Maternal Protein Restriction Before Pregnancy Affects Vital Organs of Offspring in Wistar Rats

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Epidemiologic studies indicate that undernutrition during fetal growth can have long-term effects on adult health. However, it is not known whether these effects are also associated with maternal undernutrition before conception. The objective of the present study was to examine the effect of dietary restriction before pregnancy on the vital organs and blood parameters of offspring at different time points. Wistar female rats in the restricted group were fed a diet consisting of 80 g protein/kg for 8 weeks before pregnancy and switched to 160 g protein/kg (control) from day 0 of pregnancy, while animals from the control group were fed 160 g protein/kg throughout life. The progeny were studied at birth (n = 71), at 94 days (n = 20), and at 180 days (n = 16). Weight gain during pregnancy was significantly lower ($P < .01$) for dams in the restricted group. At birth, relative weight for brain was lower ($P \leq .008$), while for kidney it was higher ($P \leq .008$) in the restricted group compared to control. At 94 days, the relative weights of brain, liver, and heart were lower ($P \leq .01$ for all) in the restricted group than in the control group. However, at 180 days, only liver and kidney showed lower ($P \leq .01$ for both) relative weights. Further, in the restricted group, increases in blood glucose at 94 days and in cholesterol at 180 days were significant ($P < .01$ for both) in the offspring. The results thus indicate that maternal undernutrition before conception not only affected growth of vital organs, but also resulted in increased levels of glucose and cholesterol in the offspring at adulthood.

RECENT EVIDENCE suggests that many adult degenerative diseases such as hypertension, ischemic heart disease, diabetes mellitus, and cardiovascular diseases may originate from an adverse environment both in utero and during early life.[1-4] However, the relationship between maternal undernutrition, size at birth, and adult diseases is not yet clearly understood. Studies in humans show that the relationship differs among adolescents[5] and among women from low socioeconomic class,[6] who often have poor nutritional status before conception. This is seen even in developed countries like Austria, where women are thin mainly because of cosmetic undernutrition.[7] In fact, a study from rural Maharashtra, India, reported that size at birth was strongly predicted by maternal pre-pregnancy nutritional status.[8] Therefore, the role of maternal nutrition, especially preconception nutritional status, in determining the risks for adult diseases is a subject of immense interest. Clearly, animal models, which are useful for dietary manipulations[9] during critical periods, are essential in resolving these issues.

Studies in rats have largely dealt with dietary restrictions either during pregnancy[10] alone or during the entire period of pregnancy and lactation.[11,12] These studies revealed effects that appear to be organ-selective and sex-dependent[12] and suggested a long-term programming influence on glucose and lipid metabolism,[9,13] as well as hypertension,[14] in adult life. However, dietary restrictions prior to conception are rarely studied. Because undernutrition before implantation is known to lead to a high incidence of failure to maintain pregnancy,[15] it becomes necessary to understand the effects of dietary restriction before conception on growth and development of the surviving offspring. The present study therefore examines the effect of undernutrition prior to pregnancy on organ growth and adult blood variables such as cholesterol and glucose.

MATERIAL AND METHODS

Diet

Two isoenergetic cereal pulse-based diets containing 160 g protein/kg and 80 g protein/kg were formulated using National Institute of Nutrition (NIN) guidelines[16] (Table 1). As pulses are major sources of protein in Indian vegetarian diets, the Bengal gram was considered in formulating the diets. It contains 20.8 g protein, 5.6 g fat, 59.8 g carbohydrate, and 372 kcal (1,556 kJ) per 100 g. Food was pressure cooked daily, analyzed for moisture, protein content using the Kjeltec auto analyzer (Tecator, Hoganas, Sweden), and fat content using the Soxhlet extraction method, and energy was calculated.[16,17] All rats had free access to feed and deionized water.

Animals

Virgin female Wistar rats obtained from Hindustan Antibiotics (Pune, India), having initial body weights of 130 to 150 g, were maintained at 24°C on a 12-hour light/dark cycle. Rats were acclimated to the 160 g protein/kg diet for 1 month. Rats in the weight range of 180 to 200 g were then selected for breeding. Mating was confirmed by sperm-positive vaginal smear. The pregnant females (n = 11) were individually caged and observed throughout gestation, and were allowed to deliver spontaneously. Pups were separated according to sex on day 21. Twenty female rats were randomly selected and then divided into 2 groups each: control (n = 10) and restricted (n = 10). However, only 9 rats in the control group and 6 rats in the restricted group conceived and were considered for the study.

