CLINICAL STUDY

Pre-Pregnancy Weight and Weight Gain During Pregnancy are Important Determinants in the Endocrine Modulation of Fetal Growth Restriction

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Abstract

Objective: Pre-pregnancy weight, nutritional status, and the amount of weight gained during pregnancy, are extremely useful indicators of fetal growth and outcome. Suboptimal maternal nutrition could have a direct effect on the organs of the developing fetus and/or affect the endocrine milieu in the maternal feto-placental unit resulting in "fetal growth restriction" which may be a significant risk factor for adult onset disease. We investigated endocrine adaptation by the fetus to overcome the growth disadvantage caused due to poor weight gain in pregnancy as a result of maternal nutritional restriction.

Materials and Methods: We examined the quantitative variations in hormonal profiles in paired maternal and cord blood samples obtained from mothers and their neonates who were classified based on maternal weight gain during the entire pregnancy.

Results: 1) A total of 37.4% mothers gained less than 6 kg during the entire pregnancy. 2) Anthropometric parameters measured in the mothers indicate that these mothers were nutritionally restricted both prior to and during pregnancy. 3) We observed increased levels of growth hormone, placental lactogen, prolactin and thyroxine (T4) and decreased levels of insulin in the cord blood of neonates and decreased insulin and TSH levels in maternal blood in the study group (<6 kg weight gain during pregnancy) as compared to the control group (>6 kg weight gain during pregnancy).

Discussion: Our results suggest that insufficient weight gain in pregnancy due to suboptimal maternal nutritional status results in fetal adaptation to a growth restricted environment by modulation of the pituitary-thyroid axis thereby altering the endocrine milieu resulting in significant "fetal growth restriction".

Keywords: pre-pregnancy weight, weight gain during pregnancy, intrauterine growth restriction, low birth weight, endocrine function, maternal malnutrition

Özet

Hamilelik Öncesi Kilo ve Hamilelikte Alınan Kilo Fetal Büyüme Kısıtlanmasının Endokrin Modülasyonunda Önemli Belirleyicilerdir

Amaç: Hamilelik öncesi kilo, beslenme statüsü ve hamilelikte alınan kilo fetal gelişme ve prognoz açısından çok önemli göstergelerdir. Suboptimal bir maternal beslenme, gelişmekte olan fetüsün organlarına doğrudan etki edebilir ve/veya fetal büyüme kısıtlanmasına yol açabilecek maternal feto-plasental ünitedeki endokrin ortamı bozabilir. Bu da, yetişkinlikte ortaya çıkan hastalıklar için önemli bir risk faktörü olabilir. Bu çalışmada, maternal beslenme bozukluklarının bir sonucu olarak yetersiz kilo alımının neden olduğu fetal büyüme kısıtlılığının üstesinden gelebilmek için fetüsün endokrin adaptasyonu incelenmiştir.

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Materyal ve Metot: Gebelik boyunca maternal kilo alımı temelinde gruplandırılmış anne ve yenidoğanlardan alınan maternal ve kordon kan örneklerinin hormonal profillerindeki kantitatif değişkenlikler incelenmiştir.
Sonuçlar: 1) Annelerin %37.4'ü gebelikleri boyunca 6 kg'dan az kilo almıştır. 2) Annelerde ölçülen antropometrik parametreler, bu annelerin gebelik öncesinde ve esnasında beslenme yetersizliğine maruz kaldıklarını göstermektedir. 3) Kontrol grubu ile karşılaştırdığımızda (gebelik boyunca kilo alımı >6 kg), çalışma grubundaki (gebelik boyunca kilo alımı <6 kg) yenidoğanların kordon kanında artmış seviyede büyüme hormonu, plasental laktojen, prolaktin ve tiroksin (T4) ve daha düşük seviyede insülin, maternal kanda da daha düşük seviyede TSH saptanmıştır.
Tartışma: Çalışmamız, suboptimal maternal beslenmenin yol açtığı yetersiz kilo alımının, hipofiz-tiroid aksının modülasyonu aracılığıyla fetal büyümenin kısıtlanması şeklinde yanıt oluşturmakta; bunun sonucu olarak da endokrin dengeler değişerek ciddi "fetal büyüme kısıtlanması" ortaya çıkmaktadır.

Anahtar sözcükler: gebelik öncesi kilo, gebelik sırasında kilo alımı, intrauterin büyüme kısıtlanması, düşük doğum ağırlığı, endokrin fonksiyon, maternal yetersiz beslenme

Introduction

A healthy body weight promotes general health, reduces the risk of developing some diseases and is a major positive influence on the management and outcome of pregnancy. Pre-pregnancy weight and nutritional status, and the amount of weight gained during pregnancy are extremely useful indicators of fetal growth and outcome. Therapeutic interventions of these parameters potentially reduce the incidence of low birth weight (LBW) and growth restricted infants (1). The World Health Organisation (WHO) recommends that women in developing countries gain at least 1 kg per month during the last two trimesters of pregnancy, resulting in a weight gain of at least 6 kg. Although attainable and realistic, most women in developing countries still do not gain the recommended amount of weight (2-6). Prepregnancy weight and pregnancy weight gain are independent and completely additive and/or subtractive in their effect on birth weight, together accounting for a difference of up to 1000 grams in birth weight (5). The height and weight of mothers from developing countries like India are lower by almost two standard deviations as compared to mothers from the developed countries. Additionally, birth weight and placental weight of neonates are also significantly lower in neonates born to these mothers. According to the most recent estimates, 15.5% of all births, or more than 20 million infants worldwide, are born with LBW. The incidence of LBW in developing countries is 16.5% which is more than double in developed nations. More than 95% of low birth weight babies are born in developing countries, and this high prevalence of LBW neonates is a major public health problem (7).

