Maternal fatty acids in pregnancy, *FADS* polymorphisms, and child intelligence quotient at 8 y of age[1–3]

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ABSTRACT

Background: Brain tissue is selectively enriched with highly unsaturated fatty acids (FAs). Altering the maternal FA status in pregnancy may improve fetal neural development with lasting consequences for child development.

Objective: We explored whether maternal FAs in erythrocytes, either measured directly or indirectly by maternal *FADS* genetic variants, are associated with child intelligence quotient (IQ).

Design: Linear regression analyses, adjusted for 18 confounders, were used to investigate the associations in 2839 mother-child pairs from the population-based Avon Longitudinal Study of Parents and Children cohort.

Results: Low levels of arachidonic acid (20:4n−6) were associated with lower performance IQ (−2.0 points; 95% CI: −3.5, −0.6 points; $P = 0.007$, increased $R^2 = 0.27\%$), high levels of osbond acid (22:5n−6) were associated with verbal IQ (−1.8 points; 95% CI: −3.2, −0.4 points; $P = 0.014$, $R^2 = 0.20\%$), and high levels of adrenic acid (22:4n−6) were associated with verbal IQ (−1.7 points; 95% CI:−3.1, −0.3 points; $P = 0.016$, $R^2 = 0.19\%$). There was some evidence to support a negative association of low docosahexaenoic acid (DHA; 22:6n−3) with full-scale IQ ($R^2 = 0.15\%$). Novel weak associations were also observed for low levels of osbond acid ($R^2 \leq 0.29\%$) and *FADS* variants with opposite effects for intron variants and variants in the promoter region such as rs3834458 ($R^2 \leq 0.38\%$).

Conclusions: These results support the positive role of maternal arachidonic acid and DHA on fetal neural development, although the effects on child IQ by 8 y of age were small (0.1 SD), with other factors contributing more substantially. The endogenous synthesis of these FAs by *FADS* genes, especially *FADS2*, may also be important. The replication of these results is recommended. *Am J Clin Nutr* 2013;98:1575–82.

INTRODUCTION

Nutritional deficiencies or excesses in the fetal environment can have long-lasting influences on health and development. During pregnancy, the mother is the sole source of nutrients, and attention has been focused on the long-chain highly unsaturated fatty acids [HUFAs[4], also referred to as long-chain PUFAs (LC-PUFAs)] such as arachidonic acid (AA; 20:4n−6) and DHA (22:6n−3) in the maternal diet. These fatty acids (FAs) in the omega-6 and omega-3 series with ≥4 unsaturated bonds are believed to have key roles in neurodevelopment by influencing cell signaling, gene expression, cell fluidity, reducing oxidative stress, and glucose metabolism (1–3).

One of the most-consistent associations of maternal diet with HUFAs, such as DHA and EPA (20:5n−3), has been fish or seafood consumption (4, 5). Benefits of these foods have also been reported for child intelligence quotient (IQ) from ages 6 mo to 9 y, especially for verbal IQ (6–8). Together, these results have been consistent with the notion that HUFAs in seafood are important to neural development. However, fatty fish also contain other nutrients such as iodine and vitamin D (9). The current evidence for associations of these nutrients with IQ is conflicting for vitamin D (10, 11) but much stronger for iodine (12, 13). These results have increased the likelihood that any effect of seafood on IQ may not be totally because of HUFAs.

Other evidence has come from studies of fish-oil supplementation. The results tended to split into 2 groups. At younger ages, perhaps ≤4 y old, there may be a positive benefit to cognitive performance (14, 15). In contrast, by 7 y of age, any previous benefit had disappeared (16, 17). However, some of these interventions were not specific and contained other nutritional constituents such as vitamin D (9).

The use of maternal FAs, rather than inferring changes to these FAs via seafood consumption or supplementation, has the advantage of evaluating contributions from a broad range of FAs. To our knowledge, 3 studies have shown associations of DHA with specific subtests of cognitive performance rather than measures of global IQ (16–18).

