



## Conference on ‘Diet, gene regulation and metabolic disease’ Plenary Lecture

# Developmental programming of energy balance regulation: is physical activity more ‘programmable’ than food intake?

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Extensive human and animal model data show that environmental influences during critical periods of prenatal and early postnatal development can cause persistent alterations in energy balance regulation. Although a potentially important factor in the worldwide obesity epidemic, the fundamental mechanisms underlying such developmental programming of energy balance are poorly understood, limiting our ability to intervene. Most studies of developmental programming of energy balance have focused on persistent alterations in the regulation of energy intake; energy expenditure has been relatively underemphasised. In particular, very few studies have evaluated developmental programming of physical activity. The aim of this review is to summarise recent evidence that early environment may have a profound impact on establishment of individual propensity for physical activity. Recently, we characterised two different mouse models of developmental programming of obesity; one models fetal growth restriction followed by catch-up growth, and the other models early postnatal overnutrition. In both studies, we observed alterations in body-weight regulation that persisted to adulthood, but no group differences in food intake. Rather, in both cases, programming of energy balance appeared to be due to persistent alterations in energy expenditure and spontaneous physical activity (SPA). These effects were stronger in female offspring. We are currently exploring the hypothesis that developmental programming of SPA occurs via induced sex-specific alterations in epigenetic regulation in the hypothalamus and other regions of the central nervous system. We will summarise the current progress towards testing this hypothesis. Early environmental influences on establishment of physical activity are likely an important factor in developmental programming of energy balance. Understanding the fundamental underlying mechanisms in appropriate animal models will help determine whether early life interventions may be a practical approach to promote physical activity in man.

### Metabolic imprinting: Epigenetics: DNA methylation: Nutrition

#### Developmental programming, epigenetics and obesity

During critical periods of embryonic, fetal and early postnatal development, environmental influences can

affect mammalian development, leading to permanent changes in the regulation of energy balance. There is increasing acceptance of the idea that such developmental programming of energy balance regulation is an

**Abbreviations:** SL, small litters; SPA, spontaneous physical activity.

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important factor in the current worldwide obesity epidemic<sup>(1,2)</sup>. To guide the exploration of underlying biologic mechanisms, 15 years ago Waterland & Garza<sup>(3)</sup> proposed the mechanistic construct of metabolic imprinting to describe adaptive responses to early nutrition that are characterised by a limited period of sensitivity during development (a 'critical window'), a persistent effect lasting through adulthood, a specific and measurable outcome and a dose–response or threshold relation between exposure and outcome. Of the five potential mechanisms they elaborated<sup>(3)</sup>, the potential for metabolic imprinting to occur via induced alterations in epigenetic gene regulation has received the most attention.

Epigenetics is the study of mitotically heritable alterations in gene expression potential that are not caused by changes in DNA sequence<sup>(4)</sup>. First recognised for their importance in genomic imprinting, X-inactivation and silencing of retrotransposons<sup>(5)</sup>, epigenetic mechanisms are now recognised to play a key role in stabilising gene expression potential in differentiated cells<sup>(6)</sup>. Various molecular mechanisms are known to participate in epigenetic regulation, including DNA methylation, covalent modification of histone proteins, autoregulatory DNA-binding proteins and non-coding RNA. Our research focuses on DNA methylation, which in mammalian somatic tissues occurs predominantly at cytosines in CpG dinucleotides. CpG methylation is recognised as the most stable epigenetic mark<sup>(7)</sup>, and is hence an excellent candidate mechanism of metabolic imprinting. Indeed, during critical periods of early development, nutrition and other environmental exposures can induce alterations in DNA methylation that persist to adulthood, in both rodents<sup>(8–11)</sup> and human subjects<sup>(12,13)</sup>.

Human and animal model data show that epigenetic dysregulation can cause obesity. As recently reviewed<sup>(14)</sup>, adult-onset obesity in cloned mice<sup>(15)</sup> suggests epigenetic causality in obesity, and individually variable hyperphagic obesity of isogenic heterozygous agouti viable yellow ( $A^{vy}$ ) mice, which correlates with  $A^{vy}$  methylation<sup>(9,16)</sup>, provides a clear example of epigenetic dysregulation causing obesity. In human subjects, sporadic cases of Prader–Willi syndrome (a neurodevelopmental syndrome characterised by hyperphagic obesity, among other symptoms)<sup>(17)</sup> are caused by aberrant hypermethylation and epigenetic silencing of a region of chromosome 15.

