9 (5.0)	15–44	11,768
Pre-pregnant weight (kg)	61.6 (10.9)	30–149	11,189
Height (m)	1.64 (0.07)	1.24–2.01	11,725
Pre-pregnant BMI (kg/m^2)	22.9 (3.8)	12.5–54.7	11,067

BMI = Body mass index

Table 2
The reported frequencies of the number of portions of seafood being eaten by the 3509 pregnant women for whom total blood mercury was also available.

Type of seafood	Not at all	Once in 2 weeks	1–3 times per week	4 + times per week
White fish	18.6%	40.3%	39.8%	1.4%
Oily fish	42.5%	33.0%	23.2%	1.2%
Shellfish	79.8%	16.3%	3.6%	0.2%

when referring to fish we are meaning white and oily fish only.

2.3.2. Dental amalgam

Information was available concerning the number of amalgam fillings the mother had in her mouth at the start of pregnancy, whether she visited the dentist during pregnancy, and if so whether she had fillings removed or inserted (see Golding et al., 2016b for details).

2.4. Offspring outcome measures used

2.4.1. At birth

Birthweight, birth head circumference and crown-heel length. The methodology used for these measurements are described in detail elsewhere (Golding et al., 2022).

2.4.2. Pre-school development

Measures of early child development were collected from detailed structured questionnaires completed by the study mothers on the whole cohort using assessments based on the Denver Developmental Screening Test (DDST) (Frankenburg and Dodds, 1967). The validity of these measures was shown by comparison of the scores using these maternal reports with independent tests of the children using trained psychologists (Wilson 2003). A description of items making up each scale can be found in the Appendices to Iles-Caven et al. 2016. Other preschool measures of development included the MacArthur Communication Development Inventory (MCDI) (Feldman et al., 2000) which comprised measures of vocabulary comprehension and social activity at 15 months of age.

2.4.3. Measures of IQ

At 8 years of age the Weschler Intelligence Scale for Children WISC-III^{UK} (Wechsler et al., 1992) was used to assess cognitive function. Versions of this scale are the most widely used individual cognitive ability tests worldwide. A short form of the measure was employed in ALSPAC where alternate items (always starting with item number 1 in the standard form) were used for all subtests, with the exception of the coding subtest which was administered in its full form. Using this format, the length of the session was reduced, and children were less likely to tire. Such shortened forms of the WISC have been used successfully in several studies (e.g. Stricker et al., 1968; Finch and Childress, 1975). All tests were administered by members of the ALSPAC psychology team. The data were used to create three scales: Verbal IQ, Performance IQ and Total IQ (see Golding et al. 2017 for further details).

2.4.4. Scholastic abilities

(see Golding et al., 2019 for details) in brief, a variety of measures were used to identify skills in reading, spelling. mathematics (tests to assess reasoning in mathematical problems independently of their computation skills (Nunes et al., 2009)) and scientific reasoning (Bryant et al., 2015).

2.4.5. Signs of atopic disorders

Mothers had completed questionnaires at frequent intervals during the child's early years concerning the frequency with which the child had signs of wheezing on the chest, and indications of a rash that was likely to be due to eczema (see Shaheen et al., 2004 for details).

2.4.6. Blood pressures

The offspring were physically examined every two years between the ages of 7 and 17. Among other measures, systolic and diastolic blood pressures were recorded.

2.5. Methods of literature review

Publications were identified using: (a) Google Scholar with the key words 'ALSPAC' and 'mercury'; (b) assessing publications in the ALSPAC publication files using the keywords mercury, dental, and fish. Subsequent checks were undertaken using the names of authors known to have used the various estimates of mercury exposure within ALSPAC including S. Shaheen; P. Grandjean; J. Daniels; J. Golding; C.M. Taylor; J. Julvez.

2.6. Statistical methodologies

In the results section detailed below we describe the conclusions from the different adjusted statistical analyses undertaken. The factors adjusted for depended on the outcome and the age of the individual as shown in Appendix A. In this paper we have concentrated on whether there were any adjusted results that would allow us to determine whether there were differences between the relationships of Hg levels with outcome, dependent on whether or not the mother ate fish during pregnancy. Often the data were published in the paper but a P value for interaction was not presented. Presence of a significant interaction ($P < 0.05$) between the groups of fish-eaters and non-fish eaters was therefore assumed when both the effect size (mean difference or odds ratio) of one group was outside the 95% confidence interval of the other and vice versa.

