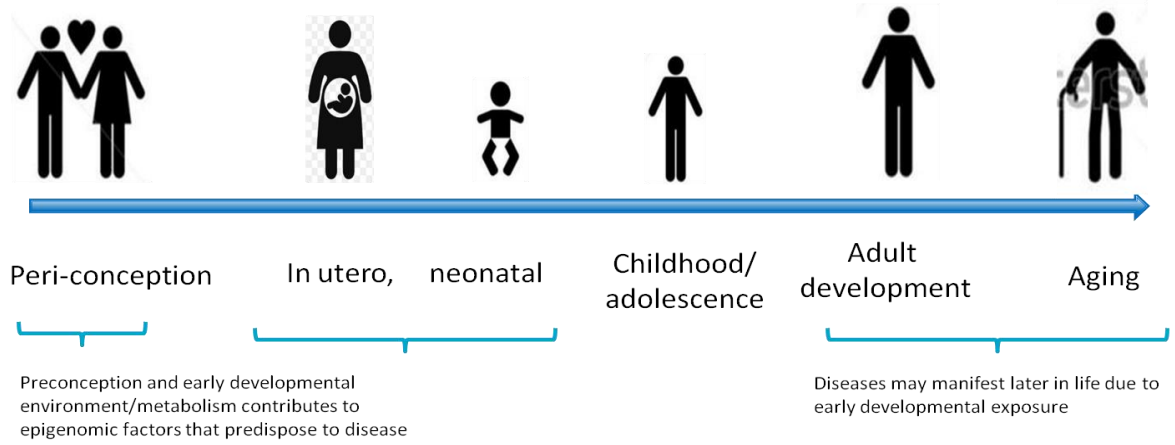
EpiSCOPE Final Report Summary

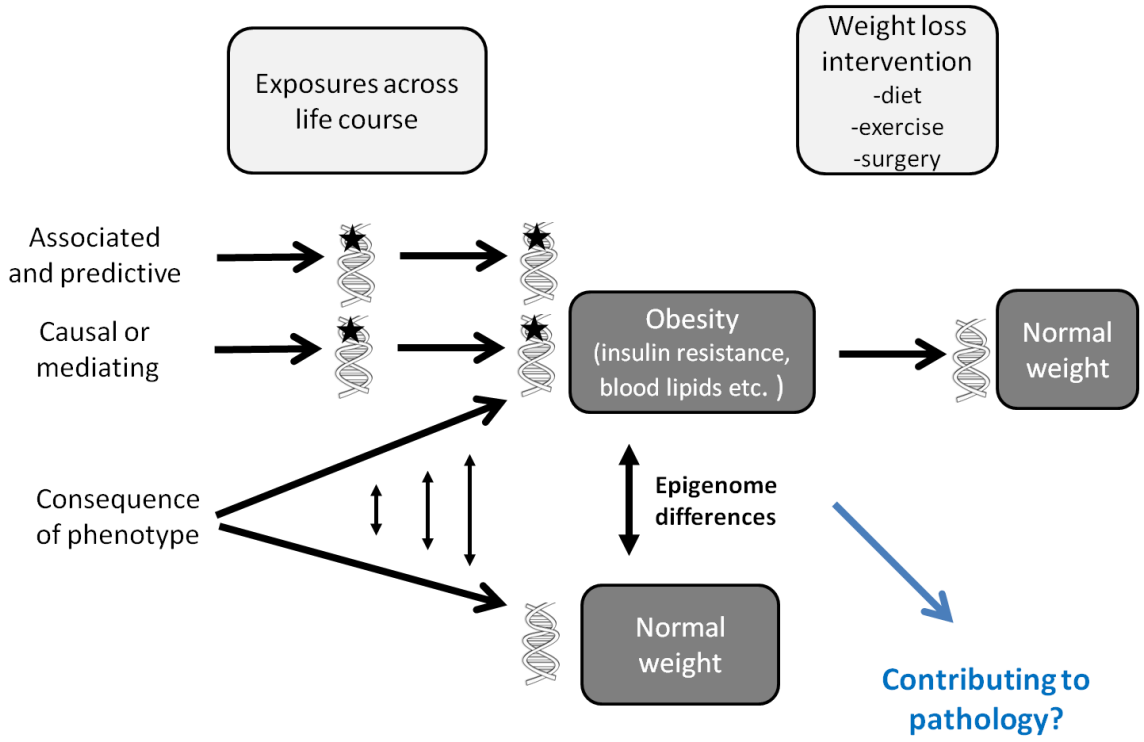
Early Nutrition, the Epigenome and the Prevention of Disease

