

## 3<sup>rd</sup> College of Paediatrics and Child Health Lecture – The Past, the Present and the Shape of Things to Come...

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### Abstract

The growth trends of Singapore children spanning 5 decades are reviewed, based on 8 anthropometric studies from 1957 till 2002. The heights of pre-school children and school age children appear to have optimised according to their genetic potential, but the weights and body mass indices of children still appear to be increasing from 6 to 18 years for both sexes, probably as a consequence of increasing affluence. This trend is reflected in the increasing obesity prevalence in school children over the past 30 years, and the concomitant increased morbidity associated with the metabolic syndrome, necessitates further research into the causes of obesity. Barker's hypothesis first suggested that changes in the intra-uterine environment can cause fetal adaptations which persist into adulthood, and are responsible for many chronic diseases of adult life. More recently, intense research in the field of epigenetics suggests that the environment can also influence the phenotype through gene expression, through modification of DNA methylation and histones which, in turn, influences gene expression. The challenge for the future is to determine if there are clear epigenetic changes, which are responsible for the increased prevalence of childhood and adolescent obesity, and whether these changes are transmitted through generations. Unravelling these epigenetic mechanisms may be the key to the prevention of obesity and the metabolic syndrome.

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**Key words:** Body mass index, Epigenetics, Height, Weight

### Introduction

In the post-war days of the 1950s, Singapore children were faced with problems of malnutrition and infectious diseases. There was poverty, overcrowded housing and lack of hygiene, and the social conditions were apparently appalling.<sup>1</sup> However, social and economic conditions slowly improved, as the government systematically improved the standard of living, and emphasised health education. From the late 1960s, school health teams were sent out to schools to screen children for growth and general health problems. Over the subsequent decades, social and nutritional conditions have also improved considerably. However, with increasing affluence, other problems have changed the landscape of diseases affecting children and adolescents of today and the future.

### Singapore Anthropometric Trends

The aim of this first segment examines how the improved

standard of living has affected the secular trend of growth in Singapore children over the past 5 decades.

### Preschool Children

The methodology employed a review of the 4 major growth studies reported for pre-school Singapore children from 1957 to 2000, which spans 5 decades of growth.<sup>2-4</sup> Table 1 documents the 4 major anthropometric studies performed in the history of Singapore. The 2 data sets for 1988 and 2000 had the largest sample size and representativeness of the population was ensured. Hence, the mean heights for these 2 studies were used for direct statistical comparison, using the paired *t*-test for significance.

Although the studies varied in methodology, a crude comparison clearly demonstrates an increase in mean heights for boys and girls from the 1957 to 2000 (Fig. 1a). However, on examining the 2 most recent data sets in 1988 and 2000,

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Table 1. Anthropometric Studies in Singapore

Anthropometric study	Sample size	Sample population
<b>A. Preschool Children (0-6 years)</b>		
1. Millis J (1957) <sup>2</sup>	526	Children of Malay constables (at police quarters): Whitley, Pearl's Hill, Maxwell Rd, Hill St, Rochore
2. Wong HB (1972) <sup>3</sup>	4419	District 3
3. Yap MA (1988) <sup>4</sup>	13,565	10 government schools, 21 nurseries, 18 kindergartens
4. Emmanuel S (2000)	19,249	All Singapore polyclinics
<b>B. School Age Children (7-18 years)</b>		
1. Wong HB (1975) <sup>5</sup>	5039	District 3
2. Lyen KR (1988)	*	*
3. Rajan U (1993)	34,030	All school children
4. Deurenberg-Yap (2002)	13,863	24 schools (10 primary, 10 secondary, 4 junior college)

St: Street; Rd: Road; \*Data unavailable

there were no significant differences in the mean height for preschool boys and girls as the graphs superimposed, suggesting that heights of preschool Singapore children have optimised according to their genetic potential.

From 1957 to 2000, the mean weights for preschool children also demonstrated a significant increase from 5 years onwards for both boys and girls (Fig. 1b), and there was also a significant increase in body mass index (BMI) for children in 2000 as compared to 1988 from the age of 5 years onwards, in both boys and girls (Fig. 1c).

