Clinical Endocrinology (2005) **62**, 728–737 doi: 10.1111/j.1365-2265.2005.02287.x

ORIGINAL ARTICLE

Could associations between breastfeeding and insulin-like growth factors underlie associations of breastfeeding with adult chronic disease? The Avon Longitudinal Study of Parents and Children

Richard M. Martin*, Jeff M. P. Holly†, George Davey Smith*, Andrew R. Ness‡, Pauline Emmett‡, Imogen Rogers‡ and David Gunnell* for the ALSPAC Study Team‡

**Department of Social Medicine, University of Bristol, †Department of Surgery, University of Bristol, Bristol Royal Infirmary, and ‡Unit of Paediatric and Perinatal Epidemiology, Department of Community-based Medicine, University of Bristol, Bristol, UK*

Summary

Objective The influence of infant feeding method (breast/formula) on growth factor levels could underlie associations of breastfeeding with childhood growth and risk factors for cardiovascular disease. We investigated associations of having been breastfed with serum IGF-I and IGFBP-3 in childhood.

Methods Prospective birth cohort study (subsample of the Avon Longitudinal Study of Parents and Children, UK) based on 871 children born in 1991/1992 who underwent clinical follow-up and blood tests at age 7–8 years. A total of 488 (56%) children had complete data.

Results In children with complete data, the age- and sex-standardized IGF-I levels of those who were partially or exclusively breastfed were 6·1 and 13·8 ng/ml higher, respectively, than those who were never breastfed (increase in IGF-I levels per category of breastfeeding exclusivity: 7·1 ng/ml; 95% CI: 0·3–13·9; $P = 0·04$). In models also controlling for birthweight, gestational age, mother's age, and socioeconomic and dietary factors, the breastfeeding–IGF-I association was attenuated (regression coefficient: 3·3 ng/ml; −4·2–10·7; $P = 0·4$); further adjustment for IGFBP-3 made little difference (regression coefficient: 4·1 ng/ml; −2·8–10·9; $P = 0·2$). There was little evidence for an association between breastfeeding and IGFBP-3 or the molar ratio IGF-I/IGFBP-3.

Conclusions The positive association between breastfeeding and IGF-I could be due to residual confounding or to chance. Nevertheless, the magnitude of the fully adjusted effect estimate and the novelty of the association suggest that larger studies should now be conducted to confirm or refute the hypothesis that variations in IGF-I by infant feeding mode explain associations of breastfeeding with health in later life.

Correspondence: Richard M. Martin, Department of Social Medicine, University of Bristol, Canynge Hall, Whiteladies Road, Bristol BS8 2PR, UK. Tel.: 0117 9287321; Fax: 0117 9287236; E-mail: richard.martin@bristol.ac.uk

(Received 21 January 2005; returned for revision 17 February 2005; finally revised 14 March 2005; accepted 24 March 2005)

Epidemiological studies suggest that people who were breastfed are taller in childhood[1] and adulthood[1–3] and have reduced risk of type 2 diabetes,[4,5] cardiovascular disease[6] and cardiovascular disease risk factors[7–12] in later life. The mechanisms underlying these associations are unclear. Nutrition plays an important role in regulating IGF-I in infancy.[13] In preterm infants, IGF-I levels are positively associated with neonatal protein ($r = 0·40$; $P < 0·1$) and energy intakes ($r = 0·45$; $P < 0·001$),[14] suggesting that very early nutritional intake influences IGF-I levels. In childhood, the GH–IGF-I axis plays a key role in the regulation of growth, and levels of IGF-I are associated with childhood height.[15–17] Greater prepubertal growth is associated with more favourable cardiovascular disease risk.[18–20] High IGF-I levels are also inversely associated with insulin resistance,[21–26] atherosclerosis[22,24,27–29] and coronary heart disease mortality.[30]

The observations that breastfeeding is positively associated with linear growth[1–3] and that IGF-I is an important determinant of linear growth[15,16] suggest the possibility that an influence of infant feeding mode on the IGF-I axis during or beyond infancy could explain breastfeeding–cardiovascular disease associations in adulthood. In adults, higher milk intakes are associated with higher levels of IGF-I.[31,32] We are unaware of any published studies investigating associations of having been breastfed with the growth factor axis beyond infancy. IGFBP-3 is the main carrier protein for IGF-I and its level is a key determinant of IGF-I availability and action. We have investigated associations of breastfeeding with IGF-I and IGFBP-3 in children aged 7–8 years to determine whether variations in these factors by infant feeding mode could underlie associations of breastfeeding with cardiovascular disease risk.

Methods

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective cohort study of around 14 000 children recruited from all births in the former county of Avon, UK between April 1991 and December 1992.[33,34] The children forming the basis of this study were part of a randomly selected subcohort of ALSPAC known as 'Children in Focus' (CIF), a 10% sample drawn from the final 6 months of recruitment (i.e. births between June and December 1992) by choosing a standard number of children per week at random from the list of births within the study.[35] These children have been more intensively followed up than the other study members, including attendance at a research clinic at 7–8 years of age for a detailed assessment that incorporated blood sampling. Compared to the entire ALSPAC cohort, the CIF group had a shortfall of mothers in the lowest educational group (14% in CIF *vs.* 22% in ALSPAC), a shortfall of mothers aged under 25 years (13% *vs.* 21%), and an excess of mothers in owner-occupied accommodation. Of 1215 white, term (gestation 37–42 weeks) singletons in this subcohort, 871 attended the clinic, 693 (80%) had measures of breastfeeding and had blood taken for IGF levels, and there were complete data on all variables included in the analyses for 488 (56%) children (267 boys). Ethical approval was granted by the ALSPAC Law and Ethics Committee and local research ethics committees.

Infant feeding

Infant feeding mode was obtained from questionnaires sent to mothers when the child was 6 and 15 months of age. As in previous analyses,[36] breastfeeding was coded as 'exclusive breastfeeding beyond 2 months of age'; 'partial breastfeeding' (i.e. breastfeeding had been stopped or was nonexclusive by 2 months); and 'never breastfed'. 'Ever breastfed' denoted any breastfeeding (exclusive or partial). The duration of any breastfeeding was coded as 'never', '< 3 months', '3–6 months' and '> 6 months'. Exclusive breastfeeding was defined as no solids, milk formulae or other drinks (except vitamins, minerals, medicines and/or water).

Confounding factors

The following socioeconomic, birth and anthropometric factors have previously been shown to be associated with breastfeeding in the ALSPAC cohort and were considered potential confounding factors: maternal factors (occupational social class, educational achievement, smoking during pregnancy, prepregnancy body mass index, age at the birth of the child, height), and the age that solids were started in infancy[36] (see Table 1). These data were collected by means of postal questionnaires, self-completed by the mother antenatally or when the child was an infant. We also considered current age, sex and birthweight (adjusted for gestational age) as potential confounding factors because of their reported association with IGF levels in childhood.[15,16] Birthweight was extracted from routine hospital birth records; gestational age was estimated based on the date of the last menstrual period and findings from the routine (< 20 weeks) antenatal ultrasound scan. Breastfeeding is associated with childhood diet.[37] Based on previous analyses exploring a large number of cross-sectional associations of diet measured at age 7–8 with growth factors in the ALSPAC cohort,[17] several nutrients and food groups were considered potential confounding factors (see Table 2). Diet was assessed using a 3-day unweighed dietary record sent to the child's caregiver with an instruction leaflet 1 week before the clinic. At the clinic the carer was interviewed by a trained assistant to check for completeness and clarify any uncertainties in the diaries. The dietary records were coded using the computer program DIDO (Diet In, Data Out)[38] in conjunction with a database consisting of the 5th edition of *McCance and Widdowson's The Composition of Foods*[39] and its supplements to generate mean daily nutrient intakes and food group intakes for each child.