1. Background and rationale of EpiSCOPE Project

There has been steadily accumulating evidence that nutritional and environmental exposures in early life, including from pre-conception and through pregnancy, can impact on later life phenotypic outcomes. For humans, this includes chronic conditions such as obesity, metabolic and cardiovascular disease, while for livestock there is impact on production traits. Animal studies have provided clear evidence supporting the role of epigenetic mechanisms as one of the means through which such long term outcomes are mediated.



In relation to human health and obesity, the framework in which these questions are being addressed is outlined in the diagram below. Epigenome differences, particularly differences in DNA methylation at specific genomic sites, between lean and obese subjects have now been observed in a number of studies, including in our own work. For an epigenetic mark to contribute causally or to mediate the development of the phenotype (obesity), it must necessarily be present prior to phenotype development. Other epigenetic differences that may be associated with later obesity, but not be causative, still have potential to be used as predictive biomarkers. Epigenetic differences that arise early in development or in response to specific nutritional cues may be present in multiple cell types; hence we are particularly interested in the use of surrogate accessible tissues such as blood or buccal cells and markers that are shared with a metabolically relevant tissue such as adipose.



Epigenetic differences may have arisen along with and in response to the development of obesity. However, such differences may still be significant in contributing to pathologies associated with obesity and provide insights for intervention as well as biomarkers for monitoring response to interventions.

Within the EpiSCOPE project three component research areas in relation to human health and obesity have been brought together to address the following key questions:

1. To what extent can the epigenome be altered through dietary intervention prior to or during pregnancy (in sheep; Component B and humans; Component C),
2. To what extent is the phenotype and function of adipose depots defined by their epigenomes (Component A; human and Component B; sheep)
3. What are the epigenomic differences associated with obesity in both metabolically-relevant adipose tissue and in surrogates such as blood with the aim of developing predictive biomarkers of risk of obesity or metabolic disease (Component A; adults and Component C; children).

2. Executive summary of findings

Findings are presented in relation to the above questions, rather than by component research area.

2.1. To what extent can the epigenome be altered through dietary intervention prior to or during pregnancy?

- Use of a sheep model has demonstrated that the periconception weight status of the mother can significantly affect offspring chromatin epigenetic marks (especially H3K27Ac) and gene expression in adipocyte tissue, but only small changes to the DNA methylation profile were detected in this model. In a second sheep model it was demonstrated that chromatin modification changes dominate DNA methylation changes in a development transition in adipose tissue.
- Epigenome-wide array analysis has been used to characterise DNA methylation profiles of 369 children in a large Randomized Control Trial (DOMInO trial) of ω3 fatty acid supplementation across the second half of pregnancy. This is the largest epigenome analysis in a randomized controlled trial involving a nutritional intervention during pregnancy conducted to date. Our data demonstrate significant, albeit modest, changes in offspring DNA methylation across the genome in response to maternal ω3 fatty acid supplementation. Some of these DNA methylation changes persist until at least 5 years of age. The data also point to different responses of males and females to the prenatal nutritional intervention.

Both studies support the underlying hypothesis that the early nutritional environment can affect the epigenome of offspring.

The data in sheep indicates that chromatin marks may be more responsive to nutritional impact than DNA methylation.

The persistence of a proportion of epigenetic (DNA methylation) differences in children for at least 5 years supports the concept that long term impacts of environmental/nutritional exposures may be epigenetically mediated (this is one of a small number of studies for which longitudinal data are available).

In the DOMInO study the nutritional intervention was in the second half of pregnancy and it is likely that an intervention in the first half of pregnancy would show a stronger effect, potentially mediated through epigenetic changes in multiple cell lineages. However, the ethical implications around any intervention during pregnancy are daunting and we have been scoping the area of sperm health in overweight/obese men in relation to fertility and child outcomes as one in which epigenetic markers could be monitored and interventions undertaken. We are also investigating opportunities to undertake epigenetic analyses in studies involving interventions applied earlier in gestation.

