Beyond the Barker hypothesis and the thrifty genotype - The womb, ethnicity, genes and the environment - Recent perspectives on the evolution of diabetes and the metabolic syndrome in India

Nihal Thomas

Department of Endocrinology and Metabolism, CMC Vellore, Tamil Nadu, India

The increasing prevalence of diabetes mellitus in the country can be explained in part by the large changes in lifestyle that have occurred over the years over the last couple of decades. A number of studies have shown that these changes have been demonstrated predominantly in Dravidian and Aryan ethnic groups of the country. Some of these studies have not taken into account the proper definition of a rural environment. The other factors which may be responsible for the rising prevalence of diabetes and the metabolic syndrome in rural India are the impact of low birth weight and the influence of a hostile intrauterine environment (the thrifty phenotype hypothesis) and the impact of the genetic factors that may interact and influence the evolution of this condition. There was room therefore to study the body composition, physiological variables including energy expenditure, insulin resistance, micro-quanta of fat and genetic variables in these individuals.

Over the last 5 years we have made endeavors to answer some of these questions:-

1. Is the onset of diabetes and the metabolic syndrome ubiquitous and does it involve the rural Indian mongoloid population?
2. What is the pre-diabetic status and body composition of rural children and young adults in Southern India?
3. How does low birth weight influence body composition, energy expenditure, intramuscular and intra-hepatic fat, insulin sensitivity and arterial blood pressure in the Dravidian population when they become young adults?
4. What is the pattern of energy expenditure in Indians and does it influence the development of metabolic syndrome and diabetes?
5. How do genetic factors interact with the intrauterine environment and what is their impact on the metabolic syndrome and body composition after a few decades of life?

Access this article online	
Quick Response Code:	Website: www.ijem.in
	DOI: 10.4103/2230-8210.104024

The North East part of India is comprised of several ethnically variable groups of subjects who are Mongoloid in origin. Hypothetically, one may have probably have expected a slightly lower trend with regards to the prevalence of diabetes in this part of the country when compared to the Dravidian dominated Southern population.

Moreover, the socioeconomic indices and the degree of urbanization in the North East part of India may not have changed as profoundly when compared to the Southern part of the country and therefore, the impact of life style changes may be significantly less. The opportunity to answer this question arose after we were involved in a project sponsored by the World Diabetes Foundation, which was initially meant to train 100 hospitals across rural India to improve capacity building in rural areas from 2004 to 2009.

We have conducted epidemiological studies in the rural parts of Tripura and Arunachal Pradesh, through door to door visits, blood sampling and administration of diet and physical activity questionnaires. In the study that was conducted in Tripura, there were several salient features that

Corresponding Author: Prof. Nihal Thomas, Department of Endocrinology and Metabolism, CMC Vellore, Tamil Nadu, India.
E-mail: nihal_thomas@yahoo.com

stood out. Firstly, the prevalence of diabetes was relatively high in this largely rural set up- 9%.[1]

One could argue that since it was the first survey of its sort in that part of the country, there may be an element of length time bias: Wherein there could be an accumulation of cases over a period of time, thereby leading to a falsely increased number of patients with diabetes at the time of the first survey.

Nevertheless, the trends were significant, whatever the case may be. Secondly, the population that was assessed was a blend of the local tribal population and a Bengali speaking population in a ratio of 3:2. There was no statistically significant difference seen between the two ethnic groups, implying that ethnicity per se and the mongoloid race itself did not seem to confer a protective effect on the population against developing diabetes. So also the economic status did not have an influence on the prevalence of diabetes.

Thirdly, a physical activity questionnaire was administered to the subjects during the course of the survey. The result of which was that there was a reduced propensity for diabetes to occur in those subjects with a higher physical activity score: this achieved statistical significance on assessment.

More recently, we have conducted a study in Rural Arunachal Pradesh, adjacent to the border of China. From a perspective of resources, this region was far more remote and rurally oriented than the population that was studied in Tripura.