Although questionnaire data were not validated against gold standards, related variables were strongly correlated in expected directions ($P < 0{\cdot}0001$), data were shown to be highly reliable on repeated questioning, and established associations were replicated [e.g. maternal smoking was strongly socially patterned ($P < 0{\cdot}0001$) and was strongly associated with low birthweight ($P < 0{\cdot}0001$)]. Diet diaries are often used to validate/calibrate other diet instruments.[40]

Measurements and assays

Height (Leicester height measure, Child Growth Foundation) and weight (Seca 724 or 835 scales, Seca Ltd, Birmingham, UK) were measured at research clinics when the children were 4 and 8 months. At the 7–8-year follow-up, height was measured to the nearest 0·1 cm with a Harpenden stadiometer and weight in underwear was measured to the nearest 50 g using a Tanita weighing scale (Tanita UK Limited, Uxbridge, UK). We computed body mass index (BMI, kg/m^2), a measure of weight adjusted for height and a crude marker of adiposity.

At the research clinic a nonfasting blood sample was taken. Some children also attended a second clinic around 6 months later, where a fasting blood sample was taken. Fasting samples were used in preference, but where these were not available nonfasting samples were used as overnight fasting does not influence IGF levels. The fasting blood samples were taken between 1998 and 2000, and serum aliquoted and immediately stored at –80 °C for 1–3 years before analysis. All analyses were undertaken on stored aliquots that had been thawed for the first time. Serum levels of IGF-I were determined in 2001 by radioimmunoassay using a monoclonal antibody (Blood Products, Elstree, Hertfordshire, UK) and recombinant peptide (Pharmacia, Stockholm, Sweden) for standard and tracer, following iodination using the chloramines-T method. Samples were analysed following acid–acetone extraction to remove the IGF-binding proteins with an excess of IGF-II added to the extract to saturate any residual binding proteins.[41] Serum levels of IGFBP-3 were determined by radioimmunoassay using an in-house polyclonal antibody raised against recombinant nonglycosylated IGFBP-3. The assay was calibrated against recombinant glycosylated IGFBP-3 (Dr Maack, Celitrix, Santa Clara, CA, USA). Samples were analysed following a 1 : 100 dilution with antibody used at a final dilution of 1 : 20 000.[41] The molar ratio of IGF-I to IGFBP-3 was calculated by multiplying the concentration ratio by 5·33 [based on the molecular weights of IGF-I (7500) and IGFBP-3 (40 000)]. This provides a crude measure of biologically available IGF-I.[17,32] The average intra-assay coefficients

Table 1. Association of socioeconomic, birth and anthropometric factors with IGF levels

Characteristic	*N**	Regression coefficients for association of socioeconomic, birth and anthropometric factors with IGF levels†‡	
		IGF-I	IGFBP3
Categorical variables			
Sex			
Male	402	Reference	Reference
Female	339	12·8 (5·4; 20·2)§	386·6 (165·6; 607·6)
Timing of introduction of solid foods to infant			
< 3 months	105	Reference	Reference
3 months	417	5·1 (−5·8; 16·1)	−26·9 (−356·4; 302·7)
≥ 4 months	194	5·9 (−6·3; 18·1)	−19·7 (−386·7; 347·2)
Maternal social class			
Non-manual	532	Reference	Reference
Manual	89	−9·8 (−21·5; 1·9)	−80·4 (−427·2; 266·4)
Maternal educational achievement			
No 'A' levels**	400	Reference	Reference
One or more 'A' levels	322	7·0 (−0·6; 14·5)	43·1 (−182·6; 268·9)
Smoking during pregnancy			
Yes	122	Reference	Reference
No	608	−2·8 (−12·8; 7·2)	−117·4 (−416·4; 181·5)
Continuous variables			
Birthweight (kg)	738	−9·6 (−16·8; −2·4)¶	62·6 (−152·2; 277·3)
Gestational age (weeks)	741	−1·0 (−3·3; 1·4)	−6·8 (−75·6; 62·0)
Age of mother at birth of infant (years)	741	0·7 (−0·1; 1·5)	25·4 (0·9; 49·9)
Maternal height (cm)	704	5·5 (−53·4; 64·4)	−407·6 (−2177·8; 1362·6)
Maternal prepregnancy body mass index (kg/m^2)	672	−0·5 (−1·5; 0·4)	6·2 (−22·3; 34·8)
Child's height (cm)	719	2·9 (2·2; 3·6)¶	64·5 (43·7; 85·3)¶
Child's current body mass index (kg/m^2)	717	4·8 (3·0; 6·5)¶	145·6 (92·4; 198·9)¶
Child's current age (years)	741	43·1 (32·7; 53·6)¶	2501·0 (2189·7; 2812·2)¶
HbA1c (%)	392	−0·3 (−17·1; 16·4)	−446·6 (−939·5; 46·3)

*Analyses based on all subjects with IGF data. †Coefficients are difference in mean IGF-I or IGFBP-3 compared with reference group for binary categorical variables or change in IGF per unit increase in continuous variable. ‡Coefficients are adjusted for current age and sex. §$P < 0{\cdot}01$; ¶$P < 0{\cdot}001$. **'A' levels are taken in the UK by those remaining in education beyond the minimum school leaving age.

of variation (CVs) for IGF-I and IGFBP-3 were 6·7% and 3·6%, respectively, and for interassay variation, 12% and 14%, respectively. Glycosylated haemoglobin (HbA1c), measured when the children were aged 9 years, was analysed by HPLC assay on whole blood stored in haemolysant at −70 °C for up to 1 year. This storage method has been validated for HbA1c.[42]

Statistical analysis

All analyses were based on white, singleton, term (gestation 37–42 weeks) children. IGF levels of children from nonwhite ethnic groups differed from those of white children, and there were too few children from nonwhite ethnic groups with breastfeeding and IGF data ($n = 21$) to allow separate analyses.

Pearson's correlation coefficients were computed to assess relationships between IGF-I, IGFBP-3 and the molar ratio IGF-I/IGFBP-3. The molar ratio IGF-I/IGFBP-3 was log transformed to reduce positive skewness in its distribution; for this outcome we therefore report geometric means and associations with breastfeeding based on the log-transformed values. Associations of potential socioeconomic, pregnancy, anthropometric and dietary confounding factors with IGF values were examined using linear regression. Intakes of nutrients and food groups (except energy) were adjusted for energy intake using the residuals method (adjustment for overall energy intake means that dietary measures relate to nutrient composition rather than to absolute intake).[43] Comparisons of baseline data by whether ever *vs.* never breastfed were made using *t*-tests or the Wilcoxon ranksum test as appropriate.

