# Maternal essential fatty acid deficiency depresses serum leptin levels in suckling rat pups

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**Abstract** **Dietary lipid quantity and quality have recently been shown to affect serum leptin levels in adult rats. Moreover, suckling pups from dams fed a high fat diet had increased serum leptin levels. The aim of the present study was to analyze the influence of essential fatty acid (EFA) deficiency on serum leptin levels in dams and their pups during the suckling period. For the last 10 days of gestation and throughout lactation, pregnant rats were fed a control or an EFA-deficient (EFAD) diet. The levels of leptin and EFA in the serum of the dams and pups were analyzed 1, 2, and 3 weeks after delivery. In parallel, serum levels of glucose and corticosterone were analyzed in the pups. Low serum leptin levels were found in the control lactating dams during the entire lactation period compared with the age-matched nonlactating animals. The leptin concentrations in the lactating dams fed the EFAD diet were lower compared with those fed the control diet. The serum leptin levels of suckling pups from dams on the EFAD diet were markedly decreased compared with controls ($P < 0.05$). The reduced serum leptin levels could not be explained by nutritional restriction as evaluated by serum levels of glucose and corticosterone. These results indicate the importance of the EFA composition of the maternal diet for serum leptin levels in both dams and pups. EFA deficiency in lactating dams may cause long-term effects on the pups through dysregulation of leptin and leptin-dependent functions.**—Korotkova, M., B. Gabrielsson, L. Å. Hanson, and B. Strandvik. **Maternal essential fatty acid deficiency depresses serum leptin levels in suckling rat pups.** ***J. Lipid Res.*** **2001.** 42: **359–365.**

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Nutritional factors such as an adequate intake of essential fatty acids (EFA) during pregnancy and lactation are important for optimal fetal and postnatal development. Dietary deficiency of EFA in early life has long-term effects on development of the neuroendocrine (1) and immune systems (2). Dietary fatty acids (FA) may indirectly modify many cell functions by influencing membrane fluidity and permeability (3, 4). The membrane status can affect various receptor and enzyme functions, membrane transport, as well as signal transduction (5). In addition, EFA are precursors of long-chain polyunsaturated fatty acids (LCPUFA), which can act as second messengers and regulate gene expression (6). EFA are also precursors of eicosanoids, which modulate cell behavior, intercellular interactions, and intracellular signal responses (7).

It has been shown in several recent studies that in addition to the regulation of food intake and energy expenditure (8), the *ob* gene product leptin plays important roles in a variety of physiological and pathological processes. Leptin may be involved in the development of the central nervous system (9), sexual maturation (10), regulation of the hypothalamus-pituitary-adrenal axis (11), insulin homeostasis (12), and immune responses (13). The effect of leptin may be especially striking during intrauterine and neonatal growth for the development of the neuroendocrine and immune systems.

It has been shown that dietary fat quantity (14, 15) and quality (16) affect 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 (14). A diet rich in polyunsaturated fatty acids (PUFA) increases leptin levels in diet-induced obese adult rats (16). Therefore, variation in the type of diet during pregnancy and lactation might significantly modulate fetal and neonatal growth and development by leptin-associated mechanisms.

The aim of the present study was to estimate the influence of EFA deficiency on the serum leptin concentration in dams and their pups during the suckling period.

## MATERIALS AND METHODS

### Animals

Pregnant Sprague-Dawley rats (BK Universal, Stockholm, Sweden) were received on day 7 of gestation and kept in our animal

Abbreviations: EFA, essential fatty acid; EFAD, essential fatty acid deficient; LCPUFA, long-chain polyunsaturated fatty acid; PL, phospholipid; PUFA, polyunsaturated fatty acid; SGA, small for gestational age.

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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 available ad libitum. Ten days before delivery, the females were assigned to one of two groups (n = 6 in each group) receiving either a control or an EFA-deficient (EFAD) diet. Age-matched nonpregnant female rats fed the control or the EFAD diet (n = 6 in each group) were started simultaneously with the pregnant rats. Because the EFAD diet resulted in too many dead pups when starting before conception, and an EFAD diet at birth did not result in an EFA deficiency in the pups for several weeks (unpublished data), it was necessary to start the diet 10 days before birth, then continue the diet after birth and during the lactation period. In this study, therefore, the effect of EFAD on the pups is developed mainly during the lactation period. The pups were kept with their mothers until weaning on day 21. Suckling pups (n = 6) randomized from each litter were used for each time point. Body weights of dams and pups were recorded every week.