2.2. What distinguishes the epigenome and transcriptome of different fat depots?

- Comparison of the epigenomes and transcriptomes of sheep white and brown fat has characterised key genes and pathways that distinguish the two depots. Extensive changes in gene expression and chromatin organisation occurred, but remarkably these were accompanied by minimal changes in DNA methylation suggesting that brown fat and white fat may share a common progenitor cell. However, the evidence indicated that DNA

methylation changes impacting a specific group of transcription factors may initiate chromatin reprogramming. Importantly, DNA methylation changes occurred within DNA hypomethylation features which were marked by specific chromatin modifications.

- Our first DNA methylome and transcriptome profiling of purified human subcutaneous ("healthy depot") and visceral ("unhealthy depot") adipocytes provides a comprehensive basis for understanding the differences in the biology of these two depots, and the intrinsic properties of the adipocytes themselves, rather than other (e.g. immune) cells also resident in the tissue. Here, extensive differences in both DNA methylation and gene expression were evident that provide cell-type specific signatures. There was a strong enrichment for transcription factor genes among the genes that were differentially methlylated highlighting their potential role in differentiation. For a subset of genes with known roles in adipogenesis and adipocyte function, DNA methylation differences are also consistent with a role for epigenetic control of their expression.

The two Epigenome comparisons that we have performed - sheep brown and white fat and human visceral and subcutaneous fat – provide divergent pictures of epigenetic control of gene expression.

In the case of visceral and subcutaneous adipocytes, we interpret the extensive DNA methylation differences associated developmental transcription factors (homeobox genes etc), to be indicative of different developmental origins of cells in the two depots that perform many convergent functions in storage of triglycerides.

The sheep data is indicative of the presence of developmentally closely-related progenitor cells that can give rise to the divergent gene expression profiles and phenotypes of brown and white adipocytes given appropriate developmental cues. This suggests an underlying plasticity in which use of alternate phenotypic states can be mediated through chromatin changes that are likely driven by key transcription factors.

As multiple studies are continuing to show gene expression differences, and more recently epigenetic differences, between different subcutaneous adipose depots, it is becoming clear that we need to re-think the simple classification of brown fat, subcutaneous and visceral depots of white fat, (and beige fat). This categorisation system is unlikely to represent the full range of plasticity of adipose tissues.

2.3. To what extent are epigenetic changes associated with obesity, and can epigenetic marks be predictive of propensity toward obesity?

- Detailed comparison of purified adipocytes from adult subjects across a range of BMI from lean to obese has identified extensive changes in DNA methylation and gene expression that were associated with BMI. The differences in levels of methylation were larger than expected and many of the differentially methylated regions (DMRs), especially the stronger ones were associated with genes encoding transcription factors, particularly those involved in developmental processes. A small number of the DMRs in visceral adipocytes overlapped obesity DMRs seen in subcutaneous adipocytes, blood or buccal cells.
- Analysis of the DNA methylation profiles at birth (using Guthrie cards) has identified preliminary DNA methylation markers that are associated with BMI or metabolic measures at 5 years of age. Gender differences were also clearly evident in these data. . We are

currently using the epigenetic data obtained to work towards developing a DNA methylation signature (i.e. a combination of multiple marks) at birth that is predictive of the BMI at 5 years of age.

The occurrence of DMRs associated with a number of metabolic pathway genes indicates that epigenetic changes in adipocytes may underlie some of the intrinsic phenotypic changes seen in visceral adipose tissue from obese subjects

The strong signature of developmental transcription factors among the DMRs has led us to propose a model of adipose tissue expansion that involves preferential expansion or migration of a (pre)adipocyte population of a different developmental origin. This could be a significant factor in the adverse phenotype of obese visceral fat and the characterisation of these cell populations and the opportunities for different intervention approaches is an area we wish to pursue.

Data from the DOMInO study has identified candidate BMI-associated biomarkers that are being tested in DNA samples from an independent set of newborns. The overlap of some of those with BMI-associated DMRs found in adults provides hope that a useful, predictive signature of obesity/metabolic health risk can be developed.