Hospital for Children and Adolescents

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LEPTIN IN THE PERINATAL PERIOD

by

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ACADEMIC DISSERTATION

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II Hytinantti T, Koistinen HA, Koivisto VA, Karonen S-L, Rutanen E-M, Andersson S. Increased leptin in preterm infants of pre-eclamptic mothers. Arch Dis Children (Fetal and neonatal edition) 83:13-16, 2000.

III Hytinantti T, Koistinen HA, Teramo KA, Karonen S-L, Koivisto VA, Andersson S. Chronic hypoxia increases fetal leptin concentration in pregnancies of mothers with type I diabetes mellitus, Diabetologia 43:709-713, 2000.

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ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
AGA	appropriate for gestational age
BMI	body mass index
cAMP	cyclic AMP
CRH	corticotropin-releasing hormone
CSF	cerebrospinal fluid
DM	diabetes mellitus
EPO	erythropoietin
FFA	free fatty acid
FSH	follicle-stimulating hormone
GA	gestational age
G-CSF	granulocyte colony-stimulating factor
GDM	gestational diabetes mellitus
GH	growth hormone
HbA1C	glycated hemoglobin A fraction
HPA	hypothalamus-pituitary-adrenal
HPLC	high-pressure liquid chromathography
IGF-I	insulin-like growth factor I
IDDM	insulin-dependent diabetes mellitus
IL	interleukin
IUGR	intrauterine growth restriction
JAK	janus kinases
LGA	large for gestational age
LH	luteinizing hormone

LPS	lipopolysaccharide
mRNA	messenger RNA
NIDDM	non-insulin-dependent diabetes mellitus
OB-gene	leptin gene
OB-R	leptin receptor
PTH	parathyroid hormone
RIA	radioimmunoassay
SD	standard deviation
SGA	small for gestational age
TNF	tumor necrosis factor
TRH	TSH-releasing hormone
TSH	thyroid-stimulating hormone

1 INTRODUCTION

Leptin hormone, discovered in 1994 (Zhang et al), is produced mainly by adipose tissue. This hormone has turned out to be involved in multiple processes in human physiology. At first, interest was focused on the role of leptin in the control of body weight regulation and satiety (Pelleymounter et al 1995, Halaas et al 1995, Campfield et al 1995). Since then, the interactions between glucose, insulin, and leptin in various experimental and clincal conditions, e.g., in diabetes mellitus, have been under intensive study (Malmström et al 1996). It has become evident that leptin takes part in numerous other processes, such as angiogenesis and reproduction, as well (Cioffi et al 1996) (Table 1).

Gender differences have been a constant finding in leptin studies: In adults and children, independently of fat mass, females have higher leptin concentrations than males (Considine et al 1996, Hassink et al 1996), thus reflecting the regulatory effect of other factors, such as hormones, on leptin metabolism.

Animal studies have shown the presence of high levels of gene expression for leptin, and for the leptin receptor during fetal development (Hoggard et al 1997a). In humans, gestational age (Jaquet et al 1998), birth weight (Sivan et al 1997), and type of fetal growth (Koistinen et al 1997) are important determinants of cord plasma leptin concentration.

The objectives for this present study were to observe how adaptation to extrauterine life is reflected in the leptin concentrations of the neonate. We also examined the effect of hypoxia, of maternal pre-eclampsia, and of diabetes mellitus on fetal leptin concentrations.

Table 1. A brief summary of some of leptin's major functions.

Leptin secretion	Target organ	Function(s)	Ref	human (H)	animal (A)
Adipocyte	CNS	Controls appetite and body weight	Halaas 1995	H	A
	Adrenals	Decreases production of glucocorticoids	Pralong 1998	H	A
	Liver	Redistributes glucose production, fetal hematopoiesis	Rosetti 1997, Cioffi 1996	H	A A
	Pancreas beta-cells	Reduces insulin secretion	Seufert 1999	H	
	White adipose tissue	Reduces glucose uptake	Wang 1999		A
	Brown adipose tissue	Increases glucose uptake	Wang 1999		A
	Muscle	Increases glucose uptake	Wang 1999		A
	Bone marrow	Activates hematopoietic cells, T-lymphocyte-mediated immunity	Mikhail 1997, Lord 1998	H	A A
	Vascular enothelium	Stimulates angiogenesis	Sierra-Honigman 1998	H	A
	?	Initiation of puberty	Chehab 1997, Farooqi 1999	H	A
Gastric mucosa		?	Bado 1998		A
Trophoblast	Placenta/embryo	Participates in the development of placenta/embryo?	Antczak 1997, Cioffi 1996	H	A A

2 REVIEW OF THE LITERATURE

2.1.1 Leptin

Leptin (Greek leptos= thin) is a 16-kD plasma protein synthetized by adipose tissue, and during pregnancy by the placental tissue (Zhang et al 1994, Masuzaki et al 1998), and at lower levels by gastric epithelium (Bado et al 1998). The brain has also been suggested to produce leptin (Wiesner et al 1999).

