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Perinatal essential fatty acid deficiency influences body weight and bone parameters in adult male rats

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Abstract

Fetal and postnatal nutrition have long-term effects on the risk for development of diseases late in life in humans and animals. The aim of the present study was to investigate the effect of dietary deficiency of essential fatty acids (EFA) in the perinatal period on later body weight and bone mass. During late gestation and throughout lactation, rats were fed a control or an EFA-deficient (EFAD) diet. At 3 weeks of age the offspring were weaned onto an ordinary chow and followed until adult age. The mean body weight of adult rats receiving the EFAD diet during the perinatal period was significantly increased from 12 weeks of age compared to the controls ($P<0.05$). Analysis by peripheral quantitative computerized tomography (pQCT) at 44 weeks of age showed that the trabecular volumetric bone mineral density (BMD) of the femur was significantly decreased ($P<0.05$) but the cortical bone mineral content, cortical area, and cortical thickness were increased ($P<0.05$) in the EFAD group of rats. The length of the femur was not affected. In conclusion, neonatal EFA deficiency was in adult rats associated with increased body weight and significant changes in both cortical and trabecular bone. The results indicate that regulatory mechanisms related to bone mass seemed to be programmed by EFA in the perinatal period. The nature of this modulation needs to be identified.

Keywords: Maternal diet; Programming; IGF-1; Leptin; Bone mineral density

1. Introduction

Human epidemiological studies have demonstrated that reduced growth rate during fetal life and infancy is associated with decreased bone mass [1,2] and increased risk of later hip fracture, the most important clinical consequence of osteoporosis [3]. The mechanism underlying this association is believed to be programming of a range of metabolic and endocrine parameters, which control skeletal growth and consequently the risk for later osteoporosis by environmental stimuli during early development [2]. Recently, it has been shown that maternal protein restriction in rats resulted in reduction of bone area and bone mineral content and widened epiphyseal growth plate among the offspring in late adulthood [4]. However, protein restriction during early development in the rat reduces the desaturase activities and impairs the conversion of essential fatty acids (EFA) to polyunsaturated fatty acid (PUFA) [5]. Deficiency of EFA, possibly due to reduced EFA intake and impaired desaturase activity, has been observed in malnourished children [6] and may be one of the causes of low fetal growth [7]. This suggests that maternal deficiency of EFA might be one of the factors modifying the risk of later osteoporosis in the offspring.

It is well documented that dietary fat plays an important role in bone metabolism [8]. In animal models specific dietary fatty acids (FA) have been shown to modulate bone formation and bone resorption by altering prostaglandin biosynthesis [9,10]. We have previously shown that dietary deficiency of EFA in early life resulted in reduced body

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weight and length in the pre-weaning period [11,12]. In addition, maternal dietary deficiency of EFA led to significantly reduced serum levels of the adipocyte-derived hormone leptin in rat offspring at 1–3 weeks of age due to reduced weight and depressed leptin mRNA expression in white adipose tissue [12]. Leptin regulates food intake and energy expenditure and acts as an important part of a feedback loop to control body weight [13]. Leptin is also involved in numerous physiological processes and was recently shown to have direct effects on bone parameters [14,15]. These data suggest that deficiency of EFA and leptin in early life might have long-term effects on bone growth and development.

The aim of the present study was to investigate the long-term effects of EFA deficiency during the perinatal period on body weight and bone mass in adult rats.

2. Experimental procedures

2.1. Animals and diets

Pregnant Sprague–Dawley rats (BK Universal, Stockholm, Sweden) were received on day 7 of gestation and kept in our animal facility under constant conditions of humidity (70–80%), temperature (22–25 °C), and light (12-h light and dark cycle). The rats were housed individually in plastic cages with food and water ad libitum. The rats were assigned to one of two groups of seven animals receiving either a control or an EFA-deficient (EFAD) diet (AnalyCen, Lidköping, Sweden) for the last 10 days of gestation and throughout the 3 weeks of lactation (31 days of exposure to the diet). The diets differed only by lipid composition: 7% soybean oil for the control diet and 7% hydrogenated lard for the EFAD diet. The composition of the two diets has been described previously [12]. The ordinary chow (Rat standard diet, B&K Universal Ltd, Grimston, Aldbrough, Hull) contained 19% protein, 5% fat, 4% crude fiber and 5.5% ash. The total metabolizable energy of the diets was 14.0 MJ/kg.

