

## **Modulation of oral tolerance in rats by perinatal supply of essential fatty acids.**

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### **Changes in dietary fatty acids in Western diet.**

Many of the diseases of the welfare state have increased dramatically during the last decades and the low-fat diet recommendations, which have been established since long ago, have in that context been more and more criticized (1). The Western dietary recommendations have thrown suspicion on saturated fat, and in the recommendation of a high intake of polyunsaturated fatty acids (FA) there has been a shift into more polyunsaturated fatty acids (PUFA) of the n-6 series and less of the n-3 series (2-4). Many metabolic functions are depending on the ratio between the two essential fatty acids (EFA)s, depending on competition of transforming enzymes and the balance between the different products, such as prostanoids and leukotrienes, and the metabolic consequences of all those (5-8). The foetal and perinatal periods are critical phases of life which can influence the health status in adulthood. A specific stimulus or insult during this critical period may lead to developmental adaptations that permanently change structure, physiology and metabolism for pathological conditions later in life, i.e. programming (9-11).

Allergy has increased markedly in the latest decades and of special interest are observations that it might be related to dietary factors (12,13). Investigations of breastmilk and the development of oral tolerance and allergy have shown conflicting results (14,15). In this context it is of interest that the balances of macronutrients in the mother's diet have important short and long-term effects on the offspring that seem to be relevant to human diseases. Biological mechanisms underlying the protective role of breast-feeding are based on the unique composition of the milk, including EFA. The EFA are defined as FA that can not be synthesised by the body and must therefore be obtained from the diet. EFA and their derivatives, long-chain polyunsaturated fatty acids (LCPUFA), play a fundamental role for optimal perinatal development as structural elements of the membranes and as functional modulators of receptors, transports and enzymes in the membranes (5). In addition, they can also directly regulate gene expression and transcription (6-8). The necessity of an adequate supply of EFA and LCPUFA, which is determined to a large extent by the maternal diet, have mainly been studied in term and premature infants on formula feedings in relation to mental development (16,17). Over the last 50 years the n-6 EFA from oil seeds has been substantially increased in developed countries (2-4). Furthermore, the consumption of n-3 PUFA derived from plant and marine food has declined. As a result the ratio of n-6 to n-3 EFA in the Western diet has increased to 10:1-16:1 in Europe with even higher ratios in US (2,4). Since the maternal diet is the most important variable that determines milk FA composition (18,19), this shift in dietary intake of EFA results in rising concentrations of n-6 EFA and reduction of n-3 EFA levels in human milk (20). In European countries the ratio of n-6/n-3 PUFA in human colostrum lipids varies from 5 to 15 (19) and commercial infant formulas have the ratio of n-6/n-3 PUFA of 8-9 (21). The optimal daily intake of PUFA and the optimal ratio of n-6

to n-3 PUFA is not established, but in Paleolithic time this ratio was probably close to 1 (22). In mammals, variations in the ratio of n-6/n-3 EFA of breast-milk affect the development of the neural and retinal systems (23), immune responsiveness (24) and in growth rates (25). The contribution of EFA in the regulation of many physiological processes suggests that the n-6/n-3 EFA ratios in maternal milk might influence the perinatal metabolism and induce the development of pathological conditions in adulthood.

### **Development of oral tolerance.**

Oral exposure to food antigens results in induction of oral tolerance, a state of specific immunological hyporesponsiveness upon further exposure to antigens (26). Several immunological mechanisms, e.g. anergy, clonal deletion and active suppression, contribute to the induction and maintenance of oral tolerance such Active suppression is associated with the presence of regulatory suppressor cells (Treg/Th3) in the draining lymph nodes in adult rats after immunisation. These cells are triggered by a specific antigen and responsible for the release of the antigen-non-specific suppressive cytokine TGF- $\beta$ . As a consequence immune responses to other antigens in the close vicinity are decreased.