### Diets

The dams were fed one of two experimental powdered diets (AnalyCen, Lidköping, Sweden) for the last 10 days of gestation and throughout lactation. 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 is given in **Table 1**. The data on major components, salt, and vitamins have been obtained from the manufacturer. The FA composition was determined in our laboratory with the method described below. The total metabolizable energy for the diets was 13.9 MJ/kg.

TABLE 1. Composition of the control and EFAD diets

| Diet | Control | EFAD |
|---|---|---|
| | *wt%* | |
| Casein | 20.0 | 20.0 |
| Potato starch | 54.0 | 54.0 |
| Glucose | 10.0 | 10.0 |
| Cellulosal flour | 4.0 | 4.0 |
| Mineral mix[a] | 4.0 | 4.0 |
| Vitamin mix[b] | 1.0 | 1.0 |
| Soybean oil | 7.0 | — |
| Hydrogenated lard | — | 7.0 |
| Fatty acids | *mol%* | |
| 12:0 | 0.1 | 0.2 |
| 14:0 | 0.2 | 0.3 |
| 16:0 | 10.8 | 5.4 |
| 16:1 | 0.1 | 0.0 |
| 18:0 | 9.0 | 41.0 |
| 18:1 | 18.9 | 0.7 |
| 18:2 | 45.7 | 0.0 |
| 20:0 | 1.7 | 9.9 |
| 18:3 | 8.1 | 0.0 |
| 20:2 | 0.1 | 0.0 |
| 22:0 | 5.0 | 41.4 |
| 24:0 | 0.2 | 1.2 |

[a] Salt mixture containing (wt %) $KH_2PO_4$ (34.1), $CaCO_3$ (35.9), KCl (2.5), NaCl (18), $MgSO_4 \times H_2O$ (5.1), $FeC_6H_5O_7 \times 5H_2O$ (3.3), MnO (0.27), $Cu_2C_6H_4O_7 \times 2.5H_2O$ (0.06), $Zn_3(C_6H_5O_7)2 \times 2H_2O$ (0.04), $CoCl_2 \times 6H_2O$ (0.002), $KAl(SO_4)2 \times 2H_2O$ (0.008), NaF (0.025), $KIO_3$ (0.009), $Na_2B_4O_7 \times 10H_2O$ (0.002), $Na_2SeO_3 \times 5H_2O$ (0.001), and $Na_2MoO_4 \times 2H_2O$ (0.001).

[b] Vitamin mixture containing vitamin A (11.9 IE/g), vitamin $D_3$ (1.5 IE/g), vitamin $B_1$ (4 mcg/g), vitamin $B_2$ (12 mcg/g), vitamin $B_6$ (5 mcg/g), Ca-pantotenate 45% (11 mcg/g), niacin (40 mcg/g), vitamin $B_{12}$ (0.02 mcg/g), vitamin $K_3$ (7.75 mcg/g), biotin 2% (3 mcg/g), vitamin C (500 mcg/g), inositol (30 mcg/g), vitamin E (42 mcg/g), choline chloride 50% (1 mg/g), and folic acid (0.5 mcg/g).

### Determination of the FA composition of serum phospholipids

Blood samples were collected from dams and pups at 1, 2, and 3 weeks (w1, w2, and w3, respectively) of lactation between 9:00 AM and 11:00 AM. Blood samples were collected from age-matched rats at the same time. Truncal blood was collected from the pups, and blood samples were taken from the tip of the tail of the adult rats. Sera were kept frozen (−20°C) until analyses of leptin, glucose, and corticosterone levels and of FA composition of serum phospholipids (PL; the compartment reflecting the EFA status of tissue lipids). Lipids were extracted from the serum with chloroform–methanol 2:1 (v/v) containing 0.01% butylated hydroxytoluene (17). The lipids were fractionated on a single SEP-PAK aminopropyl cartridge (Waters Corp., Milford, MA, USA) by the method described by Kaluzny et al. (18) and modified by Pietsch and Lorenz (19). The fraction of PL was transmethylated in methanolic-HCL-3N at 90°C over 4 h. The FA methyl esters were extracted with *n*-hexane and, thereafter, washed with water until neutral, dried with $MgSO_4$, and then dried with nitrogen. 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, and a splitless injection was used. The injector and detector temperatures were 300°C and 250°C, respectively. The column oven temperature was programmed from 50°C 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). C21:1 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 ratio of 20:3(n-9) to arachidonate was used to define the deficiency state: a ratio of greater than 0.4 is the biochemical criterion of EFA deficiency (20).