Experimental Design

Control group. Female rats in the control group received a 160 g protein/kg diet throughout the experiment, ie, before, during pregnancy, and while lactating.

Restricted group. Female rats in this group were fed 160 g protein/kg during the first 3 months and then were shifted to a diet consisting of 80 g protein/kg for 8 weeks, after which they were kept for mating. From day 0 of pregnancy, rats in the restricted group were restarted on the 160 g protein/kg diet, which was maintained through-

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Table 1. Composition of Restricted and Control Diets

Ingredients	Protein	
	80 g/kg	160 g/kg
Wheat flour	340	650
Bengal gram (whole)	80	50
Whole milk powder	60	200
Groundnut oil	35	55
Mineral mixture*	35	35
Vitamin mixture†	10	10
Starch	440	—

*Provides (g/kg): calcium phosphate 500, sodium chloride 74, potassium citrate 220, potassium sulfate 52, magnesium oxide 24, manganese carbonate 3.5, ferric citrate 6, zinc carbonate 1.6, cupric carbonate 0.3, potassium iodate 0.01, sodium selenite 0.01, chromic potassium sulfate 0.55, sucrose 118.03.

†Provides (mg/100 g of diet): vitamin A 2,500 IU, vitamin D_3 500 IU, thiamine hydrochloride 0.5, riboflavin 0.5, pyridoxine hydrochloride 0.5, sodium pantothenate 1, nicotinamide 2.5.

out gestation as well as lactation. Thus, the restricted group was exposed to protein restriction for a period of 8 weeks prior to pregnancy. For all rats in the control and restricted groups, a daily record was kept of food given and remaining.

It was decided to deliver half of the dams (randomly selected) from each group by caesarean section on day 20 of pregnancy to obtain weights of placenta. The remaining half was allowed to deliver spontaneously. In the former case, pups were dissected at birth for determination of weights of vital organs, namely, brain, heart, liver, kidney, and pancreas, while in latter case pups were grown and studied at 94 days and 180 days for organ weights, blood glucose, and cholesterol. All weights are reported for fresh organs.

Organ Weights at Birth

Dams that were delivered by caesarean section gave birth to 41 pups in the control group and 30 pups in the restricted group. Placenta and vital organs of the pups were washed in saline, blotted, and weights recorded on a Mettler balance (Afcoset, Tokyo, Japan) with a sensitivity of 0.001 g.

Organ Weights at Later Ages

Pups from spontaneous delivery (20 control and 16 restricted) were grown and studied at adult age. Their body weights were recorded weekly. Half of the pups from the control group as well as the restricted group were randomly selected and dissected at 94 days, while the remaining half from each group was dissected at 180 days to obtain organ weights. Animal maintenance and handling were in accordance with NIN, India guidelines.[16]

Comparisons of relative weights may suffer from the limitation that they cannot reflect the compositional changes, if any, owing to dietary restriction. All of the vital organs were therefore analyzed for moisture and fat content. Organs were freeze-dried and moisture was estimated. They were ground to a powder and stored at −20°C. Fat content in the organs was determined by extraction with petroleum ether (40° to 60°C boiling point, Soxhlet fat extraction system).

Blood Samples

Dams that were delivered by caesarean section were fasted overnight and blood samples were taken the next morning via heart puncture under anesthesia with diethyl ether. Fasting blood samples were collected in the similar way for pups at 94 days and 180 days of age. Blood samples were centrifuged, and the serum was separated and stored at −20°C and subsequently analyzed using diagnostic kits for glucose[18] and cholesterol.[19]

Statistical Analysis

Results are expressed as means ± SD. Data were analyzed using the SPSS/PC+ package (Version 4.0; SPSS, Inc, Chicago, IL). The difference between the 2 groups was tested using Students *t* test at conventional levels of significance, namely, 5% or 1%. Comparisons for the restricted and control groups were made after adjusting for litter size using analysis of covariance (ANCOVA).