The factors involved in the induction or breakdown of oral tolerance in the neonatal period are poorly understood. Failure to develop immunological tolerance may lead to an immune responses resulting in allergic sensitisation to food antigens. During the neonatal period the gastrointestinal tract is exposed to a wide variety of microbial and food-related antigens. In neonatal rodents oral exposure to antigen can induce tolerance or priming depending on antigen nature and dose and maturity of the immune system (27).

One of the most powerful environmental factors during early life is the nutrition. The breast milk contains numerous factors, including PUFA, which may promote the development of the infant's immune system and affect immune responsiveness to antigens. As mentioned before, the levels of n-6 and n-3 PUFA in the breast milk are determined to a large extent by the maternal diet. Thus, variation in the PUFA intake in the maternal diet might significantly modulate neonatal development of immunological tolerance and gastrointestinal sensitisation to food antigens.

In adult animals dietary intake of PUFA has been shown to influence the tolerance induction (28). Different effects of dietary n-6 and n-3 PUFA have been demonstrated on Th1- and Th2-like responses and the mechanisms of oral tolerance to ovalbumin (OA) (29).

### **Experimental evidence of programming of oral tolerance by EFA**

We have studied the influence of EFA in the perinatal period on the development of oral tolerance in rats in two models; one comprising EFA deficient (EFAD) diet and the other with different dietary ratios of the EFA belonging to the n-3 and n-6 series. All diets had the same macro- and micronutrient composition except for the quality of the fat, which constituted 7 energy percent of the diet. The EFA deficient diet contained hydrogenated fat without any EFA or trans-FA. In the diets with different ratios, the n-6/n-3 ratio was 0.4 (n-3 diet), 9 (n-6/n-3 diet) and 216 (n-6 diet), respectively. During late gestation and throughout lactation rats were fed either a diet supplemented with EFA, or an EFAD diet. The rats were subsequently exposed to ovalbumin either as pups via the milk at postnatal days 10-16, or as adults via the drinking water at 7-9 weeks of age.

In rats, which were only exposed to these diets as adults, oral exposure to ovalbumin, lead to antigen-specific suppression of the delayed type hypersensitivity (DTH) response and IgG antibody response to ovalbumin. Tolerance to ovalbumin was observed in both the EFA-supplemented and EFAD groups, and was accompanied by reduction of DTH and IgG

antibody responses to an unrelated antigen, due to bystander suppression (30). Thus, the oral tolerance was maintained and mediated at least partly by an active suppression mechanism in the adult animals of both the dietary groups.

In the adult offspring of the dams fed the EFAD diet, neonatal antigen exposure via the milk resulted in suppression of the serum antibody levels and DTH response against OA indicating induction of oral tolerance. Higher TGF- $\beta$  mRNA levels in the draining lymph nodes suggested that these effects were mediated via Treg cells. In contrast, OA exposure of the dams fed the control diet did not result in suppressed ovalbumin responses of their offspring. Hence, these data suggest that the dietary content of PUFA is one factor important for the induction, or failure of oral tolerance.

In the following experiments we studied the effects of different n-6/n-3 PUFA ratios in the maternal diet on the induction of the neonatal oral tolerance in the rat offspring (31). As described above, rat pups were exposed to ovalbumin via the milk at postnatal days 10-16. In the adult offspring from dams receiving the n-3 diet, exposure to ovalbumin via the milk resulted in lower DTH and antibody responses against both ovalbumin and HSA, compared to those offspring on the same diet but not exposed to ovalbumin postnatally, indicating induction of oral tolerance. The lymph nodes draining the immunisation site were also less enlarged in the offsprings exposed to ovalbumin via their dams, suggesting that in these animals the tolerance was mediated at least partly by an active suppression mechanism. In contrast, the adult offspring from dams receiving the n-6/n-3 diet did not show tolerance. Further increase of the n-6 PUFA in the maternal diet was associated with induction of oral tolerance in the n-6 group of offspring. However, the bystander suppression was not observed in the offspring receiving the n-6 diet, suggesting that the oral tolerance in these animals were probably mediated by anergy. These results suggest that the ratio of the n-6/n-3 PUFA in the maternal diet might affect the mechanisms of neonatal oral tolerance and are in line with data of Harbige et al., demonstrating that dietary levels of the n-6/n-3 PUFA influence the mechanism of oral tolerance in adult mice (32).

