Developmental Perspectives on Individual Variation: Implications for Understanding Nutritional Needs

Peter D. Gluckman\textsuperscript{a}, Alan S. Beedle\textsuperscript{a}, Mark A. Hanson\textsuperscript{b}, Eric P. Yap\textsuperscript{c}

\textsuperscript{a}Liggins Institute, University of Auckland, Auckland, New Zealand; \textsuperscript{b}Developmental Origins of Health and Disease Centre, Institute of Developmental Sciences, University of Southampton, Southampton, UK; and \textsuperscript{c}Defence Research and Technology Office, Ministry of Defence, Singapore

Abstract

Genetic research has focused on identifying linkages between polymorphisms and phenotypic traits to explain variations in complex biologies. However, the magnitude of these linkages has not been particularly high. Conversely, the ability of developmental plasticity to generate biological variation from one genotype is well understood, while interest has emerged in the clinical significance of epigenetic processes, particularly those influenced by the external environment. Environmental cues in early development may induce responses that provide adaptive advantage later in life. The benefit of such responses depends on the fidelity of the prediction of the future environment. Life history and physiological changes mediated through epigenetic processes then follow, determining the later phenotype. Developmental mismatch, leading to disease, can arise from discordance between the fetal environment, which is relatively constant across generations, and the postnatal nutritional environment, which can change drastically within and between generations. Metabolic disorders represent the outcome of an individual living in an energetically inappropriate environment. Experimental and clinical evidence suggests that individual capacity to live in a given energetic environment is influenced by developmental factors acting through epigenetic mechanisms. Epigenetic biomarkers may be able to identify a risk of developmental mismatch and thus offer the opportunity for nutritional or other intervention.

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Introduction

The genetics and genomic revolutions focused attention on genes as the basis of individual variation. As has been reviewed elsewhere [1], this led to a
reduced appreciation of the role of developmental factors in such diversity. Recent progress in our understanding of developmental plasticity, particularly in comparative biology, has led to the recognition that developmental processes act through epigenetic mechanisms to have a major influence on phenotypic variation. This article reviews the extent to which these processes contribute to variation in body composition and insulin sensitivity.

An individual's phenotype and capacity to live healthily in a given nutritional environment are influenced by developmental, epigenomic and genomic profiles. In all populations the incidence of obesity and its associated disorders such as type-2 diabetes and cardiovascular disease represents a growing concern. Simplistically, this has been attributed to the changing patterns of nutrition and exercise (the nutritional transition), but there has been little consideration of individual variation in the propensity to develop obesity in a given environment. There is growing evidence that there are important developmental elements in, and several developmental pathways to, obesity. But adipose tissue is not homogeneous, there are functional differences between visceral and subcutaneous fat, and there is increasing consideration of the role of muscular and intramuscular fat. However, the relationship between different patterns of obesity and disease risk is poorly understood – for example, Singaporeans have a high rate of diabetes relative to the rate of overt obesity [2]. These considerations are critical for population and individual strategies for prevention and intervention.

A further complexity is provided by the growing recognition that different fat depots have very different properties in terms of both metabolic lability and cytokine/adipokine profile. Visceral fat is considered to be that most associated with metabolic disease [3], although new imaging techniques now raise the question of the role of intramuscular fat as a source of peripheral insulin resistance [4]. The use of magnetic resonance imaging allows accurate in-life assessment of visceral fat, and it is now apparent that smaller children already have visceral obesity at birth [5] and that some individuals can have visceral obesity without obvious subcutaneous obesity (the thin outside/fat inside (TOFI) syndrome). This appears to be more common in some populations [3]. A major defect in virtually all current population studies is the reliance on simple measures of obesity without any attempt to understand it in terms of the different fat masses.

**Genetic Variation**

The initial thrifty genotype model of Neel proposed that humans had been selected in Pleistocene environments for genes that would promote energy (fat) storage, conferring a fitness benefit in the supposed environment of ‘feast and famine’ and a high protein/low glycemic index diet. Neel proposed that in recent decades, as humans lived longer and were now exposed to
higher nutrient burdens and lower energy expenditure, this formerly adaptive trait had become maladaptive in the form of an increased burden of disease in middle age. However, this model had significant weaknesses [6], including the failure to find genes to explain the very high incidence of type-2 diabetes in some populations (e.g. Pima Indians), the extremely rapid appearance of type-2 diabetes in some populations and at a declining age, and population differences – for example, in India type-2 diabetes is appearing at nutritional levels and body compositions well within the normal range.