3. Results

3.1. Associations between maternal blood Hg levels, dietary, social and dental exposures

The total blood mercury levels of the mother were compared with the pregnant woman's reported dietary intake of 103 dietary items together with six socio-demographic variables (Golding et al., 2013). Using linear regression, the total amount of variation (R^2) in log transformed total blood Hg associated with diet was 19.8%, of which less than half (44%) was associated with seafood consumption (white fish, oily fish and shellfish). Other components of diet that were positively associated with these blood Hg levels included wine and herbal teas; dietary components with significant negative associations included white bread, meat pies or pasties, and French fries (Table 3). We concluded that although seafood is a source of dietary mercury, it appeared to explain a relatively small proportion (9%) of the variation in total blood mercury in our UK study population.

In parallel, a similar detailed analysis of the maternal total blood mercury demonstrated that socio-demographic characteristics of the mother accounted for 10.4% of the variance. The analyses demonstrated increased levels of mercury for women having their first pregnancy, older women, those of higher social class as measured using occupation, those with higher levels of education, and those who owned (or were buying) their home (Golding et al., 2013).

Dental amalgam was considered in a later paper (Golding et al., 2016b). The number of amalgam fillings in the woman's mouth at the start of pregnancy, and the numbers of such fillings that had been

Table 3
The independent dietary factors that contribute to the mother's total blood mercury: Results of linear regression of LnHg (from Golding et al., 2013).

Dietary item	10 x β (95% CI)	P
Oily fish	+1.4 (+1.1, +1.8)	< 0.0001
White fish	+1.4 (+1.0, +1.8)	< 0.0001
Shellfish	+9.0 (+2.0, +1.5)	0.0070
Alcohol pre-pregnancy	+1.2 (+8.0, +1.6)	< 0.0001
Herbal tea	+1.5 (+8.0, +2.2)	< 0.0001
'Health' foods	+1.8 (+5.0, +3.1)	0.0072
Meat pies/pasties	-8.0 (−1.3, −4.0)	0.0003
French fries	-6.0 (−9.0, −3.0)	0.0003
White bread	-1.3 (−2.0, −6.0)	0.0006
Sugar in tea	-9.0 (−1.4, −3.0)	0.0011
Baked beans	-4.0 (−7.0, −1.0)	0.0059
Milk on its own	-5.0 (−9.0, −1.0)	0.0097

extracted and inserted during pregnancy contributed to a further 6.5% of the variance of the total blood mercury. A combination of dietary and dental exposures accounted for 20.2% of the total variance, and socio-demographic factors contributed only a further 3.4%.

Using the smaller number of the umbilical cord measurements that used the Sheffield laboratory (n = 1040), a relationship between Hg level and dental amalgam exposures to the mother was not obvious ($P > 0.05$) (Daniels et al., 2007). This, however, may have been because no account had been taken of the two differing machines used for these measurements.

3.2. Maternal blood mercury levels and fetal outcomes

An assessment was made of fetal growth during pregnancy in regard to maternal Hg levels by analysing birthweight, birth head circumference and crown-heel length (Taylor et al., 2016). Analyses, adjusted for maternal age, education, parity, height, body mass index (BMI), smoking and alcohol consumption during pregnancy, as well as the gestational age and sex of the offspring. The results (Table 4) demonstrate unadjusted positive associations between Hg and all three birth measurements. However, on adjustment after stratifying for whether or not the mother ate fish, a difference was found such that if the mother ate fish there was no association between Hg and birthweight, whereas if she did not eat fish, there was a negative association; the difference between the adjusted birthweights of the offspring of the fish and non-fish eaters was statistically significant.

Other ALSPAC publications included one that used exposures to dental amalgam as a proxy for fetal exposure to mercury, but found no association with birthweight, gestation, birthweight at term or preterm delivery (Daniels et al., 2007). Another publication that considered pregnancy outcome was focussed on the possibility that maternal toxic metal levels might be related to a sex difference at birth. Taylor et al., (2014) compared maternal prenatal blood levels of lead, mercury and selenium with the sex of the offspring, but there were no differences at $P < 0.05$.

