

Effects of Promoting Longer-term and Exclusive Breastfeeding on Adiposity and Insulin-like Growth Factor-I at Age 11.5 Years

A Randomized Trial

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OBSERVATIONAL STUDIES SUGGEST that greater duration and exclusivity of having been breastfed is inversely associated with adiposity^{1,2} but positively associated with stature^{3,4} and later-life serum insulin-like growth factor (IGF)-I,^{5,6} a regulator of childhood statural growth and body composition.^{7,8} However, breastfeeding and growth are socially patterned in many settings,⁹ and observed associations between these variables are at least partly explained by confounding structures.^{1,2,10,11} Also, the relationship between breastfeeding and growth is dynamic and bidirectional because infant behavior and weight gain influence maternal feeding decisions, making reverse causation an alternative explanation for observed associations of breastfeeding duration and exclusivity with growth.^{12,13}

To overcome limitations inherent in observational studies of breastfeeding's long-term effects, we designed a

Importance Evidence that longer-term and exclusive breastfeeding reduces child obesity risk is based on observational studies that are prone to confounding.

Objective To investigate effects of an intervention to promote increased duration and exclusivity of breastfeeding on child adiposity and circulating insulin-like growth factor (IGF)-I, which regulates growth.

Design, Setting, and Participants Cluster-randomized controlled trial in 31 Belarusian maternity hospitals and their affiliated clinics, randomized into 1 of 2 groups: breastfeeding promotion intervention (n=16) or usual practices (n=15). Participants were 17 046 breastfeeding mother-infant pairs enrolled in 1996 and 1997, of whom 13 879 (81.4%) were followed up between January 2008 and December 2010 at a median age of 11.5 years.

Intervention Breastfeeding promotion intervention modeled on the WHO/UNICEF Baby-Friendly Hospital Initiative (World Health Organization/United Nations Children's Fund).

Main Outcome Measures Body mass index (BMI), fat and fat-free mass indices (FMI and FFMI), percent body fat, waist circumference, triceps and subscapular skinfold thicknesses, overweight and obesity, and whole-blood IGF-I. Primary analysis was based on modified intention-to-treat (without imputation), accounting for clustering within hospitals and clinics.

Results The experimental intervention substantially increased breastfeeding duration and exclusivity when compared with the control (43% vs 6% exclusively breastfed at 3 months and 7.9% vs 0.6% at 6 months). Cluster-adjusted mean differences in outcomes at 11.5 years of age between experimental vs control groups were: 0.19 (95% CI, -0.09 to 0.46) for BMI; 0.12 (-0.03 to 0.28) for FMI; 0.04 (-0.11 to 0.18) for FFMI; 0.47% (-0.11% to 1.05%) for percent body fat; 0.30 cm (-1.41 to 2.01) for waist circumference; -0.07 mm (-1.71 to 1.57) for triceps and -0.02 mm (-0.79 to 0.75) for subscapular skinfold thicknesses; and -0.02 standard deviations (-0.12 to 0.08) for IGF-I. The cluster-adjusted odds ratio for overweight/obesity (BMI ≥85th vs <85th percentile) was 1.18 (95% CI, 1.01 to 1.39) and for obesity (BMI ≥95th vs <85th percentile) was 1.17 (95% CI, 0.97 to 1.41).

Conclusions and Relevance Among healthy term infants in Belarus, an intervention that succeeded in improving the duration and exclusivity of breastfeeding did not prevent overweight or obesity, nor did it affect IGF-I levels at age 11.5 years. Breastfeeding has many advantages but population strategies to increase the duration and exclusivity of breastfeeding are unlikely to curb the obesity epidemic.

Trial Registration isrctn.org: ISRCTN37687716; and clinicaltrials.gov: NCT01561612
JAMA. 2013;309(10):1005-1013

www.jama.com

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follow-up study of 17 046 children participating in PROBIT (Promotion of Breastfeeding Intervention Trial).¹⁴ The intervention resulted in 2 groups with substantially different durations and exclusivity of breastfeeding, providing a unique opportunity to test, in an intention-to-treat analysis, the extent to which breastfeeding causally influences growth and its regulation.

The breastfeeding promotion intervention had no measurable effect in children aged 6.5 years (PROBIT II) on stature or physical measures of adiposity,¹⁵ but adiposity was not directly measured and the findings could have been distorted by variations in timing of the adiposity rebound by duration of breastfeeding.¹⁶ The current study (PROBIT III) provides experimental evidence on whether beneficial effects of increased duration and exclusivity of breastfeeding on growth develop later in childhood, based on direct measurements by bioimpedance of body fat and lean mass and on circulating IGF-I.

METHODS

A detailed description of the cluster-based randomization, experimental intervention, and participant eligibility in PROBIT has been published.¹⁴ Briefly, the units of randomization (clusters) were maternity hospitals and their associated polyclinics (outpatient health clinics following up both well and ill children) (FIGURE). These units were randomized to a control intervention (continuation of the breastfeeding practices and policies in effect at the time of randomization) or an experimental intervention based on the Baby-Friendly Hospital Initiative developed by the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) to promote and support breastfeeding, particularly among mothers who have chosen to initiate breastfeeding.¹⁷ The trial results are based on 17 046 healthy breastfed infants from 31 maternity hospitals and polyclinics, born at term (≥ 37 weeks gestation) in 1996-1997 and enrolled during their postpartum stay. Trial inclusion criteria required the infants to be healthy, singleton, weighing at least 2500 g at

birth, born of mothers who initiated breastfeeding and who had no condition that would interfere with breastfeeding, and to have an Apgar score of at least 5 at 5 minutes after birth.¹⁴

Between January 2008 and December 2010, the child participants were followed up at dedicated research visits by 39 specially trained pediatricians, 1 in each of 23 polyclinics and 2 who shared visits at each of the remaining 8 high-volume clinics. Training and quality assurance procedures have been described in detail previously.¹⁸ Prior to the follow-up visit, the child participants were asked to fast for at least 8 hours. During the follow-up visit, duplicate measurements were taken of the following characteristics: standing and sitting height, measured with a wall-mounted stadiometer (Medtechnika); triceps and subscapular skinfold thicknesses, measured using Lange spring-loaded skinfold calipers (Beta Technology); head, mid-upper arm, waist and hip circumferences, and upper arm length measured using nonstretchable measuring tapes; weight, percent body fat, fat mass, and fat-free (lean) mass, measured by foot-to-foot bioelectrical impedance (Tanita TBF 300GS body fat analyzer).

Body mass, fat mass, and fat-free (lean) mass indices (BMI, FMI, and FFMI) were calculated as weight, fat mass, and lean mass in kilograms divided by height in meters squared, respectively. We defined overweight as being between the 85th to 94th percentiles and obesity as being in or above the 95th percentile of BMI, based on the Centers for Disease Control and Prevention (CDC) 2000 reference data.¹⁹ In our analysis, we compared being in or above the 95th percentile (obese) vs being lower than the 85th percentile and being in or above the 85th percentile (overweight/obese) vs being lower than the 85th percentile. We computed leg length as standing height minus trunk length (sitting height minus stool height).