### Analysis of leptin, glucose, and corticosterone levels in serum

Leptin concentrations were measured by a rat leptin radioimmunoassay (RIA; Linco Research Ltd., St. Charles, MO, USA) and all samples from one experiment were analyzed in duplicates in the same assay. The intra-assay coefficient of variation (CV) at 0.25 ng/ml and 20 ng/ml was 2.4% and 1.6%, respectively. Serum glucose levels were measured by the glucose Trinder method (Sigma Aldrich Sweden AB), and serum corticosterone levels were measured by BIOTRAK rat corticosterone RIA (Amersham Pharmacia Biotech, Bucks, England) following the manufacturer's protocol. Before starting the assay, serum samples for corticosterone measurements were heated at 60°C for 30 min to displace corticosterone from cortisol-binding globulin. The intra-assay CV for the corticosterone assay was 5%.

### Statistical analysis

The data were analyzed using Mann-Whitney's U test and Friedman's test. The EFAD-fed animals were compared with the control-fed animals at each stage of the treatment. A value of $P < 0.05$ was considered statistically significant.

## RESULTS

### FA composition of serum PL in nonlactating rats

**Table 2** shows changes in FA composition of serum PL in adult nonlactating rats receiving the EFAD diet compared with animals fed the control diet. Starting at w1, significant decreases in the contents of linoleic 18:2(n-6)

TABLE 2. Serum PL fatty acid composition in the control and EFAD nonlactating rats matched by time with lactating rats at weeks 1, 2, and 3

| | Control Diet (n = 6) | | | EFAD Diet (n = 6) | | |
|---|---|---|---|---|---|---|
| Fatty Acids | 1 Week | 2 Weeks | 3 Weeks | 1 Week | 2 Weeks | 3 Weeks |
| | | | *mol%* | | | |
| 12:0 | 0.4 ± 0.1 | 0.4 ± 0.2 | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.3 ± 0.1 |
| 14:0 | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.3 ± 0.1 | 0.4 ± 0.1[a] | 0.4 ± 0.1 | 0.5 ± 0.1[a] |
| 16:0 | 16.9 ± 2.9 | 18.4 ± 1.3 | 18.8 ± 2.0 | 17.8 ± 0.9 | 21.4 ± 2.3[a] | 18.7 ± 1.0 |
| 16:1(n-7) | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0 | 0.4 ± 0.2[a] | 0.6 ± 0.2[b] | 0.8 ± 0.2[a] |
| 18:0 | 34.7 ± 2.6 | 33.3 ± 1.6 | 35.4 ± 1.7 | 33.1 ± 2.2 | 31.2 ± 1.3 | 33.0 ± 2.6 |
| 18:1(n-9) | 2.8 ± 0.3 | 2.8 ± 0.5 | 2.6 ± 0.2 | 7.1 ± 1.5[a] | 8.9 ± 1.2[b] | 9.9 ± 3.0[a] |
| 18:2(n-6) | 11.4 ± 2.0 | 14.6 ± 2.0 | 11.1 ± 1.0 | 5.0 ± 1.6[b] | 4.7 ± 0.8[b] | 4.4 ± 1.4[a] |
| 20:0 | 0.2 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.2 ± 0 |
| 18:3(n-3) | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0 | 0 ± 0[a] | 0 ± 0[b] | 0 ± 0[a] |
| 20:2(n-6) | 0.2 ± 0.1 | 0.4 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1[b] | 0.2 ± 0.1 |
| 20:3(n-9) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0.8 ± 0.6[b] | 1.3 ± 0.4[b] | 3.2 ± 3.1[a] |
| 22:0 | 1.5 ± 0.1 | 1.7 ± 0.2 | 1.7 ± 0.2 | 1.7 ± 0.1[a] | 2.1 ± 0.1[a] | 2.0 ± 0.2[a] |
| 20:4(n-6) | 22.3 ± 1.9 | 19.2 ± 2.0 | 20.7 ± 1.0 | 22.3 ± 1.2 | 19.0 ± 1.7 | 18.4 ± 3.1 |
| 24:0 | 1.8 ± 0.3 | 1.9 ± 0.2 | 2.0 ± 0.2 | 1.5 ± 0.1[a] | 1.7 ± 0.1 | 1.6 ± 0.2[a] |
| 24:1(n-9) | 1.0 ± 0.2 | 1.0 ± 0.1 | 1.1 ± 0.1 | 0.9 ± 0.1 | 1.1 ± 0.1 | 1.1 ± 0.2 |
| 22:6(n-3) | 6.2 ± 0.9 | 5.4 ± 0.6 | 5.2 ± 0.7 | 8.1 ± 1.5[a] | 6.9 ± 1.0[a] | 5.8 ± 0.8 |
| 18:1/18:2 | 0.25 ± 0.04 | 0.19 ± 0.03 | 0.23 ± 0.03 | 1.64 ± 0.74[b] | 1.97 ± 0.51[b] | 2.66 ± 1.77[a] |
| 20:3/20:4 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0.04 ± 0.03[b] | 0.07 ± 0.02[b] | 0.2 ± 0.21[a] |