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[4] Abbreviations used: AA, arachidonic acid (20:4n−6); ALSPAC, Avon Longitudinal Study of Parents and Children; FA, fatty acid; HUFA, highly unsaturated fatty acid; IQ, intelligence quotient; LC-PUFA; long-chain PUFA; SNP, single-nucleotide polymorphism.

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Although these studies have typically focused on AA and DHA, it is clear from postmortem data that cerebral tissues have different FA profiles than those of other human tissues (*see* Supplementary Table S1 under "Supplemental data" in the online issue). Therefore, in addition to the recognized higher level of DHA (22:6n−3) in the brain, neural phospholipids showed some of the highest levels of *cis* vaccenic (18:1n−7), nervonic (24:1n−9), adrenic (22:4n−6), osbond (22:5n−6), and oleic (18:1n−9) acids, with the latter being attributed to myelin in white matter (19). In contrast, very low levels of linoleic acid (18:2n−6) are observed in the brain. Although the precise functional role of the abundance and scarcity of each group of FAs might not always be known, such observations can provide a priori evidence to investigate these FAs in relation to neural developmental outcomes.

In addition, analyses of variants in *FADS* genes may provide additional insights into the role of FAs with IQ because of their associations with HUFAs (20) and their resilience to confounding and reverse causation (21). *FADS* genes encode enzymes involved in the desaturation of LC-PUFAs at positions Δ-5 and Δ-6 in the carbon chain. Together with the elongation of FAs, these genes are involved in the endogenous synthesis of HUFAs such as DHA and AA.

In this study, we investigated the role of maternal prenatal FA levels on child IQ by using the Avon Longitudinal Study of Parents and Children (ALSPAC).

SUBJECTS AND METHODS

Study population

The ALSPAC was established to explore the environmental, social, psychological and genetic factors associated with child health and development. The study recruited 14,541 pregnant women in the Bristol area who had an expected delivery date between April 1991 and December 1992. The study area comprises a mixture of rural areas, inner-city deprivation, suburbs, and moderate-sized towns as well as the city of Bristol. The cohort is broadly similar to the United Kingdom in terms of a range of demographic variables. A total of 13,988 children from the study were alive at age 1 y (22). Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the local research ethics committees.

Outcomes

Child IQ was assessed at ~8 y of age by using a short form of the Wechsler Intelligence Scale for Children (23). This measure involves 10 subtests, 5 subsets of which are related to verbal tasks, and 5subsets of which are related to performance tasks. The coding subtest, which has no stopping rules, was administered in its full form. The other 9 subtests were administered by using alternate items. Raw scores on these subtests were transformed so that the range of scores was identical to the full form. In all, 1% of children had their summed scaled scores for verbal subtests prorated because of missing one subtest, whereas the equivalent figure was 8% for performance scores.

Maternal FAs

At least one blood sample was taken for FA analysis from 5222 mothers during pregnancy with ≤6 samples being available for each mother. In this study, only one sample was used that reflected the latest gestation. Total lipids were extracted from red blood cells with the phospholipid fraction being separated by using thin-layer chromatography. FA methyl esters were extracted from phospholipids by using a hexane solution. Relative amounts of 40 FAs were estimated by using gas liquid chromatography on the basis of authenticated standards as previously described (20). Analyses were performed in 1996. The reporting of FAs in this article was restricted to the 23 most common FAs and presented as a percentage of all 40 measured FAs. Associations were expected for the following 8 candidate FAs: vaccenic acid (18:1n–7), oleic acid (18:1n−9), nervonic acid (24:1n−9), linoleic acid (18:2n−6), AA, adrenic acid (22:4n−6), osbond acid (22:5n−6), and DHA.

FADS genotypes

DNA was extracted from the white blood cells of maternal blood samples taken during pregnancy (24). Seventeen genetic variants from the *FADS* gene cluster that representing a 92-kb region on chromosome 11 were genotyped by using iPLEX chemistry (Sequenom) according to the manufacturer's protocol and a matrix-assisted laser desorption/ionization time-of-flight–based allele-detection method. Of these, variants, 11 variants were selected to provide good coverage of the *FADS* cluster, whereas 6 variants were chosen because of their inclusion in other studies of FAs (20). *See* Supplementary Table S2 under "Supplemental data" in the online issue for a description of details of the 17 variants. Genotyping failure rates ranged from 1.1% to 4.1%. Error rates on the basis of 790 duplicate samples ranged from 0% to 0.64%. There was evidence of disequilibrium for rs174570 ($P = 0.001$) and rs174579 ($P < 0.001$).