There has been a large international effort seeking genetic linkages to explain variations in body composition and the propensity to diabetes. A number of polymorphisms have been identified which confer added risk [7], but even for those with the greatest linkage the attributable risk is not great, for example, the risk allele of \( FTO \) confers an added risk of obesity of 1.67-fold [7]. Only rare forms of obesity (for example, leptin receptor deficiency) and type-2 diabetes (for example, glucokinase deficiency) have been linked to monogenic disease.

**Developmental Factors**

In the late 1980s, epidemiological studies [for review see 8] started to show linkages between birth size and later risks of cardiovascular disease and type-2 diabetes and, more recently, with body composition. A critical feature which was not initially understood was that the relationship is continuous across the full range of body sizes – this led to the recognition that later disease was not a result of a disruption of development, as initially suggested, but was the adverse outcome of the normative processes of developmental plasticity [9].

Developmental plasticity can be defined as the processes by which one genotype can lead to a range of phenotypes as a result of environmental influences acting during development. There has been an enormous surge of comparative research in developmental plasticity in recent years (particularly in the field of ecological developmental biology [1]) and only in the last 5 years have the implications for human medicine become truly apparent [10]. A major mechanism underpinning developmental plasticity is that of epigenesis, whereby DNA methylation and/or histone modification of specific genes is induced by environmental factors. There is now compelling experimental and growing clinical evidence that epigenetic factors play a major role in individual susceptibility to obesity and type-2 diabetes/insulin resistance [11].

Experimentally there is now a large amount of work showing that environmental manipulation in early life can lead to obesity, insulin resistance and endothelial dysfunction and that this is associated with epigenetic changes. Most work has been conducted in rodents, where maternal undernutrition (balanced or a low protein diet) or maternal glucocorticoid administration
lead to offspring who develop insulin resistance and/or hyperinsulinemia, leptin resistance, hyperphagia, sarcopenia, obesity (visceral), endothelial dysfunction, reduced nephron number and hypertension [12]. These rats also have alterations in their hypothalamic-pituitary-adrenal axis, altered rate of maturation (earlier puberty, as also seen in humans [13]) and altered behaviors in open field testing. This is analogous to the metabolic syndrome complex seen in humans. These changes are associated with specific epigenetic and/or gene expression changes in the liver, fat and muscle of the offspring [11]. Components of the hypothalamic-pituitary-adrenal axis are particularly affected, including changes both in the glucocorticoid receptor and in the enzyme which inactivates active glucocorticoids (11β-hydroxysteroid dehydrogenase type 2), with consequential changes for glucocorticoid-dependent metabolic enzymes such as phosphoenolpyruvate carboxykinase. Changes are also seen in transcriptional factors regulating fat metabolism such as peroxisome proliferator activated receptor-α, in factors associated with insulin action such as phosphoinositide 3-kinase and protein kinase-ζ, and in factors associated with endothelial function such as endothelial nitric oxide synthase and estrogen receptor-α. Recently, we have shown that reversal of the developmental induction of metabolic dysfunction by neonatal leptin administration in females is associated with reversal of the epigenetic changes [14].

There may be major gender effects that need consideration. Experimentally, females and males have differing sensitivities to maternal undernutrition and neonatal manipulation. As selection operates to optimize reproductive fitness, it is realistic to expect developmental plasticity to operate differently in males and females, reflecting their different reproductive strategies. Epidemiological and experimental data show that females respond to intruterine undernutrition with accelerated maturation (puberty) provided that postnatal nutrition is adequate to support pregnancy [15]. This is logical in that females can only protect fitness by maximizing their reproductive lifespan – in the expectation of a threatening environment it would therefore be appropriate to put on postnatal fat to support pregnancy and lactation at a young age, and to enter puberty early. Males in most systems, including probably Paleolithic humans, rely on dominance in a narrower period of their lifespan to maximize their fitness. Thus they may protect body size differently. This is an area of fertile research.