RESULTS

Intake of Pregnant Rats

As a result of pregnancy, mean daily feed intake (dry basis) of control dams increased from 8.11 ± 1.5 g to 9.72 ± 2.0 g during the first week of gestation. It further increased considerably (Table 2) in the last week of gestation (11.8 ± 1.5 g). The daily feed intake of dams in the restricted group before pregnancy was similar (7.9 ± 1.4 g) to that of controls, but increased substantially to 10.03 ± 1.3 g by the first week of gestation. Dams in the restricted group received higher protein from day 0 of pregnancy, when they were shifted to the control diet. The substantial increase in feed intake thus reflects the effect of protein restriction in addition to the effect of pregnancy. However, the subsequent increase in feed intake in this group was negligible. Rats in the control group thus had an energy intake of 226 kJ/d in the last week of gestation, while rats in the restricted group had an energy intake of 197 kJ/d.

Weight Gain During Pregnancy

Female rats from the restricted group had a smaller weight gain in every week of pregnancy, but the difference was largest (27.3 ± 18.7 *v* 46.4 ± 10.6 g) and statistically significant ($P <$

Table 2. Dietary Intakes During Protein Restriction and During Pregnancy

Variables	Control Group	Restricted Group
Initial no. of female rats	10	10
Dams conceived	9	6
Average intake during protein restriction		
Feed intake (g/d), dry	8.11 ± 1.5	7.9 ± 1.4
Energy (kJ/d)	155 ± 28.0	143 ± 24.7
Protein (g/d)	1.26 ± 0.2	0.7 ± 0.1*
Average intake during pregnancy		
Week 1		
Feed intake (g/d)	9.72 ± 2.0	10.03 ± 1.3
Energy (kJ/d)	184 ± 38.0	192 ± 25.5
Protein (g/d)	1.5 ± 0.3	1.6 ± 0.2
Week 2		
Feed intake (g/d)	9.9 ± 1.9	11.3 ± 1.3
Energy (kJ/d)	188 ± 35.5	213 ± 24.6
Protein (g/d)	1.54 ± 0.3	1.8 ± 0.2
Week 3		
Feed intake (g/d)	11.8 ± 1.5	10.3 ± 2.4
Energy (kJ/d)	226 ± 28.4	197 ± 32.0
Protein (g/d)	1.85 ± 0.3	1.6 ± 0.4

*$P <$ .01 for differences in control and restricted group.

Table 3. Weight Gain During Pregnancy and Birth Outcome

Variables	Control Group	Restricted Group
No. of dams	9	6
Weight gain during pregnancy(g)		
Week 1	13.97 ± 8.6	11.1 ± 6.8
Week 2	20.8 ± 7.7	14.9 ± 9.5
Week 3	46.4 ± 10.6	27.3 ± 18.7*
Total weight gain (g)	81.15 ± 9.59	53.35 ± 14.65†
Litter size	9.33 ± 1.9	7.8 ± 2.8
Litter weight (g)	35.01 ± 14.8	32.87 ± 7.79
Birth weight (g)	3.90 ± 1.71	4.64 ± 1.68

*$P < .05$ for differences in control and restricted group.
†$P < .01$ for differences in control and restricted group.

.05) only in the last week of pregnancy. The mean total weight gain during pregnancy (Table 3) for the 2 groups was significantly ($P < .01$) different (81.15 ± 9.59 g in control *v* 53.35 ± 14.65 g in restricted rats). It should be mentioned that the estimates of mean weight gains during pregnancy are more accurate for dams that were delivered by caesarean section than for those that delivered spontaneously. Thus, for rats delivered by caesarean section, mean weight gains were 78.7 g (171.1 g at conception to 249.8 g at delivery) for dams in the control group and 49.1 g (205.5 g at conception to 254.6 g at delivery) for dams in the restricted group. The higher weight gain in the control group during the third week of pregnancy may be due to the marginally higher energy and protein intake.

Litter Weight

Litter means were used as the unit of analysis. Mean litter weight for control and restricted groups did not differ significantly (35.01 ± 14.8 *v* 32.87 ± 7.79g). Mean litter size also was not statistically different between the 2 groups (9.33 ± 1.9 *v* 7.8 ± 2.8). Mean birth weight was marginally higher in the restricted group (4.64 ± 1.68 g) as compared to the control group (3.90 ± 1.71 g), despite lower maternal weight gain.