Values given as means ± SD.
[a] $P < 0.05$ from control.
[b] $P < 0.005$ from control.

and α-linolenic 18:3(n-3) acids were observed, as well as a compensatory rise in the levels of oleic 18:1(n-9), eicosatrienoic 20:3(n-9), and palmitoleic 16:1(n-7) acids. Those changes were characteristic for a changed lipid metabolism resulting from the EFAD diet (21, 22). However, there was no change in the content of arachidonic 20:4(n-6) acid in the EFAD group. The biochemical index of the EFA nutritional deficit, the ratio of 20:3(n-9) to 20:4(n-6) in the nonlactating EFAD group, was below the empiric value of 0.4 at w3 (20, 21). At w1 and w2, the proportion of docosahexoenoic 22:6(n-3) acid increased significantly in the EFAD group compared with the control group.

### FA composition of serum PL in rats during lactation

During lactation, the FA composition of the serum PL in rats fed the control diet differed significantly from that in nonlactating rats receiving the same diet (Table 2 and **Table 3**). In the serum PL of the control dams during lactation, elevated levels of 18:1(n-9), 18:2(n-6), and 18:3(n-3) acids were observed compared with the nonlactating age-matched group of rats. Simultaneously, the levels of 20:4(n-6), 22:6(n-3), stearic 18:0, behenic 22:0, lignoceric 24:0, and nervonic 24:1 acids were decreased in the serum of the dams compared with the nonlactating group of rats. At w2 to w3 of lactation, the saturated lauric 12:0 and myristic 14:0 acid levels were decreased. Three weeks after weaning, the levels of FA in the serum PL of control dams reached the levels of those in the nonlactating group of rats (data not shown).

Feeding the EFAD diet resulted in dramatic changes of the FA composition of serum PL in the lactating dams compared with controls (Table 3). A significant decrease in the level of 18:2(n-6) and 18:3(n-3) acids was observed along with an accumulation of 16:1(n-7), 18:1(n-9), 20:3(n-9), and 24:1(n-9) acids. At w1, the proportion of 20:4(n-6) and 22:6(n-3) increased significantly in the EFAD group compared with the control group. Despite this increase, the biochemical index of the EFA nutritional deficit, the triene/tetraene ratio, was above the empirical upper normal value of 0.4 in the EFAD group from w2.

### FA composition of the serum PL in the rat pups

The FA composition of PL in the serum during suckling was, in general, similar in the pups and the mothers of the control rats (Table 3 and **Table 4**), although the proportions of the saturated FA 14:0, 16:0, 22:0, and 24:0, and the levels of the LCPUFA 20:4(n-6), 20:2(n-6), and 22:6(n-3) were increased in the pups at w1. There were significant changes in the EFA composition in the serum of the pups of the EFAD group 1 week after delivery compared with the control group, followed by an even more dramatic difference 2 weeks later (Table 4). The levels of 18:0, 18:2(n-6), 18:3(n-3), and 20:4(n-6) acids were decreased along with an accumulation of 14:0, 16:0, 18:1(n-9), 20:3(n-9), 16:1(n-7), and 24:1(n-9) acids. The ratio of 20:3(n-9) to 20:4(n-6) in the EFAD group was above 0.4 from w3 on. The deficiency was also expressed in the increased 18:1/18:2 ratio. At w1, the proportion of 22:6(n-3) increased significantly in the EFAD group compared with the control group. The deficiency in the pups during the first and second week of life was less pronounced than in the dams, but became similar to that of the dams after 3 weeks of age.