A key question is over what period of development does metabolic plasticity exist? The work of Kwong et al. [16] suggests that periconceptional factors can lead to permanent effects on the offspring in rodents. At the other extreme, the term ‘programming’ was used by Lucas [17] to show long-term the effects of breastfeeding versus formula feeding, suggesting that metabolic plasticity extends well after birth in humans. Recent studies confirm that formula feeding is associated with a high risk of later obesity, insulin resistance and hypercholesterolemia [18].
**Interaction between the Prenatal and Postnatal Environments**

A key factor in rodent studies is that the obesity and metabolic phenotype is dependent on the post-weaning environment. Vickers et al. [19] showed that the effect of prenatal undernutrition and that of postnatal high-fat diets were comparable on the development of obesity and insulin resistance, but that there was a clear synergistic interaction between the prenatal poor environment and the postnatal rich environment such that those antenatally undernourished were more at risk of developing obesity and insulin resistance on a high-energy diet after birth. Indeed, for some measures such as alterations in the neuroendocrine control of appetite [20], it required both elements to be present for abnormality to be seen.

**A Framework for Understanding Developmental Pathways to Obesity and Insulin Resistance**

The epidemiological studies of Eriksson et al. [21] in Finland showed that in a population who are now in their 60s and on whom detailed growth records exist, those who developed type-2 diabetes in their life course showed an earlier adiposity rebound (the time in childhood between 2 and 6 years of age when body fat stops falling from its high levels in infancy and starts to rise again) and progressively gained weight relative to height through later childhood and adolescence. There were different patterns for those born above the mean for birth weight and below the mean – those above did not regress to the mean (unlike the bulk of the population who did not develop diabetes) but put on weight relative to height from 1 year of age, whereas those born below the mean actually were thin at birth and put on weight for height faster than controls by 3–4 years of age [22]. As this was an unbiased sample because of the nature of the data collection in Helsinki, it suggests that the pathway to diabetes in Finland (an admittedly very homogeneous population) in individuals born in the 1930s started in infancy or before, and that there may be more than one pathway. The Finnish group also showed that there were interactions with specific polymorphisms in the risk of developing insulin resistance – the association of smaller birth size with increased risk of diabetes was seen only in carriers of the high-risk Pro12Pro allele of peroxisome proliferator activated receptor-γ2 [23]. Such studies together with the experimental work point to a nexus of genomic, developmental/epigenomic and concurrent environmental factors in determining an individual’s risk of obesity and type-2 diabetes.

Current opinion [6] suggests at least two major developmental pathways to obesity and insulin resistance.
The Mismatch Pathway

This pathway involves the fetus/embryo/neonate sensing a level of poor nutrition and making developmental choices towards a thrifty trajectory involving both central and peripheral factors such as sarcopenia, a propensity to prefer a high-fat diet, hyperphagia, reduced energy expenditure, and insulin resistance [24]. This is probably underpinned by epigenetic processes and is based on the developing organism predicting it will live in a nutritionally challenged environment. If it meets instead a nutritionally rich environment, then it is mismatched and this mismatch is reflected in metabolic disease as its physiological settings are inappropriate. Experimentally, there is support for this paradigm. In the rat, leptin administration to the infant offspring of undernourished mothers confers life-long protection against a high-fat diet and is associated with reversal of the epigenetic changes, thus providing strong evidence for both the epigenetic underpinnings of this pathway and the predictive (mismatch) model [14, 25]. (Leptin is an adipokine, and we suggest that its administration tricks the neonate into thinking it is fat when it is not and thus induces a plastic choice appropriate for a nutritionally rich environment.) Notably, for some of the genes studied, epigenetic and expression responses to neonatal leptin exposure are directionally dependent on past maternal nutritional status [14]. This suggests that epigenetic and phenotypic responses to an environmental stimulus at one stage in development can be determined both in magnitude and in direction by past environmental exposure. Our studies with this model have also highlighted important gender influences (unpublished work). Furthermore, genomic polymorphisms may have effects on epigenetic changes in other genes [26] and it is reasonable to assume that polymorphisms will directly affect CpG islands. These layers of complexity embracing genomic, developmental and environmental factors provide a basis for considerable variation in gene expression affecting metabolic homeostasis.

