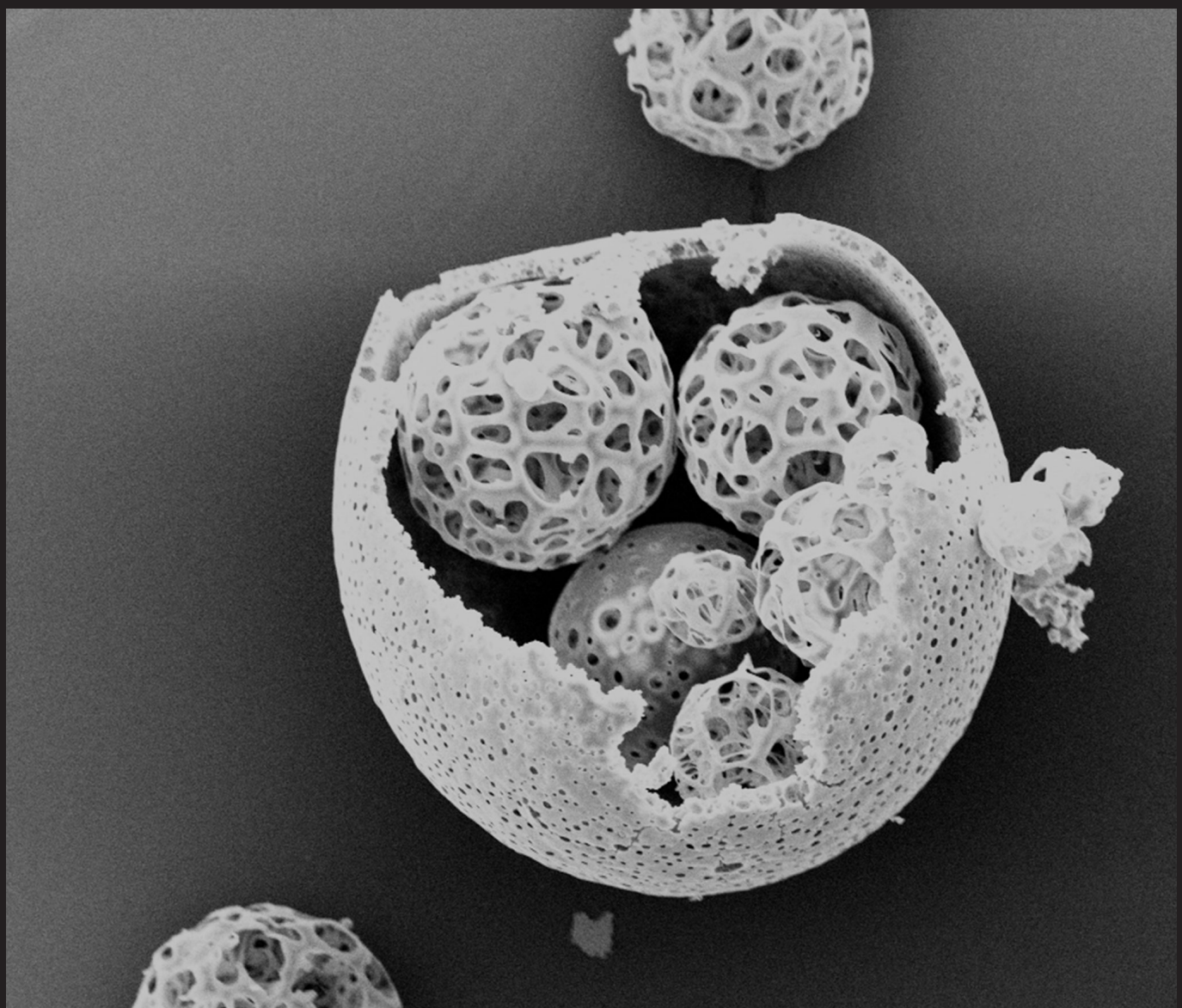
# the Translational Scientist

AUGUST 2017



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# Upfront

## Image of the Month

### Pore-fect Delivery

*August 2017*

A scanning electron micrograph of porous PLGA – poly(lactic-co-glycolic acid) – microparticles. One potential application for these biodegradable and biocompatible particles is as a carrier for delivering mesenchymal stem cells for in vivo regenerative medicine. One of the particles has been damaged during the leaching process (where the pores are created) and has ended up with smaller particles enclosed within it. The image was taken at the Nanoscale and Microscale Research Centre at the University of Nottingham.

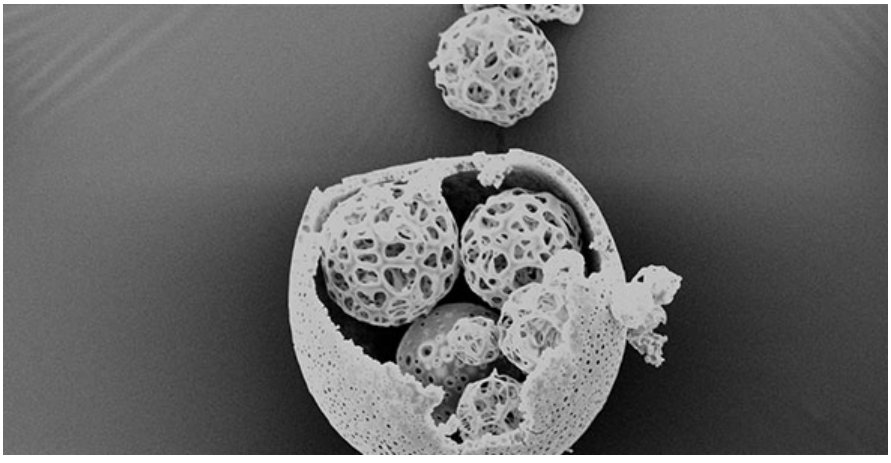

*Image credits: Sandhya Moise, School of Pharmacy, Centre for Biomolecular Sciences, University of Nottingham, UK.*

