Intake of Micronutrient-Rich Foods in Rural Indian Mothers Is Associated with the Size of Their Babies at Birth: Pune Maternal Nutrition Study[1]

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ABSTRACT One third of the Indian babies are of low birth weight (<2.5 kg), and this is attributed to maternal undernutrition. We therefore examined the relationship between maternal nutrition and birth size in a prospective study of 797 rural Indian women, focusing on macronutrient intakes, dietary quality and micronutrient status. Maternal intakes (24-h recall and food frequency questionnaire) and erythrocyte folate, serum ferritin and vitamin C concentrations were measured at 18 ± 2 and 28 ± 2 wk gestation. Mothers were short (151.9 ± 5.1 cm) and underweight (41.7 ± 5.1 kg) and had low energy and protein intakes at 18 wk (7.4 ± 2.1 MJ and 45.4 ± 14.1 g) and 28 wk (7.0 ± 2.0 MJ and 43.5 ± 13.5 g) of gestation. Mean birth weight and length of term babies were also low (2665 ± 358 g and 47.8 ± 2.0 cm, respectively). Energy and protein intakes were not associated with birth size, but higher fat intake at wk 18 was associated with neonatal length ($P < 0.001$), birth weight ($P < 0.05$) and triceps skinfold thickness ($P < 0.05$) when adjusted for sex, parity and gestation. However, birth size was strongly associated with the consumption of milk at wk 18 ($P < 0.05$) and of green leafy vegetables ($P < 0.001$) and fruits ($P < 0.01$) at wk 28 of gestation even after adjustment for potentially confounding variables. Erythrocyte folate at 28 wk gestation was positively associated with birth weight ($P < 0.001$). The lack of association between size at birth and maternal energy and protein intake but strong associations with folate status and with intakes of foods rich in micronutrients suggest that micronutrients may be important limiting factors for fetal growth in this undernourished community. J. Nutr. 131: 1217–1224, 2001.

KEY WORDS: • *India* • *maternal intake* • *food frequency questionnaire* • *green leafy vegetables* • *birth size*

One third of babies born in India are of low birth weight (<2.5 kg) (Gopalan 1994, UNICEF 1998). In addition to short-term consequences such as high infant mortality rates and childhood growth failure among survivors, low birth weight carries a long-term risk in the form of high rates of adult coronary heart disease and type 2 diabetes (Barker 1998). Low birth weight in India has been attributed to widespread maternal undernutrition. A better understanding of the relationship of birth size to maternal nutrition is critical for planning effective intervention to improve birth weight in Indian babies.

Studies that investigated the relationship between maternal nutrition and babies birth size are scarce, and those available are inconsistent (Susser 1991). This relationship is influenced by many biological and socioeconomic factors, which vary widely in different populations. For example, the relationship differs among adolescents (Scholl et al. 1994), among women from a low socioeconomic class (Hediger et al. 1994) and even in most developed countries like Austria, where women have cosmetic undernutrition (Kirchengast and Hartmann 1998). Studies of energy and protein supplementation during pregnancy have produced varying and sometimes conflicting results (Kramer 1993), although there is some evidence that supplementation may be beneficial in very marginally nourished women (Ceesay et al. 1997).

The dietary intakes of energy and protein of rural Indian mothers are low (Bhatia et al. 1981, Grover 1982, Hutter 1996, Piers et al. 1995, Rawtani and Varma 1989, Vijayalaxmi and Lakshmi 1985, Vijayalaxmi et al. 1988). The consumption of foods that are important sources of micronutrients, such as dairy products, meat, fresh fruits and green leafy vegetables (GLV),[3] is also low in rural Indian populations (Gupta and Sharma 1980). Rural Indian women are often engaged in a

[1] Supported by the Wellcome Trust, London, U.K., and the Medical Research Council, U.K.

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[3] Abbreviations used: BMI, body mass index; FFQ, food frequency questionnaire; GLV, green leafy vegetables; ICMR, Indian Council of Medical Research; MUAC, mid-upper arm circumference.

Manuscript received 5 April 2000. Initial review completed 28 July 2000. Revision accepted 2 January 2001.

high level of physical activity (Piers et al. 1995). The majority of previous studies in India have examined maternal diets in terms of quantity (macronutrients) using 24-h recall method but rarely assessed quality (micronutrients). Food frequency questionnaires (FFQ) that are likely to offer estimates of habitual intake have rarely been used in studies of pregnant women.

The assessment of maternal nutritional status requires the measurement of body composition (before and during pregnancy), determination of energy intake and workload, measurement of patterns of food intake, consumption of macronutrients and micronutrients and biochemical measurements of micronutrient status. In the Pune Maternal Nutrition Study, we set out to relate the nutritional status before and during pregnancy of women living in rural Maharashtra, India, to the birth weight and other measurements of their babies. We reported earlier (Fall et al. 1999) that the size at birth is strongly predicted by maternal prepregnancy weight and weight gain in pregnancy. Maternal height, head circumference and prepregnancy fat mass independently predicted the birth weight of the baby. The present report describes our findings in relation to maternal macronutrient intakes, intakes of micronutrient-rich foods and biochemical micronutrient status. For biochemical micronutrient status, we focused on folate, iron and vitamin C. Folate and iron have traditionally been considered important micronutrients for fetal growth, and vitamin C is crucial for iron absorption.