In humans this pathway exists in part because of the presence of maternal and/or placental disease which limits nutrient information to the fetus, in part because a surprisingly high proportion of mothers eat imprudent diets [27, 28], but primarily because of the presence of maternal constraint [29]. This is a phenomenon of particular importance in monotocous species to limit fetal growth so that the fetus can exit the pelvic canal. Thus fetal growth is not primarily regulated by the fetal genome but by maternal-placental delivery of nutrients. Maternal constraint is particularly enhanced in first-born children (who have a greater incidence of obesity [30], and the proportion of whom rises as family size falls) and in mothers of smaller stature as in many Asian populations. Because of the constitutive nature of maternal constraint, fetal environment and growth is largely limited (with the exception below), yet postnatal nutritional environments have changed rapidly. Thus the risk of mismatch grows, driven by the lower energy expenditures and greater food intakes of children and adolescents against a background of maternal
constraint favoring developmental trajectories appropriate for sparse environments.

Until recently it was assumed that maternal nutrition, unless very limited, could not affect the fetus. But nutritional variation within the normal Western dietary range can affect patterns of fetal growth [27] and cord blood IGF-1 levels [31]. A nutritional survey of women of reproductive age in the UK showed that up to 50% in the lowest educational achievement groups had diets judged as imprudent [28]. In Singapore, anecdotal evidence suggests that dieting is common in pregnancy. In Japan, the falling birth weight is associated with reduced maternal weight gain, in part due to dieting [32].

The Fetal Hyperinsulinemia Pathway

Whereas the mismatch pathway is physiological (in that it is the outcome of an evolutionarily appropriate mechanism), the offspring of diabetic or pre-diabetic mothers have larger fat mass and a greater lifetime risk of developing type-2 diabetes. In this case the mechanism is thought to be simply high transplacental glucose transfer leading to higher fetal insulin levels. Fetal insulin is weakly anabolic, in part through driving IGF-1 secretion, but is strongly adipogenic. Thus the offspring of diabetic mothers have inappropriately more fat cells, particularly subcutaneous fat, and as fat cell number is largely determined antenatally, postnatal growth in a high-energy environment will be associated with a higher risk of obesity and diabetes. This is therefore a simpler and single-system pathway.

It may however coexist with the mismatch pathway. Work from India shows that babies >2.9 kg at birth have mothers who have a high probability of developing type-2 diabetes within 5 years [33]. This suggests that these short mothers, themselves the outcome of a programmed pregnancy but now in a more nutritionally rich environment and pregnant (which is inherently an insulin-resistant state), give birth to smaller babies (with visceral obesity) because of maternal constraint and that these smaller babies are relatively obese because of maternal hyperinsulinemia. This combined pathway may underpin the growing and very high incidence of juvenile-onset type-2 diabetes in India.

Fat mothers give birth to fatter babies [34]. These are likely to have a life course similar to that of the infant of the diabetic mother, but the mechanism by which fat mothers give birth to fat babies is unclear.

Infant Overnutrition

Experimentally and clinically there is good evidence that infant over-nutrition (such as by formula feeding) can lead to a greater risk of obesity and insulin resistance/type-2 diabetes [18]. Similarly, there is as yet no clarity as to whether this is a distinct mechanism and pathway or reflects either the mismatch pathway (rapid switching in nutritional levels) or an extension of the fetal hyperinsulinemia pathway into the neonatal period (or both).
There is great confusion as to the significance of the infant pattern of growth. Some have argued that rapid infant weight gain is bad, others that it is good. Thus there is no consensus as to how to apply individual nutritional recommendations and growth curves to children of differing birth phenotypes.

Conclusions

If the logic of this article is sound, in time a key strategy may be individualized nutritional and growth curve recommendations for infants and children based on their size, gender, epigenetic profile and genotype. This is an area meriting urgent research. This discussion has highlighted the many gaps in our knowledge, yet points to the growing recognition that, in order to understand lifestyle disease, a developmental/epigenetic perspective needs to be added to the genetic and environmental dimensions.