**School Age Children**

Similar methodology was employed for the 4 major studies, from 1975 to 2002,<sup>5</sup> as shown in Table 1, which documents the sample size and population of each study. Unfortunately, the raw data for 1988 could not be traced, and heights and weights were only estimated from existing growth charts available from that study. Data from the last

Fig 1a: Mean Height by Age, 0-72 months

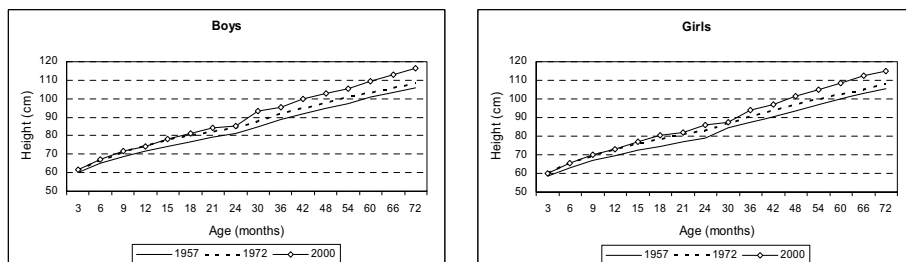


Fig 1b: Mean Weight by Age, 0-72 months

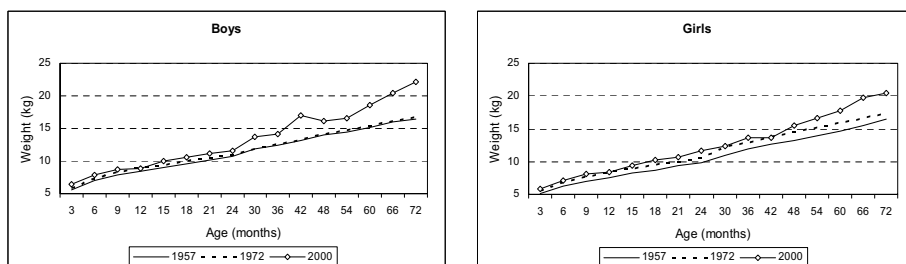


Fig 1c: Mean BMI by Age, 0-72 months

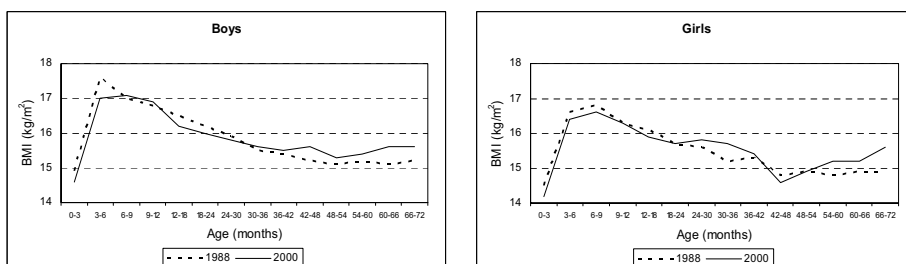


Fig. 1. Comparison of serial anthropometric indices for pre-schoolers.

Fig 2a: Mean Height by Age, 7-18 years

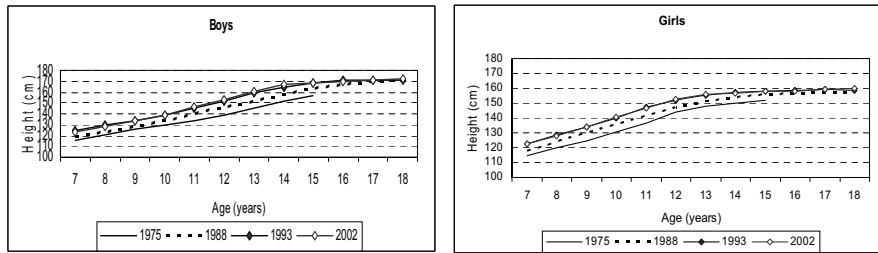


Fig 2b: Mean Weight by Age, 7-18 years

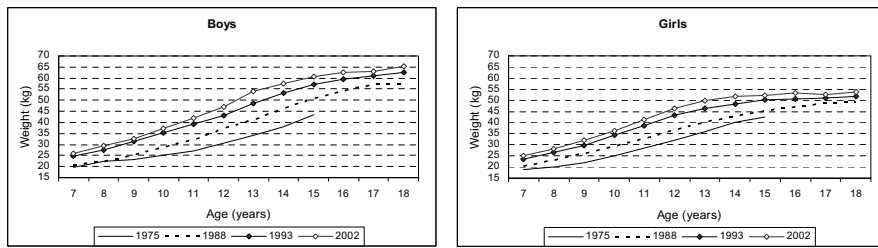


Fig 2c: Mean BMI by Age, 7-18 years

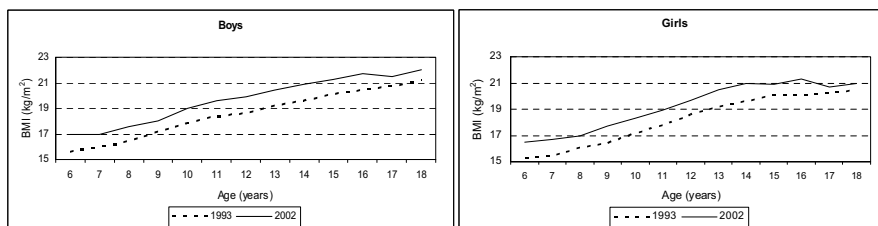


Fig. 2. Comparison of serial anthropometric indices for school-age children and adolescents.