MATERIALS AND METHODS

Mothers

The study took place in six villages, 40–50 km from Pune City, and covered a population of ~35,000. Of 2675 married eligible women (aged 15–40 y), 2466 women (92%) agreed to participate. Field workers visited them every month to record the date of their last menstrual period; women who missed two successive periods were examined with ultrasound at 15–18 wk to record sonographic gestational age (Hadlock 1990). Gestational age was derived from the last menstrual period, unless it differed from the sonographic estimate by >2 wk, in which case the latter estimate was used. Women entered the study if a singleton pregnancy of <21 wk gestation was confirmed. Socioeconomic status was assessed using a standardized questionnaire (Pareek and Trivedi 1964), which derives a composite score based on occupation and education of the head of the household, caste, type of housing and family ownership of animals, land and material possessions. The majority of women were poorly educated and belonged to subsistence farming families. All women were given 100 tablets of iron (60 mg) and folic acid (0.5 mg) at 18 wk gestation according to the National Nutritional Anemia Control Program.

During the study period, June 1994 through April 1996, 1102 women reported missing periods. One hundred twelve women reported an abortion/termination, 8 had major fetal anomalies and 3 had multiple pregnancies. Fourteen had incomplete prepregnancy anthropometry, and 168 were entered beyond 21 wk of gestation. Thus, 797 women were studied for this analysis. Ethical permission for the study was granted by the King Edward Memorial Hospital Ethical Committee and by the local village leaders.

Nutritional assessment

Anthropometric measurements. Women were measured every 3 mo to record their weight, height, skinfold at four sites and head and mid-upper arm circumference (MUAC). The last set of measurements made before pregnancy was used as prepregnancy anthropometry, and the measurements were repeated during pregnancy at 18 ± 2 and 28 ± 2 wk gestation.

Dietary intake. The conventional 24-h recall method was modified and made more objective by incorporating information on portion sizes, which were weighed at each mealtime by a trained fieldworker. Women were interviewed at 18 and 28 wk of gestation by one of four nutritionists to record the consumption of food items in chronological order from morning until dinnertime. At the time of diet survey, interviewers ensured that the woman was not fasting or had an illness and that she reported foods consumed outside the home on the day of the visit. A database of nutritive values of foods was generated by analyzing 288 distinct food preparations commonly consumed in the community. Protein was estimated in dried samples by the micro-Kjeldhal method, using the 1030 Kjeltec Autoanalyser system (Tecator AB, Hoganas, Sweden). Fat was estimated using the Soxlet method, in which food samples are subjected to continuous ether extraction for 18 h (Raghuramulu et al. 1983). Carbohydrates were estimated by subtraction. In addition to the 24-h recall questionnaire, a FFQ was administered to obtain frequency of consumption of 111 foods in 17 food categories during the preceding 3-mo period, on an 8-point scale ranging from "never" to "more than once daily." The 17 food categories were beverages, chapati/roti, rice, pulses, legumes, vegetables, GLV, chutneys, fasting foods, fruits, meat/fish, milk products, bakery items, spicy snacks, sweet snacks, festival foods and special foods. The food groups were mutually exclusive.

Biochemical measures of maternal nutritional status. In addition to dietary intakes, maternal red cell folate and serum ferritin and vitamin C concentrations were measured at 18 and 28 wk. Blood samples were taken from fasting subjects at an early-morning home visit. Samples were protected from light and transferred within 1 h via motorcycle to a local village research center, where samples were centrifuged at 1310 × *g*, and the serum was separated. Samples were transferred, at 0–4°C in a light-protected box, to the main laboratory at the King Edward Memorial Hospital in Pune. EDTA samples for red cell folate assay were diluted (1:19 by volume) with 10 g ascorbic acid/L solution and incubated for 90 min at room temperature, before being frozen at −80°C. An equal volume of 100 g metaphosphoric acid/L was added to 0.5-mL serum aliquots for vitamin C assay, before being frozen at −80°C. Samples were transferred to the United Kingdom on dry ice. Red cell folate and serum ferritin concentrations were measured in the Hematology Laboratory, Southampton General Hospital (Southampton, U.K.) with radioimmunoassays (Becton Dickinson U.K., Oxford, U.K.). Serum vitamin C concentrations were measured at the Medical Research Council Human Nutrition Research Center (Cambridge, U.K.) using an ascorbate oxidase–orthophenylene diamine assay on a Roche Cobas Bio Centrifugal analyzer (Hoffman-LaRoche, Basel, Switzerland) with a fluorescence attachment (Vuilleumier and Keck 1989).

