Intrauterine Programming of Non-Communicable Disease: Role of Maternal Micronutrients

Urmila S Deshmukh,
Himangi G Lubree,
Chittaranjan S Yajnik
Kamalnayan Bajaj Diabetology Research Centre,
King Edward Memorial Hospital
and Research Centre,
Pune, India

Introduction

Two thirds of all deaths in the world are due to non-communicable diseases (NCDs), and 80% of NCD deaths occur in low- and middle-income countries.[1] Cardiovascular diseases, obesity and type 2 diabetes (T2D) are the major contributors to the global burden of NCDs. Studies in the life course evolution of these chronic diseases have highlighted an etiological role for factors which govern intrauterine and post-natal growth. Research in this field could offer a novel solution to the "primordial" prevention of conditions which are the most prominent killers in today's world.

These novel ideas arose from a series of studies by David Barker and his colleagues in the UK. They proposed that intrauterine undernutrition initiated a number of adaptations in the fetus which increased disease susceptibility in later life, especially when post-natal nutrition tended to be "excessive".[2] A developing fetus has the ability to grow in different ways depending on the surrounding (intrauterine) environment; this ability is called the "plasticity".[3] An unfavorable environment restricts the ability of the fetus to grow "wildly" and causes a permanent structural or functional change, known as "programming".[4] India is the world's capital of low birth weight (LBW) babies, while at the same time it is evolving into one of the economic powers of the world. It was clear that research in India would shed important light on these new and exciting ideas.

Fetal nutrition, growth, birth size and programming

The original ideas in this field were based on birth weight, for which there is a large database. However, it was clear from the beginning that birth size was only a proxy for factors which affect fetal growth. These include genetic factors, maternal size, and intrauterine environment. Birth weight is not a sensitive indicator of intrauterine nutrition, nor is it specific for nutrition.[5] Animal experiments show that a brief nutritional disturbance in early pregnancy permanently alters fetal physiology without any effect on birth size.[6] Thus, birth weight studies helped focus attention on intrauterine life as an important determinant of future health, but the excitement will focus on defining the environmental factors which are the "true exposures" in this association. This is where the current research is being directed.

Possible mechanisms of programming

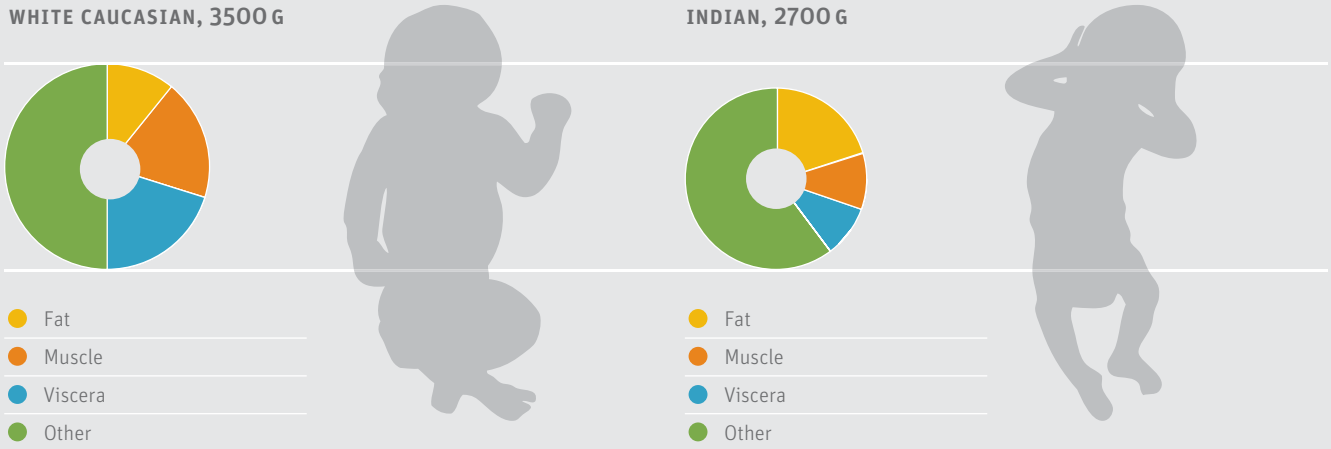
Fetal growth and development are influenced by an interaction between genetic factors and the intrauterine environment. This was beautifully shown with reference to the interaction between the glucokinase gene and maternal hyperglycemia.[7] The birth size of the newborn is influenced not only by inheritance of the gene, but also by maternal glycemia.