Relationships between breastfeeding and IGF-I, IGFBP-3 and the molar ratio IGF-I/IGFBP-3 were examined using linear regression. Preliminary age- and sex-adjusted analyses (age-adjusted analyses in sex-specific models) were performed on all children with data on both breastfeeding and growth factors ($n = 693$; 378 boys). Multivariable linear regression models were then built that controlled in turn for social factors, maternal factors, several childhood dietary factors and maternal, infant and childhood anthropometry. In extra

Table 2. Association of dietary variables with breastfeeding, IGF-I and IGFBP-3

	Breastfeeding status*		Regression of IGF on dietary factors†	
Daily nutrient and food group intake	Ever	Never	IGF-I	IGFBP-3
Energy (kcal)	1715·1 (309·5)	1635·5 (316·6)‡	0·01 (−0·01; 0·02)	0·55 (0·14; 0·96)§
Protein (g)	55·6 (12·4)	55·1 (13·9)	0·8 (0·4; 1·3)¶	6·1 (−7·8; 20·1)
Animal protein (g)	32·9 (11·3)	34·2 (13·4)	0·6 (0·2; 1·0)§	2·3 (−9·9; 14·6)
Vegetable protein (g)	22·7 (6·0)	20·8 (4·8)§	0·1 (−0·8; 1·0)	11·7 (−15·5; 38·9)
Carbohydrate (g)	231·6 (47·0)	217·3 (46·3)§	0·1 (−0·1; 0·3)	4·6 (−1·0; 10·2)
Fat (g)	69·5 (15·9)	66·9 (16·2)	−0·6 (−1·1; −0·2)‡	−16·4 (−31·3; −1·5)‡
Saturated fat (g)	28·5 (8·2)	26·8 (7·7)	−0·5 (−1·3; 0·2)	−17·9 (−1·2; 5·4)
Monounsaturated fat (g)	23·5 (5·7)	22·9 (5·8)	−1·5 (−2·7; −0·3)‡	−51·4 (−87·5; −15·3)§
Polyunsaturated fat (g)	10·7 (3·6)	10·7 (3·8)	−1·3 (−2·6; 0·1)	−13·9 (−53·8; 26·1)
Magnesium (mg)	204·1 (51·8)	189·7 (47·4)‡	0·2 (0·1; 0·3)§	4·2 (0·9; 7·5)‡
Potassium (mg)	2255·0 (558·9)	2207·0 (634·1)	0·014 (0·004; 0·024)§	0·389 (0·098; 0·681)§
Zinc (mg)	6·1 (1·7)	6·0 (1·6)	3·6 (0·6; 6·7)‡	35·0 (−56·9; 127·0)
Cows milk (g)**	233 (110–369)	240 (111·5–339·5)	2·1 (−1·9; 6·1)	119·5 (−0·4; 239·5)‡
Dairy products (g)**	300 (186–461)	291·5 (184–445)	3·1 (−1·0; 7·2)	139·6 (17·9; 261·2)‡
Fruit (g)**	67 (17–123)	50·5 (0–95)‡	2·6 (−1·2; 6·5)	27·6 (−87·7; 143·0)

Analysis based on children with data on breastfeeding, IGF measures, energy intake and the nutrient analysed ($n = 571$). *Values are means (SD) or median (interquartile range). †Values are change in IGF per unit increase in continuous variable. Regression coefficients are adjusted for current age and sex; intakes of nutrients and food groups (except energy) were adjusted for energy intake using the residuals method (adjustment for overall energy intake means that dietary measures relate to nutrient composition rather than absolute intake).[43] ** Regression coefficients are change in IGF per quartile of intake. ‡$P = 0{\cdot}05$; §$P < 0{\cdot}01$; ¶$P < 0{\cdot}001$ based on t-test comparing ever *vs.* never breastfed for normally distributed continuous variables; Wilcoxon ranksum test comparing ever *vs.* never breastfed for skewed continuous variables; Wald test for regression analysis of IGF on dietary factors.

models, IGF-I associations were, in addition to the factors listed previously, adjusted for IGFBP-3 (and vice versa). To assess confounding, age-adjusted or age- and sex-adjusted and fully adjusted models were based on 488 children (267 boys) with complete data for all confounders.

Tests for trend in these models were based on fitting values for infant feeding method (exclusive, partial or never breastfed) as a linear term to the model. The validity of the assumption of a linear relationship between breastfeeding exclusivity and IGF levels was tested using the likelihood ratio test. In view of reports of sex differences in associations between breastfeeding and later health outcomes, a sex-specific analysis was specified a priori. Likelihood ratio tests were used to test for interaction by sex.

Rapid early growth is associated with levels of IGF in childhood.[16] Slower postnatal growth patterns are associated with breastfeeding,[44] lower IGF-I levels in childhood[16] and lower levels of cardiovascular disease risk factors.[11,45,46] Among those children with available data, we investigated the influence of growth in early and mid-infancy on breastfeeding–IGF associations by including: (i) terms for birthweight and weight at 4 months in fully adjusted models ($n = 350$); and (in a separate model) (ii) terms for weight at 4 months and weight at 8 months ($n = 334$).[47] Inverse associations of breastfeeding with type 2 diabetes[4,48] and insulin resistance[10,11] and a positive association between IGF-I and insulin secretion[49] suggest that an alternative pathway linking breastfeeding with IGF levels is through an effect of breastfeeding on glucose metabolism. We also investigated the possible confounding influence of average glycaemia (indexed here by HbA1c) on breastfeeding–growth factor associations among 377 children with available data. Analyses were performed using Stata.[50]

Results

The mean age of the 693 children included in the analysis was 7·9 years (range 6·9–8·5 years) and did not differ between boys and girls ($P = 0{\cdot}3$). Overall, 235 (34%) were exclusively breastfed, 352 (51%) partially breastfed and 106 (15%) never breastfed. There was some evidence that girls (37%) were more likely to be exclusively breastfed than boys (31%) ($P = 0{\cdot}07$). Mean (SD) levels of IGF-I, IGFBP-3 and the molar ratio IGF-I/IGFBP-3 (geometric mean; 25th, 75th centile) were 148·1 ng/ml (53·7), 4871·9 ng/ml (1762·1) and 0·162 (0·127, 0·202), respectively. There was some evidence that boys had lower levels than girls of IGF-I (142·8 ng/ml *vs.* 154·4 ng/ml; $P = 0{\cdot}005$) and IGFBP-3 (4734·5 ng/ml *vs.* 5036·9 ng/ml; $P = 0{\cdot}02$), but there was no evidence of differences in the molar ratio IGF-I/IGFBP-3 (0·160 *vs.* 0·164; $P = 0{\cdot}5$). The correlations between IGF-I and IGFBP-3, IGF-I and the molar ratio IGF-I/IGFBP-3, and IGFBP-3 and the molar ratio IGF-I/IGFBP-3 were 0·51, 0·45 and −0·43, respectively.

The child's birthweight, height and BMI (factors previously associated with breastfeeding), as well as sex and current age, were associated with IGF levels (Table 1).[16] Having been breastfed was associated with later childhood intakes of energy, vegetable protein, carbohydrates, magnesium and fruit (Table 2). Childhood energy, animal protein, fat, monounsaturated fat, magnesium, potassium, zinc and cow's milk and dairy intake were associated with levels of IGF-I or IGFBP-3, or both (Table 2).[17]

Table 3. Mean (standard error) levels of IGF-I, IGFBP-3 and IGF-I/IGFBP-3 molar ratio in relation to breastfeeding

		IGF-I (ng/ml)		IGFBP-3 (ng/ml)		IGF-I/IGFBP-3 molar ratio*	
	N	Mean (SE)	*P*-value†	Mean (SE)	*P*-value†	Mean (SE)	*P*-value†
Never breastfed	106	142·3 (5·0)	–	4704·4 (147·4)	–	0·160 (0·037)	–
Ever breastfed	587	149·1 (2·1)	0·2	4902·2 (62·5)	0·2	0·162 (0·016)	0·7
Partially breastfed	352	146·9 (2·7)	0·4	4895·9 (80·9)	0·3	0·161 (0·020)	0·8
Exclusively breastfed	235	152·4 (3·3)	0·09	4911·5 (99·0)	0·2	0·164 (0·025)	0·5
Trend‡	693	5·2 (−0·4; 10·8)	0·07	85·4 (−81·9; 252·8)	0·3	0·015 (−0·027; 0·057)	0·5
Duration of breastfeeding§							
< 3 months	161	149·7 (4·0)	0·3	4957·2 (120·9)	0·2	0·160 (0·030)	0·9
3–5 months	123	143·7 (4·6)	0·8	4874·7 (138·2)	0·4	0·157 (0·035)	0·9
6 months or more	259	152·0 (3·2)	0·10	4906·8 (95·2)	0·3	0·167 (0·024)	0·3
Trend¶	649	2·1 (−1·1; 5·9)	0·17	35·7 (−69·2; 140·6)	0·6	0·016 (−0·010; 0·042)	0·2

All values are standardized for age and sex. *Geometric means (logged standard errors) are shown. †*P* for difference between the category of breastfeeding indicated in the row header and never breastfed, adjusted for age and sex. ‡Change in IGF levels per category of breastfeeding exclusivity, with never breastfed as the reference group, adjusted for age and sex. §Fewer people had information on duration of breastfeeding. ¶Change in IGF levels per category of breastfeeding duration, with never breastfed as the reference group, adjusted for age and sex.