Organ Weights at Birth

The absolute weights of all vital organs (heart, liver, kidney, brain, and pancreas) were significantly higher ($P \leq .008$) for rats in the restricted group than in the control group (Table 4). However, the relative weight of brain was lower ($P \leq .008$) for pups in the restricted group than in the control group, indicating an adverse effect. Although relative weights for other organs were higher than controls, the differences were significant ($P \leq .008$) only in the case of kidney, indicating that this organ is better protected at birth.

Organ Weights at 94 Days

Absolute organ weights (except brain) for the restricted group continued to be higher but nonsignificant at 94 days in comparison to control rats (Table 4). Brain, liver, and heart weights were significantly ($P \leq .01$ for all) lower when expressed relative to body weight. Lowering of relative organ weight thus can be attributed to the effect of protein restriction. However, comparison of relative organ weights may not reflect whether there were any changes in the composition. We therefore examined the moisture and fat content of the organs.

The relative fat weights (ie, [fat mass/body weight] × 1,000) of all organs were compared between the 2 groups. Animals in the restricted group had a higher ($P = .055$) relative fat mass (1.77 ± 0.4 g) than those in the control group (1.44 ± 0.3 g) for liver. Thus, at 94 days, although the relative weight was lower, the relative fat weight of liver was higher for the animals in the restricted group. There was no difference in relative fat weights for other organs.

Table 4. Mean Absolute Weight and Relative Organ Weights at Birth, 94 Days, and 180 Days

	At Birth*		At 94 Days†		At 180 Days†	
	Control	Restricted	Control	Restricted	Control	Restricted
No. of pups	41	30	10	10	10	6
Intake (g)			13.39 ± 2.71	16.83 ± 4.56	13.79 ± 3.63	11.89 ± 1.64
Body weight (g)	2.12 ± 0.13	3.15 ± 0.50‡	263.12 ± 56.48	341.12 ± 90.29‡	344.50 ± 96.3	377.03 ± 100.5
Absolute weight (g)						
Placenta	0.6118 ± 0.055	0.7515 ± 0.093‡				
Brain	0.1283 ± 0.010	0.1616 ± 0.009‡	1.7320 ± 0.080	1.6733 ± 0.170	1.8366 ± 0.117	1.7545 ± 0.12
Liver	0.1617 ± 0.020	0.2382 ± 0.048‡	10.5586 ± 3.117	11.1027 ± 3.644	11.6275 ± 4.15	10.5370 ± 3.02
Heart	0.0142 ± 0.0033	0.0221 ± 0.003‡	0.8548 ± 0.137	0.9474 ± 0.188	0.9439 ± 0.224	0.9757 ± 0.262
Kidney	0.0170 ± 0.003	0.0297 ± 0.005‡	1.7340 ± 0.466	2.0271 ± 0.572	1.9114 ± 0.535	1.8494 ± 0.493
Pancreas	0.0066 ± 0.002	0.0100 ± 0.002‡	0.4822 ± 0.076	0.7034 ± 0.25	0.4898 ± 0.102	0.4436 ± 0.098
Relative weight (%)						
Placenta	27.80 ± 7.04	24.5 ± 2.66				
Brain	6.10 ± 0.45	5.33 ± 0.59‡	0.69 ± 0.14	0.52 ± 0.12‡	0.57 ± 0.15	0.50 ± 0.12
Liver	7.65 ± 0.61	7.72 ± 0.94	3.96 ± 0.36	3.23 ± 0.41‡	3.33 ± 0.32	2.81 ± 0.15‡
Heart	0.67 ± 0.13	0.73 ± 0.14	0.33 ± 0.03	0.29 ± 0.03‡	0.28 ± 0.03	0.26 ± 0.02
Kidney	0.80 ± 0.11	0.97 ± 0.12‡	0.65 ± 0.05	0.60 ± 0.05	0.56 ± 0.04	0.49 ± 0.02‡
Pancreas	0.31 ± 0.07	0.33 ± 0.06	0.19 ± 0.04	0.21 ± 0.07	0.15 ± 0.03	0.12 ± 0.02

*Level of significance for this group after allowing for multiple comparisons is $P < .008$.
†Level of significance for these groups after allowing for multiple comparisons is $P < .01$.
‡Statistical significance between control and restricted group mean values at *P* levels adjusted for multiple comparisons as given above.