## Undoing the Damage

### Two common drugs appear to reverse neurological symptoms caused by fetal alcohol exposure in rats

*August 2017*

Fetal alcohol spectrum disorder (FASD) is a widespread problem – up to 11 percent of children are affected. The condition presents two main challenges: i) diagnosis can be difficult, as the stigma associated with drinking alcohol during pregnancy can cause birth mothers to obscure the truth of their alcohol consumption, ii) treatment is limited to drug and behavioral interventions that combat neurological symptoms, which include learning disabilities, and issues with memory, cognition, communication, motor skills and more.

But what if the brain damage caused by fetal alcohol exposure could be reversed? Rat studies have shown that two common drugs – thyroxine and metformin – could undo some of the problems caused by alcohol exposure in the womb (1). Thyroxine (T4) was chosen to tackle the altered thyroid function that is often observed in FASD, while metformin (a first-line

medication for the treatment of type II diabetes) addresses the glucose metabolic dysfunction.

Neonatal rats were given either metformin or thyroxine during the equivalent of the third trimester in humans (postnatal days 1–10 in rats) to increase the translational value of the study – the researchers believe that in utero treatment may be more feasible than treating newborns. Compared with adult rats exposed to alcohol in the womb and given no treatment, the animals treated with either drug showed cognitive improvements – prompting the research team to delve deeper to discover why. They found that both therapies affect the expression of DNA methyl transferase 1 (dnmt1), an enzyme that plays a role in learning and memory. Rats given a Dntm1 inhibitor showed cognitive deficits similar to rats with FASD, while rats also dosed with metformin had no FASD-like effects – demonstrating the important role of dnmt1 in brain development.

The research team is now hoping to raise funds for a clinical trial to validate their findings. "We've shown you can interfere after the damage from alcohol is done. That's huge," said Eva Redei, Professor of Psychiatry and Behavioral Sciences and Physiology at Northwestern Medicine, Chicago, USA (2). "We have identified a potential treatment for alcohol spectrum disorder."

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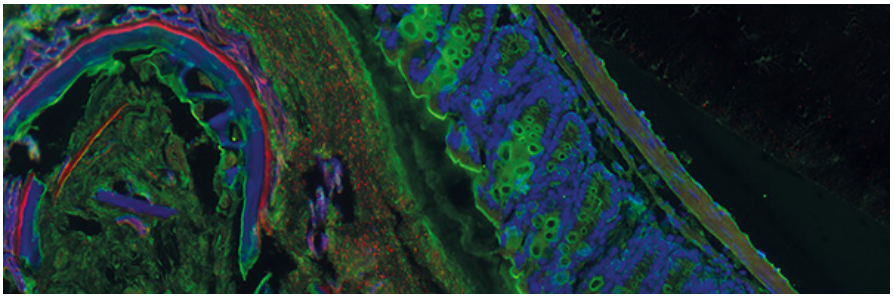

# Inside Intestinal Disease

**Sheena Cruickshank explains how examining gut mucus microbiota can lead to earlier diagnosis – or even prediction – of inflammatory bowel disease**

*August 2017*

Inflammatory bowel diseases (IBD) are relapsing and remitting chronic conditions of the gut that have a major impact on patients' quality of life. Current therapeutic approaches aim to reduce symptoms – but their effectiveness varies, and patients may develop tolerances to the drugs. As such, it's really important to have ways of assessing disease activity to better manage patients. Identifying microbial alterations associated with IBD could provide a diagnostic tool that enables us to spot disease earlier and minimize damage. It also contributes to better understanding of the underlying mechanisms associated with disease pathogenesis, allowing the design of improved therapeutic strategies.

The first line of defense against potential microbial invasion in the gut is a viscous layer of mucus that covers the intestinal epithelium. In our study (1), we excised the relevant mouse gut segment, opened it up longitudinally, and washed to remove residual luminal contents before scraping off the mucus for further testing. It's a difficult procedure to translate into human studies because most patients undergo routines such as colonic lavage before they are biopsied, resulting in samples that may not fully replicate the in situ bacterial communities. However, an in situ method of mucus sampling without colonic lavage that can be performed in the clinic was recently developed (2), so in the future it should be easier to get patient mucus samples. Testing would involve either qPCR or a form of protein analysis, such as ELISA.

Given that differences in microbiota composition start in the mucus before the onset of inflammation, our findings provide a framework for identifying temporal and spatial changes in the most relevant microbial communities that underpin subsequent development of IBD. Now, we are exploring whether the shifts in mucus microbial communities correlate with changed function and altered metabolite profiles. We are also developing methodologies to analyze microbial networks to develop better predictive strategies.