At the visit, finger prick spot samples of whole blood were collected onto Whatman 903 filter paper cards²⁰ by the

39 pediatricians who had received special training²⁰⁻²² (described previously).²³ Once the samples were collected, the cards were dried and then stored in freezers at each of the 31 polyclinic sites at -20°C until transport to the laboratory at the National Mother and Child Centre in Minsk, where they were stored at -80°C . The samples for IGF-I were stored at -20°C for a median of 1.7 months (interquartile range [IQR], 1.0-5.1) and at -80°C for a median of 18.4 months (IQR, 13.3-21.6).

We quantified circulating IGF-I from a single 3-mm diameter disc ($\approx 3 \mu\text{L}$ of blood) per child, after a single thaw, using the validated method of Diamandi et al.²⁴ Mean intraassay coefficients of variation were 6%, 7%, and 9% for low, medium, and high IGF values, respectively; the respective inter-assay values were 8%, 12%, and 16%. The Spearman correlation coefficient between 50 paired whole-blood spot vs serum IGF-I samples, collected simultaneously, was 0.93 (95% CI, 0.87-0.96). Between 98% and 105% of known quantities (250, 300, 350, 450, and 550 ng/mL) of IGF-I prepared from recombinant human IGF-I was recovered from the dried blood spots. We also demonstrated that blood spot IGF-I was stable for at least 24 months at -80°C (data available on request). IGF-I was assayed from 2 lots of reagents between January 2010 and November 2011 and, as other authors have noted, assay kits of different lot numbers have been observed to cause some variation in measured IGF-I.²⁵ To remove the potential effect of between-lot or between-run variation, we standardized values of IGF within each assay run ($n=43$) by computing z scores ($[\text{IGF value} - \text{mean for each run}] / \text{SD of the mean}$).

Audit visits were conducted to assess interobserver reproducibility of the outcome data, an important step given that blinding of pediatricians to the experimental vs control randomized group assignment was not feasible. For each of the 39 pediatricians, 1 to 5 children were randomly selected to return for remeasurement of all variables for a total of 143 audited children (108 with

baseline and repeat IGF-I values). So that all children seen in follow-up were eligible for selection, the repeated measurements were carried out after completion of primary data collection, an average of 1.3 years (range, 0.2-2.4) after the initial clinic visit. The audit was carried out by 1 of 5 Minsk-based pediatricians not involved in primary data collection and blinded to the measures obtained at the initial visit but not to experimental or control status. Because of the time elapsed between the audit and initial visits, results were compared using Pearson correlation coefficients.

PROBIT III follow-up was approved by the Belarusian Ministry of Health and received ethical approval from the McGill University Health Centre Research Ethics Board, the Human Subjects Committee at Harvard Pilgrim Health Care, and the Avon Longitudinal Study of Parents and Children (ALSPAC) Law and Ethics Committee. A parent or legal guardian provided written informed consent in Russian at enrollment and at the follow-up visits, and all children provided written assent at the 11.5-year visit.

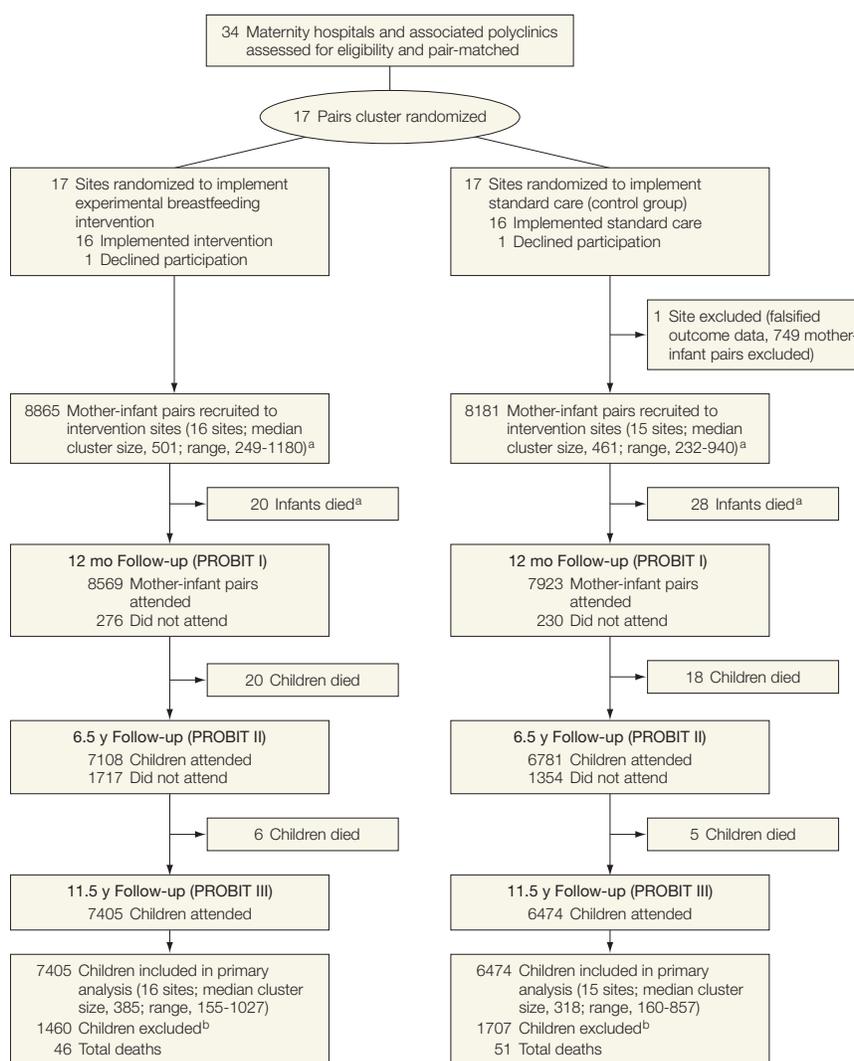
Statistical Analysis

Analyses were performed using SAS version 9.3 (SAS Institute) unless otherwise stated. Our main outcomes were measures of adiposity (BMI, FMI, FFMI, percent body fat, waist circumference, triceps and subscapular skinfold thicknesses, overweight and obesity as defined previously) and IGF-I. We also explored the effect of the intervention on other anthropometric measurements: standing height, leg length, hip circumference, waist-hip ratio, head circumference and mid-upper arm circumference. Comparisons between the experimental and control groups were based on a modified intention-to-treat analysis without imputation for missing outcome data (ie, based on the 13 879 children with observed outcomes).