Statistical analysis

Linear regression was used to analyze full-scale, performance, and verbal IQs. To assess whether associations were resilient to confounding, adjustment was made for maternal age, education, ethnicity, alcohol consumption and smoking; partner status, housing tenure, crowding index, parity, preterm gestation (<37 wk), low birth weight (<2500 g), multiple births, sex, breastfeeding, and measures of adversity (in pregnancy and during the first 2 y after birth) and child stimulation (both from the home environment and maternal interaction with the child). Maternal education was used in preference to maternal IQ because the latter was only available for a subset of mothers. Both of these measures were correlated with child full-scale IQ ($r = 0.325$ and 0.332, respectively; $n = 2675$). Maternal FAs were dichotomized to reflect either the lowest or highest quartile compared with the other 3 quartiles combined assessing deficiency or excess, respectively. Not all associations were expected to be observed. Our hypotheses were that FAs with high levels in normal brain tissue might be expected to adversely influence IQ when blood levels are low. Conversely, FAs with low neural levels might be expected to adversely influence IQ when blood levels are high, although the observed effect might depend more on which FAs they replace rather than high levels of these FAs per se.

Genetic variants of the *FADS* gene cluster were also considered as a proxy for FA levels by using an additive model (linear effect for the number of copies of the minor allele). These

associations were explored in univariable and multivariable analyses by using backward stepwise procedures. Because of negative associations of the minor alleles with HUFAs (20), we expected that these variants would be negatively associated with IQ [rs498793 was in negative linkage disequilibrium with other single-nucleotide polymorphisms (SNPs) and consequently would tend to show opposite associations with IQ compared with other SNPs].

The effect of multiple comparisons was evaluated by using Bonferroni correction, a comparison of the distribution of effects in candidate FAs and other FAs (where no associations were expected), and permutation tests for the 8 candidate FAs. Permutation tests derived an empirical distribution of results under the null hypothesis by randomizing the observations for FA data. This technique preserved the correlation between confounders and outcomes, and because the same randomization was used for all FAs within one set of 48 analyses, the correlation between FAs. In all, 10,000 permutations were generated to derive the null distribution. Analyses were performed with Stata software (version 12.1; StataCorp LP).

RESULTS

Characteristics of the sample

Data on IQ were available for 7408 children of whom 2839 children (38%) had maternal FA data, 4572 children (57%) had maternal genotype data, and 6249 children (84%) had complete data on confounders. Characteristics of the latter sample are shown in **Table 1**. All confounders except sex were univariably associated with IQ (although this result concealed opposite associations with performance and verbal IQs). Strongest independent associations were for maternal education, breastfeeding, maternal age, parity, family adversity (at 2 time points), and low birth weight that contributed an additional 4.28%, 0.89%, 0.79%, 0.66%, 0.41%, and 0.25%, respectively, to the explanation (R^2) of IQ. The total explanation for all 18 confounders was 16.82%. A comparison of children with and without maternal FA data (*see* Supplementary Table S3 under "Supplemental data" in the online issue) suggested some minor differences between these 2 groups (eg, ethnic minorities were lower in the sample used in this study at 0.9% compared with 1.9% in the sample without FA data; $P = 0.007$), although none of these differences passed correction for multiple comparisons (Bonferroni critical $P = 0.003$).