Fetal programming can be manifested in various ways. It might affect size, body composition, systems, organs and cells. It also affects physiology, sometimes without affecting size. Changes include altered setting of different enzyme systems and resetting of the endocrine axes. Endocrine mechanisms are major contributors to programming. Insulin-IGF (insulin-like growth factor) and the hypothalamic-pituitary-adrenal axis have been shown to be prominently affected.[6]

It is increasingly being appreciated that epigenetic changes are at the center of programming. These changes may be mediated by methylation of DNA, acetylation of histones and through the role of micro RNAs, all of which modify gene expression.[8,9]

"Epigenetic changes are at the center of programming"

FIGURE 1: *Thin-Fat Indian Baby.* A schematic diagram to compare the body composition of Indian and white Caucasian babies. Indian babies were ≈ 800 g lighter, muscle thin but more adipose compared to the white babies. [5,21]



The role of DNA methylation in influencing the phenotype of a growing fetus has been well demonstrated in animal models. Feeding pregnant *Agouti* mice with a methylating cocktail (vitamin B_{12} + folate + betaine + choline) changes the coat color and reduces obesity, despite inheritance of the mutation.[10] The change in phenotype is linked to methylation in the promoter region of the Agouti gene, which silences it.

Evidence from Pune studies

Research at the Diabetes Unit, King Edward Memorial Hospital, Pune has made important contributions to programming research. Our original observation was that diabetes occurred in Indians at a much lower body mass index (BMI), as compared to Europeans, and that this could in part be due to their higher central obesity and higher body fat percent, or adiposity.[11] This led to the "thin-fat" Indian concept. Many suggested that this was "genetically" determined, but we have not found any major differences in genetic associations of T2D in Indians compared to Europeans.[12] In 1991, we joined David Barker and Caroline Fall in their "fetal origins" research. The first collaborative research (Pune Children Study) confirmed that low birth weight was associated with insulin resistance as early as four years of age,[13] and that children who were born small but grew big in childhood had the highest level of risk factors for diabetes and cardiovascular disease.[14] We realized that intrauterine undernutrition could be an important contributor to the risk of adult disease. At the same time, we knew that fetal overnutrition (as in maternal diabetes) also increases the risk of obesity and diabetes in the child.[15] The stage was set to investigate the factors influencing fetal growth and programming. This was the birth of the Pune Maternal Nutrition Study (PMNS).

The PMNS was established between 1993 and 1996 in six villages near Pune, to investigate the influence of maternal body size and nutrition during pregnancy on fetal growth and its future metabolic risks.[16] We also investigated the fathers' contributions. Over 800 pregnancies were studied. Children were visited every six months for anthropometric measurements, and parents and children were investigated every six years for a detailed assessment of body composition, cardio-metabolic risk factors and neurocognitive development.

Predictors of fetal growth and birth size

Fetal growth and size are influenced by genes, parental body size, maternal nutrition and the mother's metabolic and vascular competence during pregnancy. Our measurements were guided by McCance's writings of over 50 years ago: "The size attained *in utero* depends on the services which the mother is able to provide; these are mainly food and accommodation."[17] We assessed maternal nutrition via anthropometric measurements, nutrient intake and physical activity, and by measurement of circulating nutrient levels.