We accounted for possible nonindependence of measurements within individual hospitals and their affiliated polyclinic sites (clustering) using random effects models, which permitted inference at the level of the individual child rather than at the level of the cluster (maternity hospital and polyclinic). The MIXED procedure was used

for continuous outcomes (to estimate mean differences and 95% CIs) and the GLIMMIX procedure for binary outcomes (to estimate odds ratios and 95% CIs) in SAS. The results are presented for the simple cluster-adjusted model, as well as after additional adjustment for stratum-level variables (urban vs rural and East vs West Belarus resi-

Figure. Flow Diagram of Progress of Clusters and Individuals Through PROBIT Recruitment and Follow-up Phases I, II and III



^aThe numbers of mother-infant pairs with 12 months completed follow-up and the number of infants who died before 12 months differ compared with those originally reported¹⁴ because of continued work on retrieving previously unreturned data forms from the polyclinics and continued data cleaning (intervention, 8569 pairs vs 8547 pairs originally reported; control, 7923 pairs vs 7895 pairs originally reported; 48 died vs 49 originally reported). ^bOf the 3167 not seen at PROBIT III, 913 were seen at both 12 months and PROBIT II, 483 were not seen at either 12 months or PROBIT II, 1768 were seen at 12 months but not seen at PROBIT II, and 3 were seen at PROBIT II but not seen at 12 months.

dence), and for child age at follow-up, sex, birth weight, and maternal and paternal education. Controlling for maternal and paternal height (for standing height, leg length, and IGF-I) and for measured maternal and mother-reported paternal BMI (for adiposity measures) made little material difference to the effect estimates and therefore are not presented in the main results table. The results for IGF-I were additionally controlled for the time between blood sampling to assay, as this has previously been reported to influence levels²⁵ (although we found that IGF-I from dried blood spots was stable to freezing). To determine whether results differed in boys vs girls, we also analyzed mixed models that included terms for the sex of each child and a multiplicative sex multiplied by trial arm interaction term.

In a sensitivity analysis, we investigated whether loss to follow-up influenced the results by undertaking multiple imputation to generate plausible values of missing 11.5-year outcomes and thereby including all 17 046 randomized participants in the intention-to-treat analysis.^{26,27} We used SAS imputations (Proc MI) to impute 5 values for each missing observation and combined multivariable modeling estimates using Proc MI ANALYZE in SAS.

The intention-to-treat analysis may underestimate the effect of the true exposure of interest (breastfeeding exclusivity and duration), owing to overlap in breastfeeding between the randomized groups (many intervention mothers did not exclusively breastfeed for 3 or 6 months, whereas some control mothers did). In a secondary analysis, we applied instrumental variable methods to estimate unbiased associations of the difference in breastfeeding exclusivity and duration achieved between the 2 randomized groups on our outcomes.²⁸ In this approach, we used randomization status as the instrument that is independent of any confounders of the exposure-outcome relationship, and is related to the outcomes only via the exposure (breastfeeding duration and exclusivity). We performed instrumental vari-

able estimation of continuous outcomes using the generalized 2-stage least-squares estimator implemented in the `xtivreg` command in Stata version 12.1 (Stata Corp), while accounting for clustering by study site. We performed instrumental variable estimation of binary outcomes using a random effects version of the ratio estimator of the causal odds ratio.²⁹

To assess whether we could reproduce the inverse associations of increased duration and exclusivity of breastfeeding reported in previous observational studies, we conducted observational analyses (ie, disregarding randomization status) in which we estimated the effects of the duration of any breastfeeding and of the duration of exclusive breastfeeding on the same outcomes, also accounting for clustering and the same baseline characteristics as in the expanded mixed models described previously, using multiple linear regression for continuous outcomes and multiple logistic regression for the binary outcomes. Duration of breastfeeding was classified as less than 3 months (reference standard), 3 months to less than 6 months, and 6 months or more.

Power Calculations

A priori we calculated detectable differences in outcomes based on following up 14 000 children in 31 clusters, an intention-to-treat analysis and the design effect based on a realistic value (0.01) of the intraclass correlation coefficient (ICC).³⁰ The mean detectable differences (5% significance, 80% power) in intention-to-treat analyses were 0.24 for BMI (assuming a plausible effect of longer vs shorter duration of exclusive breastfeeding of 0.6) and 4.25 ng/mL for IGF-I (assuming an effect of longer duration of exclusive breastfeeding of 10.63 ng/mL).

RESULTS

As previously reported,¹⁴ the randomization produced 2 groups with similar distributions of baseline sociodemographic and potential confounding factors. Participation in the intervention

group substantially increased breastfeeding duration and exclusivity (based on WHO definitions¹⁷) vs the control group: eg, at 3 months, infants in the intervention group were 7 times more likely to be exclusively (43.3% vs 6.4%) and twice as likely to be predominantly (51.9 vs 28.3%) breastfed. Breastfeeding, to any degree, was provided at higher rates throughout infancy for those in the intervention group vs the control group. At 6 months, however, both exclusive (7.9% vs 0.6%) and predominant breastfeeding (10.6% vs 1.6%) were low.¹⁴ Comparing the intervention vs the control group, 72.7% vs 60.0%, respectively, were still breastfeeding at any degree at 3 months, 49.8% vs 36.1%, respectively, were still breastfeeding at 6 months, and 19.7% vs 11.4%, respectively, were still breastfeeding at 12 months.

A total of 13 879 children were examined at a median (SD) age of 11.5 (0.50) years (IQR, 11.3-11.8 years), representing 81.4% of the 17 046 who were originally randomized (Figure). Of the 3167 children randomized but not followed up at 11.5 years, 97 died since randomization, 2645 were lost to follow-up, and 425 were unable or unwilling to come for their visit (Figure). Follow-up rates were similar overall in the experimental (83.5%) and control (79.1%) polyclinics, although they varied from 48% to 98%. The children followed up at 11.5 years in the experimental and control groups were similar in baseline characteristics, with small differences paralleling those seen (and previously reported¹⁴) at randomization (TABLE 1). The groups were also virtually identical in mean parental height and BMI (measured for mothers at 11.5 years follow-up and reported by the mothers for the fathers at the 6.5-year follow up).

In eTable 1 (available at <http://www.jama.com>), the audit results are summarized showing high correlations (Pearson $r > 0.80$) between initial clinic results and blinded repeat (audit) measures of weight, fat mass, fat-free mass, percent fat, subscapular skinfold thickness, hip circumference, standing

height, and mid-upper arm circumference, and substantial correlations (r , 0.73- <0.80) for waist circumference, triceps skinfold thickness, leg length, and upper arm length, but only modest correlations for head circumference ($r=0.50$) and IGF-I ($r=0.37$).

