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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 3.3.3:

Programming of muscle and adipose tissue by LC-PUFA during perinatal development in the mouse

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: DAT

OBJECTIVES AND MAIN TASKS

One of the key objectives of the EARNEST project was to assess the role of lipids in nutritional programming. Specific activity (3.3.3) of the partner in the Czech Republic, the group at the Department of Adipose Tissue Biology (Institute of Physiology of the Academy of Sciences of the Czech Republic, Prague; **DAT**) concerned characterisation *of specific role of lipids, especially long- chain polyunsaturated fatty acids (**LC-PUFA**), in the postnatal induction of uncoupling protein 3 (**UCP3**) in muscle and to reveal lasting effects of lipids in the nutrition during gestation and lactation on muscle mitochondrial function, and secretory functions and lipid metabolism of adipose tissue.* All the experiments were scheduled to be performed on laboratory mice and aimed to the characterisation of both *acute* and *lasting* effects of the early nutrition. Some of the specific tasks of the studies performed within the EARNEST project have been modified, reflecting the new results obtained by DAT and also by other laboratories during the project, as described below.

RESULTS OVER THE FULL DURATION

The early postnatal period is critical for the switch between glycolytic to oxidative metabolism in muscles and also for the formation of adipose tissue. The involvement of DAT in the EARNEST project was triggered by the original finding made by this group that biogenesis of muscle mitochondria depends on dietary lipids. Namely the finding that expression of the gene for UCP3, which is negligible in fetuses and switched on after birth by dietary fatty acids, was impaired in very preterm newborns (Brauner et al. 2003. Induction of Uncoupling Protein 3 Gene Expression in Skeletal Muscle of Preterm Newborns. Pediatric Research 53:691-697). The postnatal induction of UCP3 may affect energy homeostasis of adults, since it has been suggested that UCP3 controls mitochondrial energy conversion, is linked to lipid metabolism and diabetes, and impaired oxidation of lipids in mitochondria in muscle contributes to insulin resistance associated with ageing.

Therefore, within the EARNEST project, a hypothesis was tested whether LC-PUFA, and namely n-3 LC-PUFA contained in breast milk, could affect muscle UCP3 expression in the offspring. To this end, a model of dietary intervention during pregnancy and lactation has been established in mice, to study the effects of a mild substitution of dietary lipids by a concentrate of n-3 LC-PUFA of marine origin. I fact, the introduction of the model appeared to be very demanding and required several experiments. Due to the unexpected cannibalism of the offspring resulting from the n-3 LC-PUFA supplementation, it was necessary to titrate down the dose of n-3 LC-PUFA in the diet until no effect on the cannibalism was observed (i.e. when 5% of dietary lipids in the diet were replaced by n-3 LC-PUFA; with a total 6% wt/wt dietary lipid content). Using this original model, it was found that the administration of n-3 LC-PUFA during gestation and lactation resulted in a strong increase of the content of docosahexaenoic acid (**DHA**) and a marginal increase of eicosapentaenoic acid (**EPA**) in the milk, resulting also in the increase of the content of these two major n-3 LC-PUFA in the tissues of the neonate. However, with respect to a possible induction of mitochondrial UCP3 in the muscle of the neonate, or lasting effects of the n-3 LC-PUFA supplementation on body weight gain, or energy metabolism (evaluated by indirect calorimetry), or glycemic control of the offspring during aging (up to 6 months, after weaning to either chow low-fat diet, or high-fat diet), the results were negative. These negative results concerning the above parameters could be explained by the very low dose of n-3 LC-PUFA used for the supplementation of the diet during the perinatal development, as dictated by our attempts to control all variables, which could affect the results and lead to erroneous interpretations. Thus, in spite of the fact that the results were negative, they are important for correct interpretations of other animal studies in the field.

In contrast to the negative results concerning the effect on energy homeostasis and glycemic control using the above model, we have found that even under the conditions of very low n-3 LC-PUFA supplementation used, behavioural development of the mice was dramatically improved. Thus, when mice were exposed to the elevated dietary n-3 LC-PUFA content during lactation, and weaned onto normal Chow, their learning memory was improved compared with the control mice, when tested (using Morris water maze) at 3 weeks or 7 weeks after weaning. No differences between the forms of administration of n-3 LC-PUFA, i.e. triglycerides vs. phospholipids, were found. Better results in the cognitive function testing are accompanied by higher content of relevant fatty acids in maternal milk and subsequently in brains of the offspring, although the difference in DHA content in brain became less pronounced with the age. These original findings have been described in one of the internal EARNEST reports (Deliverable No V/019) and they are being prepared for publication.