Despite these compelling examples, it remains unclear whether epigenetic dysregulation plays a major role in the current obesity epidemic. This is due to multiple obstacles to the study of epigenetic aetiology in human subjects, including the inherent cell-type specificity of epigenetic regulation, the influence of genetic variation on epigenetic variation, the potential for reverse causality, and the overall complexity of epigenetic regulation<sup>(14,18)</sup>. For these reasons, controlled studies in isogenic mouse models now offer the best opportunities to understand how interindividual epigenetic variation influences energy balance regulation. Our recent investigations into two such models have led to surprising insights.

### Two mouse models of developmental programming of energy balance show alterations in physical activity but not food intake

The first of these is a mouse model of transgenerational effects of maternal obesity on the offspring. Fifteen years ago Levin proposed that in pregnancies complicated by maternal obesity the intrauterine environment may present an unfavourable milieu to the developing fetus, leading to aberrant development of central mechanisms for regulation of energy balance<sup>(19)</sup>. If, in this manner, maternal obesity during pregnancy promotes positive energy balance (i.e. energy storage in the form of adipose tissue) in her offspring, over successive generations this could lead to transgenerational amplification of obesity.  $A^{vy}/a$  mice provide an attractive model in which to test this because they are spontaneously hyperphagic and become severely obese as adults, but unlike most genetic mouse models of obesity, the females remain fertile. We passed the obesity-promoting  $A^{vy}$  allele through the female germline for four generations<sup>(20)</sup>. Consistent with the feed-forward hypothesis, even among this isogenic population, the offspring of fatter mothers were themselves fatter as adults. Consequently, average adult body weight and adiposity increased over successive generations. In this experiment, we also studied another four-generation lineage that was treated identically as the first, but provided a methyl-supplemented diet known to promote DNA methylation during development<sup>(9,21)</sup>. In the methyl-supplemented group, transgenerational amplification of obesity was completely prevented, suggesting a potential role for DNA methylation in this phenomenon<sup>(20)</sup>. It is important to clarify that although epigenetic mechanisms affecting body-weight regulation may be involved within each generation, we believe it unlikely that the transgenerational effect in this model reflects transgenerational epigenetic inheritance. As in many examples of transgenerational effects, recapitulation is a more likely mechanism than transgenerational epigenetic inheritance<sup>(14)</sup>.

We conducted a follow-up study in the  $A^{vy}$  model to answer two questions: (1) When is the critical period for the effect of maternal obesity (prenatal or postnatal)?; (2) Is positive energy balance in this model due to increased food intake or reduced energy expenditure? We cross-fostered newborn mice at birth between lean *ala* and obese  $A^{vy}/a$  dams to examine the effect of being exposed to an obese  $A^{vy}/a$  mother either during fetal development or during the suckling period<sup>(22)</sup>. These studies provided several surprising findings. First, we found that offspring of  $A^{vy}/a$  dams are growth restricted at birth (despite normal placental weight), suggesting placental insufficiency in  $A^{vy}/a$  dams. When offspring of obese  $A^{vy}/a$  dams were fostered to lean *ala* dams (OL offspring) they caught up to normal body weight by weaning. Most interestingly, fetal growth restriction and subsequent catch-up growth led to increased body weight and adiposity in adulthood, but only in female offspring. Overall, although these data indicate that growth restriction during prenatal development is



an essential component of the programming mechanism<sup>(22)</sup>, they do not rule out a potential critical role for catch-up growth during the suckling period.

To answer the second question, metabolic cage studies were performed to measure home cage activity, energy expenditure and food intake. The results of these studies were completely consistent with the growth and body composition data. Whereas food intake of female OL offspring was not different from that of controls, their levels of physical activity (home cage activity) and energy expenditure were reduced directly after weaning, and remained so in adulthood. In fact, the persistently blunted spontaneous physical activity (SPA) of OL females appeared to completely explain their increased adiposity as adults<sup>(22)</sup>. Notably, recent studies in three different human populations showed that perinatal undernutrition leads to increased obesity in adulthood, specifically in females<sup>(23)</sup>. Our data suggest that this may be occurring by permanent blunting of physical activity.