Physical workload assessment

The women's typical daily physical activity was recorded at 18 and 28 wk gestation using simple numeric measures in a specially designed activity questionnaire, which included farming and domestic activities. For example, domestic activities, such as cooking and washing clothes/utensils, were recorded in terms of the number of people catered for, whereas fetching water was recorded in terms of the number of trips and number of containers carried. Using published data on the energy cost of various activities (Bleiberg et al. 1981 and 1980, Lawrence et al. 1985), a weighted total daily score was derived. This score reflected as a base unit, an activity level of 1 kcal (4.184 kJ)/min for a 30-min slot of time. The questionnaire was validated in 41 women using a 1-d minute-to-minute observer maintained records. Activity scores obtained by questionnaire were significantly correlated ($P < 0.05$) with actual time spent in domestic ($r = 0.34$) and farming ($r = 0.56$) activities. The total scores were used to rank women into tertiles of "low," "medium" and "high" physical activity.

Neonatal anthropometry

Babies were measured by one of five trained fieldworkers within 72 h of birth. Birth weight was measured to the nearest 50 g using a Salter spring balance (Salter Abbey, Suffolk, U.K.); crown-heel length was measured to the nearest 0.1 cm using a portable Pedobaby Babymeter (ETS J.M.B., Brussels, Belgium). Triceps and subscapular skinfold thicknesses were measured to the nearest 0.2 mm, on the left

side of the body, using Harpenden skinfold calipers (CMS Instruments, London, U.K.). Occipitofrontal head circumference and MUAC were measured to the nearest 0.1 cm using Fiberglas tapes (CMS Instruments, London, U.K.). Abdominal circumference was measured at the level of the umbilicus in expiration. Placental weight was recorded to the nearest 5 g using Ishida scales after trimming of the umbilical cord and membranes. Interobserver and intraobserver variation studies were conducted every 3 mo to ensure quality of these measurements.

Statistical methods

Differences between group means were tested using *t* tests. Multiple regression analysis was used to examine trends in birth size according to maternal dietary intakes and biochemical data and to assess the relative contributions of other factors to the variability in birth measurements. In regressions, birth measurements, maternal measurements, energy and macronutrient intakes and erythrocyte folate, vitamin C and ferritin concentrations were analyzed as continuous variables. Intakes of specific foods based on the FFQ and socioeconomic and activity scores were analyzed as grouped variables. However, when using the regression analysis, we weighted these groupings to reflect as closely as possible the frequency of consumption per week. All analyses were adjusted for the baby's sex, gestational age at delivery and maternal parity. Maternal and neonatal skinfold measurements were skewed and required log-transformation to satisfy assumptions of normality. Analysis was carried out using SPSS/PC+, Version 5.0. Values unless otherwise stated are means ± SD.

RESULTS

Of the 797 women in the study, 12 had spontaneous abortions, 14 had late terminations and 1 died of pregnancy-induced hypertension. Seven hundred seventy infants were delivered, of whom 8 were stillborn, 9 had major anomalies detected at birth and 51 did not have birth measurements within 72 h. Thus, of 702 of normal live births, our analysis relates to 633 full-term infants.

Mothers

The mean age of the mothers was 21.4 y, and 31.6% were primiparous. They were short, light and thin (**Table 1**). Twenty-three percent of the women had a prepregnancy body weight of <38 kg, and 9% were shorter than 145 cm, considered high risk for low birth weight (Gopalan 1989). Of the women, 31.3% had a body mass index (BMI) of <17 kg/m^2, indicating severe chronic energy deficiency (World Health Organization 1995). Weight gain during pregnancy was 2.1 ± 2.8 kg up to 18 wk and 5.5 ± 2.9 kg up to 28 wk. All measurements except maternal MUAC showed a significant increase up to 28 wk' gestation.

Babies

The full-term birth weight was 2665 g (Table 1), and 28% of babies were of low birth weight (<2500 g). Even among full-term babies, birth weight increased with gestational age (r = 0.36). Birth weight, length and head circumference were greater in boys than in girls ($P < 0.01$). All measurements except length, head circumference and MUAC were smaller in babies born to primiparous than in babies born to multiparous mothers ($P < 0.05$). In our analysis of birth size in relation to nutritional data, we therefore adjusted for gestational age at delivery, baby's sex and maternal parity.

Dietary intakes

Macronutrients (24-h recall). Maternal energy and protein intakes at 18 and 28 wk were energy of 7.4 ± 2.1 and 7.0 ± 2.0 MJ and protein of 45.4 ± 14.1 and 43.5 ± 13.5 g, respectively (**Table 2**). These values are low compared with Recommended Daily Allowances for Indian pregnant women given by the Indian Council of Medical Research (1987). Carbohydrates were the main energy source (72%), whereas 10 and 18% of energy was derived from protein and fat, respectively. Most protein was derived from cereals and pulses. Only 38% of women consumed animal protein, which contributed only 15% to the daily protein intake. Activity scores at 18 wk gestation were high (82.3 ± 21.0), especially among women from farming families, and remained so at 28 wk gestation (76.6 ± 23.2).