Fetal growth is also affected by endocrine parameters like hormones and growth factors and genetic factors. Hormones and growth factors of maternal and feto-placental origin have been demonstrated to play an important role with the accretion and differentiation of fetal tissues. The peptide hormones, insulin, prolactin, placental lactogen, growth hormone and the growth factors, insulin like growth factor (IGF-I, IGF-II) and epidermal growth factor (EGF), have been shown to be of specific relevance in fetal growth and development (8-10). Additionally, the thyroid hormones stimulate protein synthesis and cell enlargement and are vital for fetal maturation. Caloric deprivation resulting in maternal malnutrition during this very critical period of development disturbs maternal thyroid function and leads to an imbalance in the fetal thyroid hormone profile, resulting in impairment of brain development and function (8). "Barker's hypothesis" suggests that maternal malnutrition could have a direct effect on the organs of the developing fetus and/or affect the endocrine milieu which could modulate the delicate hormonal balance in the maternal feto-placental unit resulting in Intra Uterine Growth Retardation (IUGR) which may be a significant risk factor for adult onset diseases (11-14). Maternal prepregnancy weight represents long-term maternal nutritional status and is responsible for approximately 13% of the variance in birth weight. On the other hand, weight gain during pregnancy, represents short-term maternal nutritional status and accounts for only 5.6% of the variance in birth weight (15-19).

Limited information is available in literature with regards to the endocrine alterations in the maternal-fetal milieu as a consequence of inadequate maternal weight gain during pregnancy or nutritional restrictions in pregnancy. In order to investigate the endocrine adaptation mechanisms by the fetus to overcome the growth disadvantage caused by maternal nutritional limitations, we examined the quantitative variations in hormonal and growth factor profiles in paired maternal and cord blood samples obtained from mothers and their neonates who were classified into two study groups based on the maternal weight gain in pregnancy.

Materials and Methods

Pregnant mothers (n=300) who were registered and being followed up in the antenatal clinic in a prominent government hospital in New Delhi, India and their newborns were the source of the clinical material for this study. The study design and the consent forms were approved by the appropripriate institutional review board and the study was conducted strictly based on their recommended guidelines. After obtaining informed consent, a detailed clinical obstetric history of the patient was documented. Gestational age was assessed from the date of the last menstrual period. At birth, cord blood (20 ml) was collected from the placental end into a sterile heparinised tube and immediately centrifuged to separate the plasma and aliquoted and stored at -20°C until further use. The maternal blood drawn (10 ml) was also similarly collected, aliquoted and stored until further analysis. Weight and length of the neonate were recorded as soon as the baby was dried

and cleaned after delivery. Anthropometric data was obtained from the mothers and the neonates. Maternal nutritional status and fetal growth status was assessed using a combination of anthropometric and biochemical parameters, like body mass index (BMI) calculated from the height and weight of the mother, mid arm circumference, abdominal girth or abdominal circumference, fundal height. Maternal weight gain during pregnancy and maternal pre-pregnancy BMI were also measured. The biochemical parameters measured to assess nutritional status were maternal and fetal total protein and serum albumin levels. The methodology used to measure total serum proteins was the standard Biuret reaction in our routine clinical laboratory using an automated sampler.

Anthropometric measures of the neonate
The following methodologies were used to measure birth weight, length of the neonates (20-22).

Birth weight measurement: The pan-type pediatric scale was used to measure birth weight of the neonate. The pan-type pediatric scale is a beam scale with non-detachable weights and is accurate within 10 grams. Birth weight was measured in grams (g).

Length measurement: To measure neonatal length, an in-house length-measuring device with a fixed headboard and a moveable footboard that was perpendicular to the surface on which the neonate was placed was used. A fixed measuring tape was attached to the surface with the zero end at the edge of the headboard. Length of the neonate was measured in centimeters (cm).

Ponderal Index (PI): Ponderal index (PI)=birth weight/birth length^3 was calculated from the neonates weight and length at birth.

Anthropometric measures of the mother
Abdominal circumference, fundal height, and mid arm circumference were measured as published earlier (20-22).
Maternal pre-pregnancy weight: Pregnant mothers are weighed during their first visit (6-8 weeks of pregnancy) to the antenatal clinic.

Maternal weight at delivery: Pregnant mothers are weighed when they are admitted into the labor room for delivery.

Women are weighed on a platform beam balance scale with movable weights. Beam balances have the highest reliability and validity and both maternal pre-pregnancy weight and weight at delivery are expressed in kilograms (kg).

Weight gain during pregnancy: Weight gain during pregnancy is expressed as the total weight gain during the entire gestational period, up until the birth of the neonate. It is expressed in kilograms and computed as follows; Weight gain during pregnancy=weight at delivery subtracted from the maternal pre-pregnancy weight.