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# Too Pregnant to Participate

## Are current ethical guidelines for clinical studies letting pregnant women down?

**By Roisin McGuigan, Associate Editor**

*August 2017*

The ethical dilemma of pregnant women taking part in clinical trials has created something of a catch-22 for the research community; the lack of knowledge surrounding pregnant women as subjects in clinical studies prevents them for taking part in studies, so the lack of knowledge is never addressed.

By analyzing 13 clinical studies, a research group from the Netherlands has identified a number of rationales for why women are excluded from clinical trials: the issue of informed consent, pregnant women's susceptibility to coercion, a heightened risk because of a lack of scientific knowledge, and the safety of the developing fetus. More broadly, pregnant women are often designated as "vulnerable" – a term typically applied to populations whose ability to make decisions for themselves in a research context is considered compromised; for example, children, or people with learning disabilities.

But how much truth is there in the notion that pregnant women are "vulnerable" or somehow unable to consent to participation in research? According to the authors, not much. "There is no immediately obvious reason to assume that pregnant women are incapacitated during pregnancy," the authors wrote (1). Tackling the issue of coercion, the notion that women will feel coerced by their own or society's wish to protect the developing fetus is problematic – and rather paternalistic, according to the authors. The lack of scientific knowledge available is certainly an issue, but one that can only be tackled by allowing pregnant women access to appropriate clinical studies. And finally, the argument regarding the vulnerability of the fetus is (or at least perhaps should be) addressed by a woman's ability to act as a surrogate for her unborn child. Women make decisions – both medical and otherwise – for their children, born and unborn, every day. Which makes the notion that the state of pregnancy renders them unable to make these decisions rather patronizing, at best.

"There is a desperate need to shift the paradigm to protect pregnant women through research, not just from research," states a linked commentary on the article (2). "With the recent emergence of the Zika crisis and the rapid pace of vaccine development, we have a crucial opportunity to demonstrate what proactive and intentional inclusion of pregnant women's interests in the R&D agenda looks like."

It is increasingly being recognized that current approaches to clinical research all too often inadequately account for gender differences (3). Many women face health issues when pregnant and are prescribed drugs to treat them. Of the 172 drugs approved by the FDA between 2000 and 2010, 97 percent had an "undetermined" risk for pregnancy – which highlights just how important it is to ensure that the latest medicines work for all patients. Allowing pregnant women to make their own decisions regarding research participation – and thus giving them more agency – is a crucial first step.

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# When Minutes Matter

## New tests can speed up the diagnosis of severe sepsis, ensuring patients get the right treatment before it's too late

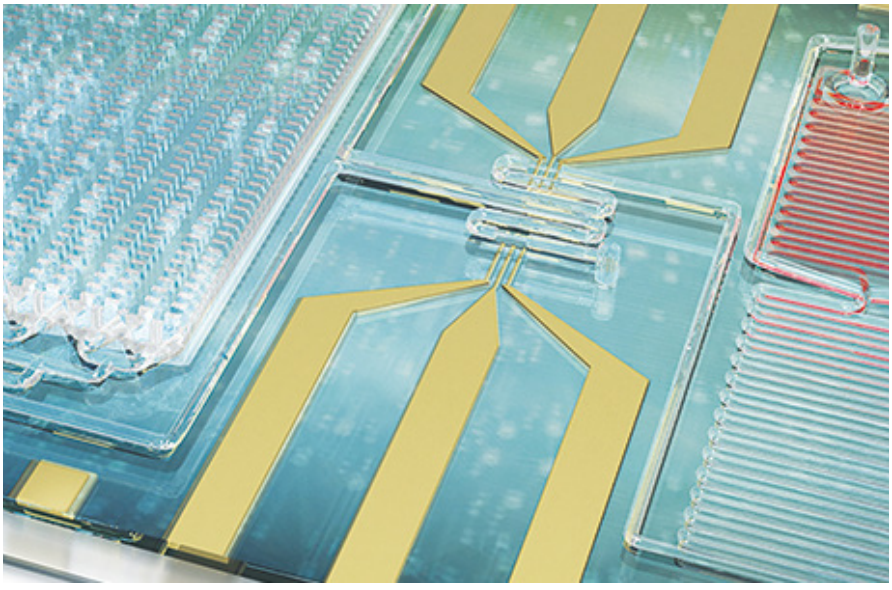
*August 2017*

Sepsis is one of the most time-critical diagnoses a hospital can make. In the most severe cases, it's estimated that patients' likelihood of survival decreases by 7.6 percent each hour that passes without effective treatment (1). But with common symptoms like fever and pain, it can be difficult to conclusively identify sepsis in a timely manner.

Fortunately, science is on the case. Two groups of researchers have recently published tests that promise rapid, reliable diagnosis of sepsis: one, a new PCR-based method, and two, a portable lab-on-a-chip device.

The first, a TaqMan-based multiplex real-time PCR detection system, probes conserved regions of the 16S rDNA gene in 10 common bacterial pathogens (2). It not only detects the organisms causing sepsis, but also positively identifies them in a matter of hours, ensuring that patients can receive appropriate antibiotic treatment as soon as possible – and freeing doctors from the need to wait a day or more for blood cultures to provide the same information.

