ABSTRACT. Objective. Leptin is a highly hydrophilic protein that circulates in plasma as a 16-kDa protein. It is produced in adipose tissue and also recently described to be synthesized by placental tissue. Plasma concentration of leptin is positively correlated to body fat mass, and administration of recombinant leptin to mice indicates that leptin participates in the regulation of food intake and energy expenditure. Leptin may have a role during initiation of human pubertal development. Gender differences have been reported among adults as well as among children, even after correction for body fat content. Little is known about variation in leptin levels during pregnancy or the level or function of leptin in the growing fetus and infants. The aim of the present study was to examine plasma concentration of leptin in pregnant women and their newborn infants during the first 3 months of life, and to relate plasma leptin concentration to body weight and gender during this period.

Materials and Methods. Among 609 women recruited to study the effect of very long-chain n-3 fatty acids during pregnancy, 180 women were selected to study leptin as well. The women were all healthy and nulli- or primiparas, and 16% were smokers. The study was randomized and double-blinded, and the participants received either 10 mL of cod liver oil (Peter Möller, avd.av Orkla ASA, Oslo, Norway) daily or the same amount of corn oil. Blood samples were taken from the mothers during pregnancy in weeks 18 and 35, and from the umbilical cord and from 4- and 14-week-old infants. The mothers’ body mass index (BMI) at 18 and 35 weeks of pregnancy was calculated by using body weight recorded within 1 week of if this was missing, by using means from weights at the closest time points before and after the sampling. The infants were weighed and measured at local health care centers. Plasma leptin concentration was measured by radio immunoassay (Linco Research, St Charles, MO) using recombinant 125I-leptin as tracer.

Results. We found no differences between the group receiving cod liver oil and the group receiving corn oil in any of the measured variables; thus, the groups are treated statistically as one. Leptin concentration in maternal plasma increased during pregnancy from 15.5 ± 9.0 μg/L (n = 175) in week 18 to 17.7 ± 10.7 μg/L (n = 166) in week 35. Mothers, pregnant with female fetuses (n = 77), had a significant increase in plasma leptin concentration, from 15.5 ± 8.8 μg/L (n = 83) at 18 weeks to 18.5 ± 10.9 μg/L (n = 80) at 35 weeks of pregnancy, whereas in mothers pregnant with male fetuses, the increase was insignificant (15.4 ± 9.3 μg/L (n = 92) to 17.0 ± 10.5 μg/L (n = 86). BMI increased during the same time period, from 24.2 ± 3.3 kg/m² to 27.8 ± 3.8 kg/m² (n = 174). There was a significant correlation between BMI and plasma leptin concentration at 18 weeks (r = 0.54, n = 169) and at 35 weeks (r = 0.45, n = 160), but we found no change in the relative leptin concentration (plasma leptin concentration/BMI) from week 18 to week 35. We found no significant difference between smokers and nonsmokers in plasma leptin concentration, neither at 18 nor 35 weeks of pregnancy. Gender differences in plasma leptin concentration was present already at birth in umbilical cord plasma (10.8 ± 9.2 μg/L for girls [n = 65] vs 7.6 ± 6.6 μg/L for boys [n = 74]). We also observed gender differences in plasma leptin concentration at 4 weeks (3.9 ± 1.8 μg/L, n = 68 vs 3.2 ± 1.8 μg/L, n = 71) and 14 weeks of age (4.9 ± 2.1 μg/L, n = 61 vs 4.1 ± 3.1 μg/L, n = 73). Plasma leptin levels at 4 and 14 weeks were lower than the level in umbilical cord plasma (n = 101). An increase in plasma leptin concentration was observed from 4 to 14 weeks of age, both for girls (n = 48) and for boys (n = 60). Leptin concentration in umbilical cord plasma correlated with birth weight (r = 0.44, n = 139), and there was significant correlation (r = 0.23, n = 124) between leptin in plasma and body weight at 14 weeks of age. We observed no correlation between maternal leptin concentration at 35 weeks of pregnancy and the birth weight of the neonates or the leptin levels in umbilical cord plasma.