Associations with maternal FAs

See Supplementary Table S4 under "Supplemental data" in the online issue for basic statistics on the 23 FAs explored in this study. Approximately 31% of the unadjusted analyses between IQ measures and the 23 FAs showed nominally significant associations ($P < 0.05$, $r^2 > 0.14\%$; *see* Supplementary Tables S5 and S6 under "Supplemental data" in the online issue). Of these, 21 associations (49%) were related to the 8 FAs expected to be associated with IQ. Most of these associations attenuated after adjustment with an average reduction in effect size of 65% including 8 associations for which the estimated effect changed sign (*see* Supplementary Tables S7 and S8 under "Supplemental data" in the online issue for comparison). As a consequence, most associations were not resilient to adjustment, but 13 associations persisted (**Table 2**). All of these associations were related to the 8 candidate FAs. Five of these associations were resilient to correction for multiple comparisons.

For the lowest quartile, the strongest association was for osbond acid (22:5n−6) with an increase in R^2 of ∼0.29%, whereby the effect was a reduction in both performance and full-scale IQs of ∼2 points. Other associations were observed for AA (20:4n−6) and DHA (22:6n−3) with reductions of ∼1.5–2 points and increases in R^2 of 0.27% (performance IQ) and ∼0.15% (both verbal and full scale IQs), respectively, although DHA failed correction for multiple comparisons.

In contrast for the highest quartile of FAs, although associations also persisted, they generally were not in the expected direction. Hence, adrenic (22:4n−6), vaccenic (18:1n–7), and osbond acids had negative effects of ∼1.5 points with increases in R^2 of 0.19% (verbal IQ), 0.19% (performance IQ), and 0.20% (verbal IQ), respectively (Table 2). Similarly, high levels of linoleic acid (18:2n−6) were positively, not negatively, associated with verbal IQ. However, note that vaccenic and linoleic acids failed correction for multiple comparisons. Oleic acid tended to have similar absolute effect sizes for performance and full-scale IQ, but the evidence that supported these results was weaker ($P < 0.081$).

Although linear effects can provide more-powerful results, in these data, there were no associations with IQ if the FAs were modeled as a linear covariate. This result suggested that neural developmental effects occurred mainly at one extreme or the other. When effects occurred at both extremes as in the case of osbond and vaccenic acids, polynomial contrasts of the quartiles suggested that these effects were more consistent with a quadratic (an inverted-U shape) relation rather than linear (*see* Supplementary Table S9 under "Supplemental data" in the online issue). AA also showed similar relations. An exploration of associations at suboptimal levels of IQ via logistic regression by using the worst quartile of IQ did not contribute to the overall findings. Similar effects were observed but generally with a lower statistical power.

Conventional multiple-comparison adjustment (Bonferroni critical $P = 0.001$) suggested that all of these results were null. However, it is important to note that this adjustment assumed all 48 comparisons were independent. For these data, the average correlation was 0.74 between IQ outcomes and 0.26 between FAs. Other evidence from the clustering of the 13 significant adjusted results ($P < 0.05$) in the 8 candidate FAs, whereas no significant results were observed in the other 15 FAs and from permutation tests (*see* Supplementary Table S10 under "Supplemental data" in the online issue; overall test for the observed set of 48 results, $P = 0.015$), which suggested that these effects were not solely results of chance. Taken together, these results were consistent with weak systematic effects in candidate FAs.

Associations with maternal genotypes

Only rs968567 had a univariable association with IQ (*see* Supplementary Table S11 under "Supplemental data" in the online issue), but its association suggested the minor allele had a beneficial effect contrary to our expectation because this allele was associated with a reduced level of highly unsaturated omega-3 and omega-6 FAs (20). Only 27% of the 51 associations

TABLE 1
Characteristics of the sample used in this study (n = 6249)[1]