Litter size was adjusted to 10 pups per litter. Half of the pups randomly selected from each litter were studied for effects of dietary EFA-deficiency in the perinatal period [12]. The rest of the pups were studied for possible long-term effects of the perinatal dietary FA. At 3 weeks of age, serum FA composition of the offspring (n=9/group) was analyzed and reflected the dietary intervention of the dams (Table 1). At 3 weeks of age, male pups (n=7/group) were randomly selected from each litter, weaned onto ordinary chow ad libitum and followed until 44 weeks of age. Body weight was recorded regularly. Blood samples for phospholipid (PL) FA composition analysis were taken from the tip of the tail of the rats at 3 and 18 weeks of age. Blood samples for leptin and insulin-like growth factor-I (IGF-1) analyses were taken from the rats at 3, 18 and 30 weeks of age in fed state. Sera were kept at −20 °C. The study was approved by the Animal Ethic's Committee of Göteborg University.

Table 1
Fatty acid composition of the serum phospholipids of the control and EFAD rats at 3 and 18 weeks of age

Fatty acids (mol%)	3 weeks		18 weeks	
	Control diet (n=9)	EFAD diet (n=9)	Control diet (n=7)	EFAD diet (n=7)
12:0	0.7±0.2	1.1±0.2*	0.05±0.04	0.04±0.03
14:0	1.3±0.2	3.0±0.3**	0.7±0.09	0.7±0.07
16:0	22.8±1.0	29.8±1.2**	29.5±0.7	29.2±1.4
16:1n-7	0.2±0.1	1.6±0.4**	1.9±0.2	2.2±0.3
18:0	22.6±1.0	19.3±0.8**	20.8±1.4	20.7±1.4
18:1n-9	4.5±0.5	19.9±3.5**	5.5±0.3	5.7±0.7
18:2n-6	26.5±1.5	6.9±1.9**	16.0±0.9	14.6±1.0
18:3n-6	0.1±0.0	0.11±0.03	0.1±0.0	0.1±0.0
20:0	0.24±0.05	0.16±0.05*	0.18±0.03	0.16±0.03
18:3n-3	0.32±0.07	0.06±0.05**	0.42±0.05	0.42±0.04
20:2n-6	0.55±0.1	0.26±0.05**	0.23±0.04	0.19±0.02
20:3n-9	0.09±0.04	6.2±1.2**	0.03±0.03	0.05±0.02
20:3n-6	0.42±0.1	0.67±0.2**	0.5±0.2	0.6±0.1
22:0	0.65±0.05	0.47±0.19*	0.3±0.05	0.25±0.03
20:4n-6	14.0±0.8	6.5±1.8**	18.1±1.6	18.9±1.9
20:5n-3	0.10±0.0	0.12±0.04	0.2±0.0	0.24±0.05
24:0	1.0±0.1	0.7±0.2**	0.82±0.05	0.76±0.07
24:1n-9	0.6±0.04	0.92±0.1**	1.2±0.1	1.2±0.1
22:6n-3	3.1±0.5	2.2±0.5**	3.4±0.3	3.9±0.4
n-6/n-3	12.0±1.4	5.8±0.8**	8.9±0.6	8.2±0.8
18:1n-9/18:2n-6	0.17±0.02	3.2±1.3**	0.35±0.04	0.39±0.06
20:3n-9/20:4n-6	0.01±0.0	1.1±0.4**	0.0±0.0	0.0±0.0

Values are mean±S.D. Results with * and ** are significantly different, P<0.05 and P<0.01, respectively, from the control values.