Abdominal circumference: A simple circumference measurement was taken at the mid-abdominal line using a non-stretchable centimeter tape. Since fetal movements could result in distortion of the abdominal shape, single measurements of the abdominal circumference could be inaccurate, therefore we took a minimum of three separate measurements of abdominal circumference, and a mean value of all three measurements was noted in centimeters (cm).

Mid-Arm Circumference (MAC): MAC is used as an indicator for both screening acute adult under nutrition and for estimating prevalence of under nutrition at a population level. To measure MAC, we used a non-stretchable centimeter tape, measurements were taken on the non-dominant arm bent at a 90° angle with palm facing up, the midpoint between the acromium and olecranon processes was chosen as the measurement mark point. The distance around the arm at this mark point was recorded to the nearest 0.1 cm. Clothing was pushed up above the shoulder or removed so that it did not interfere with MAC measurement.

Fundal height: The fundal height is a rough estimate of fetal size and is measured as the distance between the pubic bone and uterine fundus. The fundal height measurement in centimeters (cm) was taken with a non-stretchable tape measure from the top of the pubic bone to the fundus of the uterus.

Study design
The World Health Organisation (WHO) recommends that women in developing countries gain at least 1 kg per month during the last two trimesters of pregnancy, resulting in a weight gain of at least 6 kg during the entire pregnancy. Based on this recommendation, we classified the mothers in the study into two groups Group I (Study group, weight gain during pregnancy is <6 kg) and Group II (control group, weight gain during pregnancy is ≥6 kg). Only term pregnancies were included in the study, a gestational period of 38-42 weeks was considered normal and neonates born before 38 weeks were considered pre-term, while those beyond 42 weeks were considered post term. Exclusion criteria of the study included both pre- and post term neonates and their mothers and any other clinical disorders known to predispose the mothers to LBW births excepting malnutrition and/or anemia. Pregnant mothers with clinical disorders such as pre-eclampsia, renovascular disease, chronic hypertension, vasculopathy from diabetes, drug abuse, genetic or congenital abnormalities, inborn errors of metabolism and toxic exposure were excluded from the study.

To examine the endocrine profile of mothers and their neonates in Group I and Group II, an array of hormones and growth factors relevant to fetal growth and development, like Growth Hormone (GH), PRoLactin (PRL), insulin, human Placental Lactogen (hPL), thyroid hormones: (T_4: thyroxine, T_3: tri-iodo-thyronine, rT_3: reverse-tri-iodo-thyronine, TSH: thyroid stimulating normone), Insulin- like Growth Factor-1 (IGF-1) and Epidermal Growth Factor (EGF) were assayed in both cord blood and maternal blood using a combination

of in-house radioimmunoassays and commercially available kits. The standards, iodination material for preparation of the radiolabelled antigen, and the specific antisera were obtained from the National Hormone and Pituitary Program (NHPP) of the National Institute of Arthritis, Diabetes and Digestive and Kidney diseases (NIADDK), NIH, Bethesda, MD and the assays were performed using a precise protocol provided by this program. GH, PRL, hPL were assayed using the reagents from NIH while Insulin, IGF-1, TSH and EGF were assayed using a commercially available kits from Amersham Inc. (Piscataway, NJ, USA). All the assays were highly specific and sensitive with a intra and inter assay coefficient of variation less than 10%. The minimum detectibility of the GH, PRL, hPL, Insulin, IGF-1 and EGF assays was 0.23 ng/ml, 0.4 ng/ml, 0.015 mU/L, 0.4 mU/ml, 0.078 ng/ml and 0.06 ng/ml, respectively. The minimum detectibility of the T_3, T_4, rT_3 and TSH assays was 2.5 pg/ml, 5 pg/ml, 1 pg/ml, and 0.03 µU/ml, respectively.

Statistical analysis

Statistical analysis was done using ANOVA. Multiple comparisons between the study groups were performed using a Bonferroni adjustment. The tables contain the *p*-values for these pair wise group comparisons. All data analysis was done using the normal control group as the reference group. Additionally, multivariate regression analysis was done to investigate relationships between weight gain and the various anthropometric, biochemical and endocrine parameters. The statistical software used was Analyze-It (version 1.73) (Analyze It for Microsoft® Excel® Leeds, UK)

Results

A total of 300 cases were enrolled in the study. Only term pregnancies with an uneventful perinatal period were included in the study. The patient population was representative of the majority of the urban and rural, low and middle socioeconomic class of the society. The mean gestational age of the mothers was 39.13±1.03 weeks. Gestational age was assessed from the date of the last menstrual period. The mothers in the study were classified into two groups Group I consisting of n=112 (study group, weight gain during pregnancy is <6 kg) and Group II consisting of n=188 (control group, weight gain during pregnancy is ≥6 kg). The mean age of the mothers in the two groups was 20.7±1.8-years.