Characteristic	Value	Change in IQ	Association with IQ (P)	
			Univariable	Multivariable
Maternal education [n (%)]				
Low	1227 (19.6)	0 (Ref)	5.9×10^{-166}	1.8×10^{-68}
Medium	2231 (35.7)	6.08		
High	2791 (44.7)	12.95		
Maternal ethnicity [n (%)]				
White	6175 (98.8)	0 (Ref)	3.5×10^{-4}	0.0013
Black	39 (0.6)	−8.67		
Asian	35 (0.6)	3.85		
Maternal alcohol consumption in pregnancy [n (%)]				
Never	346 (5.5)	0 (Ref)	6.9×10^{-11}	0.024
Stopped	2412 (38.6)	5.46		
Still drinking	3491 (55.9)	5.61		
Maternal smoking in pregnancy [n (%)]				
Never	3507 (56.1)	0 (Ref)	1.9×10^{-12}	0.732
Stopped	1199 (19.2)	−0.17		
Still smoking	1543 (24.7)	−3.20		
Parity [n (%)]				
0	2908 (46.5)	0 (Ref)	2.3×10^{-18}	2.1×10^{-11}
1	2258 (36.1)	−1.39		
≥2	1083 (17.3)	−4.70		
Current partner [n (%)]				
No	75 (1.2)	0 (Ref)	1.6×10^{-4}	0.406
Yes	6174 (98.8)	6.45		
Housing tenure [n (%)]				
Mortgaged	5171 (82.7)	0 (Ref)	4.1×10^{-48}	0.032
Council	447 (7.2)	−10.40		
Other	631 (10.1)	−2.67		
Crowding index (persons/room) [n (%)]				
<1	5445 (87.1)	0 (Ref)	1.4×10^{-28}	0.219
1+	804 (12.9)	−6.13		
Preterm birth (<37 wk) [n (%)]				
No	5933 (94.9)	0 (Ref)	0.0020	0.656
Yes	316 (5.1)	−2.62		
Low birth weight (<2500 g) [n (%)]				
No	5986 (95.8)	0 (Ref)	2.4×10^{-9}	1.6×10^{-5}
Yes	263 (4.2)	−5.52		
Multiple births [n (%)]				
No	6103 (97.7)	0 (Ref)	9.4×10^{-5}	0.058
Yes	146 (2.3)	−4.81		
Sex [n (%)]				
M	3116 (49.9)	0 (Ref)	0.781	0.575
F	3133 (50.1)	−0.10		
Breastfeeding [n (%)]				
Never	1137 (18.2)	0 (Ref)	8.7×10^{-76}	3.4×10^{-15}
<3 mo	1670 (26.7)	4.03		
≥3 mo	3442 (55.1)	8.72		
Maternal age at birth of child (y)	29.33 ± 4.48[2]	0.58	5.5×10^{-45}	2.0×10^{-14}
Family adversity				
In pregnancy	1.01 ± 1.37	−1.89	4.9×10^{-45}	0.0029
0–1 y of age	1.42 ± 1.71	−1.40	4.7×10^{-38}	0.045
Home score at age 6 mo	8.19 ± 2.19	0.70	1.1×10^{-16}	0.054
Parenting at age 6 mo	10.52 ± 1.48	1.03	3.5×10^{-16}	0.308
Verbal IQ, child at age 8 y	107.57 ± 16.77	—	—	—
Performance IQ, child at age 8 y	100.08 ± 17.06	—	—	—
Full scale IQ, child at age 8 y	104.66 ± 16.47	—	—	—

[1] Change in IQ reflects results from univariable analyses either for each category compared with the reference category or for a one-unit increase of continuous variables. P values were based on linear regression analyses of an average of verbal and performance IQ. IQ, intelligence quotient; Ref, reference category (effect size constrained to zero).

[2] Mean ± SD (all such values).

TABLE 2
Linear regression analyses of child IQ on maternal FAs dichotomized as lowest or highest quartile adjusted for 18 confounders (n = 2514–2523)[1]