Conclusion. The leptin levels of the mothers increased during pregnancy and correlated to BMI, but the relative leptin concentration (plasma leptin concentration/BMI) did not change. Our findings demonstrate that gender differences in plasma leptin concentrations already are present at birth. A reduction of 61% in plasma leptin concentration was found from birth to 4 weeks of age. The increase in plasma leptin concentration from 4 to 14 weeks of age can be explained by the increase in weight during the same period. Together with the recent observation that leptin mRNA is expressed in placenta, our present results indicate that placenta may contribute to the high level of leptin found in umbilical cord plasma and suggest a role for leptin in intrauterine growth and development. Pediatrics 1998;101(3). URL: http://www.pediatrics.org/cgi/content/full/101/3/e12; leptin, neonates, pregnancy, growth, gender.

ABBREVIATION. BMI, body mass index.
Leptin is a protein encoded by the ob gene and secreted in proportion to adipocyte size and number.\textsuperscript{1,5} The hormone has a molecular weight of 16 kDa, and it probably executes its effects by binding to receptors found in the hypothalamus and several other tissues.\textsuperscript{3,4} The concentration of leptin in plasma is positively correlated with body mass index (BMI) and even better with the percentage of body fat.\textsuperscript{3,4} Administration of recombinant leptin to mice indicates that leptin participates in the regulation of food intake and energy expenditure.\textsuperscript{1,8-10} Although the biologic importance of leptin remains uncertain in humans, it has been observed that gender differences among adults\textsuperscript{11-13} as well as among children, even after correction for body fat content.\textsuperscript{14} It also has been reported that leptin may act as a signal triggering puberty in boys,\textsuperscript{15} suggesting that leptin is a permissive signal to the reproductive system.\textsuperscript{16}

Plasma leptin levels in pregnant women in late pregnancy have been studied by Schubring\textsuperscript{17} and Butte\textsuperscript{18}; however, little is known about variation in leptin levels during pregnancy or the level or function of leptin in the growing fetus and infants. Because of the dramatic change in environment from the amniotic cavity to the extrauterine life, there are marked changes in hormonal and metabolic conditions of newborn infants. Leptin may play an important role in energy metabolism; therefore, we wanted to describe the levels of this newly discovered hormone in infants. The present study was performed in pregnant women receiving cod liver oil or corn oil as food supplements to examine whether these oils could increase growth or psychomotor development, as suggested in earlier studies.\textsuperscript{19,20} So far, there is very little information on how dietary factors influence leptin concentrations. The purpose of the present study was to measure leptin levels in plasma of pregnant women and their newborn infants, and relate leptin values to body weight, gender, and dietary intake of cod liver oil compared with corn oil.

### Study Group

Pregnant women were recruited from two hospitals in the Oslo, Norway, area, related to routine ultrasound scan examination at 16 to 18 weeks of pregnancy. The women were healthy, between 19 and 35 years of age, and nulli- or primipara. The present study was part of a large investigation in which pregnant women were supplemented with either cod liver oil or corn oil to examine the effect of very long-chain n-3 fatty acids during pregnancy. The participants signed a written consent, and the study was approved by the local ethics committee. Among 609 women recruited to the large study, 180 were selected who had complied to the study. The participants received either 10 mL of cod liver oil (Peter Möller, avd av Orkla ASA, Oslo, Norway) daily or the same amount of corn oil and the same amount of fat-soluble vitamins. The study variation was 8.0%.

### Plasma Leptin Concentration

The blood samples were kept at ~70°C until analysis. Leptin was measured in plasma by a competitive radio immunoassay (Linco Research, St Charles, MO) using recombinant \textsuperscript{125}I-leptin as tracer.\textsuperscript{5} The intraassay variation was 5.5%, and the interassay variation was 8.0%

### Statistics

Results are presented as means ± SD if not stated differently. Neither leptin nor BMI was distributed normally; therefore, leptin was transformed to log (plasma leptin concentration), and BMI was transformed to 1/BMI. Two-tailed student’s t test was used to compare groups, eg, gender, smoking, and supplementation of fatty acids. Paired t test was used to compare individual changes over time. To evaluate correlations between plasma leptin concentration and other parameters, Pearson’s test was used on transformed data. $P < .05$ was considered significant.

