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 3.3.4:

Programming of glucose intolerance and obesity by PUFA and LC-PUFA during pregnancy/lactation in the guinea pig

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: Nestlé

The objective of the present activity was to investigate the effect of dietary fats during pregnancy and lactation on programming of obesity and glucose intolerance related outcomes. Four studies were conducted during this activity evaluating dietary interventions in the guinea pig. This was chosen as a suitable animal model as fat deposition occurs during gestation, thus resembling the window of adipose tissue development of the human infant.

Study 1: Effect of neonatal supplementation with arachidonic acid on fat mass development and adipose tissue prostaglandins of guinea pigs.

The role of arachidonic acid (AA) on the development of adipose tissue is controversial since its metabolites (i.e., prostaglandins) can either stimulate or inhibit preadipocyte differentiation in vitro. In this study, we evaluated the effects of early postnatal supplementation of AA on body weight and adipose tissue development in guinea pigs. Male newborn guinea pigs were fed for the first 21d of life with diets (milk and pellet) supplemented (+AA) or not (–AA) with AA (1.2% of total fatty acids). From day 21 to day 105 both groups were fed a chow diet. The 21-days-old + AA pups showed a twofold higher AA accretion in phospholipids associated with a two- to six fold increase in several prostaglandins, such as 6-keto $PGF_{1\alpha}$ (the stable hydrolysis product of PGI_2), $PGF_{2\alpha}$, PGE_2, and PGD_2 in adipose tissue, compared with the –AA group. No difference in fat pad and body weight, aP2, and leptin gene expression in adipose tissue, fasting plasma glucose, free-fatty acids, and triglyceride concentration was observed between groups at day 21 or day 105. These results show that dietary supplementation of AA during the suckling/weaning period increases prostaglandin levels in adipose tissue but does not influence early fat mass development in the guinea pig.

Study 2: Effect of dietary alpha-linolenic during early life on adiposity of the adult guinea pig.

The composition of dietary fatty acids (FA) during early life may impact adult adipose tissue (AT) development. We investigated the effects of alpha-linolenic acid (ALA) intake during the suckling/weaning period on AT development and metabolic markers in the guinea pig. Newborn guinea pigs were fed a 27%-fat diet (w/w %) with high (10%-ALA group), moderate (2.4%-ALA group) or low (0.8%-ALA group) ALA content (w/w % as total FA) until they were 21 days old (d21). Then all animals were switched to a 15%-fat diet containing 2% ALA (as total FA) until 136 days of age (d136). Adipose tissue dynamics (triglyceride synthesis rate and cell proliferation rate) were measured by 2H2O labelling during the last five days of life. At d 21, ALA and docosapentaenoic acid concentration decreased in plasma triglycerides with lower ALA intake, but total body fat mass was not different at this time point. Adipose tissue TG synthesis rates and proliferation rate of adipose cells were unchanged between groups at d21, but hepatic de novo lipogenesis was 2-fold increased in the 0.8%-ALA group. In older guinea pigs, the 0.8% ALA group showed a 15% increase in total fat mass (d79 and d107, $p < 0.01$) and epididymal AT weight (d136). This group also tended to show higher insulinemia compared to the 10%-ALA group. In addition, proliferation rate of cells in the subcutaneous AT was higher in the 0.8%-ALA (15.2 +/- 1.3% new cells/5d) than in the 10%-ALA group (8.6 +/- 1.7% new cells/5d, $p = 0.021$) at d136. AT eicosanoid profiles were not associated with the increase of AT cell proliferation. These results show that a low ALA intake during early postnatal life promotes an increased adiposity in the adult guinea pig.

Study 3: Effect of dietary docosohexaenoic acid during early life on adiposity of the adult guinea pig.

The objective of the present study was to investigate the effects of AA or DHA supplementation during both the gestation and suckling/weaning periods on adipose tissue development in the guinea pig. After mating, female guinea pigs were fed one of the following diets: LA/ALA ratio of 10 without LC-PUFA supplementation (10:1); 2.6% AA (10:1+AA) with 2.4% DHA (10:1+DHA); or high in saturated fat (SAT). The dams and the pups received this diet during gestation and until the pups reached 21 d of age. From d21 to

d105 of age, the offspring were fed a chow diet. Due to an unexpected high newborn mortality, especially in the 10:1+AA group (83%), the effects of AA supplementation could not be evaluated.

Offspring of mothers supplemented with DHA showed same body weight but a significant reduced fat mass at birth than those from non-supplemented mothers (15.0 ± 0.5% in 20:1 vs 12.5 ± 0.4% in 20:1+DHA). This fat mass difference was not anymore significant at d21 (13 ± 0.7% vs 11.2 ± 0.3%) and did not persist later in life (20.6 ± 1% vs 21.8 ± 0.5%) due to a rapid catch fat between d21 and d63 of age.

To our knowledge, this is the first demonstration of the anti-adipogenic effects of DHA during gestational development. The benefit of reducing gestational fat mass development of the healthy infant is uncertain, however, dietary DHA supplementation of the obese or diabetic mother may benefit the macrosomic infant by limiting its excess of fat mass at birth. This remains to be demonstrated

Study 4: Development of an animal model of maternal overnutrition

Infants born to obese mothers or mothers suffering from gestational diabetes have increased body fat than those born to mothers with normal BMI. The health consequences of this increased fat mass remain to be elucidated. The objective of this study was to develop a guinea pig model of maternal over nutrition characterized by increased body fat at birth, and evaluate its body fat accumulation after a high fat diet challenge. We tested the following maternal interventions: glucose intraperitoneal injection (mGI) during early gestation (20 and 21 d); high fat + high sucrose diet (mHFS), or high fat diet (mHFD) during pregnancy and lactation. A group of gestating dams fed with a control diet was included as a reference (mREF). Not enough pups were born in mGI and mHFS, and therefore these two groups were removed from the study. Pups born in mHFD and mREF remained with their mothers until d 21, and afterwards were fed with a high fat diet until 103 d. Measurements of body composition by nuclear magnetic resonance were taken at 21, 55 and 97 d and blood samples for glucose and insulin measurements were taken at 21 and 55 d; deuterium water was injected intraperitoneally 5 days before euthanasia and then given as drinking water in order to measure adipose tissue dynamics (cell proliferation, de novo and triglyceride synthesis). mHFD caused a an increase in body fat content of 2.9 and 2.5% at d 2 and 21, respectively. After being weaned to a high fat diet, no differences were observed between the two groups at d 54 or 97. Adipose tissue dynamics revealed decreased fractional cell proliferation rate in retroperitoneal adipose tissue of mHFD pups and decreased fractional synthesis rate of palmitate and triglycerides in subcutaneous adipose tissue of mHFD.

Conclusions

These studies aimed to explore the programming effects of dietary fatty acids on adipose tissue development in the guinea pig. Our results indicate that increased adiposity is promoted by low linolenic acid intake but not by high AA intake, thus, quantity of fatty acids is as relevant as n-6/n-3 ratio in the diet. The guinea pig was a good model to evaluate effect of maternal nutritional interventions in adiposity of the newborn as indicated by the last two studies. The effects of maternal DHA on adiposity of the newborn are promising and its benefits in a macrosomic model should be further evaluated.