	Verbal IQ		Performance IQ		Full scale IQ	
Quartile and FA	*B* (95% CI)	*P*	*B* (95% CI)	*P*	*B* (95% CI)	*P*
Lowest						
18:1n−7	−1.40 (−2.78, −0.01)	0.048	0.18 (−1.28, 1.64)	0.810	−0.70 (−2.05, 0.66)	0.316
18:1n−9	−0.52 (−1.88, 0.85)	0.460	−0.79 (−2.24, 0.65)	0.281	−0.65 (−2.00, 0.69)	0.339
24:1n−9	0.53 (−0.84, 1.90)	0.451	0.04 (−1.41, 1.49)	0.955	0.32 (−1.03, 1.66)	0.642
18:2n−6	−0.30 (−1.72, 1.12)	0.675	−0.81 (−2.31, 0.68)	0.286	−0.61 (−2.00, 0.78)	0.389
20:4n−6	−0.83 (−2.23, 0.56)	0.240	−2.03 (−3.50, −0.57)	0.007	−1.54 (−2.91, −0.18)	0.026
22:4n−6	−0.37 (−1.76, 1.02)	0.600	−0.95 (−2.42, 0.52)	0.205	−0.69 (−2.06, 0.67)	0.320
22:5n−6	−1.43 (−2.81, −0.06)	0.041	−2.11 (−3.56, −0.66)	0.004	−1.95 (−3.30, −0.61)	0.004
22:6n−3	−1.48 (−2.89, −0.07)	0.040	−1.25 (−2.74, 0.24)	0.099	−1.52 (−2.91, −0.14)	0.031
Highest						
18:1n−7	−0.57 (−1.98, 0.84)	0.427	−1.74 (−3.22, −0.26)	0.021	−1.25 (−2.63, 0.13)	0.076
18:1n−9	−0.72 (−2.20, 0.75)	0.337	−1.50 (−3.05, 0.06)	0.059	−1.29 (−2.73, 0.16)	0.081
24:1n−9	−0.32 (−1.71, 1.07)	0.650	−0.03 (−1.49, 1.43)	0.969	−0.21 (−1.57, 1.15)	0.762
18:2n−6	1.39 (0.02, 2.76)	0.047	0.77 (−0.68, 2.22)	0.296	1.24 (−0.10, 2.58)	0.071
20:4n−6	−1.05 (−2.43, 0.34)	0.138	−1.26 (−2.71, 0.20)	0.092	−1.18 (−2.53, 0.18)	0.089
22:4n−6	−1.69 (−3.07, −0.31)	0.016	−0.69 (−2.15, 0.77)	0.354	−1.37 (−2.72, −0.01)	0.048
22:5n−6	−1.75 (−3.16, −0.35)	0.014	0.26 (−1.23, 1.74)	0.734	−0.93 (−2.31, 0.45)	0.185
22:6n−3	−0.32 (−1.70, 1.06)	0.650	−0.05 (−1.50, 1.41)	0.951	−0.18 (−1.53, 1.17)	0.797

[1] High levels of linoleic acid (18:2n−6) and low levels of all other FAs were expected to have negative associations with IQ. Effect sizes are IQ points for the difference between the lowest or highest quartile for each FA compared with the other 3 quartiles combined. $P < 0.02$ was significant after adjustment for multiple comparisons (*see* Table S10 under "Supplemental data" in the online issue for permutation tests). FA, fatty acid; IQ, intelligence quotient.

occurred in the expected direction. In multivariable analyses, a number of variants were associated with IQ but most notably rs3834458, which was associated with all IQ measures (**Table 3**). This SNP was also associated with IQ, which suggested a beneficial association for the minor allele. Other SNPs were generally associated with the expected negative effect for the minor allele, although rs968567 was an exception to this general pattern. This SNP is proximal to rs3834458 with both SNPs located in the intergenic/promoter region on chromosome 11. Some care should be taken when interpreting effect sizes because of the high linkage disequilibrium between variants with greater emphasis placed on the direction rather than magnitude of effects.

Missing data

Missing data consisted of 2 main types. The first type consisted of 4569 children with IQ data but no FA data and, conversely, 2192 children with FA data but no IQ data. It was considered that the imputation for these children, for whom data were completely missing for either outcomes or main predictors, would be unlikely to provide reliable estimates. The second type consisted of partially missing data on confounders for the 2839 children with both IQ and FA data. For 2530 children (89%), there were complete data, and for the other children, missing data represented 16% of data points for the 18 confounders. The analysis of 30 imputed data sets produced very similar results to those shown in Table 2, and the biggest change was for DHA (*see* Supplementary Table S12 under "Supplemental data" in the online issue).