Leptin is a product of the obese (ob) gene. Mice inheriting mutant copies of this gene from both parents (ob/ob mice) exhibit marked obesity, low energy expenditure, hyperphagia, glucose intolerance, insulin resistance, and sterility. Both peripheral and central exogenous leptin administration to these mice lowers their body weight, body fat percentage, food intake, and serum concentrations of glucose and insulin; it restores sterility, and increases body temperature, metabolic rate, and activity levels (Zhang et al 1994, Chehab et al 1996, Pelleymounter et al 1995, Campfield et al 1995, Halaas et al 1995). Similar metabolic and behavioral disturbances are present in mice inheriting two mutant copies of the diabetes (db) gene (db/db mice). In this case, however, administration of exogenous leptin does not correct those disturbances (Halaas et al 1995). This is due to a mutation in the db gene which codes for the leptin receptor (OB-R) necessary for signal transduction (Chen et al 1996).

The ob gene encodes a 4.5-kilobase adipose tissue mRNA with a highly conserved 167-amino acid open reading frame. The amino-acid sequence is 84% identical between human and mouse (Zhang et al 1994). In humans, the ob gene has been localized to chromosome 7q31.3 (Green et al 1995), and to 7q32.1 (Geffroy et al 1995).

The circulating leptin concentrations reflect body fat content in mice and in adult human beings (Frederich et al 1995, Maffei et al 1995). Leptin is secreted in pulsatile and circadian fashion with a noctural rise in lean and obese patients, and in patients with non-insulin-dependent diabetes mellitus (Licinio et al 1997, Sinha et al 1996). The half-life of the hormone is approximately 25 minutes, and the rate of leptin clearance from plasma is a mean 1.50 $\pm$ 0.23 ml/kg/min. Rate of leptin production seems to be directly related to adiposity. A combination of greater leptin production per unit of body fat, and increased production from expanded total body fat mass, rather than alterations in leptin clearance, accounts for the increase in plasma leptin concentrations observed in obese humans

(Klein et al 1996). Kidneys play a substantial role in leptin removal from plasma by taking up and degrading the peptide. Such renal leptin uptake may account for approximately 80% of all leptin removal from plasma (Meyer et al 1997). No leptin clearance has been observed in pulmonary or splanchnic beds (Jensen et al 1999).

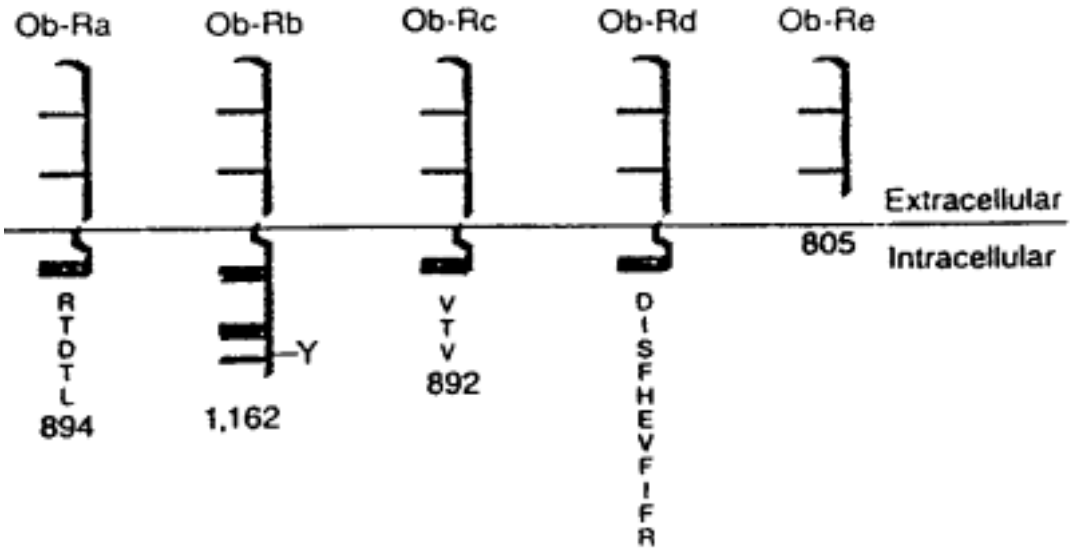
In humans, only a few leptin mutations have been found thus far. However, leptin deficiency has been identified due to a missense leptin gene mutation (Strobel et al 1998). Patients carrying this mutation present with a clinical picture of morbid obesity, hypogonadism, sympathetic dysfunction, alterations in growth hormone (GH), and in parathyroid hormone (PTH)-calcium function, and hyperinsulinemia. They also seem to have a highly increased risk for childhood mortality due to infections. Despite their obesity, however, these patients do not have risk factors for cardiovascular disease such as hypertension, impairment of lipid metabolism, or hyperglycemia (Montaque et al 1997, Ozata et al 1999).