2 studies in 1993 and 2002 remained unpublished (although the raw data sets are available). These 2 studies had the largest sample size, and representativeness of the population was ensured.

The height curves for Singapore school children aged 7 to 18 years are represented on Figure 2a. As with the preschool children, the mean heights of boys and girls have significantly increased from 1975 till 1993, but have not increased significantly from 1993 as compared to the most recent study in 2002. This suggests that the present heights of school age Singapore children have probably reached their optimal genetic potential, as a consequence of significantly improved living standards, better nutrition and better medical care for children.

However, the weights of boys continue to increase significantly from the 1975 through to 2002 (Fig. 2b), a trend which was also seen in the school age girls, although this was not as pronounced, but nonetheless still significant. As expected, the BMI for school age children is significantly higher for the 2002 study, as compared to the 1993 study

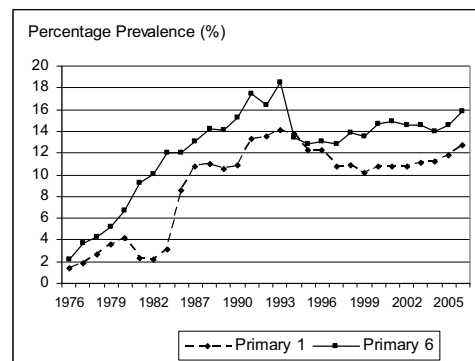


Fig. 3. Significant increase in percentage prevalence of obesity in primary 1 and primary 6 school children over the past 30 years.

(Fig. 2c). This is a likely consequence of increasing affluence.

On summarising all the 8 data sets:

1. The heights of pre-schoolers and school age children appear to have optimised according to their genetic potential.
2. The weights of children still appear to be increasing from 6 to 18 years for both sexes.

3. The body mass indices also appear to be increasing in tandem with this trend in weight increase.

### Prevalence of Childhood and Adolescent Obesity

Figure 3 summarises the prevalence of obesity obtained from the School Health Service and Health Promotion Board over the past 30 years. In 1976, the prevalence of obesity (defined as body weight more than 120% of the standard weight for height) was only 1.4% for Primary 1, and it increased 9-fold to 12.7% by 2006. Similarly, the obesity prevalence was 2.2% in Primary 6 students 30 years ago, and it increased 7-fold to 15.9%.

The prevalence of childhood and adolescent obesity in Singapore mirrors the global epidemic of obesity in both adults and in children. This is often referred to as the “globesity” epidemic, which poses one of the greatest threats to human health because of the consequences of obesity which include the metabolic syndrome (glucose intolerance, hypertension, dyslipidaemia), steatohepatitis, obstructive sleep apnoea and self image issues which affect mental health.

### What is the Role of Genetics and the Environment to Obesity?

In the energy equation, weight gain occurs when caloric intake exceeds energy expenditure. Research has shown that identifiable molecular genetic abnormalities presently account for <5% of obese individuals, and obesity is mostly polygenic with susceptibility conferred via complex genetic factors. Clearly, genes have a permissive role and interact with the environment to promote obesity. Hence, it seems that the environment has a significant role to play in the causation of obesity, as it has been argued that genes do not change so quickly in a few decades.

More recently, there has been increasing research into the field of epigenetics, which represents the missing link between genetics and the environment. In essence, it describes how the environment can influence the phenotype through modification of gene expression.

### What is Epigenetics?

Epigenetics is the study of heritable alterations in gene expression, which are not caused by changes in DNA sequence.<sup>6</sup> It is a new emerging concept in human biology, and refers to how the environment can influence the phenotype through gene expression. It occurs in humans, and is now an area of intense research, as it may provide pivotal clues to the fetal origins of obesity and the metabolic syndrome.

Fetal growth traditionally depends on both the genes and the environment, including the supply of nutrients from the mother. In a suboptimal environment, the baby adapts to the reduced nutrients and re-distributes the nutrition and

cardiac output to vital organs such as the brain.