Table 4
The associations (regression coefficient and 95%CIs) of total blood Hg in relation to continuous measures at birth of weight, head circumference and crown-heel length (adapted from Table 2 of Taylor et al., 2016).

OUTCOME	All women b (95%CI)	Fish-eaters b (95%CI)	Non-fish eaters b (95%CI)
Birthweight (g)			
Unadjusted	**+ 17.9(+0.8, +33.9)**	+ 4.3(−13.5, +22.0)	-4.2(−54.4, +46.1)
N	3853	2923	500
P	**0.029**	0.638	0.871
Adjusted[*]	-3.1(−18.9, +12.8)	-1.5(−18.6, +15.7)	**-58.4(−113.8, −3.0)**
N	2692	2324	354
P	0.705	0.867	**0.039**
Birth head-circumference (cm)			
Unadjusted	**+ 0.05(0.00, +0.10)**	+ 0.03(−0.02, +0.08)	0.00(−0.16, +0.16)
N	3342	2585	439
P	**0.033**	0.206	0.998
Adjusted	+ 0.01(−0.04, +0.06)	+ 0.01(−0.04, +0.06)	-0.05(−0.24, +0.15)
N	2376	2055	311
P	0.628	0.628	0.630
Crown-heel Length			
Unadjusted	**+ 0.10(+0.03, +0.17)**	+ 0.06(−0.03, +0.14)	+ 0.08(−0.16, +0.31)
N	3297	2548	437
P	**0.005**	0.179	0.523
Adjusted	+ 0.02(−0.06, +0.09)	+ 0.02(−0.07, +0.10)	-0.08(−0.33, +0.17)
N	2345	2026	310
P	0.637	0.709	0.540

[*] significant difference between the offspring of fish and non-fish eaters

3.3. Cognitive outcomes of the offspring and Hg in maternal blood

Estimates of fetal mercury exposures were compared with a variety of cognitive outcomes measured during the child's development (Table 5). In early infancy there were two measures from the MCDI completed by the mothers (when their child was aged 15 months) and a total development measure (the Griffiths) after examination by trained psychologists at 18 months. Although there were no associations between the MCDI and either the cord tissue Hg level or fish consumption, for the Griffiths test there were positive associations with maternal fish consumption. No assessments of interactions with cord tissue Hg level were considered.

Preschool children were assessed by their mothers using the DDST on four occasions between 6 and 42 months; for each age four subtests were carried out (fine and gross motor coordination, communication and social skills) and a total development score calculated. The relationship between maternal blood Hg level and total score was positive (i.e. better development with increasing Hg) at two of the four ages. However, of the 20 tests of interaction between maternal blood Hg level, fish intake and DDST, only one was significant (i.e. no more than would be expected by chance).

The pattern changed once the tests were of school age children. There were no indications of adverse associations of cognition with maternal Hg level, as measured by the WISC IQ, which had been administered to the 8-year-old children by trained psychologists. This was found when the Hg was taken from maternal blood (Golding et al., 2017) as well as from levels in the umbilical cord (Julvez et al., 2013).

There were significant interactions with maternal blood levels such that the prenatal Hg level of the mother was positively associated with the IQ level of her offspring if the mother had eaten fish in pregnancy, but not if she had not. This interaction was true of five cognitive outcomes: full IQ, performance IQ, mathematical comprehension, science comprehension and social cognition (Table 5 and Fig. 2). For example, for high levels of a measure of the social cognition trait (which is closely associated with autism), the Hg level of the mother who had not eaten fish was associated with increased likelihood of the child having high scores on the trait in comparison with the child of the mother who had eaten fish where there was no association with Hg level.

3.4. Other associations

Apart from the measures of cognition outlined above there were no similar interactions with 6 different measures of behaviour at 7 ages from 4 to 17 years; nor were there any such associations with signs of atopy such as wheezing attacks and eczema. In contrast there were interactions with blood pressure measurements such that there was a decrease in *systolic* pressure at ages 11 and 15 with increasing Hg levels if the mother ate fish, but an increasing *diastolic* pressure with increasing maternal Hg levels if the mother ate fish (Table 5).