The awareness of diabetes in the study population was found to be as low as 21%. Majority of subjects (58%) had a normal BMI and adequate physical activity (88%). The prevalence of smoking (72%) and alcohol consumption (49%) was found to be rather high amongst the study population. Blood glucose screening revealed that 7.5% had diabetes, 13% had impaired fasting glucose and 6% had impaired glucose tolerance. These studies indicate that prevalence of diabetes is increasing rapidly in Mongoloid populations of rural North East India.[2]

Adjacent to our institution, Raghupathy and Antoniswamy have shown that in Gudiyattam Taluk in Vellore district, the prevalence of glycemic disorders was high in those below the age of 30 years. The data was obtained from systematic screening performed in one of India's oldest birth cohorts- the Vellore birth cohort which was initiated in 1970, by a visionary biostatistician. From the data that was collected in 2003, they reported an 18% prevalence of IGT in the urban region with a 14% prevalence in the rural area, indicating that rural areas are showing a rapid progression of prediabetes, which may be comparable to that of urban areas.

In contrast, the PODIS study conducted in rural Karnataka in 2005 cited a prevalence rate of 3.7% for diabetes above the age of 25 years and 2.8% for impaired glucose tolerance, one of the lowest prevalence being cited in recent times.

Indeed, one should be cautious in interpreting data which may show superabundance in the prevalence of diabetes and pre-diabetes in a so called rural or urban region. Indeed, we should always refocus on the question and figure out as to whether the survey done in a genuinely rural part of the country; the definition of the term 'rural' includes parts of a region which do not have a clustered population in excess of 50,000 living in a market town and a designated population of less than 999 per square mile. Updated census figures should be available for the region concerned. Rapid changes in population demographics that could occur between the time of established census data being released and the time of the survey on glycemic disorders being conducted may lead to scientific misinterpretation of the data which is generated. These factors are seldom taken into account when performing a survey for chronic disease prevalence in India.

The other fallacies that come into the picture include epidemiological quirks like lead time bias where first time screening may include a cumulative number of subjects who were in fact cases of diabetes belonging to a previous decade. Length time bias as a form of selection bias where in a statistical distortion of results occurs leading to an incorrect conclusion about the data. Length time bias can occur when the lengths of intervals analyzed by selecting intervals that occupy randomly chosen points in time or space. This process favors longer intervals, thus skewing the data. Thus, there is an over representation of IFG and IGT in screening surveys which does not necessarily indicate subsequent progression of disease. The newer cut off of 100 mg/dl for impaired fasting glycemia leads to earlier detection of the disease and may enhance the long term prognostic outcome of prediabetes.

We have also explored rural adolescent school children around Vellore and examined their body composition and metabolic parameters. We identified a simple method utilizing skin fold thickness measurements in predicting metabolic anomalies in children. Hyperglycemia was significantly associated with abdominal skin fold thickness and other metabolic parameters.[3]

There was a 20% prevalence of pre-diabetes and a 60% prevalence of dyslipidemia in 1,350 rural adolescents and young adults. Anthropometric predictors of metabolic abnormalities as classified by International Diabetes Federation definition were utilized.

The waist circumference (OR 1.56, 95% CI 1.0to 2.43: $P < 0.01$) and the abdominal skin fold thickness (OR 1.44, 95% CI 1.02 to 2.04, $P < 0.01$) above the third quintile cut-offs were found to be significantly associated with metabolic abnormalities. The sensitivity of either one of these measurements in predicting metabolic abnormalities was 66.1% with a negative predictive value of 82.8%. Hyperglycemia was significantly associated with an abdominal skin fold thickness over the fourth quintile alone (OR 1.63, 95% CI 1.24 to 2.1). All anthropometric measurements correlated well with elevated triglycerides and hypertension.

When considering the Indian population in rural areas, nutrition is still less than satisfactory in some parts of the country. Hence, mothers during the course of pregnancy may be substantially underfed in terms of protein intake. The consequence of such an event is the development of small, low birth weight babies in some situations. These babies, according to Barker- who first proposed this hypothesis in 1990, are more prone to diabetes and the metabolic syndrome, when they grow up; in fact, the quantum of fat in their bodies exceeds that of the normal birth weight subjects. The prevalence of low birth weight in India is pandemic- 26% of the babies born in rural India, even in the region surrounding Vellore, the prevalence is as high as 17%. In general South Asians have a lower birth weight and are more insulin resistant than their European counterparts. When comparing babies born in the United Kingdom with Indian babies- Indian babies are approximately - 800 g lighter, 30 mm shorter, 0.3 kg/m3 thinner, have less muscle but a much higher content of adiposity. The natural tendency of a mother is to overfeed a low birth weight baby, and attempt to deliberately increase the size of the infant. She may seldom realize that overfeeding in itself would substantially increase the risk of diabetes for these infants later on in life.