The main results are shown in TABLE 2. There was a moderate degree of within-polyclinic clustering (the tendency for measurements on children attending the same polyclinic to be more similar to each other than to children attending other polyclinics¹⁸) for triceps skinfold thickness and head circumference (intra-class correlation coefficients, ICC, ≥ 0.10), but a low degree of clustering for the other measures. Mean BMI, FMI, percent body fat, waist circumference, and the prevalence of overweight and obesity were slightly higher in the experimental vs control groups, but the cluster-adjusted CIs were consistent with chance and rule out any important protective effect (lower values) on adiposity. The cluster-adjusted mean differences in our main 11.5-year outcomes between experimental vs control groups were: 0.19 (-0.09 to 0.46) for BMI; 0.12 (-0.03 to 0.28) for FMI; 0.04 (-0.11 to 0.18) for FFMI; 0.47% (-0.11% to 1.05%) for body fat; 0.30 cm (-1.41 to 2.01) for waist circumference; -0.07 mm (-1.71 to 1.57) for triceps; -0.02 mm (-0.79 to 0.75) for subscapular skinfold thicknesses; and -0.02 standard deviations (-0.12 to 0.08) for IGF-I. The cluster-adjusted odds ratio for overweight/obesity (BMI ≥ 85 th percentile vs <85 th percentile) was 1.18 (95% CI, 1.01-1.39) and for obesity (BMI ≥ 95 th vs <85 th percentile) was 1.17 (95% CI, 0.97-1.41). In exploratory analyses, there was little evidence that the intervention affected standing height, leg length, waist-hip ratio, or head or mid-upper arm circumference. A weak positive effect on hip circumference was observed in the fully adjusted model (mean difference, 0.81 cm; 95% CI, 0.09-1.53), but not the cluster-adjusted model.

These conclusions were unaltered after further adjusting for baseline po-

tential confounders (Table 2) or using multiple imputed outcomes (eTable 2). There was little evidence of interaction by sex (all interaction P values $>.10$ except for subscapular ($P=.03$) and triceps ($P=.05$) skinfold thickness).

In observational analyses (TABLE 3 and eTable 3), increased duration of exclusive breastfeeding was positively associated with body mass, fat mass and fat-free mass indices, hip circumference, head and mid-upper arm circumference, and overweight/obesity. Results

Table 1. Baseline and Follow-up Characteristics

Variable	Group, No. (%)	
	Experimental (n = 7405)	Control (n = 6474)
Measured at child's birth		
Maternal age, y		
<20	1022 (13.8)	832 (12.9)
20-34	6062 (81.9)	5374 (83.0)
≥ 35	321 (4.3)	268 (4.1)
Maternal education		
Completed university	1047 (14.1)	841 (13.0)
Advanced secondary or partial university	3558 (48.1)	3559 (55.0)
Common secondary	2489 (33.6)	1892 (29.2)
Incompleted secondary	311 (4.2)	182 (2.8)
Paternal education		
Completed university	990 (13.4)	789 (12.2)
Advanced secondary or partial university	3082 (41.6)	3305 (51.1)
Common secondary	2938 (39.7)	2040 (31.5)
Incompleted secondary or unknown	395 (5.3)	340 (5.3)
Stratum-level variable		
East, urban	2501 (33.8)	1953 (30.2)
East, rural	1085 (14.7)	1041 (16.1)
West, urban	2335 (31.5)	1252 (19.3)
West, rural	1484 (20.0)	2228 (34.4)
Older siblings living in household		
0	4369 (59.0)	3530 (54.5)
1	2466 (33.3)	2353 (36.4)
≥ 2	570 (7.7)	591 (9.1)
Maternal smoking during pregnancy	183 (2.5)	106 (1.6)
Male sex	3771 (50.9)	3370 (52.1)
Birthweight, mean (SD), g	3443 (417)	3442 (423)
Duration of exclusive breastfeeding, mo		
<3	4042 (54.6)	6023 (93.0)
3-<6	2878 (38.9)	404 (6.2)
≥ 6	485 (6.5)	47 (0.7)
Duration of any breastfeeding, mo ^a		
<3	2055 (31.2)	2493 (41.3)
3-<6	1680 (25.5)	1522 (25.2)
≥ 6	2852 (43.3)	2019 (33.5)
Measured at age 6.5 y, mean (SD)		
Paternal height, cm ^b	176 (7)	176 (7)
Paternal BMI ^c	25.7 (3.3)	25.7 (3.3)
Measured at age 11.5 y, mean (SD)		
Maternal height, cm ^d	164 (6)	164 (6)
Maternal BMI ^e	26.3 (5.5)	26.7 (5.6)

Abbreviation: BMI, body mass index.

^aBased on 6587 children in the experimental group and 6034 in the control group.

^bBased on 5894 children in the experimental group and 5843 in the control group measured in PROBIT II.

^cBased on 5887 children in the experimental group and 5825 in the control group measured in PROBIT II. BMI is calculated as weight in kilograms divided by height in meters squared.

^dBased on 7351 children in the experimental group and 6426 in the control group measured in PROBIT III.

^eBased on 6250 children in the experimental group and 5501 in the control group measured in PROBIT III. BMI is calculated as weight in kilograms divided by height in meters squared.

Table 2. Modified Intention-to-Treat Analysis (Without Imputation) Showing Differences in Adiposity Measures, Insulin-like Growth Factor-I, Circumferences, and Height Comparing Intervention vs Control Groups at Mean Age 11.5 Years (N = 13 879)

Outcome	Groups				Intraclass Correlation Coefficient	Difference, Mean (95% CI)	
	Intervention (n = 7405)		Control (n = 6474)			Cluster Adjusted	Further Adjusted for Baseline Factors ^a
	No.	Mean (95% CI)	No.	Mean (95% CI)			
BMI, kg ^b	7402	18.32 (18.25 to 18.39)	6464	18.05 (17.97 to 18.12)	0.01	0.19 (−0.09 to 0.46)	0.16 (−0.02 to 0.35)
FMI, kg ^b	7342	3.40 (3.35 to 3.45)	6427	3.24 (3.19 to 3.29)	0.01	0.12 (−0.03 to 0.28)	0.10 (−0.02 to 0.22)
FFMI, kg ^b	7348	14.92 (14.89 to 14.95)	6426	14.83 (14.80 to 14.87)	0.02	0.04 (−0.11 to 0.18)	0.04 (−0.06 to 0.14)
Body fat, %	7345	17.38 (17.20 to 17.56)	6433	16.78 (16.59 to 16.97)	0.01	0.47 (−0.11 to 1.05)	0.36 (−0.09 to 0.81)
Waist circumference, cm	7402	64.68 (64.50 to 64.87)	6471	64.77 (64.58 to 64.97)	0.09	0.30 (−1.41 to 2.01)	0.57 (−1.09 to 2.23)
Triceps skinfold thickness, mm	7399	13.62 (13.47 to 13.76)	6470	14.20 (14.05 to 14.36)	0.13	−0.07 (−1.71 to 1.57)	−0.14 (−1.85 to 1.56)
Subscapular skinfold thickness, mm	7399	9.07 (8.95 to 9.19)	6470	9.16 (9.02 to 9.30)	0.03	−0.02 (−0.79 to 0.75)	−0.14 (−0.88 to 0.60)
IGF-I (z-score) ^c	6668	0.01 (−0.02 to 0.03)	5712	−0.01 (−0.03 to 0.02)	0.02	−0.02 (−0.12 to 0.08)	−0.03 (−0.12 to 0.06)
Hip circumference, cm	7402	78.38 (78.20 to 78.56)	6471	77.38 (77.18 to 77.57)	0.03	0.82 (−0.20 to 1.84)	0.81 (0.09 to 1.53)
Waist-hip ratio	7402	82.56 (82.42 to 82.70)	6471	83.73 (83.59 to 83.88)	0.25	−0.44 (−2.63 to 1.75)	−0.12 (−2.21 to 1.96)
Standing height, cm	7403	150.39 (150.2 to 150.6)	6471	149.16 (149.0 to 149.4)	0.05	0.52 (−0.78 to 1.82)	0.42 (−0.39 to 1.23)
Leg length, cm	7395	72.01 (71.90 to 72.13)	6460	71.28 (71.17 to 71.40)	0.05	0.59 (−0.22 to 1.40)	0.54 (0 to 1.07)
Head circumference, cm	7402	53.61 (53.57 to 53.65)	6471	53.50 (53.46 to 53.54)	0.10	0.20 (−0.18 to 0.58)	0.25 (−0.13 to 0.62)
Mid-upper arm circumference, cm	7399	22.12 (22.05 to 22.18)	6467	21.95 (21.88 to 22.02)	0.03	0.19 (−0.19 to 0.57)	0.18 (−0.13 to 0.49)
BMI ≥85th percentile ^d		1192 (16.1) ^e		910 (14.1) ^e	NA	1.18 (1.01 to 1.39) ^f	1.17 (1.01 to 1.35) ^f
BMI ≥95th percentile ^d		397 (5.4) ^e		304 (4.7) ^e	NA	1.17 (0.97 to 1.41) ^f	1.16 (0.98 to 1.39) ^f