4. Discussion

This paper has summarised the results from the various papers that have used data from the ALSPAC longitudinal cohort study. There have been no studies that have shown an overall increase in adverse outcome with increasing level of exposure to Hg in pregnancy. Indeed, if the mother had eaten fish during the pregnancy, although adjustments had been made for a variety of different socioeconomic and biological factors, there were beneficial associations between prenatal levels of Hg and a number of outcomes, in contrast with the associations when the mother did not eat fish.

Although each of the papers quoted here was focussed on a single outcome (or type of outcome), we acknowledge that when considered together it is appropriate to allow for multiple testing. In all there were

Table 5
Details of the publications concerning mercury exposure during pregnancy and outcome to the child after birth. Adjusted results are for the relationship between Hg levels and child outcomes; they are only quoted if $P < 0.05$.

Author	Tissue type	Fish interaction considered	Outcome measure	Age	N	Adjusted Results
Preschool cognition						
Daniels et al., 2004	Umbilical cord (Sheffield)	No	MCDI: vocabulary, Social activity Griffiths Total Development	15 m 15 m 18 m	1054	Hg: NS Fish +ve Hg: NS
Daniels et al.,. 2007	Dental amalgam	No	MCDI: vocabulary, Social activity Griffiths Total Development	15 m 15 m 18 m	1054	Hg: NS Fish +ve Hg: NS
Golding et al., 2016a	Blood	Yes	Total DDST: 4 ages	6–42 m	~3000	Hg: +ve association at 6 m; 42 m No interaction with MFE
Iles-Caven et al., 2016	Blood	yes	4 subsections of DDST at 4 ages	6–42 m	~3000	No interactions with MFE except gross motor at 42 m where association was more +ve when MNFE
School age cognition						
Golding et al., 2017	Blood	Yes $P < 0.05$ $P < 0.05$	IQ: Full scale IQ: Performance	8 y 8 y	2062 2062	Hg: +ve if MFE; less +ve if MNFE. Hg: +ve if MFE; less +ve if MNFE.
Hibbeln et al., 2018	Blood	Yes $P < 0.05$ $P < 0.05$	Reading; spelling Math Science	7–13 y 11–12 y 11–12 y	1578 1585	NS Hg: +ve if MFE; less +ve if not
Julvez et al., 2013	Umbilical cord (Denmark)	Omega-3 from seafood $P = 0.04$	IQ: Full-scale IQ: Verbal IQ: Performance	8 y	1311	Hg interaction with omega-3 from seafood for Performance IQ
Julvez et al., 2013	Umbilical cord (Denmark)	No	IQ: Full-scale IQ: Verbal IQ: Performance	8 y	2261*	Hg: NS
Behavior						
Golding et al., 2016c	Blood	yes	6 measures of behaviour at 7 ages	4–17 y	1599+	NS
Golding et al., 2018	Blood	Yes $P < 0.05$	Sociability Coherence Repetitive Social cognition	4 y 9 y 5 y 7 y	1991	Higher Hg for child with poor social cognition, if MNFE
Other outcomes						
Gregory et al., 2016	Blood	Yes Interaction for 3 of 12 measures	Systolic b.p. Diastolic b.p.	6 ages 7–17 y	1102+	Systolic: increase with Hg if MNFE at 11 but decrease at 15 y Diastolic: decrease at 15 y if MNFE
Shaheen et al., 2004	Umbilical cord (Sheffield)	No	Wheezing Eczema	0–6 m 30–42 m 18–30 m	2044 2044 2173	Hg: NS Hg: NS Hg: NS

DDST = Denver Developmental Screening Test; MCDI = MacArthur Communication Development Inventory; MFE = Maternal fish eater; MNFE = Mother does not eat fish; NS = $P > 0.05$;
*includes sample used in Julvez et al., 2013

96 outcomes considered – thus one would expect 4.8 to be P< 0.05 by chance. However, if we confine the analyses to those where data to test for interaction was available (observed 89), 10 (expected 4.95) had a significant association, 9 being in the same direction (i.e. increasing ability with increasing maternal Hg when the mother had eaten fish but not when she was not a fish-eater).