Why are these infants more prone for such a problem?

A collaborative venture at Vellore has unraveled some of the factors in assessing the pathogenesis of low birth weight induced diabetes. A population based cohort of 60 low birth (LBW) weight subjects and 60 normal birth weight (NBW) subjects born at term was taken from a rural part in the of North Arcot district where Vellore town is located. The subjects were 18 to 22-years old at the time of recruitment.[4]

Several indices have been used in epidemiological studies to measure and insulin resistances were assessed, including a hyperinsulinemic euglycemic clamp study (using an M value). Assessment of total energy expenditure was performed using an Actiheart device and resting energy expenditure using an indirect calorimeter.

Considering the presence of a high prevalence of the metabolic anomalies in the young, we then pursued to test the impact of the Barker hypothesis on male subjects in a 20-year-old birth cohort from rural Tamil Nadu. We explored the characteristics of 60 normal (NBW) and 60 low birth weight individuals (LBW). They were healthy, but had a mean BMI of 19.5 km/m2 and the age of 18-22 years. Men with LBW were shorter (167+/-6.4 cm vs. 172+/-6.0 cm, $P < 0.0001$), lighter (51.9+/-9 cm vs. 55.4+/-7 kg, $P < 0.02$) and had a reduced lean body mass (42.1+/-5.4 kg vs. 45.0+/-4.5 kg, $P < 0.002$) when assessed by DXA (Dual Energy X-ray Absorptiometry), compared with NBW controls. The individuals who were LBW had significantly lower bone mineral content. After adjustment for height and weight, the LBW subjects had increased diastolic blood pressure (77+/-6 nnHg vs. 75+/-6 mmHg, $P < 0.01$). Five LBW subjects had impaired glucose tolerance. *In vivo* insulin secretion and peripheral insulin action were similar in both the groups.[5] Mothers of the LBW subjects were 3 cm shorter than the control mothers. It is for the first time that in ethnic rural Indian subjects that hyperinsulinemic euglycemic clamp studies have been performed and deconvolution curves been analyzed. Based on these findings, we have evidence to suggest that the intrauterine environment has an impact on body composition and some metabolic parameters. However, the physical environment and lifestyle changes, when absent- temper the impact of the Barker hypothesis per se.

We also assessed, through NMR (Nuclear Magnetic Resonance) Spectroscopy the intrahepatic and intramuscular fat content. The results were intriguing. The low birth weight subjects had median hepatic fat levels of 0.272 [0 - 14.27 nmol] and the normal birth weight subjects had median hepatic fat levels of 0.707 [0 -9.16 nmol] when estimated by MR spectroscopy. The difference in the hepatic fat levels among LBW and NBW subjects was statistically significant ($P = 0.04$). It quanta of fat in the liver was negligible in a large number of subjects, partly explained by their extremely low BMI (Hitherto most previous studies have been performed in subjects with much higher BMIs in Caucasian populations and also at an older age). The difference in the intra and extra myocellular fat among LBW and NBW subjects was not statistically significant when estimated by MR Spectroscopic measurements.

Liver fat positively correlated with intramyocellular fat content, waist circumference, waist hip ratio, total body fat (by DXA), total cholesterol, LDL, SGPT and insulin levels in the IVGTT and C-Peptide levels during the clamp, independent of birth weights. Intramyocellular fat (IMCL) positively correlated with waist circumference, total fat, total cholesterol, triglycerides, LDL and some insulin levels during the clamp. Extramyocellular fat (EMCL) positively correlated with liver fat, waist circumference, total fat, and some insulin levels following an oral glucose tolerance test, independent of birth weights. EMCL negatively correlated with the M-value independent of birth weights, probably indicating that the evolving decline in insulin sensitivity was probably due to progressive accumulation intramuscular fat. Though there was no significant derangement in glycemic parameters in most patients, this accumulation of intramyocellular fat may well be a predictor for a future decline in glycemic metabolism, which may evolve with age-indicating the importance of lack of insulin sensitivity in the muscle in this population.