Abbreviations: BMI, body mass index; FMI, fat mass index; FFMI, fat-free mass index; IGF-I, insulin-like growth factor-I, NA, not applicable.

^aAdjusted for stratum-level variables (urban vs rural and East vs West Belarus), child age at follow-up, sex, birthweight, and maternal and paternal education.

^bBMI, FMI, and FFMI were calculated as weight, fat mass, and lean mass in kilograms divided by height in meters squared, respectively.

^cIGF-I levels presented as z-scores (standardized within assay run) and coefficients additionally controlled for time between sampling and assay analysis date.

^dBased on Centers for Disease Control and Prevention 2000 reference data¹⁹ vs <85th percentile.

^eData are reported as No. (%).

^fData are reported as odds ratio (95% CI).

Table 3. Observational Associations of Duration of Exclusive Breastfeeding With Adiposity Measures, Insulin-Like Growth Factor-I, Circumferences, and Height at Age 11.5 Years (N = 13 879)

Outcome	Cluster-Adjusted, Difference, Mean (95% CI), mo ^a			P Value for Trend	Further Adjusted for Baseline Factors, Difference, Mean (95% CI), mo ^b		P Value for Trend
	3 to <6 mo (n = 3282)	≥6 mo (n = 532)			3 to <6 mo	≥6 mo	
BMI ^c	0.14 (0 to 0.28)	0.48 (0.20 to 0.77)	.001	0.13 (0 to 0.26)	0.49 (0.21 to 0.76)	.001	
FMI ^c	0.03 (−0.06 to 0.13)	0.30 (0.09 to 0.50)	.02	0.02 (−0.07 to 0.12)	0.29 (0.09 to 0.49)	.03	
FFMI ^c	0.09 (0.02 to 0.15)	0.20 (0.06 to 0.33)	<.001	0.09 (0.03 to 0.14)	0.20 (0.08 to 0.33)	<.001	
Body fat, %	0.10 (−0.25 to 0.45)	0.94 (0.21 to 1.67)	.05	0.04 (−0.29 to 0.37)	0.89 (0.19 to 1.59)	.08	
Waist circumference, cm	0.01 (−0.34 to 0.36)	0.74 (0.01 to 1.47)	.20	−0.05 (−0.40 to 0.29)	0.70 (−0.02 to 1.42)	.32	
Triceps skinfold, mm	0.11 (−0.16 to 0.39)	0.66 (0.09 to 1.22)	.05	0.06 (−0.21 to 0.33)	0.59 (0.04 to 1.15)	.12	
Subscapular skinfold, mm	0.01 (−0.24 to 0.26)	0.58 (0.07 to 1.10)	.14	−0.02 (−0.27 to 0.22)	0.56 (0.05 to 1.07)	.22	
IGF-I (z-score) ^d	0.02 (−0.02 to 0.07)	0.04 (−0.05 to 0.14)	.23	0.01 (−0.03 to 0.05)	0.03 (−0.06 to 0.13)	.44	
Hip circumference, cm	0.22 (−0.14 to 0.57)	1.09 (0.36 to 1.82)	.01	0.14 (−0.19 to 0.48)	1.02 (0.32 to 1.72)	.02	
Waist-hip ratio	−0.22 (−0.46 to 0.02)	−0.19 (−0.68 to 0.31)	.10	−0.20 (−0.43 to 0.03)	−0.13 (−0.61 to 0.34)	.15	
Standing height, cm	0.18 (−0.16 to 0.53)	0.03 (−0.69 to 0.75)	.46	0.01 (−0.31 to 0.32)	−0.08 (−0.74 to 0.57)	.90	
Leg length, cm	0.07 (−0.15 to 0.29)	0.03 (−0.42 to 0.48)	.63	−0.02 (−0.22 to 0.18)	−0.03 (−0.45 to 0.40)	.84	
Head circumference, cm	0.10 (0.02 to 0.17)	0.16 (0.01 to 0.31)	.003	0.07 (0 to 0.14)	0.15 (0.00 to 0.29)	.01	
Mid-upper arm circumference, cm	0.11 (−0.02 to 0.24)	0.37 (0.09 to 0.64)	.01	0.09 (−0.04 to 0.21)	0.36 (0.09 to 0.63)	.01	
BMI ≥85th percentile, OR (95% CI) ^e	1.15 (1.02 to 1.29)	1.36 (1.07 to 1.72)	.002	1.14 (1.02 to 1.29)	1.37 (1.08 to 1.74)	.002	
BMI ≥95th percentile, OR (95% CI) ^e	1.07 (0.89 to 1.29)	1.53 (1.08 to 2.18)	.05	1.08 (0.89 to 1.30)	1.58 (1.11 to 2.25)	.03	

Abbreviations: BMI, body mass index; FMI, fat mass index; FFMI, fat-free mass index; OR, odds ratio.

^aThe reference standard for calculating the cluster-adjusted difference at 3 to less than 6 months and at 6 months or greater is less than 3 months (n=10 065).

^bData were adjusted for stratum-level variables (urban vs rural and East vs West Belarus), and for child age at follow-up, sex, birthweight, and both maternal and paternal education.

The reference standard for calculating these further adjusted differences at 3 to less than 6 months and at 6 months or greater is less than 3 months.

^cBMI, FMI, and FFMI were calculated as weight, fat mass, and lean mass in kilograms divided by height in meters squared, respectively.