In 1991, Hales and Barker<sup>7</sup> described the fetal origins of adult disease, which states that fetal adaptations to the intra-uterine environment are the origins of important chronic diseases of adult life. Extensive human epidemiological data indicate that nutrition during critical periods of development can influence adult susceptibility to chronic disease, including components of the metabolic syndrome. Hales and Barker<sup>7</sup> reported the associations of Hertfordshire, which showed for the first time that people who had low birth weight were more insulin resistant, and had higher rates of impaired glucose tolerance and the metabolic syndrome in later life.

The effects could also be replicated in the experimental animal model: Reducing the mother’s food intake around the time of conception and during pregnancy in the rat, could induce persistent changes in gene expression and metabolism. This is the concept of fetal programming, which refers to the physiological adaptations in development that lead to life-long changes in structure and function of the body. It is as though the baby receives a forecast of the nutritional environment it will encounter after birth from the mother, and changes its physiology and metabolism accordingly. The adaptation is beneficial, so long as the baby continues to be poorly nourished in childhood and adulthood. However, a mismatch between the predicted environment and the actual environment may lead to the consequence of chronic disease. Hence, the adaptation becomes harmful if there is a mismatch with increased food intake, decreased energy expenditure and the development of obesity, as there will be increased insulin resistance, combined with a reduced capacity to secrete insulin, because of impaired pancreatic  $\beta$ -cell development. All this leads to impaired glucose tolerance, insulin resistance syndromes and type 2 diabetes mellitus.<sup>7</sup>

Waterland and Garza<sup>8</sup> also proposed the term “metabolic imprinting” to encompass these adaptive responses to early nutrition characterised by susceptibility limited to a critical period of development, with persistent effects lasting into adulthood. However, the specific biological mechanisms underlying these phenomena have not been characterised. Recently, epigenetics has been proposed as an important mechanism, which contributes to metabolic imprinting.

Epigenetics operates through mechanisms that define the conformational change in chromatin which determines gene silencing or expression. These include:

- (a) DNA methylation [the addition of a methyl group to islands of cytosine-phosphodiester-guanosine (CpG) dinucleotides, occurring within the gene promoters]. Methylation which is associated with silencing of the associated gene, restricts transcription while demethylation allows gene expression.<sup>9,10</sup>

(b) Histone modification by methylation, acetylation, phosphorylation also changes the structure, and determines the accessibility of DNA for active transcription.

## What is the Significance of Epigenetics?

### I. Imprinted Genes

Imprinted genes are among the most well understood candidates of epigenetic transcriptional modification. Imprinted genes are a subset of approximately 80 known genes in humans, which display mono-allelic expression (expression only occurs from a single parental allele). The control regions for genomic imprinting usually contain a differentially methylated CpG island in which one parental allele is methylated and the other, unmethylated. This allows for epigenetic modification.<sup>11</sup>

An increasing number of growth regulatory genes are controlled by this epigenetic phenomenon of genomic imprinting. The methylation and histone alterations and expression of the single allele of an imprinted gene is parent-of-origin dependent. Epigenetic changes have resulted in the abnormal expression of imprinted genes in Prader Willi Syndrome, Angelman syndrome and Russel Silver syndrome.<sup>12</sup>

### II. Epigenetics and Early Life Periods

#### A. The peri-conceptual period

Early nutrition can influence DNA methylation because mammalian 1-carbon metabolism, the source of all biologic methylation reactions, is very dependent on diet methyl donors such as choline and methionine, and co-factors of folate, vitamin B12, B6.<sup>13</sup> From animal experiments, rats fed a diet deficient in folate exhibited a change from normal DNA methylation to global DNA hypomethylation. Folate supplementation led to enhanced DNA methylation.<sup>14</sup>

Epigenetic changes have also been described in the model of the yellow agouti mouse.<sup>15,16</sup> The agouti gene results in a yellow pigment, rather than the black pigment. Maternal dietary methyl supplementation with folic acid, vitamin B12 and choline increased methylation at the agouti gene promoter, and shifted the coat colour distribution of the offspring toward the black colour. Interestingly, the yellow mice were more obese, were more insulin resistant and developed diabetes mellitus.

If rat mothers had severe protein restriction during gestation, their offsprings were found to have increased pancreatic apoptosis, leading to smaller mass of pancreatic  $\beta$  cells.<sup>17</sup> This can also disturb the development of the endocrine pancreas in the next generation. It is believed that epigenetic alterations induced by nutritional influences during early development can be perpetuated to affect gene

expression, metabolism and susceptibility to phenotype and chronic diseases in later life.