The resting energy expenditure (REE) constitutes 65% to 70% of the total energy expenditure. Various equations are present to compute the resting energy expenditure (REE). Our investigations with regards to resting energy expenditure in the cohort of rural 19-year-old men were largely unremarkable between the LBW and the NBW groups. However, we performed cross-sectional study was done in 30 elite male weightlifters aged 17-28 years, competing at the National and International levels. Anthropometric and body composition was measured. REE was measured on *30 male professional weightlifters aged between 17-28 years* using indirect calorimetry and compared with the 8 formulas predicted by Harris-Benedict, *Mifflin-St Jeor, FAO/WHO/UNU, ICMR, Cunninghams, Owen, Katch-McArdle and Nelson.*[6] The Pearson correlation coefficients between mREE and the anthropometric variables showed positive significance. All 8 predictive equations underestimated the REE of the weightlifters when compared with the mREE. The highest mean difference was 636 kcal/day (Owen, 1986) and lowest difference was 375 kcal/day (Cunninghams, 1980). Multiple linear regression done stepwise showed that LBM was the only significant determinant of REE in this group of sportspersons. A new equation using LBM as the independent variable for calculating REE was computed. REE for weightlifters = -164.065 + 0.039 (LBM) [C.I. -1122.984, 794.854]. This new equation reduced the mean difference with mREE by 2.36 + 369.15 kcal/day (S.E = 67.40).

The significant finding of this study was that all the prediction equations underestimated the REE, which is important from an Indian perspective. The LBM was the sole determinant of REE in this population. In the absence of indirect calorimetry, the REE equation developed by us, using LBM is a better predictor for calculating REE, at least in the professional male weightlifters of this region.

The thrifty phenotype has had its say, but the question arises as to whether there are differences in the genetic impact on Indian ethnic groups when compared to their western counterparts? We ventured further, by using the physical variables of one of India's oldest birth cohorts-the Vellore birth cohort, and exploring some aspects of their genetic make-up.

From a genetic perspective, our group has differences in the pattern of influence of genes on the Indian phenotype versus what is seen in Europe. Recently, two birth weight-lowering loci on chromosome 3 (near CCNL1 and ADCY5) were identified in a genome-wide association study, the latter of which is also a type 2 diabetes locus. Therefore, we tested the impact of these genetic variants on birth weight and adult glucose/insulin homeostasis in a large Indian birth cohort. Adults (n = 2,151) enrolled in a birth cohort (established 1969-73) were genotyped for rs900400 (near CCNL1) and rs9883204 (ADCY5). We tested associations between these genetic variants and birth weight, anthropometry at infancy, childhood, adolescence and adulthood, and with adult fasting glucose and insulin concentrations.

The average birth weight in this population was 2.79 ± 0.45 kg and was not associated with genetic variation in CCNL1 (P = 0.94) or ADCY5 (P = 0.76). Allele frequency for the birth weight-lowering variants were similar compared with Western populations. There were no significant associations with growth or adult weight. However, both variants displayed significant effects on adult estimates of insulin resistance (HOMA-IR: P = 0.01 and P = 0.008, respectively). The low birth weight in Asian Indians living in India (could be different if living in Europe) is not explained by genetic variations near CCNL1 and ADCY5 and the absent modulation of birth weight implies that non-genetic factors predominate. However, relationships with adult insulin resistance reinforce the argument for common genetic denominators for low birth weight and insulin resistance.

The association between genetic variants in *FTO* (rs9939609) and near MC4R (rs17782313) with obesity- and type 2 diabetes (T2DM)-related traits. The *FTO* locus displayed significant associations with obesity-related anthropometric traits. There was a per allele increase of 1% for BMI, waist circumference, hip circumference and waist-hip ratio.[7]

Obesity associations for the *MC4R* locus were weak, but significant signal for height was present different from that seen in Aryan Indian from the Northern part of India and European populations. The effect on obesity-related traits for *FTO* was seen in adulthood, but not at younger ages. The loci also showed associations with increased blood glucose, which was lost on BMI adjustment. The effect of *FTO* on obesity-related traits was driven by an integrated urban environmental influence.