Anthropometric and biochemical measures of maternal and fetal growth and nutritional status

Nutritional status of the mothers enrolled in the study was assessed using both anthropometric and biochemical measurements (Table 1). Our results show that maternal pre-pregnancy weight (kg) (46.01±4.61, p=0.0013) was significantly lower in (Group I) mothers who gained <6 kg weight during pregnancy as compared to the normal control group of mothers (47.76±5.32). Similar trend was observed with respect to maternal weight at delivery, where maternal weight at delivery (kg) (49.96±4.79, p=1.23E^{-13}) was significantly lower in Group I mothers as compared to the normal control group of mothers (56.33±6.28). Maternal midarm circumference (cm) (20.41±2.79, p=5.0E^{-06}) in mothers from the study Group I, was significantly lower as compared to the normal control group of mothers (22.18±3.23). No significant difference in maternal height was observed between the two groups of mothers. These results indicate that maternal pre-pregnancy nutritional status and pregnancy weight gain also affect the survival and health of the newborn. Although total weight gain during pregnancy is variable among women with good pregnancy outcomes and because the perinatal outcomes are multi-factorial in origin, weight gain is not a perfect diagnostic criteria for fetal wellbeing, therefore additional measures to assess fetal wellbeing such as maternal abdominal circumference and maternal fundal height were done. Our results show that maternal abdominal circumference (cm), (84.30±5.46, p=0.000001) was significantly

Table 1. Maternal anthropometric and biochemical parameters in the study groups

Maternal nutritional status	Group I (Weight gain <6 kg) (n=112)	Group II (control) (Weight gain ≥6 kg) (n=188)
Anthropometric parameters		
Maternal pre-pregnancy weight (kg)	46.01±4.61	47.76±5.32 (p=0.0013)*
Maternal weight at delivery (kg)	49.96±4.79	56.33±6.28 (p=1.23E^{-13})*
Maternal height (cm)	154.9±4.78	154.70±4.73 (p=0.34, NS)
Maternal mid-arm circumference (cm)	20.41±2.79	22.18±3.23 (p=5E^{-06})*
Pre-pregnancy BMI (kg/m^2)	19.19±2.07	19.97±2.19 (p=0.0011)
Weight gain during pregnancy (kg)	3.96±1.05	8.56±2.48 (p=2.9E^{-21})*
Maternal fundal height (cm)	29.99±2.67	30.95 ±3.21 (p=0.002)*
Maternal abdominal circumference (cm)	84.30±5.46	88.19±6.95 (p=0.000001)*
Biochemical parameters		
Total protein (mg/dl)	7.13±0.92	7.02±1.26 (p=NS)
Serum albumin (mg/dl)	3.75±0.67	3.66±0.82 (p=NS)
Hemoglobin (g/L)	9.87±1.66	10.08±1.38 (p=NS)*

* denotes statistically significant differences between the study group and the control group

Table 2. Fetal anthropometric and biochemical parameters in the study groups

Fetal nutritional status	Group I (Weight gain <6 kg) (n=112)	Group II (control) (Weight gain ≥6 kg) (n=188)
Anthropometric parameters		
Birth weight (gr)	2382.7±367.5	2886.6±348.9 (p=3.2E^{-20})*
Neonatal length (cm)	49.03±2.17	49.69±1.85 (p=0.0038)*
Ponderal index (cm)	2.02±0.30	2.35±0.28 (p=0.0001)*
Weight placenta (gr)	364.2±99.35	449.5±110.91 (p=3.4E^{-24})*
Birth weight percentile (%)	26.80±22.02	58.61±23.61(p=5.2E^{-25})*
Biochemical parameters		
Total protein (mg/dl)	7.08±0.89	7.20±1.15 (p=0.16, NS)
Serum albumin (mg/dl)	3.92±0.64	4.11±0.79 (p=0.015)*
Hemoglobin (g/L)	12.52±1.98	12.90±1.26 (p=NS)

* denotes statistically significant differences between the study group and the control group

lower in mothers in group I as compared to the normal control group of mothers (88.19±6.95). Similar trend was observed with respect to maternal fundal height (cm) (29.99±2.67, p=0.002) which was significantly lower in the study group as compared to the normal control group (30.95±3.21).

Table 2 outlines the differences in fetal growth status between the study group and the control group. Our results showed significantly lower neonatal birth weight (g) (2382.7±367.5, p=3.2E^{-20}) in Group I, as compared to the control group (Group II) (2886.6±348.9). Predictably, the placental weight (g) was significantly lower (364.2±99.35, p=3.4E^{-24}) in Group I, as compared to the control group (Group II) (449.5±110.91). Neonatal length (cm) was significantly lower (49.03±2.17, p=0.0038) in Group I, as compared to the control group (Group II) (49.69±1.85) and consequently Ponderal Index (PI) which is a good measure of asymmetrical fetal growth and computed from neonatal birth weight and length, was also significantly lower (2.02±0.30, p=0.0001) in Group I, as compared to the control group (Group II) (2.35±0.28). The average birth weight percentile (%) of neonates born to mothers who gained <6 kg weight during the entire pregnancy was 26.8%, p=5.2E^{-25} as compared to the birth weight percentile (58.6%) in neonates born to mothers who gained ≥6 kg weight during the entire pregnancy. These results suggests that a significant degree of fetal growth retardation is evident in the neonates born to mothers who gained <6 kg weight during the entire pregnancy, and its appears that is fetal growth retardation is a consequence of not only nutritional restriction during pregnancy but also due to poor nutritional status prior to pregnancy as evident by a low pre-pregnancy BMI. We measured the serum albumin and total protein levels as a biochemical markers of nutritional status in the maternal blood and cord blood in the two