References

Discussion

Dr. Isolauri: Regarding the current practice that particularly obese pregnant women should try to lose weight during pregnancy and they are advised to gain less weight, significantly less weight, than the normal weight women. What are the consequences on the child?

Dr. Gluckman: I think the first thing is when they are losing weight. The available evidence, and I refer you to a meta-analysis [1] published recently, is that the most important determinant of pregnancy outcome, if birth weight and gestational length...
are used as the measures of outcome, is of course the weight and body mass index of the woman at conception. There is a lot of advantage in trying to encourage women who are obese to think about their body composition before they conceive. I know it is difficult to ask but increasingly I think that it is needed. If genetic mechanisms are involved, we are still to learn the extent to which early nutritional stimuli, such as around the periconceptional period, are potentially more dramatic in their long-term effects than later nutritional stimuli, but that may or may not be the case. All the work is still pointing a lot to late effects as much as early effects, so I just don't know the answer. When I worked with the World Health Organization committee on optimizing the outcomes of pregnancy a couple of years back, we came to the conclusion that there must be a minimal weight gain during pregnancy that must be recommended, and that would appear to be at least 10 kg, potentially 12 kg. I do believe therefore the issue of dieting in pregnancy is very complex, very poorly understood, and needs to be supported by appropriate research, for instance using epigenetic biomarkers as one of the outcomes because birth weight is such a crude index. So at the moment my personal bias, which is bias without data, is women should be encouraged to lose weight before they conceive, certainly not right at the beginning of pregnancy, and that they should not be so severely undernourished that they don't gain 10–12 kg during pregnancy.

**Dr. Walker:** I am fascinated by the persistence of change that goes from one generation to the other which you alluded to, which has been shown in humans by Dick Guerrant in Brazil and Andrew Prentice in Africa. Do you have any thoughts about the potential mechanisms by which this occurs?

**Dr. Gluckman:** It is difficult because we have to separate female and male effects if we are talking about more than one generation. If we are talking about females we can go to F2 without any particularly complex mechanisms needing to be involved because the egg of F2 is actually exposed to the F0 environment because it was formed at the time the fetus of F1 was in fact in utero. In terms of males it is a bit more complicated. F2 transmission but not F3 generation has been shown through both the male and female. Then there is this business in the male with fungicide toxin going through the F3 and F4 generation by male line transmission. How is trans-generational transmission of epigenetic marks maintained? It does not need to be through methylation marks themselves being maintained, although for some genes the methylation mark is not entirely wiped out at meiosis, for many it is, but there are data showing that, even at the 2-cell stage, methylation marks are still present on H19, for example, despite the claims of others that it is completely wiped out. My own bias is more likely to involve, in particular, small RNAs. Small RNAs can transfer across generations, small RNAs may be the way in which the epigenetic markers are maintained from generation to generation. Again the data don't exist; it is a hot topic.

**Dr. Koletzko:** You alluded to the observation in India that people born with low birth weight and growing fast tend to have a very high rate of abdominal obesity, high body fat content with the same BMI, and a high risk of type-2 diabetes. It is very plausible that this has to do with early life events. You also showed the fascinating results from Eriksson and coworkers clearly showing that in this case the Pro12α-polymorphism of PPARγ2 has a huge predictive effect on birth weight association. When this is seen, would there not be an enormous opportunity to better understand what is really happening if we were able to apply some of these new developments, even doing genome-wide association studies comparing populations in India and the Western world to try to decipher the extent to which these associations are modified by genetic variation? One would expect that there should be very different susceptibility sub-groups.