### Body weights

The mean body weight of the adult nonlactating rats receiving the EFAD diet did not differ from that of the control nonlactating group. Similar mean body weights were found in the lactating dams during the whole lactation pe-

TABLE 3. Serum PL fatty acid composition in the control and EFAD lactating rats at 1, 2, and 3 weeks of lactation

| | Control Diet (n = 6) | | | EFAD Diet (n = 6) | | |
|---|---|---|---|---|---|---|
| Fatty Acids | 1 Week | 2 Weeks | 3 Weeks | 1 Week | 2 Weeks | 3 Weeks |
| | | | *mol%* | | | |
| 12:0 | 0.4 ± 0.1 | 0.2 ± 0 | 0.2 ± 0.1 | 0.5 ± 0.1 | 0.4 ± 0.1[b] | 0.3 ± 0.1 |
| 14:0 | 0.3 ± 0.1 | 0.2 ± 0 | 0.2 ± 0.1 | 0.5 ± 0.1[b] | 0.5 ± 0.1[b] | 0.5 ± 0.1[a] |
| 16:0 | 17.8 ± 1.3 | 20.5 ± 1.3 | 21.3 ± 1.6 | 21.9 ± 0.9[b] | 23.9 ± 2.4[a] | 22.0 ± 2.5 |
| 16:1(n-7) | 0.2 ± 0 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.9 ± 0.3[b] | 1.0 ± 0.2[b] | 0.9 ± 0.3[b] |
| 18:0 | 26.8 ± 2.4 | 26.6 ± 2.7 | 26.0 ± 1.8 | 25.2 ± 3.4 | 26.6 ± 2.3 | 26.5 ± 2.1 |
| 18:1(n-9) | 5.3 ± 0.5 | 5.7 ± 0.6 | 6.1 ± 0.8 | 13.7 ± 2.5[b] | 16.4 ± 3.0[b] | 17.1 ± 3.7[b] |
| 18:2(n-6) | 25.4 ± 1.9 | 26.0 ± 1.4 | 26.4 ± 1.6 | 7.8 ± 0.4[b] | 4.5 ± 1.1[b] | 5.1 ± 1.5[b] |
| 20:0 | 0.1 ± 0 | 0.2 ± 0 | 0.2 ± 0 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| 18:3(n-3) | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.2 ± 0.1[a] | 0 ± 0[b] | 0 ± 0[b] |
| 20:2(n-6) | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1[a] | 0.1 ± 0.1[a] | 0.2 ± 0.1 |
| 20:3(n-9) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 1.8 ± 1.0[b] | 5.9 ± 3.2[b] | 8.0 ± 5.0[b] |
| 22:0 | 0.9 ± 0.1 | 0.9 ± 0.1 | 1.0 ± 0.2 | 1.2 ± 0.1[b] | 1.8 ± 0.4[b] | 1.9 ± 0.4[b] |
| 20:4(n-6) | 15.7 ± 1.2 | 13.9 ± 0.4 | 12.5 ± 1.3 | 18.5 ± 1.4[a] | 12.5 ± 3.7 | 11.3 ± 4.5 |
| 24:0 | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.2 | 0.8 ± 0.1 | 1.3 ± 0.5 | 1.3 ± 0.4 |
| 24:1(n-9) | 0.7 ± 0.1 | 0.5 ± 0.1 | 0.6 ± 0.1 | 1.0 ± 0.2[a] | 1.2 ± 0.1[b] | 1.5 ± 0.3[b] |
| 22:6(n-3) | 4.8 ± 0.3 | 3.5 ± 0.2 | 3.4 ± 0.4 | 5.6 ± 0.4[a] | 3.9 ± 0.9 | 3.2 ± 1.2 |
| 18:1/18:2 | 0.21 ± 0.01 | 0.22 ± 0.02 | 0.23 ± 0.02 | 1.77 ± 0.32[b] | 3.74 ± 0.88[b] | 3.86 ± 2.2[b] |
| 20:3/20:4 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0.1 ± 0.06[b] | 0.54 ± 0.36[b] | 1.1 ± 1.27[b] |

Values given as means ± SD.
[a] $P < 0.05$ from control.
[b] $P < 0.005$ from control.

riod, unrelated to their diets (data not shown). The mean weight of the pups from the dams receiving the control diet gradually increased from 15.2 ± 1.0 g at w1 to 50.8 ± 3.8 g before weaning. The rate of weight gain in the suckling pups from dams on the EFAD diet was significantly reduced, being 12.0 ± 0.9 g at w1 and 26.0 ± 6.9 g before weaning ($P < 0.05$).