Table 3. Endocrine parameters measured in the cord blood

Cord blood	Group I (Weight gain <6 kg) (n=112)	Group II (control) (Weight gain ≥6 kg) (n=188)
Peptide hormones		
Growth hormone (ng/ml)	38.57±25.13	33.88±20.51 (p=0.05)*
Prolactin (ng/ml)	301.8±58.1	276.17±28.9 (p=NS)
Insulin (mU/ml)	13.13±9.4	18.39±10.9 (p=0.016)*
Placental lactogen (mU/L)	82.98±15.2	55.25±11.0 (p=0.001)*
Growth factors		
IGF-I (ng/ml)	223.5±98.3	237.5±123.2 (p=NS)*
EGF (ng/ml)	4.65±3.85	3.90±2.63 (p=NS)*
Thyroid hormones&Thyroid stimulating hormone		
T_4 (μg/dl)	8.75±3.33	8.04±3.01 (p=0.039)*
T_3 (ng/dl)	44.06±20.52	53.06±25.48 (p=0.0007)*
rT_3 (ng/dl)	208.44±95.65	225.1±100.85 (p=NS)
TSH (μU/ml)	4.85±3.42	5.27±4.07 (p=NS)

* denotes statistically significant differences between the study group and the control group

Table 4. Endocrine parameters measured in the maternal blood

Maternal blood	Group I (Weight gain <6 kg) (n=112)	Group II (control) (Weight gain ≥6 kg) (n=188)
Peptide hormones		
Growth hormone (ng/ml)	14.531±12.4	13.61±12.83 (p=NS)
Prolactin (ng/ml)	363.7±316.2	449.7±275.3 (p=NS)
Insulin (mU/ml)	43.92±19.1	74.01±17.7 (p=0.003)*
Placental lactogen (mU/L)	2974.8±1250.3	3029.8±1647.6 (p=NS)
Growth factors		
IGF-I (ng/ml)	125.2±28.2	141.3±53.61 (p=NS)
EGF (ng/ml)	8.2±3.5	8.08±2.44 (p=NS)
Thyroid hormones&Thyroid stimulating hormone		
T_4 (μg/dl)	10.071±3.18	9.71±3.13 (p=NS)
T_3 (ng/dl)	122.4±42.8	121.1±38.9 (p=NS)
rT_3 (ng/dl)	29.6±12.6	29.18±12.74 (p=NS)
TSH (μU/ml)	1.72±1.03	2.18±1.5 (p=0.02)

* denotes statistically significant differences between the study group and the control group

study groups and the data indicates that there was no significant difference in the maternal and cord blood total protein levels in the two groups (Table 1, 2), however significantly lower serum albumin (mg/dl) (3.92±0.64, p=0.01) levels were observed in neonates who were born to mothers who gained <6 kg weight during the entire pregnancy, as compared to the respective normal controls (4.11±0.79). Total hemoglobin levels were measured in the maternal and cord blood as an index of hematological status of the mothers and their neonates. No significant differences in the hemoglobin levels were observed in the paired maternal and cord samples in study group as compared to the control group.

Endocrine parameters in cord blood in the two study groups

We measured the levels of the peptide hormones (GH, PRL, insulin, HPL), growth factors (IGF-1 and EGF) and thyroid hormones (T_3, T_4, rT_3 and TSH) levels in the cord blood and maternal blood in the two study groups (Table 3, 4). Data (mean±SD) shows no statistically significant differences in levels of Prolactin (PRL), the growth factors IGF-1 and EGF or the thyroid hormones (rT_3 and TSH) between the two groups of neonates. However, a significant increase in GH levels (ng/ml) (38.57±25.13, p<0.05) was observed in the cord blood of neonates in Group I as compared to the control group (33.88±20.51). Additionally, significantly higher levels of cord blood HPL (mU/L) (82.98±15.2, p<0.001) were observed in the neonates in Group I as compared to Group II (55.25±11.0). However, the cord blood insulin levels (mU/ml) were significantly lower in neonates in the study group (13.13±9.4, p<0.016) as compared to the neonates in the normal control group (18.39±10.09). As regards the thyroid hormones cord blood T_4 levels (μg/dl) were significantly higher in the Group I neonates (8.75±3.33, p<0.03) as compared to the neonates in the control group (8.04±3.01), while an inverse trend was seen with respect to T_3 levels (ng/dl), where cord blood T_3 levels were significantly lower in the Group I neonates (44.06±20.52, p<0.0007) as compared to the neonates in the control group (53.06±25.48).

Endocrine parameters in maternal blood in the two study groups

Data shown in Table 4 indicates no statistically significant differences in maternal GH, HPL, PRL, IGF-1, EGF and thyroid hormones (T_3, T_4, rT_3) and TSH levels in mothers who gained <6 kg weight during the entire pregnancy (Group I) as compared to mothers who gained ≥6 kg weight during the entire pregnancy Group II (control mothers). Significant differences were observed in the Insulin levels (mU/ml) which were significantly lower (43.92±19.1, p<0.003) in Group I mothers as compared to Group II mothers (74.010±17.7) and the TSH levels (μU/ml) were significantly lower (1.72±1.03, p<0.02) in Group I mothers as compared to the Group II mothers (2.18±1.5) (Table 4).

Table 5 outlines the results of our regression analysis and Figure 1 shows the significant correlations between weight gain in pregnancy in some of the key anthropometric and endocrine parameters.