**Dr. Gluckman:** I will be honest and the answer is you might be right. The first problem is there has been remarkable inconsistency in these SNP relationships. For
example Eriksson himself cannot confirm that relationship in the different populations of Finns, and it has certainly not been confirmed elsewhere. On the other hand, SNPs in some populations do show relationships to obesity, insulin resistance, hyperphagia, the MC4 receptor polymorphisms, the perilipin polymorphisms, other PPARα polymorphisms, and so forth. I have done a lot of work in Singapore because Indians and Chinese live along side each other there and have a differential incidence of 3 times: the Indians have 3 times the incidence of type-2 diabetes in Singapore compared to the ethnic Chinese. It comes no where near explaining the differences in incidence despite the large amount of full genome-wide scanning that has been done in Singapore across the so-called Singaporese diabetes studies to look at this. Yes, it may be there, and yes, there might be polymorphisms defined, I have no doubt they will exist. But I see no reason why they would have evolved because the change in environment leading to these diseases has to be sustained, and it doesn't exist. It has to have been there for founder effects and these populations haven't been isolated long enough for founder effects to be clear. In my opinion, in evolutionary terms, it is actually very hard to find an explanation. On the other hand, if you buy into the concept of the development of plasticity that says it is a fundamental thing of all plants and most lower animals, that one changes one's phenotype in later life in a physiological and adaptive sense in relationship to early signals, and at least part of that is mediated by epigenetic processes, then one would expect to find epigenetic processes at the heart of it. But there is another overlay and that is where I think the polymorphisms come back into play, most polymorphisms we look at in terms of those within the expressed region of the gene. Of course many genes have a lot of polymorphisms in the promoter regions of the genes, we haven't looked at those to nearly the same extent, and the extent to which they will alter the capacity of epigenetic processes to lead to one outcome or another is not at all known. There are actually very little data in the literature which relate polymorphisms in promoter regions to alterations in epigenetic expression. To follow this through we first have to understand more about the epigenetic processes, but in doing so we are clearly going to have to understand a lot more about polymorphic variation, not just expressed sequences but non-expressed sequences of the genome which we have not been looking at to nearly the same extent, and that is where I think your question is probably correct.

Dr. Lagercrantz: I strongly believe in and support the Barker hypothesis. But if you look at the curve for birth weight and metabolic syndrome, it is U-shaped, therefore if a child has a very high birth weight it will also develop metabolic syndrome. There are strong data supporting that the famine in Holland resulted in metabolic syndrome, but what about the famine in Leningrad?

Dr. Gluckman: The answer is look at the literature. Part of the problem is everybody has focused on the extremes. If one looks at the normal range of birth weight in good studies, there is a consistent relationship between birth weight within the normative range for that population and outcomes. The problem is the U-shaped curve is real in the modern world. 150 years ago there were not many gestational diabetics and therefore there was not this large number of very large birth weight babies. If you look at older populations versus modern populations, multiple pathways come into play. Just imagine a U; a U can have a narrow shape or a broad shape. In the United States or New Zealand or, I suspect, Finland or Sweden, below about 2,800 g birth weight the risks of later obesity and type-2 diabetes rise as birth weight falls. That is also true in India. But looking at the other end of the U, the right-hand shape, in your country and mine, up to a birth weight of about 3,600–3,800 g there is no increased risk, that seems to be a reasonably optimal birth weight. Above 4,000 g, perhaps 4,200 g, the risks change but they have a different pattern; it is now a pattern due to maternal gestational diabetes and all the effects of maternal obesity through mechanisms we don't understand.
But in India at 2,900 g where the mothers are very short, the risk starts to rise again. In other words the U has a very narrow base and above a birth weight of 2,950 g the mother has a very high risk of having undiagnosed gestational diabetes and has a greater than 60% chance of developing type-2 diabetes within 4 years of the pregnancy, and that of course reflects the fact that she herself was born small, has now grown up in a nutritionally enriched environment, and is at greater risk of getting disease. The evidence is pretty overwhelming that the relationships exist if you understand them within a population context regarding the history of that population. Now the outcome issue, if you look at obesity and type-2 diabetes in sarcopenia, the evidence is pretty strong, because fundamentally they are related on a life history concept to the outcome. Gillman et al. [2] recently did calculations suggesting that 50% of the attributable risk of obesity relates to pregnancy, lactational and early infant factors. There is no doubt if one looks at the Eriksson data that by the age of 3 to 4 to 5 years these children are behaving differently and at birth these children already had visceral obesity. But when you come to cardiovascular disease, which is of course where Dr. Barker first started, it is much more complex. Proximate factors like smoking and other lifestyle factors appear to be much more important because cardiovascular disease is a lot further from metabolic programming than in fact are metabolic studies in metabolism itself. I agree with others that for cardiovascular disease what Dr. Barker originally stated is probably more difficult to tease out.

References