During the work on the project, it became apparent that AMP-activated protein kinase (**AMPK**), the intracellular energy sensor and the major controller of energy metabolism in cells, could be involved in lasting effects of the perinatal nutrition. AMPK, once phosphorylated due to an increase in the cellular AMP/ATP ratio or other stimulus, activates ATP-producing processes while switching off ATP consuming pathways. Activation of AMPK in skeletal muscle in response to contraction results in increased glucose uptake and fatty acid oxidation. Therefore, two types of studies were performed on mice within the EARNEST project, which were focused on the possible involvement of AMPK in the control of muscle function by lipids during gestation and during early postweaning period, and physiological consequence for muscle and whole body metabolism.

First, with respect to the postnatal induction of UCP3 in muscle by nutritional lipids, which is presumably required for the activation of lipid catabolism and which is impaired in the very premature newbors (see above), experiments were performed in mice to reveal whether AMPK is involved in the induction of UCP3. Using normal mice, it has been found that in both glycolytic and oxidative muscle, expression of AMPK positively correlates with expression of several genes of energy metabolism, with the strongest correlation found in the cases of UCP3 and Glut-4 genes. Furthermore, using a unique model of mice with a genetic disruption of the alpha2 subunit of AMPK, it was conclusively demonstrated that AMPK is involved in the control of UCP3 gene expression in the oxidative but not in the glycolytic type of skeletal muscle. These results are important concerning the mechanism of the postnatal activation of lipid metabolism in skeletal muscle by nutrition and suggest a major role of AMPK in imprinting of lean and obesity-resistant phenotype. These resulsts were published in Pediatric Research (Publication No 1).

Second, the involvement of AMPK in the muscle in the effect of dietary lipids during early post-weaning period on muscle lipid catabolism was studied, while comparing the response of obesity-resistant A/J and obesity-prone C57BL/6J (B/6J) mice. Experiments were performed on male mice born and maintained at 30 ^{o}C. Four-week-old mice were randomly weaned onto a low-fat (LF) or HF diet for 2 weeks. In the A/J LF mice, cold exposure (4 ^{o}C) resulted in hypothermia, while the A/J HF, B/6J LF and B/6J HF mice were cold-tolerant. Cold-sensitivity of the A/J LF mice was associated with a relatively low whole-body energy expenditure under resting conditions, which was normalized by HF diet. In both strains, HF diet induced uncoupling protein 1-mediated thermogenesis, with a stronger induction in A/J mice. Only in A/J mice: (i) HF diet augmented activation of whole body lipid oxidation by cold; and (ii) at 30 ^{o}C, oxygen consumption, total content and phosphorylation of AMP-activated protein kinase (AMPK), and AICAR-stimulated palmitate oxidation in soleus muscle was increased by HF diet in parallel with significantly increased leptinemia. Gene expression data in soleus muscle of the A/J HF mice indicated a shift from carbohydrate to

fatty acid oxidation. Our results suggest a role of muscle nonshivering thermogenesis and lipid oxidation in the obesity-resistant phenotype of A/J mice and indicate that HF diet could induce thermogenesis in oxidative muscle, possibly via the leptin-AMPK axis, especially during the early postweaning period. Thus, both AMPK-dependent muscle lipid catabolism, and secretion of leptin from adipose tissue are possibly involved in the differential metabolic response to dietary lipids in two strains of mice, which differ in propensity to obesity. These results were published (Publication No 3). The involvement of leptin, and especially milk-born leptin, which is transferred to neonates during lactation, in the imprinting of lean phenotype is described in the paper in press (Publication No. 5).

CONCLUSIONS

Studies conducted on mice during the EARNEST project further characterised the role of dietary lipids in the effects of early nutrition on metabolism and behavioural development. The most important findings are listed below:

- The postnatal induction of the gene for UCP3 in skeletal muscle, which is involved in the activation of lipid catabolism during the early postnatal period by nutritional lipids, requires functional AMPK – the major intracellular regulatory pathway controlling energy metabolism. These results suggest the role of AMPK in imprinting of “healthy” phenotype by lipids in early nutrition.

- Consistently with the above finding, it was also demonstrated the leptin-AMPK regulatory axes is involved in the induction of lipid oxidation and thermogenesis in skeletal muscle by nutritional lipids during the early postweaning period, depending also on the genetic background of the mice.

- Very mild increase in the nutritional intake of n-3 LC-PUFA during gestation and lactation, resulting in a preferential increase of the content of DHA in milk and also in tissues of the newborn (including brain phospholipids), exerted lasting improvement of behavioural development of the mice. These results were quite surprising, because the beneficial effect of n-3 LC-PUFA could be detected in the context of the supplementation of a diet optimized for mice breeding (including the optimization of the lipid content and lipid composition of the diet). Therefore, a novel physiological murine model was developed to study lasting effects of dietary lipids administered during the perinatal period on the neural development.

JOURNAL PUBLICATIONS WITH ACKNOWLEDGEMENTS FOR THE EARNEST PROJECT – WHOLE PERIOD

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