Discussion

Maternal nutritional status is a proximate determinant of neonatal nutritional status. Maternal malnutrition could have a direct effect on the organs of the developing fetus and/or affect the endocrine milieu in the maternal feto-placental unit resulting in IUGR which may be a significant risk factor for adulthood diseases. In developing countries like India, majority of the LBW neonates are term but are IUGR (23). Fetal growth is largely controlled by several variables such as socio-economic factors, genetic factors and metabolic factors that include the availability of oxygen and glucose and the

endocrine changes that results from the modulation of these complex variables (24-26). In recent years, pioneering epidemiological studies by Barker DJ et al. have opened up an exciting new area of investigation that has focused on the long-term adult consequences of fetal nutrient deprivation and IUGR (11-14). Weight gained earlier in pregnancy primarily contributes to maternal reserves, and is secondarily due to the growth of the placenta, breasts, uterus and increased amniotic and extra cellular fluids. After 20 weeks the fetus begin to increase dramatically in size and many investigators agree that weight gain in the second and third trimester is of greater importance for ensuring fetal growth than weight gain during the first trimester. LBW has long-term physiological consequences, and a woman born as a LBW infant herself may have difficulty developing a placenta that will provide adequate nutrition to her own fetus (16,27).

All of the anthropometric parameters measured in the mothers (except for maternal height) were indicative that the mothers who gained less than 6 kg weight gain during the entire pregnancy were nutritionally restricted both before and during pregnancy. Data in Table 2 on the anthropometric measurements of fetal growth indicated that neonates born to mothers who gained less than 6 kg weight gain during the entire pregnancy were growth restricted as indicated by significantly lower birth weight, neonatal length, PI, placental weight and birth weight percentile as compared to the control group. Although we did not observe any change in total protein levels measured in the paired cord and maternal blood in the two groups (Group I and Group II) of neonates and their mothers, we observed a small but significant reduction in serum albumin levels in the cord blood of neonates born to Group I mothers. Serum albumin concentration is commonly used as an index of nutritional status. During normal pregnancy, approximately 1000 g of weight gain is attributable to protein, half of which is distributed in the fetus and the placenta, while the remaining half is distributed as uterine contractile protein, breast glandular tissue, plasma protein, and hemoglobin. Decreased plasma albumin levels in the cord blood of Group I neonates are indicative of limited availability of nutrients for the growing fetus in these group of mothers who gained less than 6 kg weight gain during the entire pregnancy. The growth restriction of these neonates was reflected in these significantly lower birth weight percentile as compared to the control group of neonates who were well above the 50% birth weight percentile. To determine the birth weight percentile the neonates were classified based on the intrauterine growth curve validated by the National Neonatology Forum of India and approved by the Indian Council of Medical Research, and which was first validated by Gopalan C, Singh M et al. followed by more updated versions by Mohan M (15,23,28,29). These growth curves are used as a standard for determining the adequacy of weight gain of the fetus during gestation. Comparison with western literature indicates a divergence or flattening of the growth curve of Indian babies around 37 weeks of gestation most likely reflecting the effect of maternal nutritional constraints. The growth curves used were adjusted for maternal parity, fetal sex, socioeconomic status, and rural and urban populations. Thus, these data suggest that poor maternal nutritional status and consequently insufficient weight gain in pregnancy were the primary cause of fetal growth restriction in the neonates belonging to Group I.

The hormones and growth factors evaluated in the present study are known to play an important roles in the tissue accretion and differentiation in the fetus in several mammalian species (30,31). The perinatal period presents a highly synchronized sequence of metabolic and endocrine events which are very important for normal growth and development of the fetus (8-10,30,31). Significantly higher levels of placental lactogen and GH levels were observed in the cord blood of neonates born to Group I mothers, while significantly lower levels of cord blood insulin was observed in the same group. We also observed a statistically significant increase in the cord blood T_4 levels and a significantly decrease in T_3 levels in Group I neonates (Table 3). Our maternal endocrine data shows a significant decrease in insulin levels and TSH levels in mothers who gained <6 kg weight during the pregnancy. Human placental lactogen, which is synthesized and secreted by the syncytiotrophoblast cells of the placenta, is a member of the placental lactogen, growth hormone, prolactin gene family. Several studies indicate a role for human placental lactogen in the regulation of fetal growth, and its is speculated that the growth-promoting actions of placental lactogen are mediated by stimulation of IGF production in the fetus and by increasing the availability of nutrients to fetal tissues. Several proteins like apolipoprotein A1, retinoic acid, vitamin D_3, and even thyroid hormones, are implicated in the regulation of placental lactogen expression. Increased GH levels as those observed in the cord blood of Group I neonates may lead to increases in placental transport capacity, and may cause anabolic effects in the mother which may result in limiting fetal substrate supply and therefore prevent an increase in fetal growth (30,31).

Thyroid hormones are critical to growth and development of the human fetus. We and other investigators have documented that malnutrition during pregnancy induces a reduction of serum T_3 and TSH , this results in reciprocal up regulation of T_4 production by the thyroid gland (31). It is speculated that reduced synthesis of thyroid hormone (T_3) and TSH in growth retarded fetuses may be due to hypoxic suppression of thyroid function, or a hypothyroid state with a consequent decrease in metabolic rate and oxygen consumption, which would be a beneficial adaptation of the fetus to the stimulus of maternal malnutrition (10). Therefore we speculate this beneficial adaptation may be occurring in the growth restricted neonates. Additionally, increased levels of prolactin are often observed in hypothyroidism resulting from low iodine intake, therefore the increased prolactin levels in the cord blood of the Group I neonates may be a consequence of thyroid hormone dysregulation that occurs due to maternal nutritional restriction. Placental restriction of fetal growth is known to increase circulating thyroid hormone concentrations and particularly T_4 activation (31).

