Professor Jonathan R Seckl
Moncrieff-Arnott Professor of Molecular Medicine &
Executive Dean
College of Medicine and Veterinary Medicine
University of Edinburgh

Developmental programming - how your parents' environment before you were born impacts on your and your children's risk of disease

Glossary

Developmental programming – decribes the process through which a stimulus or insult during a critical window of fetal or infant development elicits permanent responses that produce long-term changes in tissue structure or function

Epigenetics – is the study of heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence.

Glucocorticoids – are a class of steroid hormones that bind to the glucocorticoid receptor, which is present in almost every animal cell. The name glucocorticoid (glucose + cortex + steroid) derives from their role in the regulation of the metabolism of glucose, their synthesis in the adrenal cortex, and their steroidal structure. Glucocorticoids are part of the feedback mechanism in the immune system that turns immune activity (inflammation) down.

Stress – typically describes a negative concept that can have an impact on one's mental and physical well-being.

Overview

In this lecture Professor Jonathan Seckl describes how a range of environmental factors 'programme' offspring for the whole of their lifespan. The outcomes of a range of maternal challenges e.g. malnutrition, post traumatic stress disorder (PTSD), hardship are similar in their effects on the biology of offspring. As such maternal stress, and its hormonal mediators, is an important influence on offspring vulnerability. Epigenetic alternations most likely lie behind these effects with the brain being particularly sensitive to such programming. While genes do not explain all, these effects can persist into a second generation. Improving the maternal environment might help to avoid or minimise the likelihood of such programming. Research is beginning to suggest ways in which the negative effects of such programming might be reversed.

Summary

Professor Seckl began his presentation with a reminder of the basic biology associated with stress. When faced with very stressful situations the basic response is that of 'fight or flight'. In addition to the stimulation of adrenalin or nor adrenalin the body also produces powerful hormones, known as glucocorticoids, in response to stress. These are released into the blood stream over the course of 30 minutes to an hour during a stress episode. They mobilise fuel, and increase blood pressure and generate euphoria to focus the body on surviving the immediate danger. They also inhibit functions which are not immediately necessary to survive the threat for example inflammation, healing, digestion, bone formation, reproduction, detailed learning and memory.

Glucocorticoids also inhibit the activity of the hippocampus, that part of the brain associated with detailed learning and memory and stimulate the amygdale – the part of the brain associated with response to fear. This activity is very useful and highly adaptive. It can however become problematic if the body is chronically stressed and the acute response lasts for a much longer time. To illustrate this point he described a patient with Cushing's disease in which the glucocorticoid response was always present due to the presence of a tumour. High blood pressure had become a permanent state and pathological condition, and the increased and sustained level of glucocorticoids flooding the bloodstream had resulted in diabetes, suppressed reproduction and infertility and a build-up of fat around her abdomen. These symptoms receded after the benign tumour was removed.

He began the main part of his lecture by suggesting that most people would agree that health and wellbeing resulted as a combination of 'nature and nurture', the adult environment and individual genetic make-up. While he agreed that there is truth in this view, he suggested it does not tell the whole story.

To illustrate, he drew upon the extensive literature on identical twin studies. He explained that in such studies there was a high degree of concordance between identical twins across a number of key physical diseases, for example high blood pressure, heart disease, diabetes and some mental health problems, for example depression and schizophrenia. If one twin has the disease the other is likely to have it or to get it. However a substantial proportion, about 30-40%, are not concordant. This is often held to illustrate the effect of environment on health. However, studies which compared identical twins separated at birth and brought up in different environments showed that they had similar levels of concordance for illness as those brought up together. He implied that this suggested the environment before or at birth was an important factor in later experience of health and illness.

Citing the extensive data collected by the meticulous recording of birth weight by midwife and NHS services over the twentieth century he showed, controlling for other factors such as class, that there is a significant inverse relationship between placental/birth weight and onset of adult diseases such as type two diabetes, metabolic syndrome and depression. These effects are large and have been replicated internationally with large cohorts. These effects occur within the normal

range of birth weights. Research looking at the stress hormone cortisol, a major glucocorticoid, has identified higher levels of cortisol in 64 year old men with low birth weight.

Research has shown that a low birth weight baby (in the normal range) has a threefold risk of contracting type two diabetes and high blood pressure and a twofold increase of early mortality from heart disease.