^dIGF-I levels presented as z-scores (standardized within assay run) and coefficients additionally controlled for time between sampling and assay analysis date.

^eOdds ratios were calculated by comparing against BMI values of lower than the 85th percentile as a reference standard. BMI percentiles are based on Centers for Disease Control and Prevention 2000 reference data.¹⁹

Table 4. Instrumental Variable Estimates of the Unbiased Associations of Duration of Exclusive Breastfeeding on Body Mass Index (Continuous), Waist Circumference, Overweight and Obesity (Binary), and Standing Height at Age 11.5 Years

	Difference, Mean (95% CI)			
	Cluster Adjusted ^a		Further Adjusted for Baseline Factors ^b	
	3 to <6 mo	≥6 mo	3 to <6 mo	≥6 mo
BMI ^c	0.46 (−0.36 to 1.27)	0.81 (−0.51 to 2.13)	0.37 (−0.25 to 0.98)	0.71 (−0.37 to 1.79)
Waist circumference, cm	0.79 (−4.03 to 5.61)	1.55 (−5.49 to 8.59)	1.51 (−3.46 to 6.48)	2.59 (−6.60 to 11.78)
Standing height, cm	1.60 (−2.39 to 5.59)	2.15 (−3.99 to 8.30)	1.30 (−1.01 to 3.61)	1.59 (−1.37 to 4.56)
BMI ≥85th percentile, OR (95% CI) ^d	1.61 (0.99 to 2.63)	1.94 (0.86 to 4.39)	1.52 (1.05 to 2.17)	1.71 (0.96 to 3.04)
BMI ≥95th percentile, OR (95% CI) ^d	1.51 (0.86 to 2.67)	1.98 (0.73 to 5.37)	1.45 (0.90 to 2.34)	1.80 (0.82 to 3.94)

Abbreviations: BMI, body mass index; OR, odds ratio.

^aThe reference standard for calculating the cluster-adjusted difference at 3 to less than 6 months and at 6 months or greater is less than 3 months.

^bAdjusted for stratum-level variables (urban vs rural and East vs West Belarus), and for child age at follow-up, sex, birthweight and both maternal and paternal education. The reference standard for calculating these further adjusted differences at 3 to less than 6 months and at 6 months or greater is less than 3 months.

^cBMI is calculated as weight in kilograms divided by height in meters squared.

^dOdds ratios were calculated by comparing against BMI values of lower than the 85th percentile as a reference standard. BMI percentiles are based on Centers for Disease Control and Prevention 2000 reference data.¹⁹

were similar for duration of any breastfeeding (eTable 4). The results of the instrumental variable analyses (TABLE 4), which provide estimates of the unbiased associations of exclusive breastfeeding for 3 months or longer vs less than 6 months and 6 months or longer vs less than 3 months (and therefore directly comparable to estimates from observational studies), are in line with the inference that increased duration and exclusivity of breastfeeding provides no important beneficial effects on the study outcomes.

COMMENT

The results from this large cluster-randomized trial indicate that the experimental intervention to promote increased duration and exclusivity of breastfeeding did not reduce continuous measures of adiposity nor reduce the prevalence of overweight or obesity in children aged 11.5 years, despite causing large increases in the duration and exclusivity of breastfeeding. The results are similar to those we obtained in children aged 6.5 years.¹⁵ The new data thus extend our observations to older children and include more direct measures of fat and lean mass.

Our findings also concur with the evidence provided by other authors attempting to systematically assess unbiased and unconfounded associations of breastfeeding on adiposity. An individual-participant meta-analysis provided empirical evidence that previ-

ously reported associations of having been breastfed with both continuous measures of adiposity and overweight/obesity may have arisen as a result of residual confounding, selective reporting, publication bias, or some combination of all of these factors.^{1,2} In low- and middle-income countries (such as Brazil and Hong Kong)^{9,11,31} or older Western cohorts,^{3,4,32,33} with confounding structures that are neutral or opposite to those currently seen in high-income countries, inverse associations of breastfeeding with adiposity are not consistently observed. An inverse association of breastfeeding with obesity was not seen in a matched sibling-pair analysis that was restricted to comparisons of infants from the same families (and hence socioeconomic background),¹⁰ while a larger study reported effect estimates consistent with the null from both the sibling pair- and full cohort analysis.³⁴

We emphasize that the breastfeeding promotion intervention was designed to increase the duration and exclusivity of breastfeeding, not its initiation (initial breastfeeding was an inclusion criterion). Our findings may not, therefore, apply to comparisons of initiating breastfeeding vs formula, the comparisons most frequently described in the literature. Nonetheless, many previous studies have reported dose-response inverse associations of the duration and/or exclusivity of breastfeeding with adiposity.³⁵ Our findings do not support those associations.

We observed a positive association of the intervention with overweight/obesity, although the magnitude was small. As previously reported, the PROBIT intervention was associated with faster weight gain during the first 3 months of infancy, although these differences were no longer present by 12 months of age.¹³ We also reported a nonsignificant positive association with overweight/obesity at 6.5 years of age.¹⁵ It is possible that PROBIT mothers randomized to receive the breastfeeding promotion intervention, knowing their infants were entirely dependent nutritionally on their breast milk, deliberately increased the frequency and duration of feeds, leading to the faster weight and length gains we observed in the first 3 months of life. Explaining the disappearance of these differences by 12 months and their reappearance (for weight) in later childhood, however, would require a combination of metabolic programming in infancy, followed by a prolonged latent period. In our view, a more likely explanation is that the positive association observed in later childhood arose by chance.

Our 2 trial cohorts were created by randomization at the time of birth (not by the mother's choice), which resulted in substantial differences between those cohorts in the duration and degree of breastfeeding. Coupled with our high rates of follow-up over 11.5 years, the intention-to-treat analysis minimizes the confounding and re-

verse causality biases that plague observational studies.³⁶ We also minimized measurement bias by assessing infant feeding contemporaneously, strictly adhering to WHO definitions¹⁷ of breastfeeding duration and exclusivity, and using specific measures of body fat distribution (waist and hip circumferences) and body composition (skinfold thicknesses, fat mass and fat free mass measured by bioimpedance).^{37,38}

To estimate the unbiased effects of the experimental breastfeeding promotion intervention, we used an intention-to-treat rather than a per-protocol analysis, as it is well known that per-protocol analyses of randomized trials, in which participants are grouped according to the intervention they received rather than that to which they were randomized, may be seriously biased. The estimates provided by the intention-to-treat analysis are the most robustly estimated expected average effects on the anthropometric and adiposity outcomes of the experimental breastfeeding promotion intervention. However, because of the substantial overlap in breastfeeding duration and exclusivity in the 2 randomized groups, these average effects may considerably underestimate the differences in outcome caused by increased duration and exclusivity of breastfeeding. We therefore estimated the magnitude of the unbiased and unconfounded associations with anthropometry and adiposity using instrumental variables analysis, which supported our inference that increased duration and exclusivity of breastfeeding was not associated with the outcomes of interest. The CIs of the instrumental variables analyses were wide, however, so we cannot exclude the estimates reported in observational studies.¹⁻³

Despite our large sample size, the precision of the observed differences (the width of the CIs) was only modest because of clustering of both the intervention (a tendency for more similar outcomes among children within a polyclinic) and measurement (a tendency for measurements made within a clinic to be more similar than measurements between clinics).⁴⁰ Never-

theless for most measurements, we could rule out differences as small as 0.15 to 0.20 standard deviations. For triceps skinfold thickness and head circumference, with ICCs of at least 0.1 (indicating an important degree of clustering within a polyclinic¹⁸), we could not rule out differences as large as 0.35 standard deviations. The point estimates for all adiposity measures were in the opposite direction to those hypothesized, however, and for overweight and obesity, the lower limit of the CI excludes an important protective effect of breastfeeding.

