FOOD-CT-2005-007036

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 3.1.4:

Impact of time window of maternal malnutrition and catch-up growth on the programming of atherogenesis and obesity

Period covered from 15.04.2005 to 14.10.2010

Start date of project: 15.04.2005 Duration: 5,5 Years

Organisation Name of Lead Contractor for this report: UCL, Professor Claude Remacle

Activity 3.1.4: Impact of time window of maternal malnutrition and catch-up growth on the programming of atherogenesis and obesity.

In the previous research contract NUTRIX-QLK1-200-00083 with the European Commission we showed in a rat model that malnutrition during early life favours the development of metabolic disease because deficiency in the pancreas, glucose intolerance, obesity and hypertension were observed at adulthood in the progeny.
Our objective in this program was to determine the effect of a maternal protein restriction that influence birth weight, followed by a rapid catch-up growth, on the development later in life of obesity and cardiovascular diseases in mice and to understand the mechanisms which link programming of obesity and cardiovascular disease. For that purpose, pregnant mice were fed with a control (C) or a low protein diet (LP) throughout gestation. The LP diet was isocaloric to the C diet. In order to force the catch-up growth, the litter size of the LP group was limited to 4 instead of 8 in the control group. In addition, at weaning, male mice were fed either a normal diet or a hypercaloric high fat diet (HF).

To reach our objective, the research project was divided in four idems. We first analysed the development and the occurrence of obesity throughout life and investigated the metabolic status of the offspring *in vivo*. Then characterized the adipose tissue collected *in vivo* and verified *in vitro* if the precursor cells that will give birth to adipocytes feature a higher rate of proliferate and differentiate more. In a third part, we verified if hypertension apppeaerd at different ages in adulthood. Lastly, have investigated if the mismatch between foetal nutrition and postnatal nutrition which leads to obesity was associated with a programming of atherogenesis.

The HF diet induced more obesity in the offspring that were growth retarded at birth due to protein deprivation during foetal life and that had a rapid catch-up growth. Such offspring had more fat tissue. The increased observed obesity was however not due to an increased daily calorie intake. These results corroborate human data that have already highlighted the detrimental effect of the catch-up growth for the development of obesity later in life. At 9 months corresponding to middle years in human, the offspring that had received the LP diet during gestation were significantly hyperglycaemic hypercholesterolemia, and hyperleptinemia independently of the postnatal diet, testifying of the foetal programming. Using a microarray designed to study the expression of 89 genes involved in adipose tissue differentiation/function, we demonstrated that the expression profile of several genes were dependent upon the maternal diet. Among the diverse genes showing altered expression, we have identified genes encoding several enzymes involved in lipid metabolism. These results indicated that offspring submitted to early mismatched nutrition exhibited alterations in adipose tissue gene expression that probably increases their susceptibility to overweight when challenged after weaning with a HC diet.
After that, we studied the *in vitro* proliferation and differentiation of rat preadipocytes originated from the C and LP offspring at weaning (28 days), before the HF diet challenge, to investigate whether catch-up growth after prenatal protein restriction *per se* may program adipose precursor cells leading to development of increased adipose tissue mass. Proliferation and differentiation were assessed across time of the culture. At the beginning of the culture, preadipocytes from LP offspring featured a higher proliferation rate which led to more precursor cells. Markers for adipocytes differentiation were less expressed at that time. At later stages of preadipocyte culture, although no difference was observed in lipid

accumulation of C or LP cultures, the mRNA expression of *leptin* (a marker of full adipocytes differentiation) was enhanced in LP cells. The results suggest that prenatal exposure to a LP diet followed by rapid catch-up growth is associated with a higher rate for proliferation of adipocyte precursors that later will differentiate in mature adipocytes contributing thereby to the observed increased obesity in the LP offspring.
In the third part of our project, we investigated whether an early mismatched nutrition produced by catch-up growth after foetal protein restriction could induce the appearance of hypertension in adult male mice. Blood pressure (BP) and heart rate (HR) were assessed by telemetry in conscious unrestrained mice of the same strain as described previously. We found that as expected, postnatal HF diet increased significantly blood pressure (BP) and hearth rate (HR) in 3-month old offspring and that no difference due to the maternal diet was observed. When analysed later at 9 months, we demonstrated that maternal LP diet induced a significant higher BP and HR and an altered circadian rhythm in addition to higher plasma corticosterone concentration in LP offspring. Lastly, the possible precipitation or increased risk to develop atherosclerosis after poor intra-uterine nutrition followed by early catch-up growth was verified in adult offspring. Due to the fact that normal mice do not develop spontaneously atherosclerosis, atherosclerosis plaque area was measured in aortic root sections of LDLr-/- mice, a transgenic mouse. Although this LP offspring showed higher plasma total cholesterol than control offspring, atherosclerosis assessed in aortic roots of 6-mo old mice featured increased plaque area due to HF feeding but not due to early mismatched nutrition. These results indicate a long-term effect of early mismatched nutrition on the appearance of hypertension independently of obesity, while no effect on atherosclerosis was noticed at this age.

In conclusion, catch-up growth after growth retardation due to foetal protein restriction is critical because it favours the development of obesity by increasing preadipocyte proliferation and by changing expression of genes playing a role in the differentiation and the function of the adipose tissue, in particular, genes involved in the lipid metabolism. High fat diet after weaning precipitates the development of obesity. In addition offspring of protein restricted mother overfed during suckling period features hypertension and this occurs independently of the development of obesity.

The results of this activity have led to the publication of 3 papers:

Bol V, Desjardin F, Reusens B, Balligand JL and Remacle C (2010).Does early mismatched nutrition predispose to hypertension and atherosclerosis in male mice. PLoS ONE 5(9): e12656.
doi:10.1371/journal.pone.0012656.

Bol V, Delattre A, Reusens B, Raes M, Remacle C (2009) Forced catch-up growth after fetal protein resytriction alters the adipose tissue gene expression program leading to obesity in adult mice. Am. J. Physiol Regul Integr Comp Physiol 297: R291-299.

Bol V, Reusens B, Remacle C (2008) Postnatal catch-up growth after fetal protein restriction programs proliferation of rat preadipocytes.
Obesity, 16 12, 2760–2763