Among the 693 subjects with measures of breastfeeding and IGF, there was some evidence of a relationship between increasing exclusivity of breastfeeding and levels of IGF-I at age 7–8 (Table 3). Exclusively breastfed children had higher mean (SE) IGF-I levels [152·4 ng/ml (3·3)] compared with never breastfed children [142·3 ng/ml (5·0)] ($P = 0·09$). In age- and sex-adjusted analyses, for each increase in category of breastfeeding exclusivity (never, partial and exclusive) there was on average a 5·2 ng/ml increase (95% CI: −0·4–10·8; $P = 0·07$) in levels of IGF-I. There was little statistical evidence that the association of breastfeeding exclusivity and IGF-I differed between boys (coefficient: 8·8 ng/ml; 95% CI: 0·8–16·7; $P = 0·03$) and girls (coefficient: 1·5 ng/ml; 95% CI: −6·4–9·5; $P = 0·7$) (*P* for interaction: 0·2). There was little evidence for an association between duration of any breastfeeding and levels of growth factors (*P* for trend: 0·17). There were no associations of breastfeeding with the molar ratio IGF-I/IGFBP-3 or with IGFBP-3.

Table 4 gives the regression coefficients from models based on the subset of 488 children with complete data to assess possible confounding by birthweight, gestational age, mother's age, mother's educational attainment, age at introduction of solids, and dietary variables in childhood (intake of energy, vegetable protein, animal protein, carbohydrate, fat, magnesium, potassium, zinc, cow's milk, dairy products and fruit). In this subset of the cohort, exclusively breastfed children had higher mean IGF-I levels than never breastfed children (mean difference: 13·8; 95% CI: –0·6–28·1). In age- and sex-adjusted models in this subset, for each increase in category of breastfeeding exclusivity (never, partial and exclusive) there was on average a 7·1 ng/ml increase (95% CI: 0·3–13·9; $P = 0·04$) in levels of IGF-I.

In fully adjusted models, the point estimate remained positive but the association between breastfeeding exclusivity and IGF-I was somewhat attenuated and the adjusted association could have arisen by chance (coefficient per increase in category of breastfeeding exclusivity: 3·3 ng/ml; 95% CI: −4·2–10·7; $P = 0·4$). The association was slightly stronger when additionally controlled for IGFBP-3 (coefficient: 4·1 ng/ml; 95% CI: –2·8–10·9; $P = 0·2$). The most important confounding factor was mother's educational attainment, which was positively associated with offspring IGF-I levels and which reduced the association between breastfeeding exclusivity and IGF-I by 27%, from 7·1 ng/ml in the age- and sex-adjusted model to 5·2 ng/ml. Controlling in addition for maternal social class, smoking in pregnancy, maternal height and prepregnancy BMI made little difference to effect estimates in the subset of children with these data ($n = 406$). There was no association between duration of any breastfeeding and levels of growth factors in fully adjusted models.

There was little evidence that glycaemia measured by HbA1c was a confounder of associations between breastfeeding and IGF-I. The age- and sex-adjusted regression coefficient in 377 children with data on HbA1c was 1·3 ng/ml per category of breastfeeding exclusivity. After including HbA1c in the model the coefficient was 1·2 ng/ml. In fully adjusted models excluding birthweight and weight at 4 months on 350 children with available data, the increase in IGF-I per category of breastfeeding exclusivity was 4·1 ng/ml (95% CI: −4·6–12·9; $P = 0·4$). After including birthweight and weight at 4 months in fully adjusted models, there was a 6·5 ng/ml (95% CI: –2·4–15·3; $P = 0·15$) increase in IGF-I per category of breastfeeding exclusivity. The inclusion of weight at 4 months and weight at 8 months in fully adjusted models made little difference to effect estimates. Controlling for the child's height and BMI, possible factors on the pathway linking breastfeeding with IGFs, made little difference to effect estimates relating breastfeeding exclusivity and IGF-I (coefficients = 3·1 and 3·6 per category of breastfeeding, respectively).

Discussion

In this population-based cohort study, the age- and sex-standardized IGF-I levels of 488 children with complete data who were partially or exclusively breastfed were 6·1 and 13·8 ng/ml higher, respectively, than those who were never breastfed (*P* for trend: 0·04). The effect

Table 4. Multivariable models showing mean differences (95% CIs) in levels of IGF-I, IGFBP-3 and IGF-I/IGFBP-3 molar ratio in relation to breastfeeding ($n = 488$)

	IGF-I (ng/ml)		IGFBP-3 (ng/ml)		IGF-I/IGFBP-3 molar ratio	
	Regression coefficient (95% CI)*	*P*-value†	Regression coefficient (95% CI)*	*P*-value†	Regression coefficient (95% CI)*	*P*-value†
Simple models (adjusted for age and sex)						
Never breastfed	0·0	–	0·0	–	0·0	–
Ever breastfed	9·2 (−3·9; 22·2)	0·17	167·5 (−225·7; 560·6)	0·4	0·032 (−0·058; 0·121)	0·5
Partially breastfed	6·1 (−7·6; 19·7)	0·4	175·6 (−236·4; 587·7)	0·4	0·012 (−0·081; 0·105)	0·8
Exclusively breastfed	13·8 (−0·6; 28·1)	0·06	155·5 (−276·5; 587·5)	0·5	0·060 (−0·038; 0·158)	0·2
Trend§	7·1 (0·3; 13·9)	0·04	54·4 (−150·2; 259·0)	0·6	0·034 (−0·012; 0·080)	0·15
Duration of breastfeeding ($n = 451$)¶						
< 3 months	12·1 (−3·3; 27·6)	0·12	356·2 (−109·3; 821·7)	0·13	0·015 (−0·09; 0·121)	0·8
3–5 months	4·7 (−11·9; 21·3)	0·6	27·0 (−472·8; 526·8)	0·9	0·029 (−0·084; 0·142)	0·6
6 months or more	11·8 (−2·5; 26·2)	0·11	120·2 (−311·8; 552·3)	0·6	0·056 (−0·042; 0·154)	0·3
Trend††	2·5 (−1·8; 6·7)	0·3	−15·8 (−144·6; 113·1)	0·8	0·019 (−0·010; 0·048)	0·2
*Fully adjusted models***						
Never breastfed	0·0	–	0·0	–	0·0	–
Ever breastfed	5·4 (−8·1; 18·8)	0·4	27·4 (−382·5; 437·3)	0·9	0·033 (−0·060; 0·126)	0·6
Partially breastfed	4·7 (−9·1; 18·5)	0·5	70·6 (−350·1; 491·3)	0·7	0·021 (−0·073; 0·117)	0·7
Exclusively breastfed	7·0 (−8·4; 22·5)	0·4	−78·3 (−549·0; 392·4)	0·7	0·060 (−0·046; 0·167)	0·3
Trend§	3·3 (−4·2; 10·7)	0·4	−61·7 (−289·1; 165·7)	0·6	0·032 (−0·020; 0·083)	0·2
Duration of breastfeeding ($n = 451$)¶						
< 3 months	12·4 (−3·0; 27·9)	0·12	258·7 (−210·7; 728·1)	0·3	0·038 (−0·069; 0·145)	0·5
3–5 months	0·2 (−16·8; 17·2)	0·9	−222·5 (−737·0; 292·1)	0·4	0·043 (−0·075; 0·160)	0·5
6 months or more	4·1 (−11·4; 19·7)	0·6	−182·6 (−653·4; 288·1)	0·4	0·060 (−0·047; 0·168)	0·3
Trend††	−0·5 (−5·3; 4·2)	0·8	−117·1 (−261·1; 26·9)	0·11	0·017 (−0·016; 0·050)	0·3