The birth weight of babies was not related to maternal energy intakes, proportion of energy from animal products or physical activity at 18 and 28 wk gestation. Because energy intake and physical activity were interrelated (r = 0.24, P

TABLE 1

Anthropometric measurements of the rural mothers before pregnancy and at 18 and 28 wk gestation and of their full-term babies[1]

Parameter	Maternal anthropometry			Neonatal anthropometry (n = 633)
	Prepregnancy (n = 633)	18 wk gestation (n = 633)	28 wk gestation (n = 594)	
Weight, *kg*	41.7 ± 5.1	43.8 ± 5.0	47.3 ± 5.2	2.665 ± 0.358
Height, *cm*	151.9 ± 5.1	—	—	47.8 ± 2.0
BMI,[3] *kg/m*^{2}	18.1 ± 1.9	18.9 ± 1.8	20.5 ± 1.7	2.5 ± 0.5[2]
Head circumference, *cm*	52.3 ± 1.5	—	—	33.1 ± 1.2
MUAC, *cm*	22.5 ± 1.8	22.3 ± 1.7	22.7 ± 1.7	9.7 ± 0.9
Skinfold, *mm*				
Subscapular	10.9 ± 4.0	12.4 ± 4.1	13.6 ± 4.4	4.2 ± 0.9
Triceps	8.9 ± 3.5	9.2 ± 3.3	9.8 ± 3.5	4.2 ± 0.9
Biceps	4.3 ± 1.8	4.3 ± 1.7	4.6 ± 1.8	—
Suprailiac	10.0 ± 5.2	13.2 ± 5.8	15.8 ± 6.6	—
Placental weight, *g*	—	—	—	360.0 ± 76.4

[1] Values are means ± SD.
[2] Ponderal index (kg/m^3).
[3] BMI, body mass index; MUAC, mid-upper arm circumference.

TABLE 2

Daily nutrient intake (modified 24-h recall) and physical activity scores of rural mothers at 18 and 28 wk gestation[1]

	18 wk gestation (n = 627)[2]	28 wk gestation (n = 609)[2]
Nutrient intakes		
Energy, *MJ/d*	7.40 ± 2.1	7.00 ± 2.0*
Protein, *g/d*	45.4 ± 14.1	43.5 ± 13.5*
Fat, *g/d*	34.9 ± 14.8	32.4 ± 14.0*
Animal protein,[3] *g/d*	7.0 ± 4.7	6.7 ± 4.5
Carbohydrate, *% energy*	72.1 ± 5.1	72.3 ± 5.0
Protein, *% energy*	10.3 ± 1.0	10.4 ± 1.1
Fat, *% energy*	17.5 ± 4.8	17.2 ± 4.6
Daily activity scores[4]		
Farming women	82.3 ± 21.0	76.6 ± 23.2*
Nonfarming women	43.6 ± 14.7	43.5 ± 14.6

[1] Values are means ± SD. * Different from 18 wk gestation, $P < 0.05$.
[2] Data were not available for 6 women at 18 wk and 24 women at 28 wk gestation.
[3] This relates to the 38% of women who ate animal proteins (meat, fish, eggs or milk products, excluding milk in tea/coffee) on the 24-h recall day.
[4] Number of farming women at 18 wk gestation was 481 and at 28 wk gestation 405, non-farming women at 18 wk gestation 146 and at 28 wk 204.

< 0.01 and 0.14, $P < 0.01$ at 18 and 28 wk gestation, respectively), we examined the association of energy intakes with birth weight after taking maternal size (prepregnancy weight, height and BMI) and maternal physical activity into account. There was no significant relationship. Similarly, protein and carbohydrate intakes (either absolute or as a percentage of total energy) were unrelated to birth measurements. In contrast, higher fat intake at 18 wk gestation was associated with greater neonatal length ($P < 0.001$) and triceps skinfold thickness ($P < 0.01$). After adjustment for maternal size and social status, fat intake at 28 wk gestation was associated only with birth length ($P < 0.05$).

Food groups (FFQ). Of the 17 food groups assessed using the FFQ, significant relationships with birth size were found with GLV, fruits and milk products.

GLV. The GLV eaten frequently (more than once a week) in this community were fenugreek leaves (57% of women), spinach (33%), coriander (16%) and colocasia (15%). The frequency of consumption of GLV at 28 wk was strongly related to all birth measurements (**Table 3**). These relationships remained significant after adjustment for prepregnancy weight (or height and BMI), energy intakes, physical activity score, weight gain during pregnancy and socioeconomic status (**Table 4**). An increase in frequency of consumption from one group to the next higher group was associated with an increase in birth weight of 19 g [95% confidence interval (CI), 8–30] after adjustments for all of these factors. The trend with birth weight was stronger (value of partial regression coefficient increased to 30 g; 95% CI, 13–47) among the lightest mothers, those with a prepregnancy weight below the lowest tertile (40 kg). The odds ratio for delivering a low birth weight baby was 0.43 (95% CI, −0.12 to 0.99) in mothers who ate GLV at least every other day compared with 1.0 in mothers who never ate them.