Osteoporosis is also more prevalent in this group as are depression and schizophrenia. There is no trade off with other forms of illness as overall mortality in this group is elevated. For example, people in this group as just as likely to contract cancer as those with higher birth weights.

Highlighting Weismann's work on butterflies in the nineteenth century, Professor Seckl went on to show that the environmental conditions in which an individual develops may have a long lasting and persistent effect. In Weismann's butterflies the environmental conditions affected their colour. In reptiles the external temperature at which eggs are incubated determines their sex.

This is termed developmental programming. Professor Seckl highlighted this as a key point of his lecture and explained it as follows.

During development an influence from the environment hard wires biology for the lifespan. It operates in specific windows of sensitivity or periods of time during development (and not in others). It is ubiquitous in the animal kingdom. It is likely to be adaptive. He invited the audience to imagine giving birth to a baby in a period of famine or war. In such times having a smaller baby probably increases the survival chances of both mother and baby. It will grow quickly and its higher blood pressure ensures it will not die at the first scratch. Its behaviour is akin to that of a child with attention deficit hyperactivity disorder. It is poor at sitting still and paying attention, but is well adapted to dangerous volatile situations. It matures quickly and is capable of passing on its genes in a situation where longevity is unlikely. This is an important observation as it suggests that the degenerative diseases of old age are reflective of our way of life rather than the underlying biology which is still characterised by 'fight or flight' responses.

How did this happen?

One suggestion is that this phenomenon is genetic – that the genes associated with low birth weight cause diseases later in life. The shortcoming of this explanation is that in identical twins, the twin with the lower birth weight exhibits higher levels of illness and disease than its heavier sibling. Genes alone do not therefore explain this phenomenon. The impact of social class on health is significant and well known and studies control for this.

A common explanation is that the effect derives from maternal malnutrition. In studies which have used data from human atrocities such as the enforced starvation of the Dutch during World War II and the tens of millions of deaths associated with the deprivations of the Great Leap Forward in China in the late 1950s and early 1960s. Birth weights of babies born during these times are shown to be 200-300 grams less on average than at other times. The babies born with low birth weight have higher levels, in adulthood, of heart disease, blood pressure, schizophrenia and depression.

What is the link? In the 1960s doctors noticed that pregnant women who received glucocorticoid treatment for other illnesses tended to have smaller babies. From this observation, the hypothesis was developed that exposure to cortisol during pregnancy explained the link between low birth weight and later disease.

Work with rat populations confirmed this hypothesis but it was also noted that low birth weight had no lasting effect unless the female rats were exposed to cortisol during the third trimester of pregnancy. However, the effect of exposure in last trimester was very strong and long lasting. Thirty years after a single exposure to cortisol in a randomised control trial investigation of past-natal respiratory distress, the women involved in the trial engaged in a follow-up study reported in the Lancet. This follow up study found that the children exposed in the womb resulted in a significant increase in insulin resistance (and therefore at greater risk of diabetes)

What is the underlying biology which gives rise to this effect?

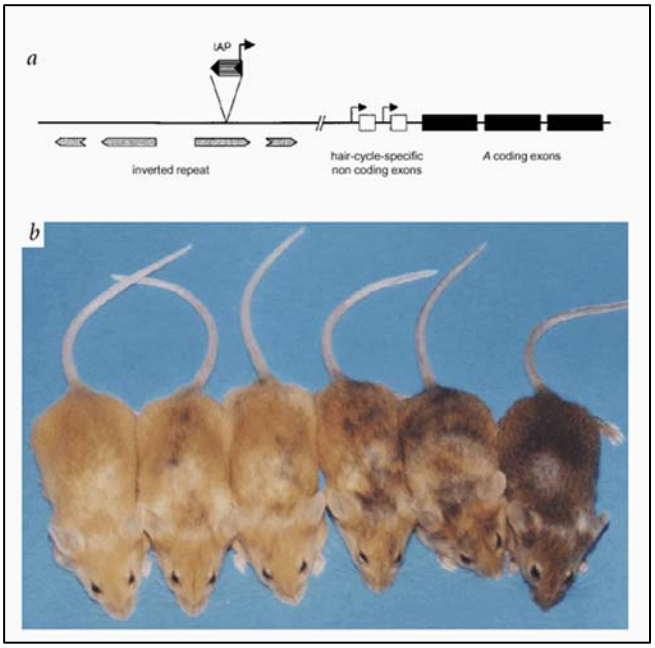
Indications are that increasing levels of glucocorticoid in maternal blood in response to stress, incrementally reduce the hormone 11B-hydroxysteroid dehydrogenase in the placenta. This latter hormone acts as a barrier, protecting the foetus from the stress hormones. However, the weakening of the barrier allows the stress hormone cortisol to pass through the placenta. Cortisol then acts to alert the foetus to the possibility of a stressful environment. One of the effects of this is to allow changes in the level of the expression of certain genes within cells. This is the story of epigenetics.