*Mean difference (95% CI) in IGF levels comparing breastfeeding category indicated in the row header minus never breastfed. †*P* for difference between the category of breastfeeding indicated in the row header and never breastfed. §Change in IGF levels per category of breastfeeding exclusivity, with never breastfed as the reference group. ¶Fewer people had information on duration of breastfeeding. **Controls for age, sex, birthweight, gestational age, mothers age, mother's educational attainment, age at introduction of solids, intake in childhood of energy, vegetable protein, animal protein, carbohydrate, fat, magnesium, potassium, zinc, cow's milk, dairy products and fruit. ††Change in IGF levels per category of breastfeeding duration, with never breastfed as the reference group.

estimates were somewhat attenuated, however, in fully adjusted models controlling for pregnancy, socioeconomic and dietary variables (differences: 4·7 ng/ml and 7·0 ng/ml for partially or exclusively breastfed infants, respectively, *vs.* those formula-fed) and the differences could have arisen by chance (*P* for trend: 0·4). Breastfeeding and IGF-I were associated with dietary and socioeconomic factors. It is also possible, therefore, that the observed positive association between breastfeeding and IGF-I reflects residual confounding and that breastfeeding is simply a marker for other dietary or behavioural influences on IGF-I.

The main strength of this study is that there was no reliance on recall of exclusivity or duration of breastfeeding and the detailed prospective data allowed the exclusion of solids, milk formulae and other drinks from the definition of breastfeeding exclusivity. Furthermore, we were able to examine and control for a large number of pregnancy, socioeconomic and dietary variables. Selection bias in the 488 children included in the final analysis seems unlikely as the age-adjusted results on this subsample with complete data were similar to age-adjusted results on all 693 subjects with breastfeeding and IGF data.

Possible biological relevance of findings

The sample size remaining in the fully adjusted analyses may have been too small to detect real and important differences between infant feeding groups. The size of the differences in IGF-I levels by infant feeding mode were similar to differences in mean IGF-I levels reported in one study between subjects with incident ischaemic heart disease (199 ng/ml) and controls without ischaemic heart disease (194 ng/ml)[30] even in the fully adjusted models. In that study there were strong associations of IGF-I with ischaemic heart disease.[30] The size of the age- and sex-adjusted difference in IGF-I levels between exclusively breastfed and bottle-fed children (10 ng/ml) was similar to differences in mean IGF-I levels between men with and without prostate cancer (11 ng/ml) in a Swedish case–control study reporting significant differences in IGF-I between those with and without prostate cancer ($P = 0·02$).[51] In terms of growth, a separate study in children aged 7 years found that a difference in IGF-I of 1·7 ng/ml was associated with a 1 cm greater height.[15] In the current study, a 1 cm greater height was associated with a 2·9 ng/ml (95% CI: 2·2; 3·6)

higher IGF-I level (Table 1). Although circulating concentrations of IGF-I are relatively very high (around 150 ng/ml), the vast majority of this IGF is in ternary complexes unavailable to the tissues; in the tissues, optimal activation of cell receptors occurs at 10–20 ng/ml.[52] If the IGF axis is the mechanism underpinning breastfeeding–height associations, the small difference in IGF-I associated with breastfeeding found in the current study is in line with our previous publication showing that breastfeeding is associated with an approximately 1–2 cm difference in height when compared with exclusive bottle feeding.[1]

IGF measurement issues

The coefficients of variation for the IGF assays suggest modest measurement error, which is most likely to have been nondifferential (equal in both groups being compared), as breastfeeding status was measured 7–8 years before the IGFs were measured and the researchers undertaking these assays did not know the breastfeeding status of the children. Such error would make differences between groups less marked than if IGFs were measured with greater precision, thus attenuating observed associations between infant feeding mode and IGF (rather than explaining them).[53] Samples were stored at −80 °C for 1–3 years before analysis, which could theoretically affect IGF concentrations, a possible source of measurement error. However, we recently found little evidence of degradation in samples stored frozen for up to 21 years[54,55] and others have shown strong associations of IGF-I with breast and prostate cancer after longer storage times.[56,57] Any potential measurement error due to sample degradation is most likely to be nondifferential (as breastfeeding status did not influence storage time), thus attenuating observed associations.[53]

In a subsample of the study participants, surveyed at a mean age of 8·3 years (around 4 months after the clinic), almost all the girls (95% Tanner stage 1 according to pubic hair) and boys were prepubertal. The very small proportion of girls who had entered puberty when the IGF levels were taken, and the fact that there was no evidence that effect estimates differed between boys (almost none of whom had entered puberty) and girls (P for interaction = 0·3), suggests that the results are not explained by alterations in IGF levels as a result of puberty.

Diet in infancy and childhood and subsequent IGF levels

There is some circumstantial evidence from previous research to suggest that further studies of the long-term impact of breastfeeding on the IGF axis are worth pursuing. Some authors postulate that raised protein concentrations in formula compared with breastmilk may stimulate higher IGF-I secretion in formula-fed babies[13,58] and explain the faster early growth of formula-fed infants shown in observational studies.[59,60] It is feasible that breastfeeding may be associated with both increased IGF-I levels in children at age 7–8 years (as we have observed) and with lower levels of IGF-I in these individuals at the time of breastfeeding during the first months of life. We have recently observed that IGF-I levels were lower at age 25 years in individuals who received milk-supplementation to their diets as children up to the age of 5 years.[61] Individuals who were exposed to famine during childhood were reported to have higher IGF-I levels some 50 years later in life.[62] In the 65 years follow-up of the Boyd–Orr cohort,[12] milk intake in childhood was inversely associated with IGF-I levels in old age (Martin, unpublished data). These studies would be compatible with increased nutritional intake in infancy, primarily protein intake, causing a direct increase in hepatic IGF-I production, which then feeds back to suppress pituitary GH output with a long-term resetting of the pituitary resulting in lower IGF-I levels if the nutritional intake reverts. A relatively low IGF-I level at the time of breastfeeding would be compatible with the slower infant growth rate at that time[44] and a consequent resetting up of the pituitary, due to less feedback, could then result in a relatively high IGF-I level subsequently at age 7–8 years.