We went further to address the genetic effect on obesity-related traits among Indian adolescents, we investigated the association between two common variants; rs9939609 (*FTO*) and rs17782313 (*MC4R*) with body mass index (BMI), weight, height, waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), body fat percentage and glucose traits in a cross-sectional cohort of 1,230 adolescents [mean (SD) age, 17.1 (1.9) years; [males: 917 (75%), females: 309 (25.2%)] from a homogenous population from South India. The variant at the *FTO* locus was found to be associated with waist-hip ratio (WHR) but, not with overall obesity in this population. There was no significant association observed for measures of obesity with *MC4R* variant rs17782313. We conclude that a common variant of *FTO* is associated with body fat distribution during early growth in Indian adolescents and may predispose to obesity and metabolic consequences in adulthood.[8] To our knowledge, this is the first replication study of these loci among Indian adolescents. Further, our cohort is a well-characterized and drawn from a homogenous population, allowing for assessment of the genetic variants in relation to multiple measures of obesity.

A low power (60%) may explain the non-significant association with BMI, however it should be emphasized that BMI is not a reliable indicator of obesity in young Indians, who are known to have a lower lean body mass and higher subcutaneous fat. Deviations from HWE could probably be due to enrichment of homozygosity, which is the case in consanguineous parentage (26%). Exclusion of individuals born to consanguineous parentage also showed deviations from HWE, which probably is related to the enrichment of homozygosity since inter-familial marriages might have been practiced in the earlier generations in this population. We did not perform multiple-correction testing, due to high *priori* assumptions of the association of these well-known genetic variants with obesity traits.

Diabetes and the metabolic syndrome in the rural Indian context are ubiquitously on a increase. These studies from an epidemiological, physiological and genetic perspective are foundational in helping us understand the complex interaction between the thrifty phenotype, the thrifty genotype and the environment in rural India.

References

1. Lau SL, Debarma R, Thomas N, Asha HS, Vasan KS, Alex RG, *et al*. Healthcare Planning in North-East India: A Survey on Diabetes Awareness, Risk Factors and Health Attitudes in a Rural Community. J Assoc Phys India 2009;57:305-9.
2. Nanniah A, Wagmere A, Milton P, Samuel P, Thomas N. Awareness and attitude towards diabetes in the rural population of Arunachal Pradesh, North East India. Indian J Endocrinol Metab 2012; 16:S83-6.
3. Vasan SK, Thomas N, Christopher S, Gitanjali FS, Paul TV, Sanjeevi CB. Anthropometric Measurements for the Prediction of the Metabolic Syndrome: A Cross-sectional study on Adolescents and Young adults from Southern India. Heart Asia 2011;3:2-7.
4. Thomas N, Grunnet LG, Poulsen P, Christopher S, Spurgeon R, Inbakumari M, *et al*. Born with low birth weight in rural Southern India - What are the metabolic consequences 20 years later? Eur J Endocrinol 2012;166:647-55.
5. Thomas N, Asha HS, Spurgeon R, Christopher S, Mohan VR, Inbakumari I, *et al*. Type 2 diabetes in rural India- new paradigms in its epidemiology and evolution. J Indian Med Assoc 2009;107:785-90.
6. Joseph M, Prema L, Inbakumari M, Thomas N. Energy expenditure studies amongst South Indian professional weightlifters. Indian J Nutr Diet 2012;49.
7. Vasan SK, Fall T, Neville MJ, Antonisamy B, Fall CH, Geetanjali FS, *et al*. Associations of variants in *FTO* and near *MC4R* with obesity traits in South Asian Indians. Obesity 2012;20:2268-77.
8. Vasan SK, Thomas N. Developmental origins of adult metabolic disease: The Indian scenario, driving towards a unified hypothesis. Indian J Endocrinol Metab 2012;16:493-5.

Cite this article as: Thomas N. Beyond the Barker hypothesis and the thrifty genotype - The womb, ethnicity, genes and the environment - Recent perspectives on the evolution of diabetes and the metabolic syndrome in India. Indian J Endocr Metab 2012;16:S142-6.

Source of Support: Nil, **Conflict of Interest:** None declared