The second test takes a unique approach – instead of looking for the cause of infection, it detects the patient's immune response (3). How? The device takes a complete white blood cell count, a neutrophil count, and measures levels of the CD64 neutrophil cell surface marker. As the immune response increases, so do these numbers, giving doctors a rapid heads-up that the patient's condition is deteriorating. In some cases, the immune response can spot sepsis even before the causative pathogen is detectable in the blood.



*The lab-on-a-chip system that uses a patient's immune response to diagnose sepsis.Credit: Janet Sinn-Hanlon*

"We think we need both approaches," said Rashid Bashir, senior author on the latter study. "Detect the pathogen, but also monitor the immune response. (4)"

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# Translating Science into Signs

## One student's mission to boost scientific sign language

**By Roisin McGuigan, Associate Editor**

*August 2017*

Liam Mcmulkin, a second year life sciences student at the University of Dundee, UK, was facing a translational problem; as a deaf student, the dearth of complex scientific terms available in British Sign Language (BSL) was making life difficult. "My interpreter has to fingerspell words. Can you imagine sitting in a class, with your lecturer unable to pronounce complex scientific terms?" asks Mcmulkin. "They would have

*Liam McMulkin using BSL in the lab.*

to speak out an individual letter a time. For example, D-E-O-X-Y-R-I-B-O-N-U-C-L-E-O-T-I-D-E. How frustrating would it be to have these complex terms spelled out individually over twenty times in an hour-long lecture? Also, fingerspelling can be misleading – deoxyribonucleotide and deoxyribonucleoside are spelled almost the same – and that could result in a potential hazard to students. The lack of complex scientific terms in BSL also means it's rare to see users communicating with each other about science."

So, Mcmulkin decided to do something about it and embarked on summer project, funded by the Robertson Trust's Self-Development Award, to develop over 100 new signs for scientific jargon, which will be made available on the Scottish Sensory Centre website. But the impact could be wider, as the problem is not just a BSL one: "It's a trending thing at the moment in the deaf community across the world," says Mcmulkin. "I have a great connection with other deaf people internationally on social media, and people are talking about this problem. I also know there have been some events held this year across the world where deaf academics meet and talk about how to improve deaf people's education, exchanging ideas. From this, I think more and more countries are setting up their own projects."

"I rarely meet other deaf people with interests in science, and I believe it's because deaf people have limited access to science education" Mcmulkim says. "For scientists – and BSL users in general – having signs for complex scientific terms means better communication between one another, making the exchange of information quicker and more accurate. After my workshop, I will able to talk via sign language about somitogenesis, immunoprecipitation, acrosome reaction, and so on, without having to rely on fingerspelling."

# Features

## Babies' Breath

### New research reveals a potential noninvasive predictor of bronchopulmonary dysplasia risk in preterm infants

*By Jegen Kandasamy*

### At a Glance

- Preterm infants, especially those requiring prolonged oxygen therapy, are at risk of developing bronchopulmonary dysplasia
- None of the proposed genetic or cytokine biomarkers for BPD risk have been replicated upon closer study
- A new type of biomarker – mitochondrial function – may be more successful, and can be noninvasively tested in umbilical cord blood cells
- If validated, mitochondrial function testing could help doctors determine which infants need modified respiratory support

*August 2017*

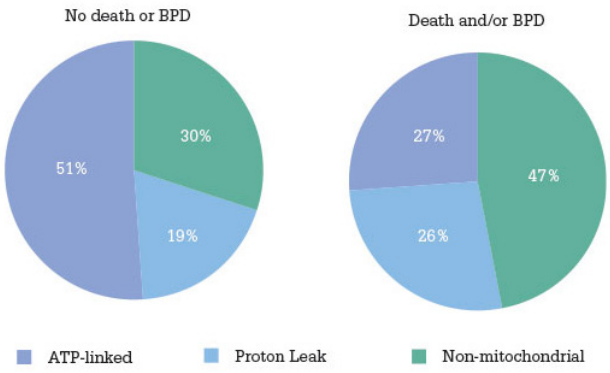
Many prematurely born infants struggle with breathing, and as many as half may develop bronchopulmonary dysplasia (BPD), a lung function abnormality that stresses the infants' underdeveloped lungs and can result in lifelong chronic or even fatal disease. Reactive oxygen species (ROS) arising from prolonged oxygen therapy in babies who are unable to breathe sufficiently on their own interfere with the lungs' maturation and may mean that the terminal saccules – vital for gas exchange during breathing – don't develop correctly. But is there any way to predict which preterm infants may develop BPD, and therefore, which might need modified respiratory support? Until recently, the answer has been "no" – but a new type of biomarker may change that.

### Why we did it

Over the last few years, many medical professionals have hypothesized that pulmonary vascular dysfunction may be an important causative factor in the development of BPD in preterm infants. Even though evidence has now emerged regarding the central role of mitochondria in hyperoxia-related tissue injury – a key pathogenic factor for prematurity-related pulmonary disease – mitochondrial function is a relatively novel and under-investigated area in this disease process.

Human umbilical venous endothelial cells (HUVEC) have often been used as model systems to study the role of vascular function and pathology in the pathogenesis of several diseases, including diabetes, atherosclerosis and hypertension. But, until our study, they had never been used to investigate endothelial function as a risk factor for diseases to which the infants from whom they are obtained are susceptible in their earliest days.