2.2. Fatty acid analysis

Total lipids from the serum of the rats were extracted according to Folch et al. [16] and fractionated on a single SEP-PAK aminopropyl cartridge (Waters Corp., Massachusetts, USA) according to the method described previously [11]. The fractions of PL were transmethylated in methanolic-HCl-3N at 90 °C over 4 h. The FA methyl esters were separated by capillary gas–liquid chromatography in a Hewlett-Packard 6890 gas chromatograph equipped with a 30-m×0.25-mm SP-2380 column; film thickness 20 μm. Helium at 2.0 ml/min was used as carrier gas. The injector and detector temperatures were 300 and 250 °C, respectively. The column oven temperature was programmed from 50 to 230 °C at a heating rate of 20 °C/min up to 180 °C, and thereafter 2 °C/min. The separation was recorded with HP GC Chem Station software (HP GC, Wilmington, DE). The FA 21:1 was used as internal standard and the FA methyl esters identified by comparison with retention times of pure reference substances (Sigma Aldrich Sweden AB, Stockholm, Sweden). The ratios of eicosatrienoic (Mead) acid 20:3 (n-9) to arachidonic 20:4 (n-6) acid and of oleic

18:1(n-9) to linoleic 18:2 (n-6) acid were used to define the deficiency state: ratios greater than 0.4 and 1.5, respectively, were used as the biochemical criterion of EFA deficiency [17].

2.3. Analysis of leptin and IGF-1

Leptin concentrations in serum were measured by a rat leptin radioimmunoassay (RIA; Linco Research Ltd., St. Charles, MO, USA) and all samples from one experiment were analyzed in duplicate in the same assay. Serum IGF-1 levels were measured by double antibody IGF binding protein-blocked RIA using a commercial kit (Mediagnost, Tubingen, Germany).

2.4. Peripheral quantitative computerized tomography (pQCT)

Computerized tomography was performed with the Stratec pQCT XCT Research M (Norland; v5.4B) operating at a resolution of 70 μm as previously described [18]. Trabecular volumetric bone mineral density (BMD) was determined with a metaphyseal pQCT scan of the distal femur and defined as the inner 45% of the total area. We have previously demonstrated that the trabecular volumetric BMD as measured on the inner 45% of the total area using pQCT is well correlated to trabecular bone volume as a ratio to total bone volume (BV/TV) as measured by histomorphometry [19]. Cortical volumetric BMD, cortical cross-sectional area, periosteal circumference, endosteal circumference, cortical thickness, cross-sectional moment of inertia and cross-sectional moment of resistance were determined with a mid-diaphyseal pQCT scan of the femur. Periosteal circumference, endosteal circumference and cortical thickness were calculated using the Stratec software version 5.40. The total bone area and the bone marrow area were in that calculation assumed to be circular. The periosteal circumference, according to that method, is equal to the circumference of the total area, the endosteal circumference is the outer circumference of the bone marrow cavity and the cortical thickness is the difference between the periosteal and endosteal radius. If the quality of the bone is unchanged then the bone strength as measured by four-point bending test is directly proportional to the cross-sectional moment of inertia [20,21]. Thus, the cross-sectional moment of inertia indicates the bending strength and is dependent on the periosteal and endosteal diameters of bone cross section. Cortical moment of resistance is an indicator of the resistance to torsion as calculated from the outer dimensions of the bone cross section.

2.5. Statistical analysis

Data were analyzed using Mann–Whitney's U test. The animals fed the EFAD diet during the perinatal period were compared to those fed the control diet at each stage of the study. Values are given as mean±S.D. if not otherwise indicated. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Body weight

The mean body weight of the offspring of the dams on the EFAD diet was significantly reduced compared to the control offspring at 3 weeks of age (Fig. 1A). All rats were weaned onto ordinary chow. At 12 weeks of age the offspring in the EFAD group had significantly increased body weight compared to the controls and this difference was even more pronounced at 44 weeks of age ($P<0.01$) (Fig. 1B). The results indicated that deficiency of EFA during the perinatal period was followed by an accelerated weight gain in the adult rats.