The trial was carried out in Belarus, rather than North America or Western Europe, because at the time of randomization, maternity hospital practices in Belarus and other former Soviet republics were similar to those in North America and Western Europe 30 to 40 years ago and thus provided a greater potential contrast between intervention and control study sites. However, although different in many socioeconomic, cultural, and economic respects from North America and Western Europe, Belarus is a relatively developed country with strict hygienic standards, high immunization rates, low incidence of infection, low rates of infant and child mortality, similar types of formula feeds, and accessible health care services. The prevalence of childhood obesity in the United States (and some other Western countries) is much higher (>15% compared with approximately 5% in Belarus based on the CDC 2000 reference data⁴¹). It is possible that our results may not generalize to settings with a much higher prevalence of obesity than Belarus, although such a lack of generalizability implies an interaction between method of infant feeding and some (unknown) environmental factor in more obesogenic environments.

IGF-I was measured from dried blood spots but, in addition to the validation data presented in this article, circulating IGF-I has previously been reported to be stable when dried on filter paper (for 40 days at room temperature^{24,42} and

5 months at -20°C ⁴³) and validly and reliably measured from dried blood spots using either a commercially available radioimmunoassay⁴³ or ELISA kits from Diagnostic Systems Laboratories,^{24,42} including the kit used in this study.²⁴

CONCLUSIONS

Among healthy term infants in Belarus, an intervention to improve the duration and exclusivity of infant breastfeeding did not prevent overweight or obesity, nor did it affect IGF-I levels among these children when they were aged 11.5 years. Nevertheless, breastfeeding has many health advantages for the offspring, including beneficial effects demonstrated by our PROBIT trial on gastrointestinal infections and atopic eczema in infancy¹⁴ and improved cognitive development at age 6.5 years.⁴⁴ Although breastfeeding is unlikely to stem the current obesity epidemic, its other advantages are amply sufficient to justify continued public health efforts to promote, protect, and support it.

Author Contributions: Dr Martin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Acquisition of data: Martin, Patel, Kramer, Vilchuck, Bogdanovich, Sergeichick, Gusina, Foo, Oken.

Analysis and interpretation of data: Martin, Kramer, Guthrie, Palmer, Rifas-Shiman, Gillman, Oken.

Drafting of the manuscript: Martin, Palmer, Davey Smith.

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Statistical analysis: Martin, Guthrie, Palmer, Rifas-Shiman.

Obtained funding: Martin, Kramer, Gillman, Oken.

Administrative, technical, or material support: Martin, Patel, Guthrie, Vilchuck, Bogdanovich, Sergeichick, Gusina, Oken.

Study supervision: Martin, Kramer, Vilchuck, Bogdanovich, Sergeichick, Gusina, Foo, Davey Smith, Oken.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Martin reports presenting an invited talk for the Nestle Nutrition Institute. Dr Kramer reports receipt of an institutional grant from the Canadian Institutes of Health Research and receipt of unrelated meeting expenses from Nestle Nutrition Institute. Dr Foo reports receipt of a consulting fee support for travel to meetings from the University of Bristol. Ms Guthrie and Drs Gillman and Oken report receipt of an institutional grant from the National Institutes of Health (NIH). Dr Oken reports presenting an invited talk for Nestle Nutrition Institute and also receipt of travel reimbursement.

Funding/Support: This study was supported by grants from: the European Union, Early Nutrition Programming Long-term Efficacy and Safety Trials (FOOD-DT-2005-007036); Canadian Institutes of Health Research (MOP-53155); and NIH (R01 HD050758). Dr Oken was supported by US National Institute of Child Health and Development (K24 HD069408). Drs Martin, Palmer, and Davey Smith work within the CAiTE Centre, which is supported by the Medical Research Council (G0600705) and the University of Bristol.

Role of the Sponsors: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Online-Only Material: eTables 1 through 4 are available at <http://www.jama.com>.

Additional Contributions: We are grateful to the cohort members, their parents and the study pediatricians and auditors who participated so willingly in the study. We acknowledge Jennifer Thompson, MPP, Harvard Medical School and Harvard Pilgrim Health Care Institute, for assistance with data analysis. Ms Thompson was not compensated in association with her work on this article.