Maternal body size, body composition and weight gain during pregnancy

The average mother in the PMNS was 21 years old, weighed 42 kg (BMI 18.1 kg/m²), and ate ≈1,700 kcal and 45 g proteins per day during pregnancy. The newborns weighed on average 2,700 g with a ponderal index (PI) of 24.1 kg/cm³; 28% were LBW (<2,500 g).[18]

Babies of heavier mothers were larger in all aspects, and babies of taller mothers were longer. Maternal fat measurements influenced the baby's weight and skin folds. It is interesting that

paternal size predominantly influenced skeletal measurements, while the baby's adiposity was predominantly determined by maternal factors. Short and fat mothers gave birth to the most adipose babies, suggesting an intergenerational influence of maternal early life "growth retardation" and the mother's subsequent weight gain on body composition of the growing fetus.[17] One more interesting finding was that babies born to multiparous women had higher skin folds and a higher abdominal circumference than those born to primiparous women.[19]

"A gain in maternal tissue during early weeks is an important determinant of fetal growth"

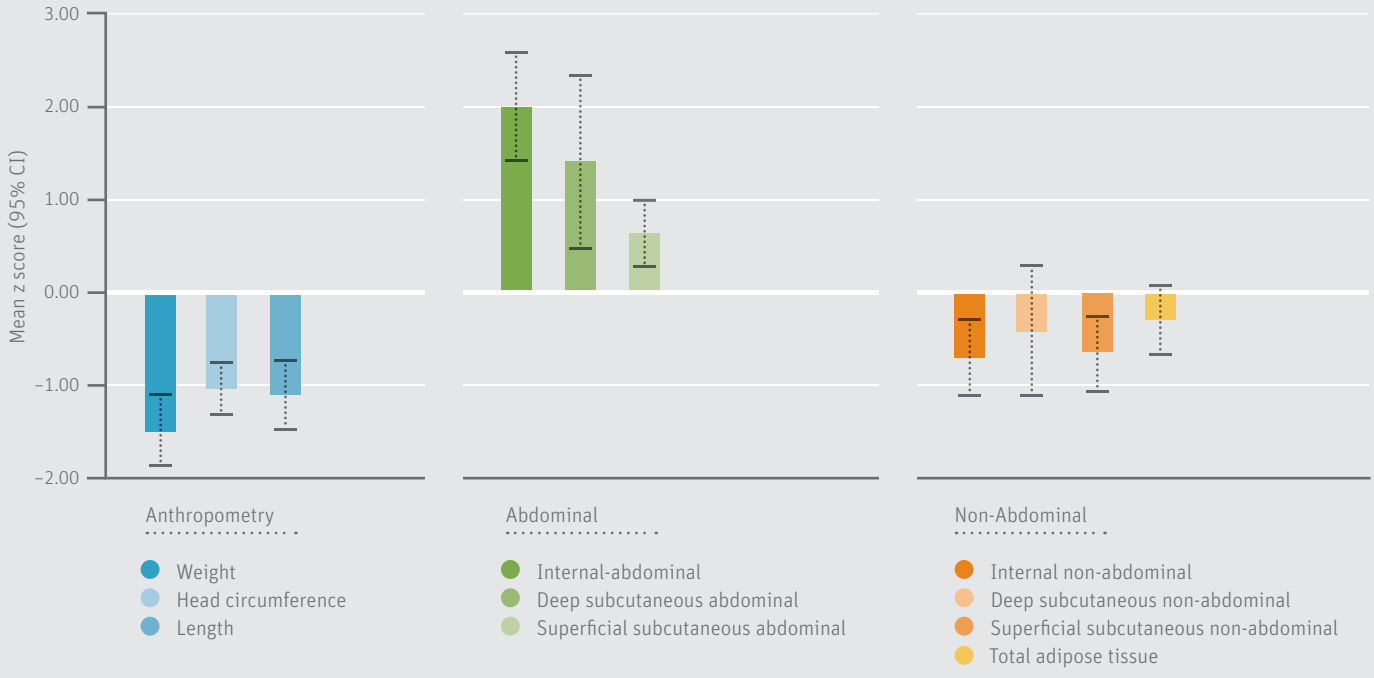
Maternal weight gain during the first 18 weeks influenced all neo-natal measurements, indicating that a gain in maternal tissue during early weeks is an important determinant of fetal growth. Placental volume measurement at 18 weeks' gestation was also an independent determinant of fetal growth, highlighting the role for this important organ.[20]