### Serum leptin levels in the rats and their pups

Low serum leptin levels were found in the control lactating dams during the whole lactation period. The concentrations of leptin during the feeding with the control diet varied from 0.59 ± 0.13 ng/ml at w1 of lactation, with a significant increase to 0.82 ± 0.15 ng/ml at w2 ($P < 0.05$) and 0.61 ± 0.13 ng/ml just before weaning (**Fig. 1B**). Nonlactating animals fed the control diet and tested for the same period of time showed significantly higher concentrations of leptin, ranging from 1.91 to 2.74 ng/ml (Fig. 1A). The serum leptin levels in the pups from the dams with the control diet increased slightly from 1.09 ± 0.67 ng/ml at the end of the first week to 1.86 ± 1.33 ng/ml before weaning (not significant; Fig. 1C).

TABLE 4. Serum PL fatty acids in the control and EFAD rat pups at 1, 2, and 3 weeks of age

| | Control Diet (n = 6) | | | EFAD Diet (n = 6) | | |
|---|---|---|---|---|---|---|
| Fatty Acids | 1 Week | 2 Weeks | 3 Weeks | 1 Week | 2 Weeks | 3 Weeks |
| | | | *mol%* | | | |
| 12:0 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.5 ± 0.2[a] | 0.4 ± 0.2 | 0.4 ± 0.2 |
| 14:0 | 1.0 ± 0.1 | 1.2 ± 0.2 | 0.9 ± 0.1 | 1.5 ± .01[b] | 1.7 ± 0.4[b] | 1.8 ± 0.6[a] |
| 16:0 | 22.2 ± 0.3 | 23.8 ± 0.5 | 22.3 ± 1.0 | 28.3 ± 1.4[b] | 29.5 ± 0.4[b] | 27.6 ± 1.6[b] |
| 16:1(n-7) | 0.1 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.4 ± 0.1[b] | 0.7 ± 0.5[b] | 1.0 ± 0.3[b] |
| 18:0 | 18.8 ± 0.9 | 20.8 ± 0.8 | 23.5 ± 0.7 | 15.0 ± 0.2[b] | 18.0 ± 1.0[b] | 19.0 ± 1.7[b] |
| 18:1(n-9) | 2.8 ± 0.2 | 2.7 ± 0.4 | 2.9 ± 0.1 | 5.4 ± 1.0[b] | 9.1 ± 3.1[b] | 15.4 ± 3.4[b] |
| 18:2(n-6) | 22.6 ± 1.1 | 23.9 ± 2.0 | 25.3 ± 2.0 | 13.0 ± 1.6[b] | 13.4 ± 3.2[b] | 8.4 ± 2.5[b] |
| 20:0 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0 |
| 18:3(n-3) | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0 ± 0[a] | 0 ± 0[b] |
| 20:2(n-6) | 0.9 ± 0.1 | 0.6 ± 0.1 | 0.5 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.2 | 0.4 ± 0.1 |
| 20:3(n-9) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0.7 ± 0.1[b] | 2.7 ± 1.0[b] | 7.9 ± 2.1[b] |
| 22:0 | 1.4 ± 0.1 | 1.1 ± 0.1 | 1.2 ± 0.1 | 1.5 ± 0.2 | 1.4 ± 0.2 | 1.1 ± 0.3 |
| 20:4(n-6) | 18.9 ± 0.9 | 16.8 ± 1.6 | 16.2 ± 0.8 | 17.9 ± 1.1 | 13.5 ± 1.2[b] | 10.4 ± 1.8[b] |
| 24:0 | 1.2 ± 0.1 | 1.5 ± 0.1 | 1.4 ± 0.1 | 1.2 ± 0.1 | 1.1 ± 0.2[a] | 1.1 ± 0.1[a] |
| 24:1 | 0.9 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.1 | 2.0 ± 0.2[b] | 1.6 ± 0.3[b] | 1.3 ± 0.1[b] |
| 22:6(n-3) | 8.6 ± 1.3 | 6.1 ± 0.7 | 4.3 ± 0.7 | 11.4 ± 1.1[b] | 6.1 ± 1.5 | 3.9 ± 0.7 |
| 18:1/18:2 | 0.12 ± 0.01 | 0.11 ± 0.02 | 0.11 ± 0.01 | 0.43 ± 0.13[b] | 0.75 ± 0.39[b] | 2.17 ± 1.46[b] |
| 20:3/20:4 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0.04 ± 0.01[b] | 0.2 ± 0.09[b] | 0.79 ± 0.36[b] |

Values given as means ± SD.
[a] $P < 0.05$ from control.
[b] $P < 0.005$ from control.