Thus the quality of FA ingested by the mother may have effects on the development of immunological tolerance to dietary antigens in the offspring.

### **Possible associated factors in programming of oral tolerance.**

The hormone leptin is mainly produced by white adipose tissue, but also by placenta (33), mammary glands (34), gastric mucosa (35) and neonatal adipose tissue (36). Leptin is known to regulate food intake and energy expenditure, but is also involved in several other physiological processes, including immune responses. It is structurally similar to IL-6 cytokines and binds to receptors, which belong to the class I cytokine receptors. Leptin stimulates proliferation and differentiation of hematopoietic cells and up-regulates monocytes/macrophage functions (37). It modifies T cell responses with increasing Th 1 (IL-2, IFN- $\gamma$ ) and suppressing Th 2 (IL-4, IL-10) cytokine production (38). Serum leptin in mice was increased related to enhanced metacholine responsiveness and IgE responses on sensitization with ovalbumin in a recent study (39). Thus, leptin might play an important role in the induction and maintenance of immune and inflammatory responses, especially vital in the perinatal period. Dietary fat quantity affects perinatal serum leptin levels. Increased maternal fat intake raises plasma leptin concentrations in neonatal rats and affects hypothalamus-pituitary-adrenal responsiveness in neonates and prepubertal rats (40).

Recently, we have shown that dietary fat quality modulates serum leptin levels in rat offspring during the suckling period (41,42). During late gestation and throughout lactation, rats were fed a control, or an EFAD diet. The weight of inguinal white adipose tissue (WAT) depots and the serum leptin levels of the EFAD offspring were significantly lower than in the

control pups during the whole suckling period, despite that milk leptin levels were higher in the EFAD dams than in the control dams at 3 wk of lactation. Furthermore, leptin mRNA was significantly increased in mesenteric lymph nodes, but the mRNA levels in inguinal WAT were reduced in the EFAD pups compared with the control pups at 3 wk of age.

We have also demonstrated the effects of dietary n-6/n-3 PUFA ratios on serum leptin levels in the postnatal period (43). During late gestation and throughout lactation the rats were fed a diet containing linseed oil (n-3 diet), sunflower oil (n-6 diet), or soybean oil (n-6/n-3 diet). As a result the ratio of n-6/n-3 PUFA in the breast milk was significantly different between the dietary groups and the same as in the diet (ref). It was also significantly different in serum phospholipids of the offspring, mean(SE) being 2.5(0.4), 8.3(0.7) and 17.5(4.4) in the n-3, the n-6/n-3 and the n-6 dietary group, respectively. Body weight, body length, inguinal fat pad weight, adipocyte size and serum leptin levels of the offspring receiving the n-3 diet were also significantly lower during the whole suckling period compared with n-6/n-3 fed offspring. The mean serum leptin levels of the n-6 offspring were between the other two groups, but not different from either group. The mRNA in the adipose tissue was significantly lower in the n-6/n-3 group compared with the other two groups at 3 weeks of age (43). No differences were observed in the milk leptin content between the groups.

### **Summary**

Our results indicate important physiological changes of both quantity and quality of EFA intake in the maternal diet as reflected in adipose tissue growth and serum leptin levels in the offspring. Parallel changes in the development of oral tolerance suggest that associations between leptin and the immune system might be of importance in the neonatal period. Interestingly, EFAD diet and a diet rich in n-3 FA perinatally induced oral tolerance, but not a diet high in n-6 FA. It would be of interest in further studies to investigate the mechanisms for a possible relation between leptin and membrane EFA in the gastrointestinal tract and the development of oral tolerance.

### **References:**