DISCUSSION

To our knowledge, this study is the largest and most comprehensive study to investigate the role of maternal FAs with child IQ by using both blood levels and genetic variants to infer associations. This study showed associations with global measures of IQ; the lowest quartile of DHA was associated with a decrease of ∼1.5 IQ points compared with that for other quartiles. This result was in contrast to that shown in previous studies with sample sizes of ∼150 (16–18), which only showed

TABLE 3
Multivariable genetic associations of 17 maternal *FADS* genetic variants with child IQ[1]

IQ and variant	*n*	*B* (95% CI)	*P*
Verbal			
rs3834458[2]	4388	3.91 (0.89, 6.93)	0.011
rs174578[2]	4388	−3.29 (−6.28, −0.30)	0.031
rs498793[3]	4388	0.81 (0.07, 1.55)	0.032
Performance			
rs174548[4]	4382	−2.03 (−3.93, −0.14)	0.035
rs3834458[4]	4382	2.62 (0.80, 4.43)	0.005
rs968567	4382	1.57 (0.25, 2.89)	0.020
rs174455	4382	−1.49 (−2.45, −0.53)	0.002
Full scale			
rs3834458[5]	4403	4.24 (1.50, 6.98)	0.002
rs174574[5]	4403	−3.67 (−6.37, −0.97)	0.008

[1] Effect sizes are IQ points per copy of the minor allele on the basis of linear regression analyses of IQ. The R^2 for models were 0.25% (verbal), 0.38% (performance), and 0.23% (full scale). IQ, intelligence quotient.

[2] Variants in strong linkage disequilibrium (r^2 = 0.940); 2.7% of the sample showed discordant genotypes.

[3] Single-nucleotide polymorphism in negative linkage disequilibrium with other variants; a positive effect size was expected

[4] Variants in strong linkage disequilibrium (r^2 = 0.812); 8.3% of the sample showed discordant genotypes.

[5] Variants in strong linkage disequilibrium (r^2 = 0.936); 2.7% of the sample showed discordant genotypes.

DHA (22:6n−3) to be associated with particular subtests. Our result for DHA was supported by the negative association for high levels of osbond (22:5n−6) and adrenic (22:4n−6) acids with IQ. Evidence from postmortem data suggested these FAs are substituted for DHA during DHA deficiency (25–27), but this substitution may inhibit secondary neurite growth and synaptogenesis (28, 29).

Low levels of AA (20:4n−6) were also associated with reduced performance IQ, which perhaps reflected changes in eicosanoid production (1) leading to abnormal electrical brain activity and inflammatory responses (30, 31). To our knowledge, this association has not been reported in previous studies of maternal FAs (16, 17). In studies that investigated cord and childhood FAs, results have been conflicting with one study that reported a positive relation (32), one study that reported a negative association (14), and a number of other studies that showed no statistical association (16, 17, 33).

The adverse results for low levels of osbond (22:5n−6) and vaccenic (18:1n–7) acids were, to our knowledge, novel but were predicted by considering the FA profile of neural tissue. Although levels of osbond acid are low in all cells compared with other FAs, they may be ≥50% higher in neural and photosensitive retinal tissue (*see* Supplementary Table S1 under "Supplemental data" in the online issue). Low levels of this FA have been associated with neurologic disorders such as schizophrenia (34) and may suggest a role in the cognitive dysfunction often associated with this disorder (35). Because its effect size in this study was larger than for any other FA, it was unlikely to be a chance event. Results for vaccenic acid, albeit nonsignificant after correction for multiple comparisons, supported the results from other studies that MUFAs promote glucose metabolism and cortical activity (36).

The prediction that low levels of oleic acid (18:1n−9) might have deleterious effects was not supported by these data. Although oleic acid appears to be important for normal brain development (37), deficiency may have been unlikely in this sample of mothers because oleic acid is abundant in most diets (38) and can be easily synthesized from stearic acid (18:0).