TABLE 3

Relationships between frequency of rural mothers' intakes of green leafy vegetables and fruits at 28 wk gestation and of milk at 18 wk gestation and neonatal anthropometry[1]

			Neonatal measurement							
Food group	Frequency	n	Birth weight	Birth length	Head circumference	MUAC[2]	Abdominal circumference	Triceps skinfold	Subscapular skinfold	Placental weight
			g	*cm*				*mm*		*g*
Green leafy vegetables, wk 28	Never	60	2571 ± 356	47.0 ± 2.0	32.6 ± 1.2	9.6 ± 1.0	28.2 ± 1.8	3.9 ± 1.4	3.9 ± 1.2	347 ± 69
	<1×/wk	175	2601 ± 341	47.5 ± 1.9	32.9 ± 1.2	9.6 ± 0.8	28.2 ± 2.0	4.0 ± 1.2	4.1 ± 1.2	354 ± 66
	>1×/wk	225	2675 ± 363	48.0 ± 2.0	33.2 ± 1.2	9.7 ± 0.9	28.6 ± 1.9	4.1 ± 1.2	4.0 ± 1.2	358 ± 82
	≥Alternate days	149	2742 ± 350	47.9 ± 1.9	33.3 ± 1.2	9.9 ± 0.9	29.1 ± 1.7	4.4 ± 1.2	4.3 ± 1.2	371 ± 81
$P1$			<0.001	<0.01	<0.001	<0.05	<0.001	<0.001	<0.05	<0.05
$P2$			<0.005	<0.05	<0.005	<0.05	<0.005	<0.001	<0.05	0.41
Fruits, wk 28	<1×/wk	44	2598 ± 340	47.5 ± 1.7	32.7 ± 1.1	9.7 ± 0.8	28.6 ± 2.0	4.1 ± 1.2	4.2 ± 1.2	352 ± 76
	>1×/wk	363	2633 ± 355	47.5 ± 2.0	32.9 ± 1.2	9.6 ± 0.9	28.5 ± 1.9	4.1 ± 1.2	4.1 ± 1.2	353 ± 75
	≥1×/d	202	2721 ± 357	48.1 ± 1.9	33.4 ± 1.2	9.8 ± 0.8	28.8 ± 1.9	4.1 ± 1.2	4.2 ± 1.2	370 ± 79
$P1$			<0.01	<0.01	<0.001	0.09	0.15	0.44	0.67	<0.05
$P2$			0.13	0.23	<0.01	0.80	0.45	0.38	0.99	0.07
Milk products, wk 18	Never	95	2643 ± 369	47.5 ± 2.0	32.9 ± 1.2	9.6 ± 1.0	28.5 ± 2.1	4.2 ± 1.2	4.1 ± 1.2	354 ± 78
	<1×/wk	134	2618 ± 356	47.6 ± 2.0	33.0 ± 1.2	9.7 ± 0.9	28.6 ± 1.7	4.1 ± 1.2	4.1 ± 1.2	348 ± 79
	>1×/wk	116	2639 ± 344	47.6 ± 2.0	33.0 ± 1.1	9.5 ± 0.8	28.5 ± 1.9	4.1 ± 1.2	4.1 ± 1.2	352 ± 71
	≥Alternate days	281	2704 ± 361	48.0 ± 2.0	33.2 ± 1.3	9.8 ± 0.9	28.8 ± 2.0	4.1 ± 1.2	4.1 ± 1.2	371 ± 77
$P1$			<0.05	<0.05	<0.01	<0.05	0.15	0.90	0.41	<0.01
$P2$			0.14	0.13	<0.01	0.11	0.52	0.48	0.46	<0.01

[1] Values are means ± SD. $P1$ values after adjustment for sex, parity and gestational age at delivery. $P2$ values after additional adjustment for prepregnancy weight, energy intake, activity, social class, weight gain up to 28 wk and relevant micronutrients and macronutrients (for green leafy vegetables, erythrocyte folate concentration; for fruits, serum vitamin C concentration; for milk products, fat intake).
[2] MUAC, mid-upper arm circumference.

TABLE 4

Multiple regression analysis of the relationship of the frequency of maternal intakes of green leafy vegetables, fruits and milk products with birth weight among rural Indian women[1]

Dependent variable	Independent parameters	Green leafy vegetable intake at 28 wk gestation			Fruit intake at 28 wk gestation			Milk product intake at 18 wk gestation		
		R^2	β	P	R^2	β	P	R^2	β	P
Birth weight, *g*	Sex, parity, gestation at birth	22.1 (1.8)	19.1	<0.001	21.4 (1.0)	7.4	<0.01	20.5 (0.7)	6.9	0.08
	Sex, parity and gestation at birth *plus* prepregnancy weight,[2] energy intake, activity, social status and weight gain up to 28 wk and micronutrients and macronutrients[3]	32.6 (1.8)	19.4	<0.001	29.8 (0.34)	4.3	0.12	29.4 (0.4)	4.8	0.06

[1] Dietary variables were grouped (as in Table 3) and were weighted to reflect as closely as possible the consumption per week. Therefore, the β coefficient represents the increase in birth weight associated with increased consumption of that food group by one time per week. Values in parentheses represent the contribution of dietary variables to R^2.

[2] Replacement of prepregnancy weight with height and body mass index did not change the relationship (R^2, β values and significance).

[3] Indicates further adjustment for other variables, including micronutrients and macronutrients: for green leafy vegetables, erythrocyte folate concentration; for fruit, serum vitamin C concentration; for milk products, fat intake. The exclusion of gestation from this regression reduced R^2 values for the three food groups to 22.0, 17.8 and 16.9%, respectively, whereas the contribution of dietary variables increased to 2.05, 0.5 and 0.4%, respectively.