We have collaborated in the past with our co-investigators at the University of Alabama's Department of Pathology on a project that used HUVEC obtained from term newborn infants to investigate mitochondrial bioenergetic differences arising from ethnicity. As a neonatal physician and a lung development and injury researcher, the approach intrigued me – and, as a result, I conceived the idea of using HUVEC obtained from preterm infants to measure endothelial function. My goal was to compare function between infants who later developed lung disease or died early versus those who survived without BPD. I was especially lucky to work at the University of Alabama at




Birmingham, one of the few centers in the United States that has all the necessary components to work collaboratively on such a project – researchers with expertise in the areas of lung development and injury and in mitochondrial and redox biology research, as well as neonatal physicians and a robust and large neonatal intensive care unit. In the end, our study spanned four years, and I'm grateful to all of the people involved; without such a broad range of skills, we could never have completed our work.

Why could the results of the project be so revolutionary? At the moment, most scoring systems that predict BPD risk rely on variables like gestational age and birth weight differences, which contribute significantly to the developmental immaturity that places preterm infants at risk of complications. Those aren't always reliable measures, though, so researchers have made numerous attempts to identify potential biomarkers of these infants' risk of lung disease. Several studies have suggested various cytokines as possible biomarkers; others have proposed genetic polymorphisms (1)(2). Unfortunately, no such link has been replicated in subsequent studies. In short, there has been no single reliable biomarker for predicting an individual's risk of developing lung disease. That's why our new discovery – that bioenergetic function (measured in cells that are relatively easy to obtain from preterm infants at the time of their birth) may be a marker for their risk of BPD – is so important. And, if successfully validated, it could improve our ability to identify prematurely born infants at increased risk before they develop significant lung injury.

## How we did it

In our study, we harvested HUVEC from the umbilical cords of 69 infants born at or earlier than 32 weeks' gestational age. We carried out bioenergetics measurements (intact HUVEC oxygen consumption) with a flux analyzer, as well as reactive oxygen species (ROS) measurements in hyperoxia-exposed HUVEC using fluorescence-based methods. Finally, we used quantitative PCR to measure damage to mitochondrial DNA.

Ultimately, we identified HUVEC bioenergetic function (measured as basal and maximal oxygen consumption under standard assay conditions) as the most significant factor that could reliably distinguish between infants with and without BPD. Fortunately, there are already several platforms that can reliably measure cellular oxygen consumption using as few as 1,000 cells – and we're currently in the process of developing a protocol that will use such systems to validate HUVEC bioenergetic measurements as a biomarker for BPD risk. The test won't hit the clinic tomorrow – validation will likely take at least two to three years – but, if successful, it would help us avoid the need for cell culture and allow us to measure endothelial mitochondrial bioenergetic function in primary cells obtained directly from the umbilical cords of infants at the time of their birth.

It's possible that, in the future, we might be able to test preterm infants for mitochondrial dysfunction in other ways. Mitochondrial genetic inheritance occurs through maternal transmission, so one particularly interesting approach would be test mitochondrial function and genetic differences using cells obtained from pregnant mothers, especially those at increased risk of preterm delivery. Sampling for this wouldn't be difficult; buccal epithelial cells obtained through oral mucosal scrapings would suffice. It's also possible to obtain human umbilical arterial endothelial cells (in addition to the HUVEC we used) from every newborn infant without the need for invasive procedures. These cells could also serve as a source of information regarding mitochondrial function and genetics.

Of course, along with the clinical test will come the question: who should be tested? In my opinion, any infant who is at risk for lung injury because of premature birth is likely to benefit from endothelial mitochondrial function testing. This is especially true for the various subgroups of infants that we particularly identified as having bioenergetic and redox dysfunction in our study – namely, those exposed to maternal and placental infection or inflammation and African-American infants.

## What's next?

Our study identifies an association between BPD risk in preterm infants

and the degree of their endothelial mitochondrial dysfunction. However, the mechanisms behind that association are still unclear and need further investigation. We have some preliminary hypotheses – for instance, endothelial mitochondrial dysfunction could cause deranged pulmonary angiogenesis by reducing nitric oxide and vascular endothelial growth factor availability. Then, the increased ROS generation from these cells could lead to the dysfunction of neighboring cells that constitute the pulmonary tree.

Because mitochondrial bioenergetic function depends on proteins in the electron transfer chain, which are derived from mitochondrial and nuclear gene expression, it's important to investigate both of these sets of genes. Variations in mitochondrial genetic haplotypes, differences in interactions between these two genomes ("mito-Mendelian genetics"), or both could impair or modify mitochondrial response to hyperoxia. Additionally, we also need to investigate mitochondrially targeted therapeutic strategies that could decrease pulmonary mitochondrial dysfunction – and thereby potentially also reduce the risk of lung injury in preterm infants. With a combination of better tests and better treatments, these babies may soon be able to breathe more easily.

*Jegen Kandasamy is Assistant Professor at the University of Alabama at Birmingham and Director of the Rare Disease and Congenital Anomalies Programs at Children's Hospital of Alabama, Birmingham, USA.*

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# Tackling Old Age Before Birth

**Understanding the factors that influence epigenetic changes throughout life offers us the potential to combat diseases like osteoporosis – before they manifest**

*By Beth Curtis*

## At a Glance

- Osteoporosis is a major culprit for fractures in the elderly, comes with a huge economic cost, and has no cure – but what if it could be prevented?
- By understanding the factors that influence health – from pre-conception into adulthood – we have the opportunity to change the course of human disease
- Studying the epigenome can provide a molecular record of life events and teach us what interventions might improve bone health
- Our studies indicate that vitamin D supplementation during pregnancy could have an effect comparable to anti-osteoporotic medications in preventing fractures in later life, if the differences observed at birth persist into older age.