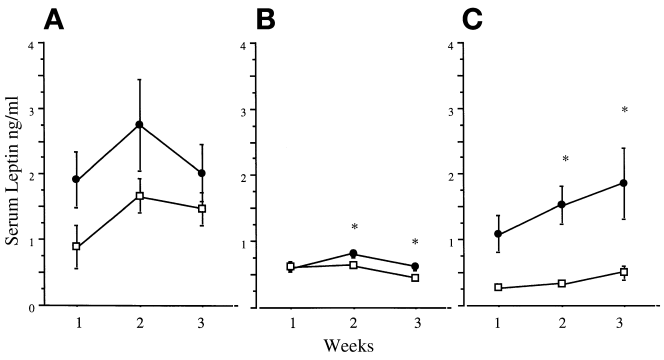


**Fig. 1.** Effect of the EFAD diet on serum leptin levels (mean ± SE) in (A) nonlactating rats, (B) lactating rats, and (C) pups during suckling. Each point represents six animals. Filled symbols represent animals on control diet and open symbols represent animals on EFAD diet. * $P < 0.05$ from control.

Feeding dams with the EFAD diet affected the leptin levels in the suckling pups from the first week of lactation and up to weaning. The leptin concentrations in the control group during this period of time were 3 to 4 times higher than those in the EFAD group (Fig. 1C). Lactating dams also had lower serum leptin levels when fed the EFAD diet compared with controls, but this only occurred during the 2nd and 3rd weeks ($P < 0.05$; Fig. 1B). In the nonlactating rats, the serum leptin levels did not differ significantly between the control and the EFAD groups (Fig. 1A).

**Serum glucose and corticosterone levels in the rat pups**

Serum glucose levels of the suckling pups from dams fed the control diet were similar to those of the offspring from dams fed the EFAD diet during the suckling period (mean ± SE): 157.7 ± 6.2 versus 133.3 ± 5.5 mg/dl at w1, 203.3 ± 7.2 versus 203.9 ± 29.1 mg/dl at w2, and 249.5 ± 9.3 versus 238.5 ± 17.0 mg/dl at w3 in control and EFAD pups, respectively. The mean serum corticosterone levels in the suckling pups were similar in the control and EFAD groups (mean ± SE): 7.5 ± 0.7 versus 7.6 ± 0.6 ng/ml at w1, 10.6 ± 0.7 versus 12.9 ± 1.5 ng/ml at w2, and 77.6 ± 26.9 versus 60.5 ± 11.5 ng/ml at w3, respectively.

## DISCUSSION

Our study shows that a deficiency of dietary EFA during pregnancy and lactation caused a marked suppression of serum leptin levels in neonatal rats. The EFAD diet also decreased the serum leptin levels in the lactating dams.

The involvement of the peptide hormone leptin in different physiological processes in the organism raises the question of the relation between the dietary fat quality and the development of disease. It was shown recently that variations in the leptin levels of adult animals might affect the development of the immune response (13) and glucose homeostasis (23). The quality of dietary fat can regulate the serum leptin levels in adult rats irrespective of adipose tissue mass (16). A high fat diet obviously influences the serum leptin levels and the responsiveness of the hypothalamic-pituitary-adrenal axis in rat pups during the suckling period (14). Following this, an inadequate dietary fat intake during the pregnancy and lactating period resulting in low leptin levels in the offspring may have significant negative effects on postnatal development of the animal.

Feeding the EFAD diet to the nonlactating animals changed the serum lipid profile, but not dramatically. On the other hand, EFA deficiency induced marked changes in the serum PL FA composition of lactating rats. In addition, during lactation, the FA composition of the serum PL of the dams receiving the control diet differed from those of the nonlactating animals. The serum PL levels of LCPUFA, 20:4(n-6) and 22:6(n-3), were significantly decreased in the lactating rats; this was accompanied by elevated serum PL levels of the precursors of LCPUFA (linoleic acid and α-linolenic acids) and oleic acid compared with the nonlactating animals. Our results showed that even when the maternal diet contained adequate levels of linoleic acid, the level of arachidonic acid in the serum of these animals remained relatively lower than in the nonlactating animals. This is in agreement with data in humans showing relative deficiencies of LCPUFA during the last trimester and lactation (24). The changes seen in plasma PL profiles suggested a significant transfer of n-3 and n-6 PUFA from the mother to the fetus and neonate (25). Deficiency of EFA in the diet during pregnancy and lactation of the dams resulted in a faster development of EFA deficiency in the lactating animals.