Remarkably, in a completely different mouse model of developmental programming of obesity, we found a similar female-specific effect on physical activity. Fostering newborn rodents to small litters (SL) is a simple method to achieve overnutrition during the suckling period. When rats or mice are fostered to SL during the suckling period, by the age of weaning they are consistently heavier than control pups fostered in normal-sized litters<sup>(24,25)</sup>. Suckling period overnutrition permanently alters body-weight regulation and glucose metabolism of SL rodents. Although all pups are provided the same diet (*ad lib*) after weaning, SL offspring remain heavier and fatter in adulthood<sup>(25,26)</sup>, and exhibit impaired glucose tolerance<sup>(24,27)</sup> and dysregulated endocrine pancreas function<sup>(27)</sup>. We performed metabolic cage studies to determine if the persistent change in energy balance regulation of SL mice is caused by altered food intake or energy expenditure<sup>(28)</sup>. At postnatal day 1 (P1), mice were fostered to SL (four pups/litter) or control litters of nine pups. As previously reported, SL males and females were heavier and fatter than control mice at weaning (P21) and remained so as adults (P180). Our metabolic studies showed no group differences at P25, but by adulthood (P180) we observed blunted energy expenditure correlated with reduced SPA in SL mice. Although similar trends were observed in both sexes, the effects were significant ( $P < 0.001$ ) only in females<sup>(28)</sup>. Hence, together with our data in the  $A^{vy}$  model of fetal growth restriction and catch-up growth<sup>(22)</sup>, these findings suggest that females are particularly sensitive to developmental programming of energy balance.

### Biological determinants and developmental programming of physical activity

Most importantly, integrating these two very different models of early nutritional influences on the development of energy balance regulation, in which both food intake and physical activity were extensively characterised by state-of-the-art methods, our results suggest that

developmental programming of energy balance may be mediated principally at the level of physical activity. This insight is particularly timely, in that modernisation is leading to a worldwide epidemic of physical inactivity<sup>(29)</sup>. Physical activity clearly has a genetic component<sup>(30,31)</sup> and plays an important role in protecting some individuals from obesity<sup>(32–34)</sup>. Based on a scholarly review of the literature on the biological basis of physical activity, Rowland proposed that each individual is endowed with a specific set point (the activity-stat) that determines his or her propensity for physical activity<sup>(35)</sup>. Although most have considered the activity-stat to be genetically determined, it could also be subject to developmental programming. Indeed, in addition to our recent mouse studies, previous studies have found evidence of developmental programming of the activity stat. Before considering those studies, it is helpful to point out that physical activity is typically classified as either voluntary exercise or SPA. Voluntary exercise is physical activity that is not directly needed for survival or influenced by any outside factors, whereas SPA is all physical activity that is not voluntary exercise, including activities of daily-living and pacing and fidgeting<sup>(32)</sup>. In rodents, running wheel activity is the universal model for voluntary exercise<sup>(32)</sup>, and home cage activity is indicative of SPA<sup>(34)</sup>.

In what we believe was the first reported animal data suggesting developmental programming of physical activity, Vickers *et al.*<sup>(36)</sup> studied a rat model of maternal undernutrition (30 % of *ad libitum* intake) throughout pregnancy, and examined effects on the offspring. They performed brief (15 min) measurements of home cage activity (SPA) when the offspring were both juveniles (P35) and adults (P145), and found reduced SPA in offspring of undernourished dams. Importantly, other than our data described above, this is the only study we are aware of that measured effects on physical activity both shortly after weaning and in adulthood. In this manner, the study demonstrated that maternal undernutrition during pregnancy caused reduced physical activity in the offspring shortly after weaning, and this persisted to adulthood. Two subsequent studies examined variants of another rat model of gestational undernutrition, assigning pregnant dams to a normal or low-protein diet during gestation<sup>(37)</sup> or gestation through weaning<sup>(38)</sup>. In the first study, offspring of control and undernourished dams were cross-fostered at birth to control dams to limit the exposure (undernutrition) to prenatal development. Voluntary exercise (wheel running) was measured over a 2-week period in adolescent (P56) offspring of undernourished dams. Both male and female offspring of undernourished dams engaged in much less voluntary exercise than control offspring<sup>(37)</sup>. The other study of maternal low-protein diet<sup>(38)</sup> differed from the first in that the exposure started at day 10 of gestation and continued through the end of the suckling period. Rather than reduced physical activity, this study reported increased motor activity in adult (P72) offspring of undernourished dams. Interestingly, as in our studies, this effect was observed only in female offspring<sup>(38)</sup>. Combining our data (discussed above) with these earlier

studies, the results of these four diverse models of developmental programming suggest that, indeed, the setting of one's activity stat can be affected by nutritional exposures during prenatal and early postnatal development. Now, the challenge is to understand how this occurs, and determine whether similar processes occur in man.