We believe that significantly lower levels of cord blood insulin observed in the cord blood of Group I neonates in our study, could alter maternal growth factor levels and that these changes may direct the metabolic and growth adaptation of the mother to pregnancy, which ensures an adequate flow of substrates to the developing fetus trying to compensate a growth disadvantage due to maternal nutritional restrictions. Both, insulin and thyroid hormones are controlled by the supply of glucose and oxygen, respectively, and they influence fetal growth, partly via IGF-I (8,9). We observed decreased IGF-1 levels in both maternal and cord blood in Group I mothers and their neonates, but no statistically significant differences were observed between the control group and study group. Nevertheless, since the fetus is known to control the placental utilization of substrates via its blood concentrations of oxygen and glucose and possibly via IGF-I, the levels of placental lactogen, can alter the stability and concentrations of IGF binding proteins and the levels of circulating IGFs. IGF-I levels are modulated by both insulin and glucose and IGF-I may increase blood glucose concentration or maternal hyperglycemia by inhibiting insulin secretion and decreasing insulin concentration as observed in both cord and maternal blood levels in neonates and mothers belonging to Group I. Deficiencies in either fetal or maternal insulin secretion during pregnancy can alter fetal growth and have important consequences for perinatal survival and postnatal morbidity.

Table 5. Multivariate regression analyses between weight gain during pregnancy and maternal-fetal anthropometric, biochemical and endocrine parameters

Multivariate regression analysis		
Relationship between weight gain vs	**F-Statistic**	***p* value**
Maternal pre-pregnancy weight	15.87	<0.0001*
Maternal weight at delivery	211.90	<0.0001*
Neonatal birth weight	263.85	<0.0001*
Ponderal Index	114.74	<0.0001*
Neonatal length	21.90	<0.0001*
Placental weight	53.58	<0.0001*
Birth weight percentile	260.48	<0.0001*
Maternal abdominal circumference	32.73	<0.0001*
Maternal mid-arm circumference	32.57	<0.0001*
Fundal height	12.94	0.0004*
Maternal hemoglobin	0.68	0.41
Cord T_4	5.69	0.017*
Maternal T_4	1.76	0.185
Cord T_3	9.55	0.002*
Maternal T_3	0.89	0.34
Cord rT3	0.91	0.34
Maternal rT_3	0.47	0.49
Cord TSH	0.21	0.61
Maternal TSH	1.95	0.09
Cord growth hormone	6.19	0.013*
Maternal growth hormone	2.11	0.14
Cord prolactin	2.50	0.11
Maternal prolactin	1.79	0.18
Cord placental lactogen	4.71	0.030*
Maternal placental lactogen	0.00	0.94
Cord insulin	2.42	0.12
Maternal insulin	6.00	0.015*
Cord total protein	0.13	0.71
Maternal total protein	3.15	0.07
Cord IGF-I	0.22	0.32
Maternal IGF-I	0.74	0.43
Cord EGF	0.69	0.88
Maternal EGF	0.74	0.43
Cord albumin	1.27	0.26
Maternal albumin	3.76	0.05*
Cord ferritin	0.04	0.84
Maternal ferritin	1.61	0.20

* denotes statistically significant differences between the dependent and independent variables. F-statics: ratio of mean square/error variance.

The endocrine modulation in Group I mothers and their neonates cannot be solely attributed to the amount of weight gained in pregnancy. Pre-pregnancy weight, nutritional status both before and during pregnancy significantly influences fetal growth and development, and all of these anthropometric measurements are interrelated. Therefore, we did a multivariate regression analysis (Table 5) to establish a correlation if any, between weight gain in pregnancy and the anthropometric, biochemical and endocrine parameters studied. Figure I shows the significant correlations between weight gain in pregnancy in some of the key anthropometric and endocrine parameters. Although other studies suggest that weight gain during pregnancy represents short-term maternal nutritional status and accounts for only 5.6% of the variance in birth weight (15-19), our results show that poor weight gain in pregnancy can significantly modulate various endocrine and anthropometric parameters. Thus, weight gain in pregnancy is a significant factor that influences both maternal and fetal well being, and is key to a favorable neonatal outcome.

Over the last several decades important information about the role of nutrition in the course and outcome of pregnancy has been assimilated (32-35). The fetus has been termed as a "parasite", who draws on maternal stores for support. However, researchers believe that a limit exists as to the ability of the fetus to drain maternal supplies. The consequences of poor maternal nutrition range from the development of amenorrhea to the stimulation of spontaneous abortion, stillbirth and congenital malformations. Limited maternal weight gain and subsequent low birth weight is the most common result of suboptimal maternal nutrition. Our study showed that the incidence of poor maternal weight gain as a result of suboptimal maternal nutrition in the population studied is 37.3%, which is an alarmingly large number of cases comprising almost 1/3rd of the subjects studied. The endocrine profile in such a data set is extremely valuable information that may lead to a better understanding of maternal-fetal physiology and about how environmental factors such as maternal malnutrition can adversely affect fetal development and the neonatal and long-term outcome. In the light of "Barker's hypothesis", which suggests that alterations in the maternal endocrine, nutritional, and metabolic environment disrupt