### Materials

#### TABLE 1. Demographic Characteristics of Mothers and Infants

<table>
<thead>
<tr>
<th>Study Group</th>
<th></th>
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<tbody>
<tr>
<td>Mothers Mean ± SD (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.6 ± 3.2 (180)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepregnancy BMI (kg/m$^2$)</td>
<td>22.0 ± 2.9 (131)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI week 18 (kg/m$^2$)</td>
<td>24.2 ± 3.5 (174)###</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma leptin week 18 (µg/L)</td>
<td>15.5 ± 9.0 (175)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI week 35 (kg/m$^2$)</td>
<td>27.8 ± 3.8 (174)***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma leptin week 35 (µg/L)</td>
<td>17.7 ± 10.7 (166)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>15.9 (170)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparas (%)</td>
<td>70.6 (180)</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Infants Boys Mean ± SD (n)</th>
<th>Girls Mean ± SD (n)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (d)</td>
<td>278.8 ± 8.6 (93)</td>
<td>278.2 ± 10.4 (87)</td>
</tr>
<tr>
<td>Placental weight (g)</td>
<td>652.1 ± 124.6 (88)</td>
<td>641.7 ± 126.0 (81)</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>51.3 ± 1.9 (91)</td>
<td>50.4 ± 2.1 (83)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3680.2 ± 435.6 (93)</td>
<td>3547.6 ± 473.0 (87)</td>
</tr>
<tr>
<td>Plasma leptin at birth (µg/L)</td>
<td>7.6 ± 6.6 (74)</td>
<td>10.8 ± 9.2 (65)</td>
</tr>
<tr>
<td>Weight 4 weeks (g)</td>
<td>5141.2 ± 720.6 (74)$$$$</td>
<td>4507.5 ± 662.1 (70)$$ $$</td>
</tr>
<tr>
<td>Plasma leptin 4 weeks (µg/L)</td>
<td>3.2 ± 1.8 (71)$$ $$</td>
<td>3.9 ± 1.8 (68)$$ $$</td>
</tr>
<tr>
<td>Weight 14 weeks (g)</td>
<td>4608.9 ± 647.2 (75)$$$$</td>
<td>6045.0 ± 658.8 (73)$$ $$</td>
</tr>
<tr>
<td>Plasma leptin 14 weeks (µg/L)</td>
<td>4.1 ± 3.1 (73)$$ $$</td>
<td>4.9 ± 2.1 (61)$$ $$</td>
</tr>
</tbody>
</table>

### Notes

$\#\# \leq .001$ compared with prepregnancy value; ** $P \leq .01$, *** $P \leq .001$ compared with week 18; $$$ P \leq .001$ compared with values at birth; $\dagger\dagger\dagger P \leq .001$ compared with 4 weeks. $^a$ measured at week 5.7 ± 1.7; $^b$ measured at week 5.0 ± 1.7; $^c$ measured at week 13.5 ± 0.8; $^d$ measured at week 13.9 ± 1.9.
RESULTS

There were no differences in plasma concentration of leptin or any other parameters in mothers or infants between the groups supplemented with cod liver oil or corn oil during pregnancy and the initial months after birth (data not shown). Therefore, the groups were treated statistically as one group. It should be noted, however, that the leptin concentration increased ($P = .005$) in plasma from mothers supplemented with cod liver oil, whereas there was no significant increase for the corn oil group ($P = .5$).

Mothers

Plasma concentration of leptin increased significantly ($P = .01$, $n = 163$) among pregnant women from $15.5 \pm 9.0 \mu g/L$ ($n = 175$) in week $17.6 \pm 1.0$ (referred to as week 18) to $17.7 \pm 10.7 \mu g/L$ ($n = 166$) in week $35.1 \pm 1.3$ (referred to as week 35) (Table 1). Mothers pregnant with female fetuses ($n = 77$) had a significant increase ($P = .002$) in plasma leptin concentration, from $15.5 \pm 8.8 \mu g/L$ ($n = 83$) at 18 weeks to $18.5 \pm 10.9 \mu g/L$ ($n = 80$) at 35 weeks of pregnancy. Among women with male fetuses, the change in leptin concentration was insignificant ($15.4 \pm 9.3 \mu g/L$ [$n = 92$] to $17.0 \pm 10.5 \mu g/L$ [$n = 86$], $P = .41$).

