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EARNEST

EARly Nutrition programming- long term follow up of Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research

Instrument: Integrated Project

Thematic Priority 5.4.3.1: Food Quality and Safety

Final public report on activity 3.1.1:

The impact of high and low protein diets on fetal metabolism; identification of credible markers of long term disease

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Final report on activity 3.1.1 covering the entire project phase

In the last century there have been dramatic shifts in the intake of protein and energy as well as a marked change in the fatty acid composition of European diets. These are believed to be associated with the rapid increase in the incidence of metabolic syndrome in the population. The interactions between protein and fat in the maternal diet are poorly understood in relation to metabolic programming in early life.

The rat low protein model has been widely used to investigate the mechanisms underlying the fetal origins of type-2 diabetes. The offspring of rats fed a low protein diet during pregnancy release less insulin when subjected to a glucose challenge compared to the offspring of dams fed a complete diet. However it is difficult to study this model as protein deficiency produces numerous adaptive responses in both dam and fetus, for example, changes in amino acid metabolism and tissue growth, which are not necessarily associated with the development of the phenotype in the offspring. Until now this has limited the value of nutrigenomic techniques (proteomics and transcriptomics), because although potentially very useful tools with which to study the mechanism, it is difficult to differentiate between the fundamental programming mechanisms and the underlying adaptive responses.

Fortunately the final phenotype of the offspring depends on the fatty acid composition of the maternal diet. Insulin action in response to a glucose challenge depends on both the protein content and the fatty acid composition of the mothers' diet. Thus the offspring of dams fed low protein diets prepared with corn oil show no change in insulin release, whereas the offspring of dams fed a low protein diet prepared with soya oil show a significant change. This result implicates the fatty acids in the n-3 and n-6 series in the programming mechanism. This interaction between fatty acids and protein provides us with a means to identify the critical mechanisms which program insulin release and glucose metabolism.

In our studies we have used this rat model to study both metabolism and gene expression during pregnancy. Rats have been fed diets containing high or low protein and prepared with either corn (no phenotype) or soya (postnatal phenotype) oils. The animals were killed at 21 days of gestation and we have analysed metabolites, gene and protein expression in the maternal and fetal livers.

The results have shown that the different oils differentially affect lipid metabolism in the dam. Soya oil is known to lower plasma triglyceride; however the results show that this is only occurring in the high protein animals, low protein diets have higher triglyceride concentrations similar to that seen in the animals fed the corn oil diets. Other metabolic parameters were unchanged. The metabolic observations were supported by corresponding changes in the patterns of gene and protein expression observed in the transcriptome and proteome of the maternal liver. The combined data were consistent with an increase in fatty acid oxidation in the maternal liver in the high protein diets prepared with soya oil but not in the low protein diets. All of these observations point to a central role for lipid metabolism and suggest that changes in the protein:energy ratios in the maternal diet may be an important factor in fetal programming.

In contrast the metabolite profile of the fetus is protected from the metabolic changes occurring in the mother. Metabolite parameters in serum were largely unchanged as a result of the altered maternal diet, with the only changes corresponding to a reduced amino acid supply as observed previously. There were substantial changes in the proteome and transcriptome and prominent amongst these were proteins associated with metabolism and protein synthesis. These are core indications of the response to a protein deficient diet and were found in both corn and soy groups. Amongst mRNAs modified by protein deficiency were a number of signalling molecules providing an insight into the regulatory mechanisms underlying the nutrient mediated control of liver growth and differentiation. The changes in these pathways were almost completely attributable to changes in the protein supply, demonstrating that the change in hepatic growth is due to a reduction in the availability of amino acids. However there was no effect of the oil type on these pathways, suggesting that changes in growth are not associated with the programming of postnatal insulin resistance.

There were also widespread changes in the expression of a number of different pathways in the fetal liver which could be attributed to the fatty acid composition of the maternal diet. There was evidence for changes in lipid metabolism, mirroring those taking place in the maternal system. The changes induced by diets rich in n-3 were far reaching affecting cholesterol, lipid and amino acid metabolism. In contrast, the diets poor in n-3 fatty acids upregulated pathways associated with insulin action, IGF signalling and cell proliferation. This suggests that maternal diets high in PUFA may have important effects on fetal growth and caution should be exercised in advocating changes to PUFA intake in human pregnancy until the mechanistic basis is understood.

Pathways associated with a postnatal insulin resistant phenotype i.e. those pathways modified by low protein diets prepared with soya oil (giving a postnatal phenotype) but not changed in the low protein diets prepared with corn oil (no postnatal phenotype), were found to involve insulin signalling. Both the PTEN (Phosphatase-Tensin Homologue) and the MAP kinase (Microtubule associated protein kinase) pathways are altered in the group of animals which would have gone on to show change in insulin action postnatally. Both of these pathways are downstream of the insulin receptor and regulate the transduction of different elements of the insulin signal. A potential mechanism linking increased lipid supplies to altered insulin signalling has been suggested by in vitro studies. Unsaturated fatty acids inhibited PTEN expression in HepG2 cells via activation of a signalling complex formed by the mammalian target of rapamycin (mTOR) and nuclear factor-kappaB (NF-kappaB). These factors are also involved in the response to amino acid deficiencies. To validate these genome wide studies we have investigated the phosphorylation of proteins downstream of the insulin receptor. The results confirmed that there were changes associated with the insulin receptor substrate (IRS-1) but not in other cell signalling pathways (AKT or ERK, which are associated with cell proliferation). These findings suggest that there are early changes in the downstream signalling pathways occurring during fetal development.

The genome wide studies were also used as the basis for the identification of potential markers. We examined a number of candidate genes and found that one protein, Lipocalin-2, was differentially expressed in the fetal liver during development.

The protein which is located at the cell surface is strongly expressed in the fetal liver during development and its expression is suppressed in the groups which go on to develop insulin resistance. It has been reported previously that animals with a targeted deletion of this gene go on to develop insulin resistance. It was therefore striking that this protein was also associated with the fetal programming of insulin resistance.

Our findings have shown that changes in lipid metabolism during fetal development are associated with the development of insulin resistance in the offspring. These results are strikingly similar to those obtained from animals fed high fat diets, consistent with a central role for lipid metabolism; however we believe that these findings suggest that changes in the protein supply can also influence the outcome. The results suggest that changes in the protein:energy balance may be critical in the programming mechanism through alterations in hepatic development. This is consistent with a model in which the “set points” for insulin action are acquired during the transition from open to closed loop regulation during fetal development.