It has been argued that breastfed babies are protected from early overnutrition and accelerated growth, and it is the slowing of very early growth that benefits the cardiovascular risk profile of breastfed babies.[63] Recent studies have also linked greater stature and raised levels of IGF-I with increased risk of haematological cancers and cancers of the breast, prostate, colo-rectum and lung.[64,65] There is little evidence, however, that breastfeeding is associated with increased risks of childhood haematological cancers[66–68] and cancers occurring in adulthood.[68–71] Given the lack of data relating breastfeeding with future levels of growth factors, and the complex regulation of the IGF axis, it may not be possible at this stage to formulate a unifying mechanism involving the IGF axis that explains both the inverse association of breastfeeding with cardiovascular disease and the absence of any strong association with cancer.

Although glycaemia (indexed by HbA1c) was not a confounder of the association between breastfeeding and IGF-I, insulin resistance remains as a possible explanation for this association, as insulin resistance is associated with breastfeeding[11] and IGF levels are regulated by insulin levels.[52] We could not explore this hypothesis further in the current dataset, and this explanation should be investigated in future studies.

Conclusions

We are unaware of any other published reports examining the long-term influence of breastfeeding on later IGF levels. The possibility that breastfed babies have clinically important higher levels of IGF-I at age 7–8 than formula-fed babies, and the increasing reports that IGF-I levels are inversely associated with cardiovascular disease risk,[21–25,27–30,72] suggest that larger prospective studies are required to confirm or refute the hypothesis that the IGF axis may be the biological mechanism linking breastfeeding with childhood growth and adult cardiovascular disease risk. The hypothesis that breastfeeding influences later IGF levels could ethically and feasibly be tested in large, randomized, controlled trials of successful breastfeeding promotion interventions with long-term follow-up.[73] Analysis of such a randomized trial on an intention-to-treat basis would provide a robust estimate of the effect of breastfeeding on IGF that should be free from the effects of confounding, which limit the interpretation of observational studies.

Contributions

R.M.M., D.G., G.D.S. and J.M.P.H. developed the hypothesis. R.M.M. undertook the initial analysis, wrote the first draft of the paper and

coordinated completion of the paper. J.M.P.H. completed the IGF assays in his laboratory. P.E. and I.R. coordinated the fieldwork and coding of the nutritional database. All authors contributed to the development of ideas, concepts, and the analytical strategy during the writing of the paper, and to the final version. R.M.M. and A.R.N. will act as guarantors.

Acknowledgements

We are very grateful to all the parents and children who took part in the study and to the midwives for their cooperation and help in recruitment. The whole ALSPAC study team comprises clinic staff, interviewers, computer technicians, laboratory technicians, clerical workers, research scientists, volunteers and managers who continue to make the study possible. R.M.M. was supported by a Wellcome Trust Research Training Fellowship in Clinical Epidemiology. This study could not have been undertaken without the financial support of the Medical Research Council, the Wellcome Trust, the European Commission, Quality of Life and Management of Living Resources Programme, contract number QLG1-CT-2000-01643, the Department of Health and the Department of the Environment. The ALSPAC study is part of the WHO-initiated European Longitudinal Study of Parents and Children. Colin Steer extracted data from the main database for this analysis. Professor Jean Golding commented on earlier drafts of the paper.

References

1 Martin, R.M., Davey Smith, G., Mangtani, P., Frankel, S. & Gunnell, D. (2002) Association between breast feeding and growth: the Boyd–Orr cohort study. *Archives of Disease in Childhood: Fetal and Neonatal Edition*, **87**, F193–F201.

2 Wadsworth, M.E., Hardy, R.J., Paul, A.A., Marshall, S.F. & Cole, T.J. (2002) Leg and trunk length at 43 years in relation to childhood health, diet and family circumstances; evidence from the 1946 national birth cohort. *International Journal of Epidemiology*, **31**, 383–390.

3 Victora, C.G., Barros, F., Lima, R.C., Horta, B.L. & Wells, J. (2003) Anthropometry and body composition of 18 year old men according to duration of breast feeding: birth cohort study from Brazil. *British Medical Journal*, **327**, 901–904.

4 Pettitt, D.J., Forman, M.R., Hanson, R.L., Knowler, W.C. & Bennett, P.H. (1997) Breastfeeding and incidence of non-insulin-dependent diabetes mellitus in Pima Indians. *Lancet*, **350**, 166–168.

5 Young, T.K., Martens, P.J., Taback, S.P., Sellers, E.A., Dean, H.J., Cheang, M. & Flett, B. (2002) Type 2 diabetes mellitus in children: prenatal and early infancy risk factors among native Canadians. *Archives of Pediatrics and Adolescent Medicine*, **156**, 651–655.

6 Fall, C.H.D., Barker, D.J.P., Osmond, C., Winter, P.D., Clark, P.M.S. & Hales, C.N. (1992) Relation of infant feeding to adult serum cholesterol concentration and death from ischaemic heart disease. *British Medical Journal*, **304**, 801–805.

7 Owen, C.G., Whincup, P.H., Gilg, J.A. & Cook, D.G. (2003) Effect of breast feeding in infancy on blood pressure in later life: systematic review and meta-analysis. *British Medical Journal*, **327**, 1189–1195.

8 Owen, C.G., Whincup, P.H., Odoki, K., Gilg, J.A. & Cook, D.G. (2002) Infant feeding and blood cholesterol. A study in adolescents and a systematic review. *Pediatrics*, **110**, 597–608.

9 Gillman, M.W. (2002) Breast-feeding and obesity. *Journal of Pediatrics*, **141**, 749–750.

10 Ravelli, A.C.J., van der Meulen, J.H., Osmond, C., Barker, D.J.P. & Bleker, O.P. (2000) Infant feeding and adult glucose tolerance, lipid profile, blood pressure, and obesity. *Archives of Disease in Childhood*, **82**, 248–252.

11 Singhal, A., Fewtrell, M., Cole, T.J. & Lucas, A. (2003) Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet*, **361**, 1089–1097.

12 Martin, R.M., Ebrahim, S., Griffin, M., Davey Smith, G., Nicolaides, A.N., Georgiou, N., Watson, S., Frankel, S., Holly, J.M.P. & Gunnell, D. (2004) Breastfeeding and coronary atherosclerosis. Intima–media thickness and plaques at 65 year follow up of the Boyd. *Journal of Epidemiology and Community Health*, **58** (Suppl. II), A22–A23.

13 Thissen, J.P., Ketelslegers, J.M. & Underwood, L.E. (1994) Nutritional regulation of the insulin-like growth factors. *Endocrine Reviews*, **15**, 80–101.

14 Diaz-Gomez, N.M., Domenech, E. & Barroso, F. (1997) Breast feeding and growth factors in preterm newborn infants. *Journal of Pediatrics, Gastroenterology and Nutrition*, **24**, 322–327.

15 Fall, C.H., Pandit, A.N., Law, C.M., Yajnik, C.S., Clark, P.M., Breier, B., Osmond, C., Shiell, A.W., Gluckman, P.D. & Barker, D.J. (1995) Size at birth and plasma insulin-like growth factor-1 concentrations. *Archives of Disease in Childhood*, **73**, 287–293.

16 Ong, K., Kratzsch, J., Kiess, W., Dunger, D. & the Alspac Study Team. (2002) Circulating IGF-I levels in childhood are related to both current body composition and early postnatal growth rate. *Journal of Clinical Endocrinology and Metabolism*, **87**, 1041–1044.

17 Rogers, I.S., Gunnell, D., Emmett, P.M., Glynn, L.R., Dunger, D.B., Holly, J.M. & the Alspac Study Team. (2005) Cross-sectional associations of diet and insulin-like growth factor levels in 7- to 8-year-old children. *Cancer Epidemiology, Biomarkers and Prevention*, **14**, 204–212.

18 Davey Smith, G., Greenwood, R., Gunnell, D., Sweetnam, P., Yarnell, J. & Elwood, P. (2001) Leg length, insulin resistance, and coronary heart disease risk. The Caerphilly Study. *Journal of Epidemiology and Community Health*, **55**, 867–872.