BMI increased during the same time period, from $24.2 \pm 3.3$ kg/m$^2$ (week $17.2 \pm 1.7$) to $27.8 \pm 3.8$ kg/m$^2$ (week $35.4 \pm 1.4$) ($P < .0001$, $n = 174$) (Table 1). There was no difference in BMI related to fetus gender. There was a significant correlation between BMI and plasma leptin concentration at 18 weeks ($r = 0.54$, $P < .0001$, $n = 169$) and at 35 weeks ($r = 0.45$, $P < .0001$, $n = 160$). No significant change was observed in the ratio plasma leptin concentration/BMI from week 18 to week 35. We found no significant difference between smokers and nonsmokers in plasma leptin concentration, neither at 18 weeks nor at 35 weeks of pregnancy, although the leptin concentration was lower in smoking than in nonsmoking women (data not shown).

Infants

In umbilical cord plasma, we found a significant difference in leptin levels for girls ($10.8 \pm 9.2 \mu g/L$, $n = 65$) versus boys ($7.6 \pm 6.6 \mu g/L$, $n = 74$) ($P = .01$) (Fig 1). A gender difference also was found at 4.3 ± 0.5 weeks of age ($3.9 \pm 1.8 \mu g/L$, $n = 68$ vs $3.2 \pm 1.8 \mu g/L$, $n = 71$) ($P = .003$) and at 13.6 ± 1.2 weeks (referred to as 14 weeks) of age ($4.9 \pm 2.1 \mu g/L$, $n = 61$ vs $4.1 \pm 3.1 \mu g/L$, $n = 73$) ($P = .006$). The gender differences were more pronounced when we evaluated the ratio plasma leptin concentration/body weight at birth, 4 weeks, and 14 weeks of age ($P = .002$, $P < .0001$, $P < .0001$, respectively).

Plasma levels of leptin at 4 and 14 weeks were lower than the level in umbilical cord plasma ($P < .0001$, $n = 101$ in both groups). However, an increase in leptin concentration was observed from 4 weeks to 14 weeks for girls ($P = .0009$, $n = 48$) as well as for boys ($P = .001$, $n = 60$) (Fig 1). There was a significant reduction in the ratio plasma leptin concentration/body weight from birth to 4 weeks of age ($P < .0001$, $n = 86$), but no change in the ratio from 4 weeks to 14 weeks of age ($n = 94$).

Leptin concentration in umbilical cord plasma was positively correlated to birth weight ($r = 0.44$, $P < .0001$, $n = 139$) (Fig 2). The correlation was significant both for girls ($r = 0.48$, $P < .0001$, $n = 65$) and for boys ($r = 0.50$, $P < .0001$, $n = 74$). When all infants were evaluated, the correlation between weight and plasma leptin level was significant at 14 weeks of age ($r = 0.23$, $P = .01$, $n = 124$). We found no correlation between the maternal plasma concentration of leptin at 35 weeks of pregnancy and the birth weight of the neonates ($n = 166$) or the placental weight (647.1 ± 125.0 g, $n = 169$). Furthermore, we observed no correlation between maternal leptin concentration at 35 weeks of pregnancy and leptin levels in umbilical cord plasma. There was no correlation between placental weight and leptin levels in umbilical cord plasma, but placental weight was positively correlated to birth weight ($r = 0.55$, $P < .0001$, $n = 169$), and there was a significant correlation between BMI at 35 weeks of pregnancy and leptin concentration in umbilical cord plasma ($r = 0.18$, $P = .04$, $n = 134$). The correlation between the mothers’ BMI in week 35 and the infants’ birth weight was significant ($r = 0.32$, $P < .0001$, $n = 174$).