Fruits. Pregnant women had relatively high intakes of "vitamin C–rich" fruits, which were freely available from trees growing in the fields. These fruits included zizapus (eaten more than once a week by 58% of women), raw tamarind (48%) and guava (40%). The frequency of consumption of fruits at 28 wk gestation was related to birth weight, birth length, head circumference and placental weight but not to measures of neonatal fat, MUAC or abdominal circumference (Table 3). These relationships remained significant after adjustment for prepregnancy weight (or height and BMI), energy intake, physical activity score and socioeconomic score but not after adjustment for weight gain (Table 4). Similar to GLV, relationships were stronger among lighter mothers. An increase in the frequency of fruit consumption from one group to the next higher group was associated with an increase in birth weight of 4 g (95% CI, −1 to 10) in the sample as a whole and 15 g (95% CI, 6–24) in women with a prepregnancy weight of <40 kg (Table 4).

Milk products. Milk was consumed mostly by women from families who owned milk-producing animals. It was consumed either with roti or rice or was drunk but rarely was consumed in the form of other milk products. Milk consumption was strongly associated with socioeconomic score ($P < 0.001$). The frequency of milk consumption at 18 wk gestation was related to birth weight, birth length, MUAC, head circumference and placental weight but not to measures of neonatal fat or abdominal circumference (Table 3). In contrast to GLV and fruits, these relationships were stronger at 18 wk' than at 28 wk gestation. They were similar at all levels of prepregnancy maternal weight and remained significant after adjustment for prepregnancy weight (or height and BMI), energy intakes, physical activity score, socioeconomic status and weight gain (Table 4).

In all of these regression analyses, the major contribution was from main confounding variables (gestation, sex and parity: ~20%). The contributions of dietary variables varied from 2 to 3% but were significant.

Biochemical indices of maternal nutrition

Concentrations of erythrocyte folate, serum ferritin and serum vitamin C [median (interquartile range)] were 868 nmol/L (687–1097), 13 μg/L (8–23) and 10 μmol/L (2–31) at 18 wk gestation and 968 nmol/L (741–1273), 10 μg/L (7–20) and 6 μmol/L (1–22) at 28 wk gestation. All three micronutrients were related to birth size (**Table 5**). The strongest and most consistent relationship was with 28-wk erythrocyte folate concentration, which was positively related to birth weight, birth length, head circumference, MUAC, abdominal circumference and placental weight but not to skinfold thickness. Higher serum vitamin C concentration at 28 wk gestation was associated with higher birth length, MUAC and abdominal circumference (Table 5), and higher concentration at 18 wk gestation was associated with higher MUAC ($P < 0.05$) and triceps skinfold thickness ($P < 0.05$). In contrast, ferritin concentration at 28 wk gestation was inversely related to birth length, MUAC and abdominal circumference (Table 5). All of these relationships remained significant after adjustment for prepregnancy weight (or height and BMI), energy intakes, physical activity, weight gain and socioeconomic status.

GLV are a rich source of folate. Higher intakes at 28 wk gestation were associated with higher erythrocyte folate concentration (1099 nmol/L in women who ate GLV at least once every other day compared with 949 nmol/L in those who never ate them, $P < 0.05$). The relationship between intake of GLV with birth weight remained significant after adjustment for erythrocyte folate concentration (Table 4). Similar analysis revealed that this relationship was also strong for all other neonatal measurements. GLV are also a rich source of iron. GLV intakes were not, however, related to serum ferritin concentration. Fruits consumed by rural mothers were rich in vitamin C, and higher fruit intakes were associated with higher serum vitamin C concentration (19 μmol/L in women who ate fruit at least once daily compared with 11 μmol/L in those who ate fruit less than once a week; $P < 0.05$). The relationship of fruit intake to birth weight was not significant ($P = 0.13$) after adjustment for serum vitamin C concentra-

TABLE 5

Relationship's between rural mothers' erythrocyte folate, serum ferritin and serum vitamin C concentrations at 28 wk gestation and neonatal anthropometry[1]