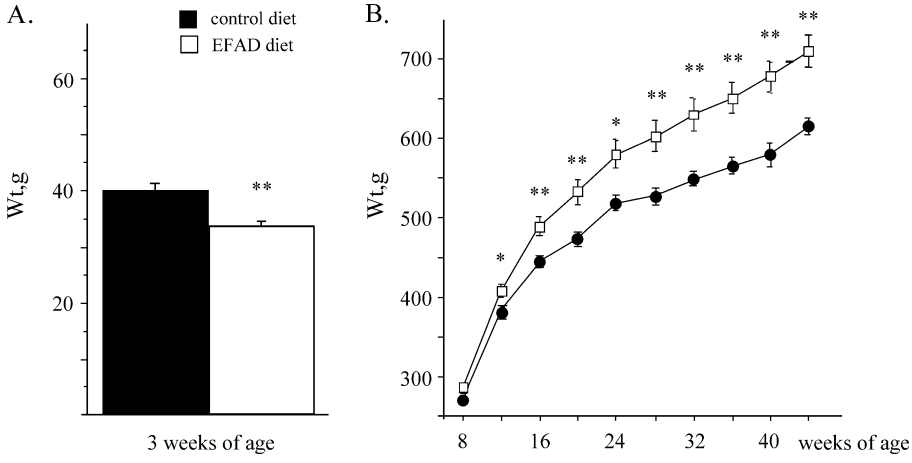

Fig. 1. Effect of EFAD diet given during the perinatal period on the body weight (mean±S.E.) of offspring (A) at 3 weeks of age and (B) of adult rats (each point represents 7 animals). Results with * and ** are significantly different, $P<0.05$ and $P<0.01$, respectively, from the control values.

Table 2
Leptin and IGF-1 in the serum of the rats fed different diets during the neonatal period

	Leptin (ng/ml)		IGF-1 (ng/ml)	
	Control diet	EFAD diet	Control diet	EFAD diet
3 weeks (n=9)	2.3±0.7	1.4±0.9*	158±28	125±27*
18 weeks (n=7)	5.1±3.1	5.3±1.7	795±83	814±102
30 weeks (n=7)	11.6±3.4	13.4±3.1	872±67	887±68

Values are mean±S.D. Results with * are significantly different, P<0.05, from the control values.

3.2. FA composition of serum PL

At 3 weeks of age, the FA composition of the serum PL in the offspring of the mothers receiving the EFAD diet was significantly different from the control group (Table 1). Especially the ratios indicating EFA deficiency were markedly different, 20:3(n-9)/20:4(n-6) in the EFAD group was 1.1±0.4 mol% compared to 0.01 mol% in the controls and the ratio of 18:1(n-9)/18:2 (n-6) was 3.2±1.3 mol% vs. 0.17±0.02 mol%. Feeding ordinary chow to the weaned rats from both groups resulted in no differences in FA composition of the serum PL in rats at 18 weeks of age. The Mead acid was not identified in any dietary group and the ratio of 18:1(n-9) to 18:2 (n-6) was 0.39±0.06 mol% and 0.35±0.04 mol% in the EFAD and the control group, respectively. Because of these results, the serum PL FA composition was not analyzed at later age.

3.3. Leptin and IGF-1 levels in serum

At 3 weeks of age, serum leptin levels in the EFAD offspring were significantly lower than in the controls (P<0.05) (Table 2). After introduction of the ordinary diet the serum leptin levels were not different in the EFAD and in the control groups of rats at 18 and 30 weeks of age. The serum IGF-1 levels were significantly reduced in the 3-week-old offspring of the dams on the EFAD diet compared to the controls (P<0.05) (Table 2). In the adult rats the serum IGF-1 levels were similar in the EFAD and in the control groups at 18 and 30 weeks of age.

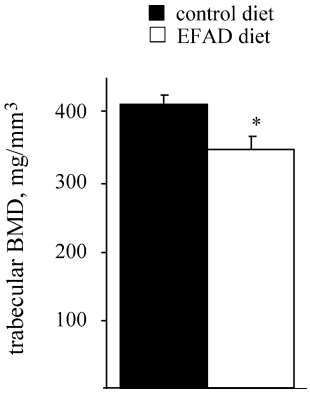

Fig. 2. Effect of EFAD diet given during the perinatal period on trabecular bone mineral density (mean±S.E.) of adult rats (each point represents seven animals). Results with * are significantly different, P<0.05, from the control values.