The "thin-fat" Indian baby

We compared the birth measurements of Indian babies with those of white Caucasian babies born in Southampton, UK. Indian babies were lighter (2.7 vs. 3.5 kg, z score -1.74), shorter (47.3 vs. 50.2 cm, z score -1.01) and thinner (PI 24.5 vs. 28.2 kg/cm^3, z score -1.62), but their sub-scapular skin fold measurements were relatively well preserved (4.2 vs. 4.6 mm, z score -0.53). At any sub-scapular skin fold thickness, Indian babies had a lower PI than that of the white Caucasian babies.[21]

In a subsequent study, we used whole body MRI to calculate body fat and its regional distribution in neonates. Compared to the larger white Caucasian babies, the Indian babies had similar whole body adipose tissue content ("thin-fat") and significantly higher absolute adiposity in all three abdominal compartments, viz internal (visceral), deep subcutaneous and superficial subcutaneous. Non-abdominal superficial subcutaneous adipose tissue was, however, lower.[22] Thus, Indian babies are more adipose and have a fat distribution that is suggestive of a higher risk of diabetes, as compared to white Caucasian babies.
(Figures 1 and 2)

FIGURE 2: *Thin-Fat Indian Baby.* Anthropometry and MRI comparison of Indian and white Caucasian babies. Despite their anthropometric smallness, Indian babies had a higher amount of fat in subcutaneous and visceral abdominal compartments. White Caucasian babies are used as reference, and z scores for Indian babies are plotted.[22] This figure is not to scale. The figure highlights relative adiposity of Indian newborns.

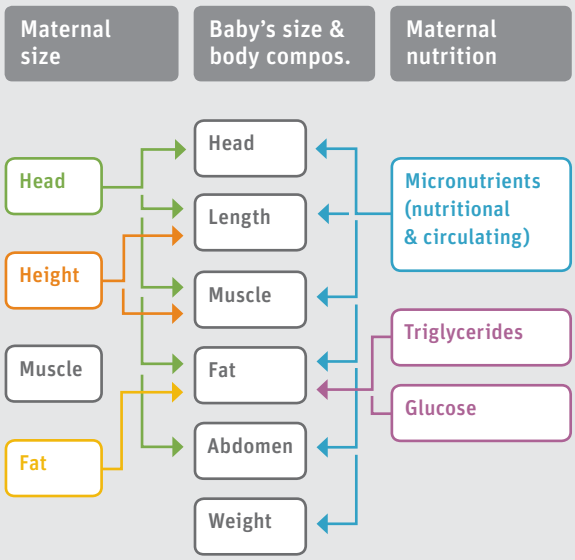


Maternal nutrition during pregnancy

In the PMNS, we measured maternal macronutrient and micronutrient nutrition, with special attention to one-carbon (1-C, methyl) metabolism, which is crucial for cell growth, differentiation and development. Maternal energy and protein intake was not associated with birth size; fat intake was weakly associated. On the other hand, the intake of micronutrient-rich foods (green leafy vegetables, milk and fruits) had a substantial effect on fetal growth. Maternal erythrocyte folate concentrations and vitamin C concentrations predicted larger neonatal size; vitamin B_{12} was not predictive.[16] Maternal plasma homocysteine concentrations predicted smaller birth weight.[23] Our results suggested an important role for micronutrients, especially for maternal 1-C metabolism in fetal growth and its body composition. **(Figure 3)**

> "The intake of micronutrient-rich foods had a substantial effect on fetal growth"