Table 5. Mean Blood Glucose and Cholesterol Values for Dams and Pups at 94 Days and 180 Days

	Glucose (mmol/L)		Cholesterol (mmol/L)	
	Control Group	Restricted Group	Control Group	Restricted Group
Dams (n)	5.61 ± 1.23 (4)	1.79 ± 0.27* (3)	4.58 ± 0.19 (4)	1.79 ± 0.33* (3)
Pups at 94 d				
Males (n)	6.24 ± 1.25 (5)	12.58 ± 3.35* (5)	2.93 ± 0.46 (5)	3.64 ± 0.61 (5)
Females (n)	6.08 ± 1.13(5)	9.56 ± 0.80* (5)	2.91 ± 0.35 (5)	2.82 ± 0.32 (5)
Pooled	6.16 ± 1.20 (10)	11.07 ± 2.86* (10)	2.92 ± 0.41 (10)	3.23 ± 0.63(10)
Pups at 180 d				
Males (n)	8.31 ± 2.53 (5)	10.04 ± 2.66 (3)	3.09 ± 0.62 (5)	4.74 ± 0.17* (3)
Females (n)	6.72 ± 3.31 (5)	9.74 ± 0.95 (3)	2.96 ± 0.49 (5)	4.36 ± 0.19* (3)
Pooled (n)	7.52 ± 3.05 (10)	9.89 ± 2.01 (6)	3.03 ± 0.56(10)	4.55 ± 0.26* (6)

*$P < .01$ for differences in control and restricted group.

Organ Weights at 180 Days

Absolute weights of all organs except heart in the restricted group were lower, but were not significant at 180 days (Table 4). Relative weights of kidney ($P \leq 0.01$) and liver ($P \leq .01$) were significantly lower in the restricted group when compared to control animals, which indicates that there was no protective effect at 180 days. The relative fat weight of kidney was also significantly ($P < .05$) lower for animals in the restricted group than those in the control group. No significant difference was observed for liver weights.

Blood Analysis

Dams from the restricted group had lower blood glucose and cholesterol levels as compared to the control group (Table 5). In contrast, pups (both male and female) born to dams from the restricted group had higher ($P < .01$) blood glucose at 94 days and higher ($P < .01$) cholesterol levels at 180 days.

DISCUSSION

Animal experiments are critical for investigating the impact of maternal undernutrition on vital organs of offspring. Most studies[10-12] investigating effects of dietary restrictions on growth of vital organs of offspring imposed restrictions exclusively during gestation or during the entire period of gestation and lactation, but rarely before implantation. Furthermore, reported studies[20] have compared relative weights of organs, whereas differences in organ composition are only speculative. In the present study, the fat content of the organs was estimated. Our study demonstrates the effect of protein restriction for 8 weeks prior to conception on organ weights and their fat content, as well as blood glucose and cholesterol levels.

The major findings of our study are discussed with consideration to some of the following points. Power calculations are possible in epidemiologic studies, but not in animal studies, as we had little knowledge about the variability in the parameter of investigation—an essential prerequisite for estimating number using power calculations. In view of the fact that reported studies[12,20] have used 5 to 6 animals per group for comparison of growth of vital organs, 10 dams were used initially in each group to obtain the desired number of pups at subsequent time points. In addition, our diets were isocaloric and as such the findings highlight the effects of protein rather than energy restriction.

Our observations indicate that undernutrition may affect the rate of conception (60% conceived in the restricted group *v* 90% in the control group). Reproductive physiology is influenced by food availability and it is reported that in female mammals when food intake is limited, reproductive attempts are suspended in favor of processes necessary for individual survival.[21] In fact, infertility is seen in a number of situations like famine.

Most reported studies have shown that protein restriction during pregnancy has a significant effect on weight gain. Thus, Marin et al[11] have shown that mean body weights of pregnant dams fed 15%, 10%, and 5% protein diets were 79%, 72%, and 58%, respectively, of control. Significantly lower weight gains during pregnancy were also observed by Desai et al[12] for dams fed 8% protein during gestation as compared to those fed a 20% protein diet. In our study, weight gains were significantly ($P < .01$) affected even when protein restriction was prior to conception and not during pregnancy. Furthermore, weight gain during pregnancy remained significantly low, despite protein repletion from day 0 of pregnancy, which highlights the importance of nutrition before conception.