EFA deficiency also developed in the pups of the animals on the deficient diet, although this effect was not so dramatic during their first 2 weeks of life. This delayed development of EFA deficiency could be explained at least partly by the pups being compensated by the mother via the milk. Because the dams did not receive an adequate amount of EFA from the food, the EFA in the milk may have been produced with involvement of EFA from maternal tissues. A gradual depletion of the mothers' resources during lactation might change the composition of the milk to become more deficient in EFA. This may subsequently influence the EFA composition of the serum PL in the pups, increasing the abnormality during the third week after delivery. The abnormal EFA levels might then cause the change in the leptin levels of the pups.

It is well established that the main source of serum leptin is mature adipocytes. The murine 3T3-L1 preadipocyte cell line can be induced to differentiate into mature adipocytes by human milk (26). The possible active factor(s) was isolated from the lipid fraction of human milk and was suggested to be fatty acids. The importance of LCPUFA precursors for adipocyte function is indicated by their specific accumulation in rat adipose tissue (27).

We observed that in the lactating dams, the serum leptin levels were significantly higher during the second and third weeks of lactation in the control group compared with the EFAD group. Other reports indicated that during lactation, the serum leptin levels in rats are down regulated (28, 29). We also found low serum leptin levels in the control lactating dams during the entire lactation pe-

riod and in the EFAD group during the second and third weeks of lactation compared with nonlactating animals. The mechanism responsible for the diminished leptin secretion during lactation is unclear, but both neuronal and hormonal factors could contribute. The already down-regulated levels of leptin during lactation were further diminished by the EFAD diet.

We observed that the pups from the dams on the EFAD diet had significantly lower leptin levels in the serum compared with the control pups. This difference was found during the entire period of suckling and could not be explained by altered nutritional status in the EFAD pups, as evaluated by glucose and corticosterone levels. As EFAD has been shown to be one factor contributing to infants born small for gestational age (SGA) (30), and it has been suggested that some of these infants may have problems with overweight later in their life (31), it is of interest that SGA newborns have decreased leptin levels, compared to both preterm infants and infants born at term (32).

There may be several mechanisms involved in the down-regulation of the leptin levels by EFA deficiency both in the dams and the pups. It has been shown that leptin concentration falls during fasting (33). Indeed, we found a considerable difference in the mean weight of the EFAD pups compared with the control group, but we did not see any difference in the serum glucose and corticosterone levels between these groups. It has been shown that serum glucose and corticosterone levels were diminished in growth-retarded undernourished rat pups (34). We assume that the low serum leptin levels in the pups fed the EFAD diet are not due to nutritional restriction. The growth retardation observed in the EFAD pups may be caused by a dysfunction of growth hormone regulation that has been demonstrated in EFAD animals (35). In this context, it is interesting to note that a low serum leptin level in children is a negative predictor for successful growth hormone therapy (36).

It has been shown that human fetuses might produce a part of the circulating leptin in their own adipocytes (37), and the *ob* gene is expressed and leptin is produced early in postnatal life in rats (38). A diet rich in PUFA is shown to increase serum leptin levels in adult rats by using a mechanism that is unrelated to changes in adipose tissue mass (16). On the other hand, pronounced EFA deficiency induced in rats fed an EFAD diet for 16 weeks after 3 weeks of age caused a significantly reduced weight gain by 20%, but no effect on fat pad weight (39). PUFA and their metabolites regulate adipocyte differentiation (40) and adipocyte gene expression (6) by affecting the nuclear peroxisome proliferator activated receptor. During the development of the EFA deficiency in the dams and the pups, we found a significant decrease of the relative concentration of linoleic, linolenic, and arachidonic acids in the serum PL of the EFAD animals compared with the controls. The EFA deficiency might affect adipocyte capacity to produce leptin.

The significantly higher level of leptin in the control pups may also be explained by consumption of leptin via the milk. It has recently been shown that leptin is present in both human and mouse milk (41, 42). Moreover, the mouse milk leptin levels clearly increased during the late lactation period (42). Further studies are in progress to investigate the effects of EFA deficiency on leptin content in milk.

In conclusion, we found a significant decrease of serum leptin levels in the pups of dams on an EFAD diet. Our data might have relevance also for some of the human SGA newborns. This suggests the importance of an adequate diet during pregnancy and lactation to attain normal levels of leptin in the offspring. Such levels may be important for a normal development of the neuroendocrine and immunological systems. The long-term effects of neonatal disturbances in leptin homeostasis need to be investigated.

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