FIGURE 3: Maternal size and nutrition influence baby's size and body composition. Maternal head circumference (a surrogate for early life growth and nutrition) is related to neonatal size, her height is related to neonatal length and muscle, and fat to neonatal fat. Maternal dietary and circulating micronutrients (folate and vitamin C) influence neonatal size, circulating glucose and triglycerides are predominantly related to neonatal fat.[16,24]



Adipocytes – more than a bag of fat: the role of adipocytokines

It is remarkable that the human newborn has the highest body fat percentage (≈15%) of all mammals, including pigs (≈2%) and sea lions (≈5%).[25] The significance of this fact is yet to be established, but it suggests that neonatal adipose tissue must have a significant role in survival. Until recently, adipose tissue was considered only to be a storehouse for triglycerides, to provide energy and mechanical and thermal insulation. We now know that it is the biggest "endocrine organ" in the body. The amount and distribution of adipose tissue influence a wide variety of physiological functions and also predispose to a variety of clinical disorders. Adipocytes secrete a number of molecules called "adipocytokines". These influence food intake and energy metabolism, the insulin sensitivity of tissues, vascular reactivity, blood clotting mechanisms and, importantly, regulate "innate inflammation". A growing number of adipocytokines are being discovered and ascribed crucial physiological roles.[26] This represents a novel link between diet, physical activity and susceptibility to a number of non-communicable disorders.

We studied one such adipocytokine, leptin, in newborn Indian and white Caucasian babies. Cord leptin concentrations (median: 6.2 ng/mL, Pune; 6.4 ng/mL, London) were comparable in the two groups, but higher in Indian babies when adjusted for the difference in birth weight.[27] Thus, the excess adiposity of the Indian babies was reflected in functional disturbances indicative of an increased risk of diabetes and related disorders.

Recently, there has been interest in other adipocytokines which influence insulin resistance and, therefore, the risk of diabetes. These include adiponectin and retinol-binding protein 4 (RBP4). Adiponectin has the highest circulating concentration of all the adipocytokines and influences insulin resistance, inflammation and other cardiovascular risk factors.[28] Low adiponectin is an important risk factor for diabetes. RBP4 transports circulating retinol and is synthesized in liver and adipose tissue. It reduces insulin sensitivity and affects glucose metabolism.[29] There is scant information on adiponectin and RBP4 concentrations in cord blood.

We measured adiponectin and RBP4 concentrations in stored cord blood samples, and investigated their associations with maternal size, nutrition and metabolic parameters and newborn size. Adiponectin and RBP4 concentrations in cord blood were lower compared to the published data on western newborns. Maternal calorie, fat and protein intake and the mother's body size were not related to cord adiponectin and RBP4 concentrations. Both adipocytokines were positively associated with the baby's body composition (adiponectin with neonatal length, and RBP4 with sum of skin folds). Cord RBP4 was positively associated with maternal intake of vitamin A rich foods, suggesting that maternal vitamin A status may influence fetal adipocyte functioning. Longitudinal follow-up of these associations is ex-

pected to reveal the long-term effects of maternal nutrition on adipocyte functioning in offspring.

Follow-up of the PMNS children

The Developmental Origins of Health and Disease (DOHaD) theory suggests that structural and functional changes in the fetus consequent upon maternal nutritional, metabolic and other influences persist in later life. There are not many human studies linking maternal nutrients with offspring body composition and risk factors for NCD. Design of the PMNS allows us to follow up the children and study the effects of fetal programming.

We found that a child's adiposity (DXA) and insulin resistance, the two major risk factors for future diabetes, were significantly related to maternal micronutrient nutrition, especially those nutrients which regulate 1-C metabolism. Maternal folate concentrations were directly related to the adiposity of the child at six years of age, and also to insulin resistance. On the other hand, low maternal vitamin B_{12} status predicted higher insulin resistance. The most insulin resistant children were born to mothers who had the lowest vitamin B_{12} but highest folate status.[30]

In addition, we found that maternal vitamin B_{12} and folate predicted a child's neurocognitive function, suggesting that the 1-C metabolism of the mother also programs the child's brain development and function.[31]