Blood nutrient	Concentration	*n*	Neonatal measurement							
			Birth weight	Birth length	Head circumference	MUAC	Abdominal circumference	Triceps skinfold	Subscapular skinfold	Placental weight
			g	*cm*				*mm*		*g*
Erythrocyte folate, *nmol/L*	<816	171	2616 ± 360	47.5 ± 1.9	33.0 ± 1.3	9.5 ± 0.9	28.4 ± 2.0	4.1 ± 1.2	4.2 ± 1.2	347 ± 78
	816–1147	171	2637 ± 369	47.7 ± 2.3	32.9 ± 1.2	9.7 ± 0.8	28.6 ± 1.7	4.1 ± 1.2	4.1 ± 1.2	356 ± 66
	≥1147	172	2727 ± 347	48.1 ± 1.8	33.3 ± 1.2	9.8 ± 0.9	28.9 ± 1.9	4.2 ± 1.2	4.1 ± 1.2	374 ± 83
P[1]			<0.001	<0.05	<0.05	<0.005	<0.005	0.19	0.64	<0.001
P[2]			<0.001	0.08	0.12	<0.01	<0.01	0.26	0.79	<0.001
Serum ferritin, *μg/L*	<8	162	2695 ± 362	47.7 ± 2.0	33.1 ± 1.3	9.8 ± 0.8	28.9 ± 1.7	4.2 ± 1.2	4.2 ± 1.2	361 ± 76
	8–16	196	2657 ± 350	47.8 ± 2.0	32.9 ± 1.2	9.7 ± 0.8	28.6 ± 1.8	4.2 ± 1.2	4.1 ± 1.2	364 ± 65
	≥16	185	2649 ± 378	47.7 ± 2.1	33.2 ± 1.3	9.6 ± 1.0	28.5 ± 2.1	4.1 ± 1.2	4.1 ± 1.2	355 ± 90
P[1]			0.20	<0.05	0.85	<0.01	<0.05	0.18	0.22	0.21
P[2]			0.18	<0.05	0.86	<0.01	<0.05	0.28	0.25	0.15
Serum vitamin C, *μmol/L*	<4	254	2647 ± 343	47.6 ± 2.0	33.0 ± 1.2	9.7 ± 0.8	28.5 ± 1.9	4.1 ± 1.2	4.1 ± 1.2	356 ± 75
	4–19	169	2679 ± 353	47.8 ± 2.0	33.1 ± 1.2	9.6 ± 0.9	28.7 ± 1.7	4.2 ± 1.2	4.1 ± 1.2	360 ± 79
	≥19	146	2688 ± 396	47.9 ± 2.0	33.1 ± 1.3	9.8 ± 1.0	29.0 ± 2.1	4.2 ± 1.2	4.2 ± 1.2	367 ± 78
P[1]			0.23	<0.05	0.70	<0.01	<0.01	0.07	0.59	0.92
P[2]			0.29	0.07	0.70	<0.05	<0.05	0.15	0.75	0.84

[1] Values are means ± SD. *P*[1] values after adjustment for sex, parity and gestational age at delivery. *P*[2] values after additional adjustment for prepregnancy weight, energy intake, activity, social class and weight gain up to 28 wk gestation.

tion (Table 4) but remained significant for head circumference. Similarly, milk was a major contributor (29%) to total fat intake, and after adjustment for fat intakes, the relationship of milk consumption with birth weight (Table 4) was not significant ($P = 0.10$) but remained significant for neonatal length ($P < 0.005$) and triceps skinfold thickness ($P < 0.01$).

DISCUSSION

We studied the size of rural Indian newborn babies in relation to their mothers' diets and circulating micronutrient concentrations in pregnancy. The strengths of our study were that it was community based, wherein prepregnancy maternal anthropometry and accurate gestational age were recorded. We also developed community-specific methods for estimating dietary intakes objectively, and we report data on the consumption of various food groups as well as nutrient intakes.

Rural mothers were thin and short, indicating that many were chronically energy deficient before conception. Although their mean weights and heights were similar to those reported from other rural areas of India (Vijayalaxmi et al. 1988), they were significantly less than those of urban affluent Indian (Devi et al. 1989, Gupta and Sharma 1980, Piers et al. 1995) and Western (Godfrey et al. 1996) mothers. The neonatal size was similar to that reported in other Indian populations (Mohan et al. 1990) but markedly smaller than that of Western babies (Godfrey et al. 1996).

Maternal intakes of energy and protein were low, ~70–75% of recommended intakes (ICMR 1987) at both time points, and showed no significant relationships with neonatal size. For energy intakes, the lack of any relationship with birth size was true for the whole study group, within subgroups of maternal prepregnancy weight and after taking physical activity into account. It was also true when women with extremely low intakes (<1.2 basal metabolic rate, $n = 150$) were excluded. These important negative findings are consistent with the slight effects on birth size of energy and protein supplementation trials in pregnancy (Kramer 1993).

Among the macronutrients, only fat intake at 18 wk gestation showed an association with birth size. Fat intake has been shown to correlate with birth weight (Doyle et al. 1982), but there are few data for fat reported as an entity separate from energy. Recent studies suggest that specific fatty acids are important for fetal growth (Crawford et al. 1989), which brings into question whether fat is a macronutrient or micronutrient.

Birth size was strongly related to intakes of GLV and fruits at 28 wk gestation and of milk at 18 wk gestation. These three food groups are particularly rich in micronutrients. Our observations therefore suggest the importance of specific micronutrients, or their combinations, for fetal growth. For example, GLV are a rich source of folate, iron, provitamin A carotenoids and antioxidants. Increased frequency of the consumption of GLV was associated with an increase in all neonatal anthropometry, and the relationship with birth size remained significant even after correction for red cell folate concentration in blood, suggesting that nutrients other than folate contribute to the relationship. Similarly, fruits are rich in vitamin C and other antioxidants, whereas milk provides high quality proteins, fat, calcium, riboflavin and vitamins A and D. It was interesting to note that a greater consumption of fruits or milk was not associated with increased measures of neonatal fat or abdominal circumference, as occurred for the consumption of GLV. Furthermore, the relationship with fruit consumption when corrected for circulating vitamin C concentration in blood did not remain significant for group as a whole but was significant only for thin mothers. Similarly, the relationship of milk consumption after adjustment for fat intake did not remain significant for birth weight but was significant for length and triceps skinfold thickness. This suggests important roles of different micronutrients in improving fetal growth.