Table 3
Cortical bone parameters of the adult rats fed different diets during the neonatal period

	Control diet (n=7)	EFAD diet (n=7)
Cortical content (mg/mm)	15.1±0.53	16.2±0.5**
Cortical density (mg/mm^3)	1.50±0.005	1.49±0.009
Cortical area (mm^2)	10.1±0.4	10.9±0.4**
Cortical thickness (mm)	0.93±0.02	0.96±0.02*
Cortical periosteal circumference (mm)	13.8±0.3	14.4±0.5*
Cortical endosteal circumference (mm)	7.9±0.3	8.4±0.6
Cortical cross-sectional moment of inertia (mm^4)	39.3±3.1	47.0±6.1**
Cortical moment of resistance (mm^3)	14.5±0.7	16.3±1.2**

Values are mean±S.D. Results with * and ** are significantly different, P<0.05 and P<0.01, respectively, from the control values.

3.4. Bone growth and bone mineral status in adult rats

No significant difference was observed in the length of the femur between the EFAD and control groups (4.34±0.08 and 4.22±0.11 cm, respectively). PQCT analysis showed that the deficiency of EFA during the perinatal period affected cortical and trabecular bone parameters in different ways in the adult offspring. The trabecular volumetric BMD in the metaphysis of the femur was significantly decreased in adult EFAD rats compared to the controls (Fig. 2). In contrast, analysis of the cortical bone parameters of the femur showed that the cortical bone mineral content (BMC) was increased by 7% in the EFAD group at 44 weeks of age (Table 3). This increase was due to an enhanced cross-sectional cortical bone area (8%), while the cortical volumetric BMD was unchanged. The significantly higher cortical thickness in the EFAD group was associated with a significantly enhanced cortical periosteal circumference (P<0.05), indicating radial cortical growth (Table 3). The changes in the cortical thickness and the position of the cortical cross-sectional bone area resulted in an increase of the cortical cross-sectional moment of inertia and the cortical cross-sectional moment of resistance, which suggested higher mechanical strength of the cortical bone in the EFAD compared to the control group.

4. Discussion

Our study showed that deficiency of EFA in the perinatal period, accompanied by low leptin and IGF-1 levels, was associated with increased weight gain and altered cortical and trabecular bone parameters in the adult offspring. Normalization of serum PL FA composition, leptin and

IGF-1 levels in the adult rats suggests that changes in weight and bone status were programmed early in life and persisted into adulthood. The changes might have been modified but were not compensated for by introduction of standard diet after weaning.

Human epidemiological data and animal studies have demonstrated that pre- and postnatal nutrition have long-term effects on future health [22,23]. Reduced growth in early life, a potential marker of the nutritional status, is a risk factor for numerous adult diseases in mammals [24]. In man, growth during early life correlates with the PUFA status [25]. A growth-promoting effect of PUFA may be related to their structural function in membrane lipids, and to their role as regulators of gene expression or as eicosanoid precursors. In contrast, dietary deficiency of EFA may be one of the causes of low birth weight of newborns [7]. In our animal model, maternal EFA deficiency resulted in reduced weight and length of neonatal rats and was associated with low serum leptin levels [11,12]. Leptin is involved in the regulation of body weight and reduced leptin levels have been observed in the cord blood of infants with low birth weight [26,27]. Thus, variations in the dietary intake of EFA might influence body weight also via leptin-associated mechanisms.

In humans, low leptin levels in early life predict catch-up growth and high rates of weight gain later in infancy [27,28]. Low leptin levels in growth-restrained infants may lead to reduced level of satiety and cause more rapid postnatal weight gain under good nutrition [28]. In the present study, we have observed that the offspring deficient in EFA, which had low serum leptin levels and reduced growth in the postnatal period [12], after weaning to the ordinary diet, had higher rates of weight gain compared to the controls. Although the offspring in the EFAD group had 20% higher body weight, we did not observe differences in serum leptin levels between the dietary groups in adulthood. The possible link between low leptin levels in the postnatal period and an increased weight gain in adult life may reflect persisting changes in leptin regulation or associated systems initiated by nutritional deficiency of EFA.