> "Our research suggests that an imbalance in vitamin B_{12} and folate nutrition and consequent disturbances in maternal 1-C metabolism may contribute to the epidemic of adiposity and T2D in India"

In the PMNS, two-thirds of mothers had low vitamin B_{12} (<150 pmol/L) status during pregnancy, and a third had raised tHcy concentrations (>10 µmol/L). Folate deficiency was rare.[29] This nutrient pattern is at least partly ascribable to vegetarian food habits and partly to the prescription of folic acid by obstetricians. Our research suggests that an imbalance in vitamin B_{12} and folate nutrition and consequent disturbances in maternal 1-C metabolism may contribute to the epidemic of adiposity and T2D in India.

Folate and vitamin B_{12} are the major methyl donors in diet, and methylation of DNA is one of the major mechanisms of regulation of gene expression (epigenetics). Methylation silences the genes and affects the phenotype. It will be important to study how an improvement in the maternal nutrition of these nutrients influences the growth of a fetus and its future health and susceptibility to disease. This will be a step forward in the "primordial prevention" of diabetes and other NCDs.

> "Future research should target the option of intervening in the young to influence the intergenerational transmission of health"

Summary

Recent developments in the field of DOHaD have thrown an interesting light on the life-course evolution of many of the chronic NCDs. It is becoming increasingly obvious that a substantial proportion of adult health is programmed *in utero*. The health of young girls in a community is of paramount importance and is a major influence on the health of the next generation. Maternal micronutrient nutrition contributes to the fetal programming of NCDs. Current ideas on preventing NCDs in the middle-aged and the elderly via difficult-to-perform lifestyle adjustments are very ineffective models. Future research should target the more promising option of intervening in the young to influence the intergenerational transmission of health. Balanced micronutrient nutrition of young mothers may be the key.

Acknowledgements

We are funded by the Wellcome Trust (London, UK); the Nestlé Foundation (Lausanne, Switzerland); The International Atomic Energy Agency (Vienna, Austria); the Department of Biotechnology (DBT), Government of India (New Delhi, India); and ***Sight and Life,*** Basel, Switzerland. Thanks are due to colleagues, collaborators, field workers, and parents and children who participated in the studies mentioned in this article.

Correspondence: ***Prof. Chittaranjan S Yajnik,*** *Diabetes Unit, 6th floor, Banoo Coyaji Building, KEM Hospital, Rasta Peth, Pune 411011, Maharashtra, India* ***E-mail:*** *diabetes@vsnl.com*