Feed intake during restriction as well as during pregnancy was comparable for control and restricted animals. These observations are similar to those reported earlier[22] when rats were fed 9% or 18% protein diets during pregnancy. In the present study, when dams from the restricted group were shifted to control diet (16% protein), intake increased significantly ($P < .05$) in the first week of pregnancy. A similar effect of protein repletion on feed intake has been reported[23] in lambs and in male rats.[24]

Our observations at birth show significantly ($P \leq .008$) lower relative weights for brain. This is in contrast to the brain-sparing effect reported by earlier researchers.[11,12] The relative weight for kidney was higher at birth, indicating protective effect. However, differing impacts on different organs were observed at later ages of the pups. Thus, at 94 days, the relative weights were lower for brain, liver, and heart. The relative fat weight of liver for animals in the restricted group was higher, which suggests some alteration in the composition of the liver. This observation assumes significance in view of the fact that in humans, protein restriction is known to result in fatty liver, as is the case in Kwashiorkor children.

At 180 days, in addition to liver, relative weights of kidney

were also significantly reduced. Nwagwa et al[20] also reported lower relative weights for kidney in offspring at 4 weeks of age born to dams fed a 9% protein diet as compared to an 18% protein diet during pregnancy. The relative fat weight too was significantly ($P < .05$) lower for kidney in the restricted group compared to the control group. A decrease in the number of nephrons during protein restriction has been reported,[25] but not a reduction in relative fat weight. No significant difference in relative fat weight was seen for other organs at 180 days.

The changes in relative fat weight in liver were observed at an earlier age, ie, 94 days, but appeared temporary, as such an effect was not seen at 180 days. However, changes in relative fat weight for kidney were observed at a later age, ie,180 days. Differing impacts on growth of vital organs at different time points in life have also been reported for protein restriction during pregnancy and lactation.[12] Our findings therefore indicate that dietary restriction prior to conception can exert a similar influence on growth and composition of vital organs.

Protein restriction during critical periods of development can lead to a reduction in the size of organs and tissues with change in the activity of enzymes associated with glucose metabolism.[12] In our study, pregnant dams from the restricted group had lower blood glucose and cholesterol levels on the day of delivery as compared to controls. This may indicate massive transport of nutrients to the fetus.[26] The fact that placental enlargement was observed in the restricted group renders support to this observation. Langley Evans et al[27] have also reported placental hypertrophy in response to undernutrition during pregnancy. These are some of the probable explanations for the lower values of glucose observed in dams on the restricted diet.

The more relevant observation in the context of the "fetal origin hypothesis" is, however, the elevated levels of glucose observed at 94 days in pups born to these dams. Pups born to mothers in the restricted group had high cholesterol levels at 180 days of age compared to those in the control group. Male rats had higher glucose and cholesterol levels compared with females, suggesting that nutritional sensitivity in males relates to the faster growth of tissues.[9] These differences are unlikely to be due to current nutritional status, as the diets of restricted and control dams differed only in the preconception period. It is worthwhile to note here that the relative fat weight of the liver was higher in the restricted group at 94 days, which may have altered its functioning. Thus, the findings suggest that undernutrition before conception may also make the fetus vulnerable to programming effects. However, it is beyond the scope of our data to estimate levels of protein/energy restriction required to induce negative effects in the offsprings; further research is needed.

The differences in relative organ weights and blood parameters of restricted and control animals remained significant even when the P value was discounted for multiple comparisons, ie, for 6 (at day 20) or 5 (at 94 and 180 days) organ groups, making $P = .008$ or $P = .01$ as the cut-off for statistical significance. Our observations suggest that undernutrition before pregnancy creates less than optimum nutrition levels in the first week—a phase of implantation (2 to 3 days) and organogenesis (9 to 12 days) in the developing fetus.

If the ratio of life spans is taken as a factor to calculate a biologic time homology between rats and humans, it can be estimated[28] that the duration of 8 weeks in the life of a rat corresponds to approximately 4 years in human life. Maternal nutrition studies have shown that pre-pregnant nutritional status is a major determining factor for birth outcome.[7,29] Studies in Jamaica have indicated that in humans, poor dietary status before conception may be a risk factor for low birth weight and elevated blood pressure in offspring.[30] The wider implication of our observations is that poor nutritional status before conception is likely to exert a long-term influence on the functioning of vital organs. These findings underscore the importance of good nutritional status before conception and indicate the need for intervention programs for young married girls in developing countries.

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