2.1.2 Leptin receptors (OB-R)

Leptin is recognized by the leptin receptor, the product of the diabetes (db) gene (Tartaglia et al 1995). The leptin receptor was identified through expression cloning strategy. The OB-R is a single membrane-spanning receptor related to cytokine receptors such as interleukin (IL)-6, granulocyte colony-stimulating factor (G-CSF), and leukocyte inhibitory factor. The OB-R also has the signaling capabilities of IL-6 type cytokine receptors (Baumann et al 1996). The OB-R has been shown to have at least five splice variants OB-R(a-e) in the mouse, figure 1, (Chen et al 1996, Lee et al 1996) and four forms in humans (Cioffi et al 1996). The extracellular forms of these are identical throughout their entire length. The OB-Rb variant encodes a receptor with a long intracellular domain that is thought to be essential for intracellular signal transduction (Tartaglia et al 1995). Human and mice OB-R DNA are 78% identical (Tartaglia et al 1995). The gene coding for OB-R is located on chromosome 4 in the mouse, 5 in the rat, and 1p in human beings (Chung et al 1996). In humans, an OB-R mutation has been identified leading to extreme obesity in childhood (Roth et al 1998). OB-Rs signal through tyrosine phophorylation of a class of transcription factors. Alternative and/or additional signaling pathways of OB-Rb (long form) involve cytoplasmic protein kinases, (Janus kinases, JAKs), and serine residues (Considine et al 1996). The obese phenotype of the db/db mouse is generated by recessive mutations in mice diabetes (db) genes. This db/db mutation leads to loss of the

carboxy-terminal region, and to a protein with a truncated cytoplastic domain in the long form of the OB-R, which renders the OB-R inactive (Chen et al 1996).

Figure 1. OB-R variants in the mouse (permission for reproduction of this figure is granted by the authors and *Nature*, Friedman et al 1998)



In mice, the expression of the OB-R mRNA is the highest in lung and kidney and lower in liver, skeletal muscle, brain, and heart (Tartaglia et al 1995). In addition, in placenta, cartilage, and hair follicles high levels of OB-R expression have been observed (Hoggard et al 1997a). In the murine, the long form is expressed predominantly in the hypothalamus. Of the peripherial tissues, the long form is expressed at significant levels only in the medulla of the adrenal, and the inner zone of the medulla of the kidney (Hoggard et al 1997b). OB-Rb has also been identified in murine and human fetal liver, and in several hematopoietic cell lines, and in adult human reproductive organs (Cioffi et al 1996). In humans, the coexpression of long and short isoforms of OB-R has been detected in brain, bone marrow, fetal liver, and spleen (Gainsford et al 1996). In human adipose tissue and gastric mucosa, OB-R isoforms have been detected also (Kielar et al 1998, Breidert et al 1999). Recently, the long form of OB-R has been detected in mice and human lung tissue and in a lung squamous cell line. Experimental data suggest that in these tissues leptin may act as a growth factor through its specific receptor (Tsuchiya et al 1999). So far, of the tissues tested, only hypothalamus expresses more long form

transcipt than the most predominant short form (OB-Rs1). The vast majority of OB-R transcipts detected in various tissues are transcripts encoding short intracellular domain forms (Tartaglia et al 1997).