19 Gunnell, D., Whitley, E., Upton, M.N., McConnachie, A., Davey Smith, G. & Watt, G.C.M. (2003) Associations of height, leg length, and lung function with cardiovascular risk factors in the Midspan Family Study. *Journal of Epidemiology and Community Health*, **57**, 141–146.

20 Gunnell, D.J., Davey Smith, G., Frankel, S., Nanchahal, K., Braddon, F.E.M., Pemberton, J. & Peters, T.J. (1998) Childhood leg length and adult mortality. follow-up study of the Carnegie (Boyd Orr) survey of diet and health in pre-war Britain. *Journal of Epidemiology and Community Health*, **52**, 142–152.

21 Halleux, C.M., Takahashi, M., Delporte, M.L., Detry, R., Funahashi, T., Matsuzawa, Y. & Brichard, S.M. (2001) Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. *Biochemical and Biophysical Research Communications*, **288**, 1102–1107.

22 Janssen J.A.M.J.L, Stolk, R.P., Pols, H.A.P., Grobb, D.E. & Lamberts, S.W.J. (1998) Serum total IGF-I, free IGF-I and IGFBP levels in an elderly population. Relation to cardiovascular risk factors and disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **18**, 277–282.

23 Janssen J.A.M.J.L. & Lamberts, S.W.J. (2002) The role of IGF-1 in the development of cardiovascular disease in type 2 diabetes mellitus: is prevention possible? *European Journal of Endocrinology*, **146**, 467–477.

24 Conti, E., Andreotti, F., Sestito, A., Riccardi, P., Menini, E., Crea, F., Maeri, A. & Lanza, G.A. (2002) Reduced levels of insulin-like growth factor-1 in patients with angina pectoris, positive exercise stress test, and angiographically normal epicardial coronary arteries. *American Journal of Cardiology*, **89**, 973–975.

25 Holt, R.I., Simpson, H.L. & Sonksen, P.H. (2003) The role of the growth hormone-insulin-like growth factor axis in glucose homeostasis 30793. *Diabetic Medicine*, **20**, 3–15.
26 Sandhu, M.S., Heald, A.H., Gibson, J.M., Cruickshank, J.K., Dunger, D.B. & Wareham, N.J. (2002) Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. *Lancet*, **359**, 1740–1745.
27 Capaldo, B., Patti, L., Oliviero, U., Longobardi, S., Pardo, F., Vitale, F., Fazio, S., Di Rella, F., Biondi, B., Lombardi, G. & Sacca, L. (1997) Increased arterial intima–media thickness in childhood-onset growth hormone deficiency. *Journal of Clinical Endocrinology and Metabolism*, **82**, 1378–1381.
28 Spallarossa, P., Brunelli, C., Minuto, F., Caruso, D., Battistini, N., Caponnetto, S. & Cordera, R. (1996) Insulin-like growth factor-I and angiographically documented coronary artery disease. *American Journal of Cardiology*, **77**, 200–202.
29 Vaessen, N., Heutink, P., Janssen, J.A., Witteman, J.C., Testers, L., Hofman, A., Lamberts, S.W., Oostra, B.A., Pols, H.A. & van Duijn, C.M. (2001) A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. *Diabetes*, **50**, 637–642.
30 Juul, A., Scheike, T., Davidsen, M., Gyllenborg, J. & Jorgensen, T. (2002) Low serum insulin-like growth factor I is associated with increased risk of ischemic heart disease: a population-based case-control study. *Circulation*, **106**, 939–944.
31 Holmes, M.D., Pollak, M.N., Willett, W.C. & Hankinson, S.E. (2002) Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiology, Biomarkers and Prevention*, **11**, 852–861.
32 Gunnell, D., Oliver, S.E., Peters, T.J., Donovan, J.L., Persad, R., Maynard, M., Gillatt, D., Pearce, A., Hamdy, F.C., Neal, D.E. & Holly, J.M. (2003) Are diet–prostate cancer associations mediated by the IGF axis? A cross-sectional analysis of diet, IGF-I and IGFBP-3 in healthy middle-aged men. *British Journal of Cancer*, **88**, 1682–1686.
33 Golding, J., Pembrey, M., Jones, R. & Alspac Study Team. (2001) ALSPAC – the Avon Longitudinal Study of Parents and Children. I. Study methodology. *Paediatric and Perinatal Epidemiology*, **15**, 74–87.
34 ALSPAC website. (2004) Available at http://www.alspac.bris.ac.uk/.
35 North, K., Carmichael, A., Bishop, J., Sadler, S. & Golding, J. (2004) ALSPAC Children in Focus. Available at http://www.alspac.bris.ac.uk/protocol/Appendix7/default.ht.
36 Martin, R.M., Ness, A.R., Gunnell, D., Emmett, P.M. & Davey Smith, G. (2004) Does breastfeeding in infancy lower blood pressure in childhood? The Avon Longitudinal Study of Parents and Children. *Circulation*, **109**, 1259–1266.
37 Von Kries, R., Koletzko, B., Sauerwald, T., von Mutius, E., Barnert, D., Grunert, V. & von Voss, H. (1999) Breast feeding and obesity: cross sectional study. *British Medical Journal*, **319**, 147–150.
38 Price, G.M., Paul, A.A., Key, F.B., Harter, A.C., Cole, T.J., Day, K.C. & Wadsworth, M.E.J. (1995) Measurement of diet in a large national survey: comparison of computerized and manual coding of records in household measures. *Journal of Human Nutrition and Dietetics*, **8**, 417–428.
39 McCance, R.A. (1991) *McCance and Widdowson's the Composition of Foods*, 5th revised and extended edition. The Royal Society of Chemistry and the Ministry of Agriculture, Fisheries and Food, Cambridge.
40 Willett, W.C. (1998) Nutritional epidemiology. In: K.J. Rothman, S. Greenland eds. *Modern Epidemiology*, 2nd edn. Lippincott-Raven, Philadelphia, 623–642.
41 Lemmey, A., Maddison, P., Breslin, A., Cassar, P., Hasso, N., McCann, R., Whellams, E. & Holly, J. (2001) Association between insulin-like growth factor status and physical activity levels in rheumatoid arthritis. *Journal of Rheumatology*, **28**, 29–34.
42 Jones, W., Scott, J., Leary, S., Stratton, F., Smith, S., Jones, R., Day, A., Ness, A. & ALSPAC Study Team (2004) Stability of whole blood at –70 °C for measurement of hemoglobin A1c (HbA1c) in healthy individuals. *Clinical Chemistry*, **50**, 2460–2461.
43 Willett, W.C. (1998) *Nutritional Epidemiology*. Oxford University Press, New York.
44 Ong, K.K., Preece, M.A., Emmett, P., Ahmed, M.L., Dunger, D.B. & the Alspac Study Team. (2002) Size at birth and early childhood growth in relation to maternal smoking, parity and infant breast feeding: longitudinal birth cohort study and analysis. *Pediatrics Research*, **52**, 863–867.
45 Singhal, A., Cole, T.J., Fewtrell, M., Deanfield, J. & Lucas, A. (2004) Is slower early growth beneficial for long-term cardiovascular health? *Circulation*, **109**, 1108–1113.
46 Singhal, A., Cole, T.J., Fewtrell, M. & Lucas, A. (2004) Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. *Lancet*, **363**, 1571–1578.
47 Lucas, A., Fewtrell, M.S. & Cole, T.J. (1999) Fetal origins of adult disease – the hypothesis revisited. *British Medical Journal*, **319**, 245–249.
48 Young, T.K., Martens, P.J., Taback, S.P., Sellers, E.A., Dean, H.J., Cheang, M. & Flett, B. (2002) Type 2 diabetes mellitus in children: prenatal and early infancy risk factors among native Canadians. *Archives of Pediatrics and Adolescent Medicine*, **156**, 651–655.
49 Ong, K.K., Petry, C.J., Emmett, P.M., Sandhu, M.S., Kiess, W., Hales, C.N., Ness, A.R. & Dunger, D.B.; ALSPAC study team. (2004) Insulin sensitivity and secretion in normal children related to size at birth, postnatal growth, and plasma insulin-like growth factor-I levels. *Diabetologia*, **47**, 1064–1070.
50 Statacorp. (2003) *Stata Statistical Software: Release 8.0.* Stata Corporation, College Station, TX.
51 Wolk, A., Mantzoros, C.S., Andersson, S.O., Bergstrom, R., Signorello, L.B., Lagiou, P., Adami, H.O. & Trichopoulos, D. (1998) Insulin-like growth factor 1 and prostate cancer risk: a population-based, case–control study. *Journal of the National Cancer Institute*, **90**, 911–915.
52 Thissen, J.P., Ketelslegers, J.M. & Underwood, L.E. (1994) Nutritional regulation of the insulin-like growth factors. *Endocrine Reviews*, **15**, 80–101.
53 Armstrong, B.K., White, E. & Saracci, R. (1994) *Principles of Exposure Measurement in Epidemiology*. Oxford University Press, Oxford.
54 Allen, N.E., Roddam, A.W., Allen, D.S., Fentiman, I.S., dos Santos Silva, I., Peto, J., Holly, J.M.P. & Key, T.J. (2005) A prospective study of serum insulin-like growth factor-I (IGF-I), IGF-II, IGF-binding protein-3 and breast cancer risk. *British Journal of Cancer*, **92**, 1283–1287.
55 Schernhammer, E. Holly, J.M., Pollak, M.N. & Hankinson, S.E. (2005) Circulating levels of insulin-like growth factors, their binding proteins, and breast cancer risk. *Cancer Epidemiology, Biomarkers and Prevention*, **14**, 699–704.
56 Hankinson, S.E., Willett, W.C., Colditz, G.A., Hunter, D.J., Michaud, D.S., Deroo, B., Rosner, B., Speizer, F.E. & Pollak, M. (1998) Circulating concentrations of insulin-like growth factor I and risk of breast cancer. *Lancet*, **351**, 1393–1396.
57 Chan, J.M., Stampfer, M.J., Giovannucci, E., Gann, P.H., Ma, J., Wilkinson, P., Hennekens, C.H. & Pollak, M. (1998) Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science*, **279**, 563–566.
58 Ketelslegers, J.M., Maiter, D., Maes, M., Underwood, L.E. & Thissen,