*August 2017*

Osteoporosis is a skeletal disorder characterized by low bone mass and loss of the normal bone microarchitecture, which leads to increased bone fragility; it causes more than 8.9 million bone fractures worldwide every year. Data suggests that, in the UK, one in two women and one in five men aged 50 are expected to have an osteoporosis-related fracture in their remaining lifetime (1). Clearly, this has a huge economic cost, and comes with considerable mortality and morbidity – hip fractures are associated with an increased mortality rate of 10–20 percent in the first year after fracture, and a significant number of people will require long term residential or nursing care. There is no cure, although the condition can be managed through lifestyle changes and medications.

But what if you could protect people from osteoporosis before they've even been born?

## Looking at the bigger picture

At the Medical Research Council (MRC) Lifecourse Epidemiology Unit, University of Southampton, we work towards improving human health at a population level, by understanding factors which influence human health and development from pre-conception, through pregnancy, and then childhood. The idea that epigenetic modifications may influence later bone health stems from the fetal programming hypothesis. It proposes a developmental model for the origins of disease, where maternal, infant and childhood nutrition, health, exposure to infections, and lifestyle permanently "program" an individual's metabolism and growth – and, in doing so, determine the pathologies of old age.

The premise of developmental plasticity (that a single genotype, influenced by specific intrauterine events, has the capability to produce different phenotypes) is now widely accepted. Specific developmental periods are known to exist when an organism is sensitive, or plastic to, its environment. Every organism aims

to develop a phenotype that is best suited to its environment. For example, a malnourished fetus will alter the structure and function of various organs – including the cardiovascular, endocrine and musculoskeletal system – to preserve neurodevelopment, and to enable its survival in the womb.

## Making connections

The first clues that the early life environment may be important in determining the risk of diseases as an adult came from studies of coronary heart disease. It was noted that the incidence of coronary heart disease in Britain was twice as high in poorer areas of the country, and in lower income groups. Studies of the rates of death from coronary heart disease throughout England and Wales were shown to parallel death rates in babies (2), and analyses of birth registries in the county of Hertfordshire, UK, were the first to show that birth weight at the lower end of the normal range was associated with higher rates of coronary heart disease and type II diabetes in later life. These findings have since been extensively replicated, in both men and women in Europe, the USA, China and India, and low birth weight was also shown to be associated with osteoporosis.

Studies from the MRC Lifecourse Epidemiology Unit, directed by Cyrus Cooper, and from elsewhere, have shown that maternal lifestyle, body build and vitamin D status during pregnancy influence childhood bone health, and so do factors in childhood including postnatal nutrition, body composition and physical activity. In addition, poor early growth in childhood has been shown to be associated with increased risks of hip fracture in old age.

But what can we do with this information? As osteoporosis becomes an increasingly pressing public health issue with the aging population, more research is being directed towards ways of preventing osteoporotic fracture (3) to understand the factors affecting the peak bone mass (PBM) attained by individuals during growth and development, and to learn which factors affect the rate of subsequent bone loss. PBM is defined as the maximum total skeletal mass accrued at the completion of skeletal development, usually in the third or early fourth decade of life, and is a key component of the mechanical strength of bone. It has been shown in mathematical modeling studies to be a more powerful predictor of the age of osteoporosis development than age at menopause or rate of subsequent age-related bone loss – for example, a 10 percent increase in PBM delays the onset of osteoporosis by 13 years. Acquiring optimal peak bone mass is therefore essential to bone health as an adult, so targeting the developmental processes that influence bone mineral accrual during early life may be the key to future prevention of the disease, and is central to our line of research (1)(3).

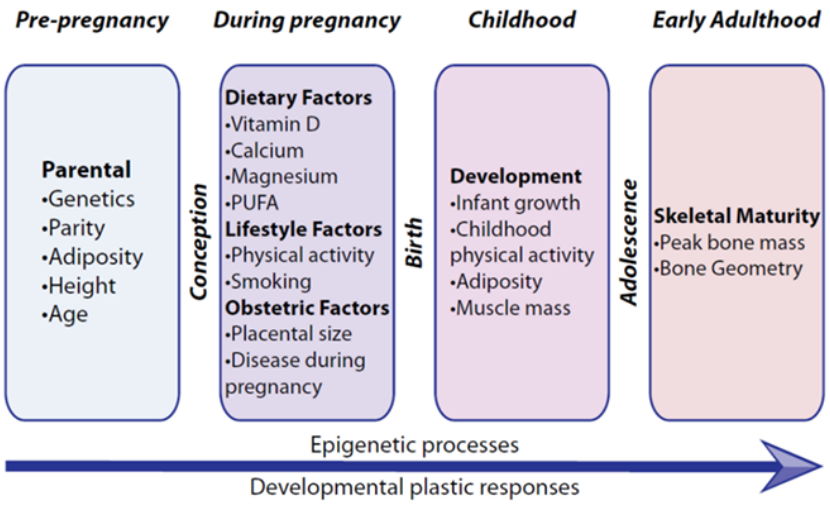


*Figure 1. A summary of influences on early bone development. The magnitude of the variation in population risk of poor bone health increases with age. Conversely, developmental plasticity decreases markedly after intrauterine and early postnatal life. PUFA: polyunsaturated fatty acids.*