#### B. Late gestation and early neonatal periods

Data from animal models have shown that late fetal and early postnatal nutrition or adverse environmental factors can also lead to altered methylation of both imprinted and non-imprinted genes, and to changes in gene expression.<sup>17</sup> In neonatal rats, a diet high in carbohydrates immediately induces hyperinsulinaemia, which then persists into adulthood without any further nutritional stimulus.<sup>17</sup> The metabolic imprinting forms a vicious cycle because these female rats spontaneously transmit their phenotype to their progeny!

Similarly, in human children and adolescents, undereating and overeating at critical periods of development may both programme carbohydrate and lipid metabolism for life, thus predisposing them to the metabolic syndrome. However, the strongest evidence of postnatal epigenetic programming comes from the Dutch Famine study. During the famine of 1944 to 1945 in the Netherlands, previously well nourished women were subject to low caloric intake and environmental stress. Pregnant women exposed to famine in late pregnancy gave birth to smaller babies, who had an increased risk of later insulin resistance. The young adult subjects conceived during the famine demonstrated higher 2-hour plasma glucose values after an oral glucose load than controls born before or conceived after the famine. Famine exposure at different stages of gestation was associated with increased risk of obesity, dyslipidaemia and coronary heart disease.<sup>18</sup>

### III. Does Epigenetics Contribute to Metabolic Syndrome X?

At present, there is no published data linking epigenetic modification to the development of the metabolic syndrome, but this field is just being intensively researched. However, there is some circumstantial evidence: Diabetic patients have reduced S-adenosylmethionine, the main physiologic donor of methyl groups, and are at risk of hypomethylation.<sup>19</sup> DNA methylation errors and hypomethylation accumulates with increasing age, possibly accounting for higher incidence of metabolic syndrome in older individuals.<sup>20</sup>

Converging data appear to support the hypothesis that individuals who suffered from inadequate nutrition and metabolic disturbances during fetal and postnatal development underwent incorrect “epigenetic programming” and developed the metabolic syndrome.

However, in order to really prove that epigenetics does contribute to the metabolic syndrome, 3 criteria must be satisfied:

1. There must be clear demonstration that environmental changes can result in the metabolic syndrome.

2. Experimental data must confirm that environmental changes can cause changes in methylation of CpG islands and acetylation of histones.
3. Experimental data must unequivocally demonstrate that these epigenetic changes cause disease components of the metabolic syndrome.

Presently, there is strong evidence for the first criterion through the epidemiological studies of Barker and the Dutch Famine study which show that early nutrition is associated with subsequent metabolic problems, but evidence for the other 2 criteria are currently lacking. If the agouti mouse model is true, and there is demonstration of environment-induced gene expression, which is clearly responsible for components of the metabolic syndrome, then the hypothesis may also prove to be true for humans. Indeed, if it can be proven that “transgenerational effects” exist, then it provides us with much food for thought, and a paradigm shift in our understanding of the pathogenesis of the metabolic syndrome:

1. As children of the past generation, are we ourselves carrying epigenetic changes of our forefathers?
2. Will obese children pass on their epigenetic changes to their progeny?
3. Will the obesity perpetuate itself, or progressively worsen with each generation?
4. We have all seen some obese children who claim to adhere to diet and exercise recommendations, but yet increase in weight and develop insulin resistance. Can they really help being obese, or has their metabolism been pre-set to lower rates?

### Epigenetics Research: The Way Forward

Clearly, deciphering the epigenetic targets and mechanisms of dysregulation by environmental exposure is the first step to translation into clinical practice. The altered epigenetic patterns may serve as biomarkers for the assessment of prognosis. If proven, then children with biochemical markers of disease may be identified to develop overt metabolic disease in adult life. Epigenetic research may provide new opportunities to develop prophylactic strategies in the form of novel diets or drugs, especially if these changes are reversible.

### Conclusion

In conclusion, this review has provided the opportunity to glance into the mirror of the past, and reminisce on the poor growth and suboptimal nutrition of the children of yesteryear. It has allowed us to assess the present situation, and how improved standards of living have optimised the heights of children, but resulted in the penalty of obesity. Finally, it has highlighted epigenetics as an important research theme for the future. The revelation that the

environment can influence gene expression and the possibility that transgenerational inheritance may occur, poses many interesting but unanswered questions for future research. Unravelling these epigenetic mechanisms may be the key to the prevention of obesity and the metabolic syndrome. How well we do it will determine the shape of things to come...

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