One of the causal pathways for the effect of micronutrients could be an increase in the gestational period. The exclusion of gestation from regression analyses in Table 4 showed marginal increase in the contribution of respective dietary variables in R^2 and keeps the relationship significant. However, its inclusion explains up to 15% of the variability in birth weight and substantially increases the overall R^2. This suggests that the effect of micronutrient intakes on fetal growth is in part mediated through the lengthening of the gestation.

Birth measurements were related to intakes of GLV and fruit at 28 wk, whereas they were related to fat and milk intakes at 18 wk. This may reflect different nutrient requirements for fetal growth at these times due to the development of different tissues at various stages of gestation (Dugdale and Payne 1975, Tanner 1989). The relationships of GLV and fruit intake to birth size were strongest in lighter and thinner women. We did not detect any significant difference in other factors (parity, macronutrient intake, blood pressure and glycemic status) associated with fetal growth in these women compared with the remainder of the group. It is likely that micronutrients may be the most important limiting nutrients in undernourished women.

We found inverse relationships between maternal serum ferritin concentration at 28 wk gestation and neonatal size. This somewhat paradoxical finding has been reported in other populations (Goldenberg et al. 1996) and might be explained by hemodilution effects due to plasma volume expansion, which is associated with increased fetal growth (Rosso et al. 1992). Because ferritin represents a stored form of iron, an alternative explanation is that a lower ferritin concentration in the mother signifies the successful mobilization of iron, making it available for fetal growth. It is interesting, in this respect, that the inverse relationships of serum ferritin concentration were strongest with neonatal MUAC (representing muscle) and abdominal circumference (representing viscera), tissues with a high iron content.

To our knowledge, this study is the first to show an association between maternal intakes of GLV, fruits and milk and size at birth . Furthermore, the significant relationship between biomarkers (erythrocyte folate and serum vitamin C) and the frequency of consumption of GLV and fruits, respectively, provides a measure of confidence in our FFQ assessment. Although we cannot assume cause-and-effect relationships in any of the associations we have described, our data suggest that improved maternal intakes of milk in early gestation and of GLV and fruit in late gestation could lead to improved fetal growth. There may be concern in attributing significance to these foods because we had conducted multiple analyses of 17 food groups. We did, however, start with the a priori hypothesis that micronutrient-rich foods may be important for fetal growth. If the P-value was corrected for these 17 food groups, making $P = 0.003$ the cutoff for statistical significance, the relationship between birth size and GLV remains significant.

There is controversy as to whether food or pharmacology is the best means of providing micronutrients (West 1996). There is some evidence that supplementation with folate in pregnancy leads to improved fetal growth (Baumslag et al. 1970, Ek 1982, Goldenberg et al. 1992, Iyengar and Rajalakshmi 1975). However, an evaluation of India's long-standing anemia prophylaxis program, with routine iron and folate supplementation to women in the third trimester of pregnancy for the past two decades, demonstrates no significant impact on birth weight (ICMR 1989). It may be that the micronutrient-rich foods discussed here provide a more effective combination of nutrients than do conventional supplements that contain only one or two micronutrients or macronutrients. Thus, food-based interventions may be more beneficial. There are limited data from controlled trials on the effects of micronutrients on fetal growth. Most have shown a small or no effect (Mathews 1996, Onis et al. 1998). The long-term implications of our findings that specific maternal food intakes are related to different components of neonatal phenotype are unknown at this stage and could be best understood by following these babies to determine infant mortality rates, childhood growth rates and long-term health. Further research, in animals and humans, is needed to identify the important nutrients provided by these key food groups and their mode of action. We also believe an intervention trial should be attempted to study the effects of increased availability of these foods for pregnant women.

ACKNOWLEDGMENTS

We are grateful to the community and to the pregnant women and their families for their cooperation. We would like to thank David Collis and the staff of the Special Hematology Laboratory (Southampton General Hospital, Southampton, U.K.) for the ferritin and folate assays and Chris Bates, Glynn Harvey and Jonathan Perkins (Medical Research Council Resource Center for Human Nutrition Research, Cambridge, U.K.) for the vitamin C assays. We also thank A. D. Agate (Director, Agharkar Research Institute), V. N. Rao (Director, the King Edward Memorial Hospital Research Center) and K. J. Coyaji (Director, Department of Obstetrics and Gynecology, King Edward Memorial Hospital) for providing the facilities for this collaborative research. We acknowledge the contributions made to the study by Arun Kinare, Monesh Shah, Asit Natekar, Manoj Chinchwadkar, Binu John, Anuja Bisht, Mahananda Bhavikatti, Poonam Gupta, Charu Joglekar, Parveen Bharucha and Vanessa Cox.

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