## Famine, bone, and epigenetics

How is optimal PBM achieved? It is, of course, governed in part by genetic factors – but there is increasing evidence that some residual variance in both PBM and future fracture risk may be explained by the environment's influence on gene expression, both in utero and in early life (see Figure 1). Such changes can be caused by epigenetic mechanisms – where gene expression is modified without changes to the DNA code itself. Epigenetic signals are essential in determining when and where genes are expressed, and the epigenome can be considered as a molecular record of events, accumulating throughout a lifetime. For example, monozygotic twins have been shown to be most similar from an epigenetic point of view at birth, with their epigenomes diverging at a slower rate if they share a common environment.

Epigenetic mechanisms include DNA methylation, histone

modification and non-coding RNA, but DNA methylation is the most widely studied. Studies of the Dutch Hunger Winter of 1944–45, a famine which occurred following the German occupation of the Netherlands, provide evidence that maternal nutrition influences offspring health in later life and suggest that the timing of the nutritional restriction is important (reviewed in 4), with women exposed to the famine during mid-to-late gestation having babies with significantly reduced birth weights. Babies whose mothers were exposed only during early gestation had normal birth weights; however, they grew up to have higher rates of obesity and cardiovascular disease than those born before and after the war, and higher rates than those exposed during mid-to-late gestation. Comparable findings are now well established in a variety of animal models where nutrition can be precisely controlled, and alterations in the methylation of a number of genes have been found, with the timing of the nutritional constraint appearing to be important.

We wanted to assess whether DNA methylation could be associated with bone health in a mother-offspring cohort, with the aim of identifying whether specific factors in pregnancy or of the maternal diet could be of importance.

## Tracking bone health

At the University of Southampton, through collaboration between scientists at the MRC Lifecourse Epidemiology Unit and the Institute of Developmental Sciences, we have a well-established pipeline for the identification of epigenetic markers of various aspects of child health, including obesity and child bone health. But to conduct this kind of cohort work, a specialist infrastructure and a large team of motivated people are needed – not just the scientists, but research nurses, radiographers, and administrative and laboratory staff too. Considerable organization is needed behind the scenes to arrange appointments, catalogue samples and log questionnaire data. It is also extremely helpful to work in a team where epidemiology can run alongside basic science: in our case, it has been of great benefit to be allied with a team of epigenetics specialists (run by Karen Lillycrop).

To assess DNA methylation, our group (led by Cyrus Cooper and Nicholas Harvey, and by Karen Lillycrop's group at the Institute of Developmental Sciences, University of Southampton) performed methylation arrays using umbilical cord tissue samples from children who were carefully phenotyped, looking for associations between DNA methylation and particular traits. In the context of bone health, we have identified various differentially methylated regions of interest – including the CDKN2A gene (known to be important in cell cycle regulation) and RXRA (involved in vitamin D signaling, amongst other functions). We were then able to use pyrosequencing to look at the methylation of DNA at the individual base (CpG site) level, and understand more about the associations between methylation and offspring phenotype.

Because we're looking at associations between DNA methylation and bone health in children, it is important for us to determine whether a

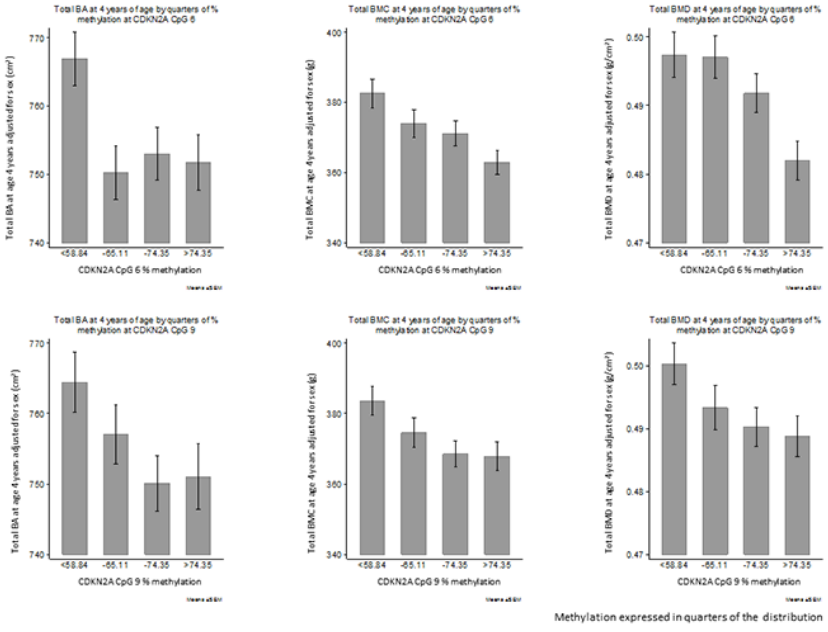


*Figure 2. CDKN2A CpG methylation in relation to bone mineral outcomes. Percentage methylation at CDKN2A CpG 6 (top) and CpG 9 (bottom) expressed in quarters of the distribution in umbilical cord tissue, and offspring total bone area (whole body minus head, cm2), bone mineral content (g), and areal bone mineral density (g/cm2) at age 4 years. Reproduced with permission from EM Curtis et al (8).*

measurement in early life (for example, bone mineral density) can be used to predict a future measurement in an individual. Tracking refers to the maintenance of a relative rank of an individual within a group, when a measurement is repeated at two or more time intervals. Therefore, evidence for high levels of tracking of the measurement or characteristic would support a higher likelihood that an early life intervention to improve the measurement would have a sustained positive effect over time.