DISCUSSION

Maternal plasma leptin levels and BMI increased from week 18 to week 35 of pregnancy (Table 1). The enhanced BMI during pregnancy may reflect the growing fetus and uterus, an increase in blood volume, and fat depots. Because leptin concentration in plasma is more strongly correlated to total fat mass than to BMI, one might expect the relative leptin concentration (plasma leptin concentration/BMI) to
decrease during pregnancy. However, we found no change in relative leptin concentrations from week 18 to week 35.

Elevated plasma leptin levels at 36 weeks of pregnancy compared with postpartum levels have been reported previously to be attributable to other factors than maternal fat mass alone. Hassink et al found similar or higher amounts of leptin mRNA in placental tissue than in abdominal fat tissue and speculated that placenta might be the major source of leptin production for the fetus. The increase in plasma leptin concentration during pregnancy might be attributable to transfer of placental leptin to maternal circulation. This hypothesis is supported by our observation that infant gender differences were reflected in a significant increase in maternal plasma leptin concentration when carrying female compared with male fetuses. According to Schubring et al, maternal plasma leptin levels did not correlate with umbilical cord plasma level or birth weight, but they found an inverse correlation between leptin levels in maternal plasma and placental weight. We could not confirm this latter finding in our present study, probably because of the delay between blood sampling at week 35 and time of delivery.

We observed that boys had only 70% of the leptin concentration compared with girls in umbilical cord plasma, as reported recently by others. The gender difference remained present at weeks 4 and 14 after birth, despite significantly lower weights among girls than boys. The smaller gender differences in leptin concentration at weeks 4 and 14 most likely cannot be explained by intra- or interassay variation, because all samples were analyzed randomly. The mechanisms behind the gender difference in plasma leptin concentration are not understood. Effects of estrogen and/or progesterone cannot explain the sexual dimorphism in adults, because postmenopausal females with hormone replacement had the same high leptin levels as those without treatment.

In umbilical cord plasma, the concentration of leptin (girls 10.8 ± 9.2 μg/L, n = 65 vs boys 7.6 ± 6.6 μg/L, n = 74) was in the same range as reported by Schubring et al (9.7 ± 9.4 μg/L in arterial cord blood, n = 27). Moreover, we observed a 61% reduction in plasma leptin levels from birth to 4 weeks of age and an increase in plasma leptin concentration from 4 to 14 weeks after birth (Fig 1), which probably is caused by a rapid increase of total body fat. Placental secretion may contribute to the high levels of leptin in umbilical cord plasma, because we observed a reduction in plasma leptin levels from birth to 4 weeks of age. The relative importance of leptin secretion from adipose tissue and placenta is unknown during fetal development. It is possible that leptin is an important factor in the regulation of growth during intrauterine life, as indicated by Hassink et al.

The high level of plasma leptin at birth also could be attributable to high expression of the ob gene in brown adipose tissue, as observed in rats.

Blood sampling of mothers and infants was performed between 8 AM and 3 PM, without overnight fasting. Although there is a marked diurnal variation in plasma concentration of leptin between 8 PM and 4 AM, there is very little change in our sampling period. We cannot exclude the possibility that the time of sampling may influence the individual leptin levels, but the large number of individuals included reduces this possibility.

There was no difference in plasma leptin levels between the group supplemented with corn oil and that supplemented with cod liver oil at any time, but we observed a significant increase in pregnant women on cod liver oil from week 18 to week 35. These observations must be evaluated further in future studies.

We found an insignificant reduction of plasma leptin levels among pregnant smokers compared with nonsmokers. Other studies suggest that smoking causes reduced level of leptin in plasma.

In conclusion, our findings show that plasma lep-
tin concentration increases in pregnant women from week 18 to week 35. Furthermore, we observed that female infants had higher leptin levels than male infants and that the levels fell from birth to 4 weeks of age before it increased moderately during the consecutive 2 months of life. These findings may suggest that leptin is produced by placenta and that this hormone is of metabolic importance during pregnancy and infancy.

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Ingrid B. Helland, Janne E. Reseland, Ola D. Saugstad and Christian A. Drevon
Pediatrics 1998;101;e12

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