REFERENCES

- Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Effect of infant feeding on the risk of obesity across the life course. *Pediatrics*. 2005;115(5):1367-1377.
- Owen CG, Martin RM, Whincup PH, Davey Smith G, Gillman MW, Cook DG. The effect of breastfeeding on mean body mass index throughout life. *Am J Clin Nutr*. 2005;82(6):1298-1307.
- Martin RM, Smith GD, Mangtani P, Frankel S, Gunnell D. Association between breast feeding and growth. *Arch Dis Child Fetal Neonatal Ed*. 2002;87(3):F193-F201.
- Wadsworth ME, Hardy RJ, Paul AA, Marshall SF, Cole TJ. Leg and trunk length at 43 years in relation to childhood health, diet and family circumstances. *Int J Epidemiol*. 2002;31(2):383-390.
- Martin RM, Holly JMP, Smith GD, et al; ALSPAC Study Team. Could associations between breastfeeding and insulin-like growth factors underlie associations of breastfeeding with adult chronic disease? *Clin Endocrinol (Oxf)*. 2005;62(6):728-737.
- Larnkjaer A, Ingstrup HK, Schack-Nielsen L, et al. Early programming of the IGF-I axis: negative association between IGF-I in infancy and late adolescence in a 17-year longitudinal follow-up study of healthy subjects. *Growth Horm IGF Res*. 2009;19(1):82-86.
- Ong KK, Langkamp M, Ranke MB, et al. Insulin-like growth factor I concentrations in infancy predict differential gains in body length and adiposity. *Am J Clin Nutr*. 2009;90(1):156-161.
- Hoppe C, Mølgaard C, Thomsen BL, Juul A, Michaelsen KF. Protein intake at 9 mo of age is associated with body size but not with body fat in 10-year old Danish children. *Am J Clin Nutr*. 2004;79(3):494-501.
- Kwok MK, Schooling CM, Lam TH, Leung GM. Does breastfeeding protect against childhood overweight? *Int J Epidemiol*. 2010;39(1):297-305.
- Nelson MC, Gordon-Larsen P, Adair LS. Are adolescents who were breast-fed less likely to be overweight? *Epidemiology*. 2005;16(2):247-253.
- Brion MJ, Lawlor DA, Matijasevich A, et al. What are the causal effects of breastfeeding on IQ, obesity and blood pressure? *Int J Epidemiol*. 2011;40(3):670-680.
- Kramer MS, Moodie EEM, Dahhou M, Platt RW. Breastfeeding and infant size. *Am J Epidemiol*. 2011;173(9):978-983.
- Kramer MS, Guo T, Platt RW, et al; PROBIT Study Group. Breastfeeding and infant growth? *Pediatrics*. 2002;110(2 pt 1):343-347.
- Kramer MS, Chalmers B, Hodnett ED, et al; PROBIT Study Group (Promotion of Breastfeeding Intervention Trial). Promotion of Breastfeeding Intervention Trial (PROBIT): a randomized trial in the Republic of Belarus. *JAMA*. 2001;285(4):413-420.
- Kramer MS, Matush L, Vanilovich I, et al; PROBIT Study Group. Effects of prolonged and exclusive breastfeeding on child height, weight, adiposity, and blood pressure at age 6.5 y. *Am J Clin Nutr*. 2007;86(6):1717-1721.
- Chivers P, Hands B, Parker H, et al. Body mass index, adiposity rebound and early feeding in a longitudinal cohort (Raine Study). *Int J Obes (Lond)*. 2010;34(7):1169-1176.
- WHO/UNICEF. *Protecting, Promoting and Supporting Breastfeeding: the Special Role of Maternity Services*. Geneva, Switzerland: World Health Organization; 1989.
- Guthrie LB, Oken E, Sterne JAC, et al. Ongoing monitoring of data clustering in multicenter studies. *BMC Med Res Methodol*. 2012;12:29.
- Ogden CL, Kuczmarski RJ, Flegal KM, et al. Centers for Disease Control and Prevention 2000 growth charts for the United States. *Pediatrics*. 2002;109(1):45-60.
- Mei JV, Alexander JR, Adam BW, Hannon WH. Use of filter paper for the collection and analysis of human whole blood specimens. *J Nutr*. 2001;131(5):1631S-1636S.
- Warnick GR, Leary ET, Ammirati EB, Allen MP. Cholesterol in fingerstick capillary specimens can be equivalent to conventional venous measurements. *Arch Pathol Lab Med*. 1994;118(11):1110-1114.
- National Committee for Clinical Laboratory Standards (NCCLS). *Blood Collection on Filter Paper for Neonatal Screening Programs, Approved Standard, National Committee for Clinical Laboratory Standards Document A4A3*. 3rd ed. Wayne, PA: National Committee for Clinical Laboratory Standards; 1997.
- Martin RM, Patel R, Zinovic A, et al. Filter paper blood spot enzyme linked immunoassay for insulin and application in the evaluation of determinants of child insulin resistance. *PLoS One*. 2012;7(10):e46752.
- Diamandi A, Khosravi MJ, Mistry J, Martinez V, Guevara-Aguirre J. Filter paper blood spot assay of human insulin-like growth factor I (IGF-I) and IGF-binding protein-3 and preliminary application in the evaluation of growth hormone status. *J Clin Endocrinol Metab*. 1998;83(7):2296-2301.
- Rowlands MA, Holly JMP, Gunnell D, et al. Circulating insulin-like growth factors and IGF-binding proteins in PSA-detected prostate cancer. *Cancer Res*. 2012;72(2):503-515.
- Horton NJ, Kleinman KP. Much ado about nothing: a comparison of missing data methods and software to fit incomplete data regression models. *Am Stat*. 2007;61(1):79-90.
- Rubin DB. *Multiple Imputation for Non Response in Surveys*. New York, NY: J. Wiley & Sons; 1987.
- Angrist JD, Imbens GW, Rubin DB. Identification of causal effects using instrumental variables. *J Am Stat Assoc*. 1996;91(434):444-455 DOI:10.1080/01621459.1996.10476902.
- Palmer TM, Sterne JAC, Harbord RM, et al. Instrumental variable estimation of causal risk ratios and causal odds ratios in Mendelian randomization analyses. *Am J Epidemiol*. 2011;173(12):1392-1403.
- Ukoumunne OC, Gulliford MC, Chinn S, Sterne JA, Burney PG, Donner A. Methods in health service research. *BMJ*. 1999;319(7206):376-379.
- Victora CG, Barros F, Lima RC, Horta BL, Wells J. Anthropometry and body composition of 18 year old men according to duration of breast feeding. *BMJ*. 2003;327(7420):901-904.
- Parsons TJ, Power C, Manor O. Infant feeding and obesity through the lifecourse. *Arch Dis Child*. 2003;88(9):793-794.
- Michels KB, Willett WC, Graubard BI, et al. A longitudinal study of infant feeding and obesity throughout life course. *Int J Obes (Lond)*. 2007;31(7):1078-1085.
- Gillman MW, Rifas-Shiman SL, Berkey CS, et al. Breast-feeding and overweight in adolescence. *Epidemiology*. 2006;17(1):112-114.
- Harder T, Bergmann R, Kallischnigg G, Plagemann A. Duration of breastfeeding and risk of overweight. *Am J Epidemiol*. 2005;162(5):397-403.
- Smith GD, Ebrahim S. Data dredging, bias, or confounding. *BMJ*. 2002;325(7378):1437-1438.
- Kipping RR, Jago R, Lawlor DA. Obesity in children, part 1: epidemiology, measurement, risk factors, and screening. *BMJ*. 2008;337:a1824.
- Goran MI. Measurement issues related to studies of childhood obesity. *Pediatrics*. 1998;101(3 pt 2):505-518.
- Monasta L, Batty GD, Cattaneo A, et al. Early-life determinants of overweight and obesity. *Obes Rev*. 2010;11(10):695-708.
- Kramer MS, Martin RM, Sterne JAC, Shapiro S, Dahhou M, Platt RW. The double jeopardy of clustered measurement and cluster randomisation. *BMJ*. 2009;339:b2900.
- Ogden CL, Carroll MD, Kit BK, Flegal KM. *Prevalence of obesity in the United States, 2009-2010*. Hyattsville, MD: US Department of Health and Human Services; 2012.
- Jones JS. Insulin-like growth factor I measurement on filter paper blood spots. *Horm Res*. 2001;55(suppl 2):80-83.
- Schütt BS, Weber K, Elminger MW, Ranke MB. Measuring IGF-I, IGFBP-2 and IGFBP-3 from dried blood spots on filter paper is not only practical but also reliable. *Growth Horm IGF Res*. 2003;13(2-3):75-80.
- Kramer MS, Aboud F, Mironova E, et al; Promotion of Breastfeeding Intervention Trial (PROBIT) Study Group. Breastfeeding and child cognitive development. *Arch Gen Psychiatry*. 2008;65(5):578-584.