Confining to the child's abilities when tested under standardised settings, with the likelihood that the measures would be more accurate, the pattern becomes clearer. This applies to birth measurements (3), IQ tests at 8 years (3), educational abilities (4), and blood pressure measurements (12). There were 22 such measurements, 8 of which showed interactions (expected 1.1; $P < 0.001$). Of these 8 outcomes, cognitive measures that involve interpretation and reasoning of various types predominated. These included:

(a) Mathematical comprehension (tests to assess reasoning in mathematical problems independently of their computation skills (Nunes et al., 2009)).
(b) Social cognition (defined as the way in which people process, remember, and use information in social contexts to explain and predict their own behaviour and that of others).
(c) Scientific reasoning (generally defined as a problem-solving process that involves critical thinking in relation to content, procedural, and epistemic knowledge (Barz and Achimaş-Cadariu, 2016).
(d) Performance IQ (designed to assess fluid reasoning, spatial processing, and attention to details (Lange, 2011). This outcome measure was found to be associated with an interaction between both maternal whole blood Hg and maternal fish eating (Golding et al., 2017), but also an interaction between the umbilical cord Hg (Danish assay) and maternal intake of omega-3 from sea food (Julvez et al., 2013). Thus, the two different methodologies came to the same conclusion with different selections of participants, different tissue samples, different statistical methods, and different confounders taken into account.

Strengths and limitations of ALSPAC results.
The strengths of the ALSPAC findings are listed below:

(1) The data used in all analyses were collected blind to the Hg levels.

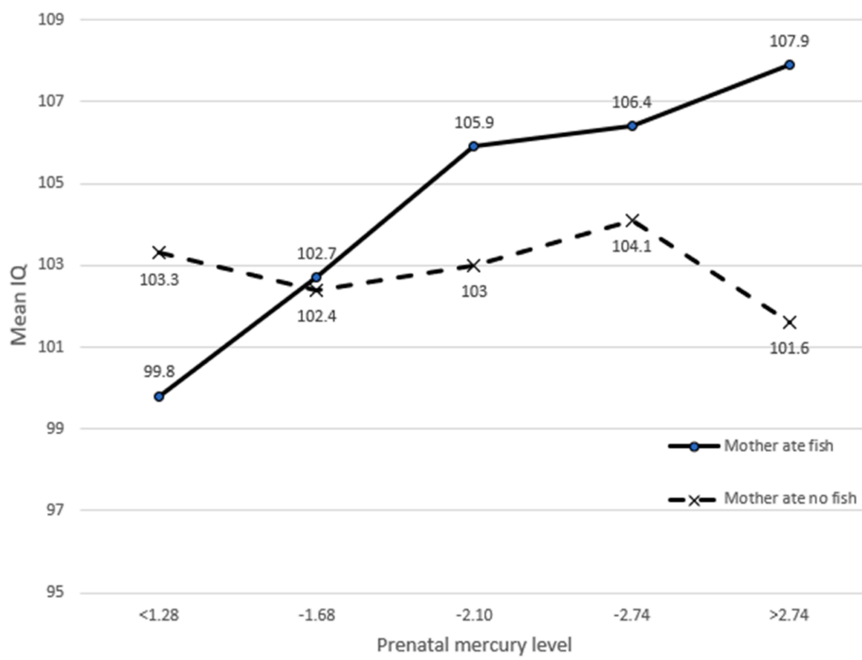

Fig. 2. The mean IQ levels found for the 8-year-old offspring for each 20th centile of maternal blood Hg level, contrasting the levels of the children whose mothers had eaten fish in pregnancy with those who had not.

(2) Compared with most other studies the numbers of individuals with fetal mercury exposures estimated and relevant outcome measures available is large.
(3) Results were stronger when the outcome measures were obtained by professionals as opposed to maternal reports.
(4) The associations between Hg measures in umbilical cord and a proxy for fish intake (omega-3 fatty acids from seafood) (Julvez et al., 2013) showed the same interaction with performance IQ as did maternal prenatal blood Hg and fish consumption.
(5) Many (but not all) of the interactions concerning fish intake and Hg associations concerned similar types of cognitive outcomes (each of which included an interpretation and/or reasoning component).
(6) ALSPAC measured many of the same psychological and cognitive domains as other studies such as the Seychelles.