Similarly, high levels of linoleic acid (18:2n−6) were hypothesized to have deleterious effects, whereas some evidence of positive effects were observed for verbal IQ. One possible explanation is that the primary function of linoleic acid is not for incorporation into neural phospholipids (39) but, rather, for other biological processes for which high levels are beneficial, such as energy consumption. Pregnancy is associated with high energy demands with the developing fetal brain requiring a disproportionate amount of total energy (40). It seems plausible that the extra demand is met by the oxidation of linoleic and other 18-carbon FAs (41, 42) producing ketones, which are an alternative to glucose as an energy source for the brain (3). This study showed some evidence that the associations of 18-carbon FAs were stronger in twins for whom higher energy demands would be expected than for singletons. Our result is in contrast to a small study of 20 infants in whom child linoleic acid was negatively associated with a developmental quotient (32).

Two studies have shown positive effects of cord or child levels of EPA (20:5n−3) on specific subtests of cognitive function (14, 16), although other studies have shown no association (17, 32, 33). In this study, we showed no association of maternal EPA with composite measures of IQ.

We also examined associations of 17 SNPs within the *FADS* gene cluster. We showed no strong evidence that individual markers were associated with IQ. But in multivariable analyses, more persistent associations were detected. We observed that SNPs in the *FADS2* promoter regions rs3834458 and rs968567 had positive associations for minor alleles with IQ. Other evidence for their positive role comes from their positive effects on transcription levels (43, 44) and from haplotype analyses. Two haplotypes, which differed only by the alleles of rs968567, both showed negative associations with HUFAs, but the genetic effect was attenuated with the haplotype that contained the minor allele, which suggested a positive effect for this allele (45). These results suggested that multivariable models are potentially separating the opposite effects of promoter and intron SNPs of the *FADS2* gene. The models differed qualitatively by the IQ measure with verbal and full scale IQs being associated only with *FADS2* SNPs, whereas performance IQ was associated with all 3 genes. Although interesting and plausible, because of the high correlation between some predictors and the stepwise procedures used, we caution against overinterpretation of these novel results, which require replication by other studies.

Additional evidence of the role of HUFAs on fetal neurodevelopment has come from studies on motor development at 3 mo of age and behavior at 10 y of age (46, 47). Both of these studies reported that lower levels of AA (20:4n−6) in cord blood were associated with adverse outcomes. In addition, DHA (22:6n−3) was negatively associated with total behavioral difficulties and hyperactivity (47). These results were consistent with those in the current study and suggested, perhaps as might be expected, that HUFAs play a wider role in neural development than just cognition.

The major potential limitation of this study was the selective dropout of ∼47% of the cohort by 8 y of age. As is common in all longitudinal studies, the more socially and economically disadvantaged have higher rates of dropout than the more advantaged leading to the reduced sample being unrepresentative of the full cohort and the target population. Although it is clear that this biased downward prevalence estimates of such indicators as low IQ, it has been shown that epidemiologic associations are more robust (48). Another potential limitation was the use of blood FAs to infer changes in the fetal brain. Some studies have shown that not all FA changes in normal compared with impaired tissue are reflected in blood lipids (49), whereas other studies have shown strong tissue and blood correlations (50). In addition, the placental transfer may further distort any associations, although strong correlations between the maternal and fetal circulations have been reported for all PUFAs considered in this study (51). Third, this study was hampered by the small effect sizes and raised the possibility that some of the discussed results might have been a result of chance.

In conclusion, these results indicate that maternal HUFAs during pregnancy are associated with child IQ at 8 y of age although effect sizes were small (∼0.1 SD). Genetic associations supported this conclusion. Most notable was the association that low levels of DHA in the maternal circulation had negative effects on child IQ ∼8 y later. From one point of view, these associations make a small contribution to the total (≤0.29%), with a more substantial proportion (∼17%) being explained by the confounders used in this study that represented other environmental and genetic factors. However, in the context that these

results reflect independent associations, the contribution of FAs and genetic variants to the explanation of IQ is similar to that of important confounders such as low birth weight. This study also showed some evidence of negative effects for osbond (22:5n−6) and vaccenic (18:1n–7) acids at both extremes and positive effects for high levels of linoleic acid (18:2n−6), which require additional investigation.

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