J.P. (1995) Nutritional regulation of insulin-like growth factor-I. *Metabolism*, **44** (Suppl. 4), 50–57.

59 Atladottir, H. & Thorsdottir, I. (2000) Energy intake and growth of infants in Iceland – a population with high frequency of breast-feeding and high birth weight. *European Journal of Clinical Nutrition*, **54**, 695–701.

60 Michaelsen, K.F., Hoppe, C., Schack-Nielsen, L. & Molgaard, C. (2002) Does an excessive protein intake early in life cause health problems such as obesity later in life? In: R.E. Black, K.F. Michaelsen eds. *Public Health Issues in Infant and Child Nutrition.* Vevey/Lipincott Williams & Wilkins, Philadelphia, 279–293.

61 Ben-Shlomo, Y., Holly, J., McCarthy, A., Savage, P., Davies, D. & Davey Smith, G. (2005) Pre and post natal milk supplementation and adult insulin-like growth factor I: Long term follow-up of a randomised controlled trial. *Cancer Epidemiology, Biomarkers and Prevention.* In press.

62 Elias, S.G., Keinan-Boker, L., Peeters, P.H., Van Gils, C.H., Kaaks, R., Grobbee, D.E. & van Noord, P.A. (2004) Long term consequences of the 1944–1945 Dutch famine on the insulin-like growth factor axis. *International Journal of Cancer*, **108**, 628–630.

63 Singhal, A. & Lucas, A. (2004) Early origins of cardiovascular disease: is there a unifying hypothesis? *Lancet*, **363**, 1642–1645.

64 Holly, J.M.P., Gunnell, D.J. & Davey Smith, G. (1999) Growth hormone, IGF-I and cancer. Less intervention to avoid cancer? More intervention to prevent cancer? *Journal of Endocrinology*, **162**, 321–330.

65 Davey Smith, G., Gunnell, D. & Holly, J. (2000) Cancer and insulin-like growth factor-I. *British Medical Journal*, **321**, 847–848.

66 Hardell, L. & Dreifaldt, A.C. (2001) Breast-feeding duration and the risk of malignant diseases in childhood in Sweden. *European Journal of Clinical Nutrition*, **55**, 179–185.

67 UK Childhood Cancer Study Investigators. (2001) Breastfeeding and childhood cancer. *British Journal of Cancer*, **85**, 1685–1694.

68 Martin, R.M., Davey Smith, G. & Gunnell, D. (2005) Do breastfed infants have an altered risk of breast cancer? In: G. Goldberg, A. Prentice, S. Filteau, K. Simondon eds. *Breastfeeding: Early Influences on Later Health*. Springer, London, in press.

69 Michels, K.B., Trichopoulos, D., Rosner, B.A., Hunter, D.J., Colditz, G.A., Hankinson, S.E., Speizer, F.E. & Willett, W.C. (2001) Being breastfed in infancy and breast cancer incidence in adult life: results from the two nurses' health studies. *American Journal of Epidemiology*, **153**, 275–283.

70 Wingard, D.L., Criqui, M.H., Edelstein, S.L., Tucker, J., Tomlinson-Keasey, C., Schwartz, J.E. & Friedman, H.S. (1994) Is breastfeeding associated with adult longevity? *American Journal of Public Health*, **84**, 1458–1462.

71 Titus-Ernstoff, L., Egan, K.M., Newcomb, P.A., Baron, J.A., Stampfer, M., Greenberg, E.R., Cole, B.F., Ding, J., Willett, W. & Trichopoulos, D. (1998) Exposure to breast milk in infancy and adult breast cancer risk. *Journal of the National Cancer Institute*, **90**, 921–924.

72 Kajantie, E., Fall, C.H., Seppala, M., Koistinen, R., Dunkel, L., Yliharsila, H., Osmond, C., Andersson, S., Barker, D.J., Forsen, T., Holt, R.I., Phillips, D.I. & Eriksson, J. (2003) Serum insulin-like growth factor (IGF)-I and IGF-binding protein-1 in elderly people: relationships with cardiovascular risk factors, body composition, size at birth, and childhood growth. *Journal of Clinical Endocrinology and Metabolism*, **88**, 1059–1065.

73 Kramer, M.S., Chalmers, B., Hodnett, E.D., Sevkovskaya, Z., Dzikovich, I., Shapiro, S., Collet, J.P., Vanilovich, I., Mezen, I., Ducruet, T., Shishko, G., Zubovich, V., Mknuik, D., Gluchanina, E., Dombrovskiy, V., Ustinovitch, A., Kot, T., Bogdanovich, N., Ovchinikova, L., Helsing, E. & PROBIT Study Group (2001) Promotion of Breastfeeding Intervention Trial (PROBIT): a randomized trial in the Republic of Belarus. *Journal of the American Medical Association*, **285**, 413–420.