The ALSPAC data concerning measures of Hg study is limited by the following:

(i) As with all observational data, there is the possibility that there were confounders that were not taken into account. However, it should be noted that there were a large number of confounders that were included, different mixtures of which showed similar adjusted associations, so this may be unlikely.
(ii) All large longitudinal studies suffer from increasing drop-out, partly due to loss to follow up, waning interest or enthusiasm, and less frequently to mortality. ALSPAC may well have lost key participants that would have changed the results. None of the study papers concerning measures of Hg have adjusted for missing data, however.
(iii) Initial funding received was for the Sheffield measurements. The assumption had been that the ICP-MS machine would be available at the start of the grant, but there was a major delay in supply of the machine and a time limit on the use of the funds, and consequently the available ICP-OES machine was used initially until the ICP-MS machine arrived. Unfortunately, there was no overlap between the assays using the two different machines, so we cannot judge how similar the results were. However, the distribution of the results (e.g. means and standard errors) differed. Subsequent funding was therefore obtained for assaying (a) the maternal whole blood at CDC, and for the Danish assays of the umbilical cord. These two assays were used for all but two of the studies reported here (see Table 5).

It should be noted that, for scientists wishing to use the data, numbers of participants quoted in this paper may change if individuals state they wish their data to be withdrawn.

5. Conclusions

Here we have summarised the data from the cohort born in a relatively industrialised area in south-west of England, comparing the outcomes of children born to fish-consuming and non-fish-eating pregnant women according to the degree to which they had been exposed to Hg in utero. Like the cohorts from the Seychelles where almost all pregnant women were fish eaters, we found that among the ALSPAC children of mothers who ate fish in pregnancy, there were positive associations between the level of Hg to which they had been exposed in utero and their abilities in complex understanding and reasoning. We have not discussed in this paper the results found in ALSPAC concerning the genetic associations with Hg (Julvez et al., 2013, 2019), or the epigenetic associations demonstrated in the Seychelles population (Ulluoa et al., 2020). These are intriguing, but whether they are related to the interactions between fish, mercury and offspring outcomes have yet to be demonstrated.

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CRedit authorship contribution statement

Jean Golding: Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Visualization, Supervision, Funding acquisition, **Caroline Taylor**: Writing – review & editing, **Yasmin Iles-Caven:** Writing – review & editing, Project administration, Funding acquisition, **Steven Gregory**: Formal analysis, Data curation.

Ethics

Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee (ALEC; IRB00003312) and the Local Research Ethics Committees. Detailed information on the ways in which confidentiality of the cohort is maintained may be found on the study website: http://www.bristol.ac.uk/alspac/researchers/research-ethics/. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. List of factors taken into account when calculating adjusted results concerning the inter-relationships between Hg in pregnancy, fish consumption and offspring outcome

Author	Confounders adjusted for
Daniels et al., 2004	ABCDGHJKLM
Golding et al., 2016a	ACEJLNOPQY
Iles-Caven et al., 2016	ABCDEGHJLNOPQY
Golding et al., 2017	ABCDEGHJLNOPQY
Hibbeln et al.,2018	ABCDEHJLNOPQY
Julvez et al., 2013	ABCEFGHRSTU
Golding et al., 2016c	ABCDEGJLNOPQY
Golding et al., 2018	ABCDEJLNOPQY
Gregory et al., 2016	ABCDEHJLNOPQY
Shaheen et al., 2004	ABCDEGJVWX
Taylor et al., 2016	ABCDGLWX

A = maternal age; B = maternal education level; C = parity; D = smoking in mid-pregnancy; E = tenure of housing; F = social class; G = sex of offspring; H = age at testing; J = breast feeding; K = dental treatment; L = alcohol consumption in pregnancy; M=HOME score of parenting; N = Family adversity index comprising 38 features including measures of maternal depression and anxiety; P = household crowding (no. persons /no. rooms); Q = stressful life events in the first half of pregnancy; R = the examiner; S = paternal education; T = maternal dietary pattern in pregnancy; U = child's dietary pattern at age 8; V = maternal atopic disease, child's head circumference at birth, attendance at day care in the first 6 months, ethnic origin, and birthweight; W = maternal body mass index; X = gestation; Y = selenium.

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