*Assessing the role of epigenetic mechanisms in regulation of physical activity: translation to human subjects*

Accordingly, a current goal is to use these models to test the overall hypothesis that developmental programming of physical activity occurs via metabolic imprinting of epigenetic mechanisms. We began by asking two key questions. The first: What tissues are involved? Since epigenetic regulation is tissue- and cell-type specific, it is important to determine which tissues and cell types are most likely to play a primary role in the imprinting mechanism. The hypothalamus is a logical starting point because, in addition to playing a key role in regulating food intake, it is also critical in the regulation of physical activity. Peripheral factors such as cardiovascular and skeletal muscle function are important determinants of physical exercise capacity but play negligible roles in SPA. SPA appears to be primarily regulated in the hypothalamus (by key molecular players including orexins, agouti-related peptide and neuromedin U)<sup>(34)</sup>. Voluntary exercise is also regulated in the lateral hypothalamus<sup>(39)</sup>, but dopaminergic signalling (particularly in the nucleus accumbens and hippocampus) is also important<sup>(32)</sup>.

This brings us to the second key question: When are the critical windows during which environmental influences are most likely to affect developmental epigenetics in the hypothalamus? Our and others' previous work indicates that epigenetic processes are most vulnerable to environmental perturbation during periods when epigenetic mechanisms are undergoing developmental establishment or maturation<sup>(18)</sup>. Hence, to begin to answer this question, we studied epigenetic development in the hypothalamus during the suckling period (which is relevant potentially to both of our models discussed earlier). Two levels of epigenetic specialisation in the hypothalamus complicate these studies. Within the hypothalamus are specialised regions called hypothalamic nuclei (such as the arcuate nucleus, paraventricular nucleus, etc.) with distinct functions, gene expression patterns, and epigenetic regulation<sup>(40–42)</sup>. Moreover, within the hypothalamus there are many different cell types; the broadest dichotomisation is neurons (which convey information by generating action potentials), and glia (which lack this ability and instead serve broad functions including structural integrity, maintenance, and immune regulation)<sup>(43)</sup>. To ask whether the suckling period is a critical period for cell-type-specific epigenetic development in the mouse, we studied P21 *v.* P0 hypothalamus, in neuronal and glia DNA separately<sup>(44)</sup>. We found dramatic neuron-specific hypermethylation during the suckling period in the mouse. Gene ontology analysis showed that these changes were strongly associated with neurodevelopmental processes. These data indicate that, in the mouse, the suckling period is a critical period for cell-type-specific

epigenetic development in the hypothalamus. An obvious next step is to determine if and when similar epigenetic changes occur in the human hypothalamus. Although milestones of brain development are generally thought to occur later in mice than in human subjects, we know very little about how the ontogeny of neuroepigenetics compares between these two species. Such data will be essential to translate findings from mouse studies to man.

## Conclusions

Metabolic imprinting of energy balance regulatory mechanisms is likely to be an important factor in developmental programming of obesity. Studying these processes in appropriate inbred animal models currently offers the best opportunity to gain fundamental insights into underlying mechanisms. Importantly, although alteration of epigenetic mechanisms in the central nervous system will almost certainly play a central role, it is imperative to emphasise that this is just one of several potential mechanisms of metabolic imprinting<sup>(3,14)</sup>. Gaining a meaningful understanding of how early nutrition affects the establishment of each individual's activity-stat will most likely require integrated analysis of developmental epigenetics in the context of other ontogenic processes that are co-ordinately affected.

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## Conflicts of Interest

None.

## Authorship

S. Z., J. E., and R. A. W. drafted the manuscript. M. S. B. and G. L. performed studies discussed in the manuscript. All authors edited and approved the final version of the manuscript.

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