Professor Seckl's interest in glucocorticoid responses to stress caused him to investigate the receptors for glucocorticoids. These reside inside individual cells and can bind to the DNA and turn individual target genes on and off. His research has shown that the foetus of a stressed or malnourished mother, is exposed to stress hormones, which reduces the placental barrier described above and alters the glucocorticoid receptor. Other exposures, such as to liquorice (which contains high levels of the same enzyme) have the same effect. This caused the receptors to increase in the liver and in body fat around the middle and in the brain, particularly in the hippocampus a brain area associated with memory, learning and the control of stress. This happens because the glucocorticoid receptor gene starts from different places in different tissues to make the receptor.

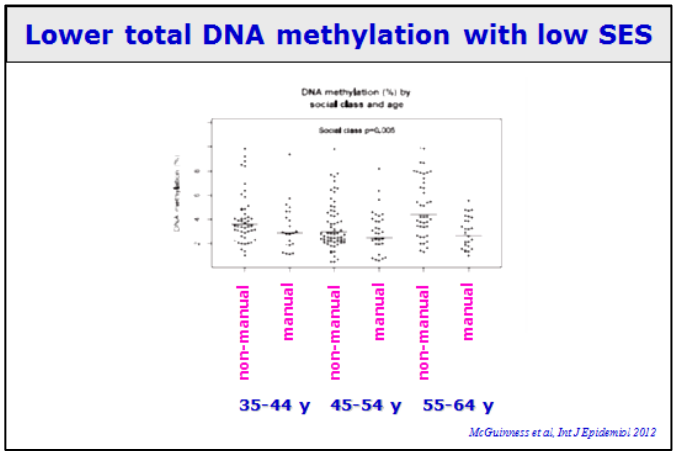
He suggested that this may be due to the presence of the glucocorticoid receptor Exon 1_7, which is highly enriched in the brain, is highly susceptible to early life events. If exposed to stress in early life the levels of the "happy" hormone serotonin reduce and the exon1_7receptor is turned off.

How are receptors turned off and how does this persist?

To answer this he suggested that a good metaphor might be the text of a book. If receptors are 'turned off' this would be akin to a portion of the page being blanked out by tippex. The words are still there, but they cannot be read. The genetic code is still there but it cannot be read. This is a process known as DNA methylation. When a methyl group is added to a gene it cannot be read. Some parts of the 'book of life' are covered up. He illustrated the point by showing a photo of a group of cloned mice. These mice are genetically identical and yet they do not look identical. They have different markings. This difference comes from the fact the mother has agouti genes, which cause banding or marking on the fur of the offspring. The yellowish colour of the genetically identical mice on the left comes from different epigenetic processes compared to the genetically identical ones on the right. It appears that sometime in the past an ancestor was infected with a retro virus, which integrates into the DNA. The body perceives the virus to be a foreign body and covers it (by methylating the DNA) providing protection from the virus. The unique fur colouring depending on exactly where in the DNA the virus integrated.



In these mice part of the methylation fails and the yellow gene is turned on. Unfortunately the gene is also associated with obesity and these mice die young. This shows the power of methylation to activate or deactivate genes early in life. If unmethylated, brain cells have more glucocorticoid receptors and regulates stress hormones more effectively thus protecting health.



For the glucocorticoid receptor, early life exposure to stress makes an enormous impact on the rate of methylation, and therefore the readability of the Exon 1_7 start point. If methylated, and unread, the production of stress hormones is less regulated. If this is then related to growing up in some of the more deprived areas of Scotland then the early life environment will have a significant impact on the foetus before birth leading to greater experience of disease later in life. He cited data from a study published by the Glasgow pSoBid group earlier this month which showed that total DNA methylation is indeed lower in groups of men with lower social class across the age ranges. (See slide page 5)

He went on to describe an investigation being led by Rachel Yehuda in the USA, working with survivors of the Nazi holocaust. The research shows that 60 years later, glucocorticoid levels are lower than expected and that the enzymes (similar to those in the placenta described above but found in the liver and the kidney of adults) are one third lower than carefully matched control populations. So those exposed are developmentally programmed for a very tough environment, experienced over months and years, where it is difficult to survive and energy is extracted from the liver and every grain of salt from the kidney. The younger the subject when exposed to the holocaust, the greater the effect on the metabolism.