Low body weight and slow growth in early life are strong predictors of reduced BMC in lumbar spine and femoral neck in early and late adulthood in humans [1,29]. These predictors might at least partly reflect EFA and leptin deficiencies, as we have shown in neonatal rats [12]. Both EFA and leptin are involved in the regulation of bone growth and bone status. Dietary FA may influence bone metabolism directly by altering eicosanoid synthesis or indirectly via a regulation of systemic hormones such as via IGF-1 [8]. Prostaglandin E_2 (PGE_2), an important product of arachidonic acid, is the major prostaglandin affecting bone metabolism. Both bone formation and bone resorption are influenced by PGE_2 and its effect on bone is dose-dependent. At low levels PGE_2 enhances bone formation [30,31], while at higher levels PGE_2 promotes bone resorption [32]. Moreover, leukotriene B_4 (LTB_4), another product of arachidonic acid, stimulates bone resorption in vivo and in vitro [33]. Reduction of the dietary ratio of n-6/n-3 FA was associated with lower production of PGE_2 and increased bone formation in growing rats [9]. In addition, diet enriched with n-3 FA prevents the loss of bone weight and strength in ovariectomized adult rats [34] and reduced bone resorption [35]. In the present study, dietary EFA deficiency influenced the ratio of n-6/n-3 EFA in serum PL of 3-week-old animals, which was twice higher in the control group compared to the EFAD group. In adult age, the EFAD rats had increased cortical BMC caused by an increased radial growth in the EFAD rats. The effect of an EFAD diet on cortical bone mass may include direct effects of FA composition or/and n-6/n-3 FA ratio on the bone tissue metabolism. In addition, dietary EFA deficiency might have an impact on bone parameters indirectly by affecting body weight.

The higher body weight in the EFAD rats would be expected to increase BMD, thus the reduction of the trabecular BMD in the EFAD rats was especially surprising. Dietary FA may also influence bone metabolism via a regulation of systemic hormones such as IGF-1 [8] or cytokines [36]. IGF-1 plays an important role in the longitudinal bone growth and the maintenance of bone mass and its synthesis is rapidly and dose-dependently induced by PGE_2 [37]. Reduction in serum IGF-1 levels was related to decreased BMD in normal children and in children with idiopathic osteoporosis [38,39]. In the present study, the lower serum IGF-1 levels in the EFAD group at 3 weeks of age might have possible impact on decreased trabecular BMD in adult rats.

In addition, reduced serum levels of leptin, which were observed in the EFAD offspring in the postnatal period, might lead to a decreased bone growth and BMD. Indeed, leptin has been shown to induce osteoblastic cell proliferation, de novo collagen synthesis, and mineralization and to inhibit osteoclastogenesis in vitro [14,15]. Thus, reduction in EFA and PGE_2 seems to promote cortical bone mass and strength, while deficiency of leptin and IGF-1 might decrease bone accretion and mineralization. Since EFA deficiency was associated with low leptin and IGF-1 in the neonatal rats, there might be a sensitive balance in this system, which probably influenced cortical and trabecular bone differently. Furthermore, EFA and derived eicosanoids might modulate other mechanisms also involved in the regulation of bone parameters.

In conclusion, neonatal EFA deficiency was associated with altered bone mass and mineralization and increased body weight in adult rats, indicating that regulatory mechanisms have been programmed early in life and were not or only partly compensated for by later ordinary feeding. The results suggest that maternal dietary intake of EFA might be one of the factors modifying the risk of later osteoporosis in the offspring. It has to be studied if restricted EFA diet after weaning might exaggerate the found effects further. The precise mechanisms by which neonatal EFA

deficiency induces long-term effects on skeletal growth and development remain to be determined.

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