The tracking of bone mineral density (BMD) and bone mineral content (BMC) has been well described in populations of different ages and ethnicities, supporting the notion that interventions to increase BMC could have sustained effects into adulthood.

We have shown that CDKN2A methylation is negatively associated with bone health (BMC, BMD and bone area) in children from the Southampton Women's Survey (SWS), a well characterized mother-offspring cohort, at the ages of four and six years (as shown in Figure 2). A similar finding was published by Nicholas Harvey, who found that RXRA methylation is negatively associated with bone health in the same group of children, and replicated this finding in another cohort. There is some evidence that this process might be mediated by vitamin D, as increased vitamin D status has been associated with lower levels of RXRA methylation (5). We are now in the process of evaluating the effects of vitamin D supplementation in pregnancy on RXRA methylation in a randomized controlled trial – MAVIDOS (6).

Quantifying exactly how large an effect a change in methylation of CDKN2A has on fracture risk is challenging, but we are working towards a better understanding. Previous studies have tried to associate birth with fracture risk; in a meta-analysis, each 1 kg increase in birth weight has been shown to be associated with a 1.49 g increase in BMC at the lumbar spine and 1.41 g at the hip (95% CI 0.91, 1.91 g) in adulthood, with the effect independent of adult weight and BMI (7). The 1.49 g increase in adult lumbar spine BMC per 1 kg increase in birth weight is equivalent to a 0.13 Standard Deviations/kg change. If such differences tracked into adulthood, this could lead to a 21 percent reduction in risk of any fracture, or a 35 percent reduction in risk of osteoporotic fracture per 1 kg increase in birth weight.

In the MAVIDOS study, supplementation was found to improve bone health in babies born in winter months. For example, for winter births, the difference in whole body BMC between treatment and placebo offspring was approximately 0.5 SD, which, if maintained into adult life, would equate to a 50 percent difference in fracture risk – an effect comparable with many anti-osteoporotic medications (6). We are currently investigating whether epigenetic mechanisms (including methylation at RXRA and CDKN2A) could underlie this observed effect. In the field of epigenetics, it is particularly important to integrate associations observed between methylation at a particular locus and a health outcome with functional studies. It is vital to try to understand the mechanisms behind the observations at a molecular level, as this helps us in the future to integrate observation, mechanisms and future interventions to improve population health.

The implications of this particular study are that a cheap and simple intervention – such as vitamin D supplementation – could potentially be used on a population level to increase peak bone mass obtained, and therefore reduce skeletal problems in old age. By better understanding the things that influence us during our growth and development, we have the power to change them – in this case, a small effect on millions of people could go on to prevent thousands of bone fractures.

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# Getting the Vaccine Message Across

## Are the common strategies used to dispel vaccine myths making the matter worse?

*August 2017*

The far-reaching and, in some cases, potentially lethal after-effects of Andrew Wakefield's fraudulent 1998 paper that linked the MMR vaccine to autism are still being felt to this day. Some parents still opt not to vaccinate their children because of a fear that vaccines are harmful. It has been described as "perhaps the most damaging medical hoax of the last 100 years" (1), and researchers are still working to reverse the changes it caused in the public perception of vaccines.

Publicizing science that combats the fictions being spread appears to be one solution – but the methods used to do so appear to be having an unintended effect; a recent survey has found that repeating false information with the aim of refuting it may actually spread misconceptions about vaccines (2).

After surveying their existing opinions, the study authors gave new vaccine information to Scottish and Italian students. One group received a booklet that focused on vaccine myths versus facts. A second group was given tables that compared the dangers posed by measles, mumps and rubella with the dangers posed by the vaccine. A third group were shown photographs of unvaccinated children with diseases along with descriptions of the symptoms, and a warning about the dangers of not vaccinating. And final control group received unrelated fact sheets on preventing medical errors and safer healthcare.

All three approaches seem like logical ways to tackle the vaccination challenge – but which ones worked best? The research team found that two strategies were actually counter-productive and reinforced false beliefs rather than dispelling them when compared with the control group. Specifically, the myths versus facts leaflets created stronger beliefs in the vaccine-autism link. The fear-based technique – exposing people to images of sick children – also increased misperceptions, and was linked to the strongest beliefs that vaccines caused side effects. The use of tables caused less damage, but also was not effective in changing people's minds about vaccination.

So how can we effectively combat vaccine myths? The authors suggest tailored and frequent interventions to increase the chance that the correct message is spread and understood within the population. However, they acknowledge that a "golden strategy" does not exist, and stated, "the independent and relative impact of each determinant of vaccination choice is complex and context-specific, varying across time, place, and vaccines."

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