To explore whether a single stressful incident would have a similar effect, the team followed up women who had been pregnant and in the twin towers on 9-11 or very nearby on the day of the terrorist attacks. They investigated 187 women and found that there was an effect, but only if the mother developed PTSD and was in the final three months of her pregnancy. This combination of environment and stage of pregnancy acts as a signal to the foetus to prepare for a very difficult environment and so the metabolism prepares for a meagre and difficult environment of material scarcity. Instead most find an environment of excess. This he argued is at least partly fuelling the obesity epidemic which we are witnessing in developed countries, particularly the USA. The biology of the baby is mismatched with the excess of the environment it suddenly finds itself in.

A further finding in this field is that this effect seems to extend itself into the next generation even in the absence of further exposure to physiological stress. This seems to pass down the male line with the father contributing to epigenetic effects. The second generation offspring might look the same but their underlying biology can be different. Epigenetic factors can be quite small with 5-10% differences in methylation yielding significant differences in birth weight. This point was illustrated by showing us cases of Beckwith Weidermann (pathologically overweight) and Silver-Russell (pathologically underweight) syndromes which are cause by methylation differences on receptors of 15-30%.

Does methylation in humans matter beyond these rare cases?

To answer this question he introduced a study of the inhabitants of Overkalix in Northern Sweden at the edge of food cultivation where good record have been kept for 120 years These agricultural records of good and bad harvest years and associated nutrition were compared over the years with disease rates. This data show that if a grandmother suffers from malnutrition at age two – reflecting her time in utero – then her grandchildren have twice the death rate from heart disease than those whose grandmothers were not so malnourished. The same relationship holds for the grandfather/grandchildren though this is related to the time just before puberty when his sperm are beginning to form.

This relationship also holds in the case of children in the holocaust sample discussed above. Even children born to survivors 15 years after the holocaust have significantly higher levels of PTSD (30%) than the general population (5-10%). This suggests that maternal holocaust exposure predicts offspring depression and that survivor PTSD predicts offspring PTSD. Offspring with no exposure to the holocaust have the same low cortisol and enzyme characteristic as their exposed mothers, suggesting a vulnerability factor. This pattern was also observed in the 9-11 maternal cohorts who did not respond to simple counselling and support, suggesting a predisposition or vulnerability programmed by early life experience. Early life events therefore help to explain why some people are resilient to shocks and others not.

What can be done about this?

Professor Seckl described the Motherwell diet introduced by a doctor to pregnant mothers in the 1960s. Mothers were asked to eat significant quantities of meat. Being poor they ate low quality meat. Looking at this cohort now they have higher levels of heart disease, metabolic syndrome, higher blood pressure and higher cortisol levels than other similar cohorts. Forty years later glucocorticoid epigenetic change, including methylation of receptor genes is higher in the offspring of this group compared to other similar groups.

A small study has shown that metformin, a common anti-diabetic drug, can reduce glucocorticoid levels in the liver by reversing methylation. Other work suggests that methyl donors (such as foliate, choline, vitamin B12, and betaine) be given by dietary supplements also seem to reverse methylation. Birth weight can also be increased by doses of serotonin which cause enzyme levels associated with protection to rise. He described these processes not necessarily recommending them as treatment, but rather to show that reversal of unwanted methylation is possible in some mammals.

Concluding summary

He concluded by summarising the key points of his lecture. He suggested that many factors in the environment can programme offspring. The outcome of various maternal challenges such as stress and infection may result in offspring with higher levels blood pressure, attention deficit hyperactivity disorder and heart disease for example. Stress hormones are central to these processes and a barrier enzyme in the placenta may play a key role in this. He also showed how early life events can persist in effect over the lifespan without changing genes and might persist into the next generation. These effects, with no further intervention, seem to disappear in the third generation. Not everything is written in the genes or epigenes.

He suggested that we do not yet know how important these early life impacts are but they may be good stable markers of individual exposures and risk. Epigenetic changes can be modified and it may be possible to personalise this effect. However this biology has survived for many hundreds of millions of years and so interfering blindly is likely to yield unwanted consequences.