References

01. The Global Status Report on Non-communicable Diseases 2010. World Health Organization. http://whqlibdoc.who.int/publications/2011/9789240686458_eng.pdf. (Accessed on 4 May 2011).
02. Hales CN, Barker DJP. Type 2 (non-insulin dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 1992;35:595–601.
03. Bateson P, Barker D, Clutton-Brock T et al. Developmental plasticity and human health. Nature 2004;430:419-21.
04. Lucas A. Programming by early nutrition in man. In: Bock GR, Whelan J, editors. The childhood environment and adult disease. CIBA Foundation Symposium 156. Chichester: Wiley; 1991. pp 38–55.
05. Yajnik CS. Obesity epidemic in India: Intrauterine origin? Proc Nutr Soc. 2004;63:387-396.
06. Harding JE. The nutritional basis of the fetal origins of adult disease. Int J Epidemiol. 2001;30:15-23.
07. Hattersley AT, Tooke JE. The fetal insulin hypothesis: an alternative explanation of the association of low birth weight with diabetes and vascular disease. Lancet 1999;353:1789-92.
08. Demerath EW, Cameron N, Gillman MW et al. Telomeres and telomerase in the fetal origins of cardiovascular disease: a review. Hum Biol 2004;76:127-46.
09. Burdge GC, Lillycrop KA, Jackson AA. Nutrition in early life, and risk of cancer and metabolic disease: alternative endings in an epigenetic tale? Br J Nutr 2009;101:619-30.
10. Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 2003;23:5293-300.
11. Yajnik CS. The insulin resistance epidemic in India: fetal origins, later lifestyle, or both? Nutr Rev 2001;59:1-9.
12. Chauhan G, Spurgeon CJ, Tabassum R et al. Impact of common variants of PPARG, KCNJ11, TCF7L2, SLC30A8, HHEX, CDKN2A, IGF2BP2, and CDKAL1 on the risk of type 2 diabetes in 5,164 Indians. Diabetes 2010;59:2068-74.
13. Yajnik CS, Fall CH, Vaidya U et al. Fetal growth and glucose and insulin metabolism in four-year-old Indian children. Diabetic Med 1995;12:330-6.
14. Bavdekar A, Yajnik CS, Fall CH et al. Insulin resistance syndrome in 8-year-old Indian children: small at birth, big at 8 years, or both? Diabetes 1999;48:2422-9.
15. Dabelea D, Hanson RL, Lindsay RS. Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. Diabetes. 2000;49:2208-11.
16. Rao S, Yajnik CS, Kanade A et al. Intake of micronutrient-rich foods in rural Indian mothers is associated with the size of their babies at birth: Pune Maternal Nutrition Study. J Nutr 2001;131:1217-1224.
17. McCance RA. Food, growth, and time. Lancet 1962;2:621-6.
18. Fall CHD, Yajnik CS, Rao S et al. The effects of maternal body composition before pregnancy on fetal growth; The Pune Maternal Nutrition Study. In: Fetal programming Influences on Development and Disease in Later life. Shaughn O'Brien PM, Wheeler T, Barker JP eds RCOG, London, 1999, Chapter 21, p231-245.
19. Joshi NP, Kulkarni SR, Yajnik CS et al. Increasing maternal parity predicts neonatal adiposity: Pune Maternal Nutrition Study. Am J Obstet Gynecol 2005;193:783-9.
20. Kinare AS, Natekar AS, Chinchwadkar MC et al. Low midpregnancy placental volume in rural Indian women: A cause for low birth weight? Am J Obstet Gynecol 2000;182:443-8.
21. Yajnik CS, Fall CHD, Coyaji KJ et al. Neonatal anthropometry: the thin-fat Indian baby: The Pune Maternal Nutrition Study. Int J Obes 2003;26:173-80.
22. Modi N, Thomas EL, Uthaya SN et al. Whole body magnetic resonance imaging of healthy newborn infants demonstrates increased central adiposity in Asian Indians. Pediatr Res 2009;65:584-7.
23. Yajnik CS, Deshpande SS, Panchanadikar AV et al. Maternal total homocysteine concentration and neonatal size in India. Asia Pac J Clin Nutr. 2005;14:179-81.
24. Yajnik CS. The lifecycle effects of nutrition and body size on adult adiposity, diabetes and cardiovascular disease. Obesity Reviews 2002;3:217-224.
25. Kuzawa CW. Adipose tissue in human infancy and childhood: an evolutionary perspective. Am J Phys Anthropol. 1998;27:177-209.
26. Antuna-Puente B, Feve B, Fellahi S et al. Adipokines: the missing link between insulin resistance and obesity. Diabetes Metab 2008;34:2-11.
27. Yajnik CS, Lubree HG, Rege SS et al. Adiposity and hyperinsulinemia in Indians are present at birth. J Clin Endocrinol Metab 2002;87:5575-80.
28. Whitehead JP, Richards AA, Hickman IJ et al. Adiponectin-a key adipokine in the metabolic syndrome.Diabetes Obes Metab. 2006;8:264-80.
29. Yang Q, Graham TE, Mody N et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005;436:356-362.
30. Yajnik CS, Deshpande SS, Jackson AA et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: The Pune Maternal Nutrition Study. Diabetologia 2008;51:29-38.
31. Bhate V, Deshpande S, Bhat D et al. Vitamin B12 status of pregnant Indian women and cognitive function in their 9-year-old children. Food Nutr Bull. 2008;29:249-54.