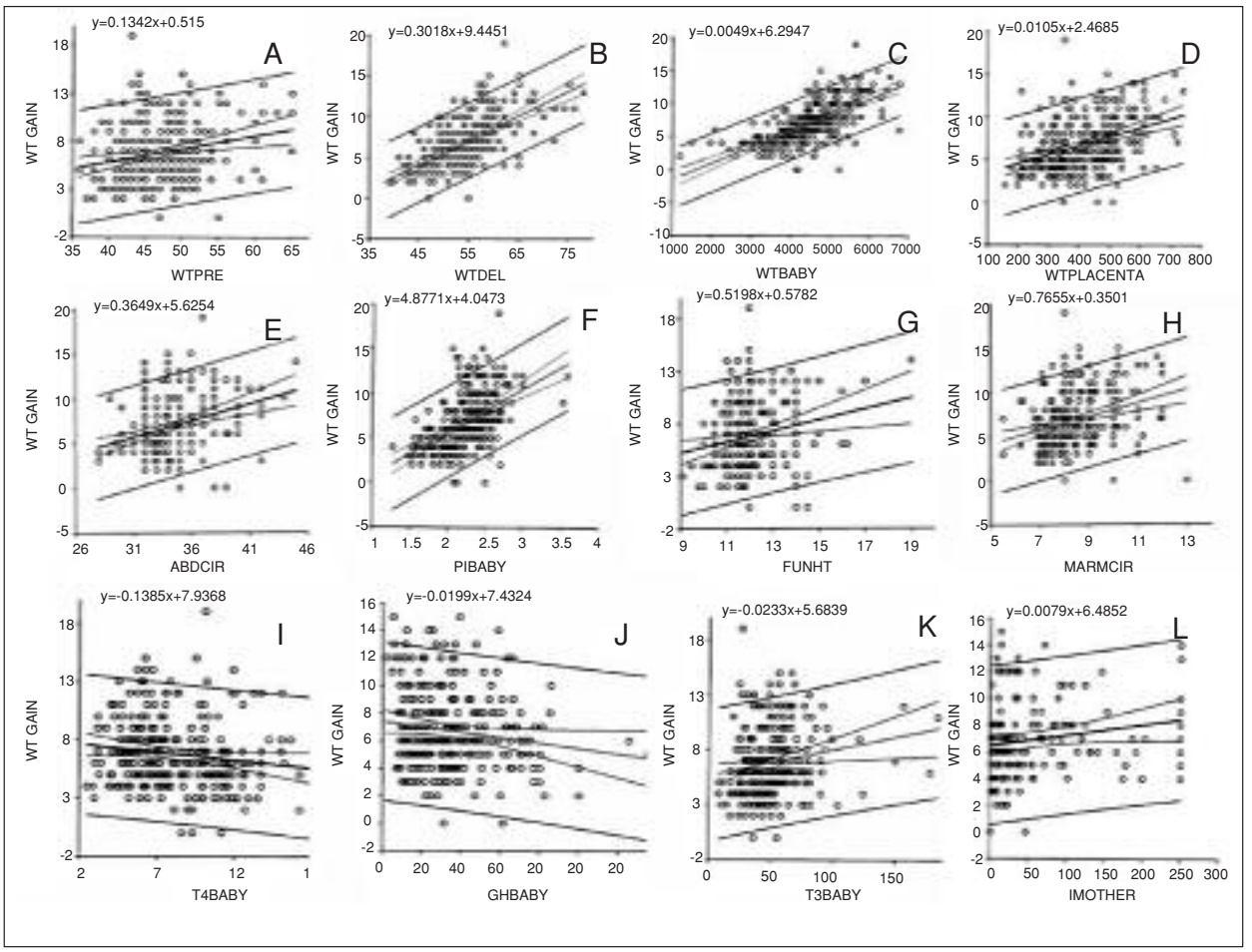

Figure 1 [A-L]: Graphical representation of multiple regression analysis between weight gain during pregnancy confirming an unbiased relationship between these parameters.

the developmental trajectory of the fetus, and can lead to adult diseases, more research studies such as ours needs to be done to evaluate the complex relationship between maternal nutritional status, fetal growth retardation, postnatal catch-up growth, and early markers of future adult diseases. Monthly monitoring of weight gain in pregnancy may not be feasible for many developing country settings, but a minimum of two measurements can be taken at least one month apart anytime during the second or third trimester. Lack of weight gain of approximately 1 kg per month or weight loss between two consecutive measurements is very detrimental to the fetus and/or mother and requires immediate action. Where weight-gain monitoring is not feasible at all, screening with measurements that require only one contact with a woman, such as prepregnancy weight (or weight-for-height or arm circumference) is still predictive of pregnancy outcome (16,18,27,31). There is an urgent need for research to assist in the development of pregnancy weight-gain charts which establish appropriate weight-gain curves for women in developing countries. These charts should be clear about the outcomes they are intended to predict and prevent, such as LBW or mortality. Improving pre-pregnancy weight and weight gain during pregnancy are effective strategies which reduce and prevent LBW and adequate nutritional supplementation will result in LBW neonates to experience catch-up growth and achieve normal postnatal growth.

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