Short OB-R forms may play a role in leptin transport and in clearance, but also be a source of soluble receptor (Tartaglia et al 1997). In fact, OB-Re, which is truncated at the extracellular domain, has been demonstrated to generate leptin-binding activity in the circulation (Liu et al 1998).

2.1.3 Leptin-binding proteins

Leptin has been found to bind competitively to at least three serum macromolecules with molecular masses of ~85, ~176, and ~240 kDa in rodents and ~176 and ~240 kDa in humans (Houseknecht et al 1996). Also 80’ or 100-kDa size binding proteins have been found (Sinha et al 1996). A possible role for the binding proteins is to facilitate the transport of leptin across the blood-brain barrier to its hypothalamic (or other) site(s) of action (Houseknecht et al 1996). Leptin-binding proteins may possibly be soluble forms of leptin receptors. This feature is typical of a number of members of the cytokine family (Muller-Newen et al 1996).

In nonobese humans and mice, a significant portion of endogenous leptin is bound. With increasing severity of obesity and increasing circulating leptin levels, leptin "spills over" into the circulating free, presumeably bioactive protein, pool. The percentage of free leptin is also stongly related to the degree of obesity (Houseknecht et al 1996, Sinha et al 1996).

That changes in free leptin are rapid and significant during fasting and refeeding suggests that bound and free leptin behave as different compartments in such physiological alterations (Sinha et al 1996). During pregnancy, no significant difference in free or bound leptin levels exists between normal and insulin-dependent diabetic subjects, but the latter have significantly higher soluble leptin receptor levels (Lewandowski et al 1999).

2.1.4 Leptin and cytokines

Leptin, which is thought to be ancestrally related to the cytokines (Bornstein et al 1998), is suggested to participate in the modulation of the immune and cytokine response to inflammation. Indeed, the full-length leptin receptor has the signaling capabilities of IL-6-

type cytokine receptors (Baumann et al 1996). In mice, the administration of tumor necrosis factor (TNF), IL-1, and leukocyte inhibitory factor, but not IL-10, IL-4, or IL-2, produces a prompt and dose-dependent increase in serum leptin levels and leptin mRNA expression in fat. However, after administration of *Escherichia coli* lipopolysaccharide (LPS), leptin levels rose (Sarraf et al 1997). IL-1 is essential for leptin induction by LPS, but IL-6 is not (Faggioni et al 1998). Sensitivity to LPS-induced mortality is significantly greater in ob/ob mice; however, this sensitivity was reversed after treatment with leptin (Faggioni et al 1999). Ob/ob and db/db mice, as well as normal mice treated with leptin receptor antagonist, exhibit increased sensitivity to the lethal effect of TNF. Exogenous leptin affords protection to TNF in ob/ob, but not in other mice (Takahashi et al 1999). In vitro, both in mice and men, the TNF produced by adipocytes can inhibit leptin production through the TNF-type I receptor, suggesting the presence of autocrine or paracrine regulation of leptin in mouse and human adipose tissue (Yamaguchi et al 1998). Interestingly, in healthy humans in vivo, subcutaneous abdominal adipose tissue has been shown to release IL-6, but not TNF (Mohamed-Ali et al 1997).

In diabetic as well as in healthy humans, TNF levels are independently associated with circulating leptin levels (Mantzoros et al 1997). Repeated TNF infusions have resulted in an increase in serum leptin levels, levels which return to baseline within 24 h after cessation of TNF infusions, suggesting that leptin levels are under the control of TNF (Zumbach et al 1997). Equally, IL-1 infusions have been shown to increase serum leptin concentrations in a dose-dependent fashion. However, after prolonged treatment, tachyphylaxis of the leptin response appeared (Janik et al 1997).

In survivors of sepsis, there exists a negative relation beween IL-6 and leptin. This is of potential importance, as high IL-6 levels have been associated with poor outcome in critically ill patients, and relatively low leptin levels may impair sympathetic system and immune functions (Torpy et al 1998).

Both ob/ob and db/db mice have impaired T-lymphocyte immunity. Leptin has been found to increase T-helper lymphocyte 1 and suppress T-helper lymphocyte 2 cytokine production. Administration of leptin also reversed the immunosuppressive effects of acute starvation. These findings suggest a role for leptin in linking nutritional status to cognate cellular function, and provide a molecular mechanism to account for the immune dysfunction observed in starvation (Lord et al 1998).

2.2.1 Leptin and the central nervous system: leptin resistance, control of appetite, and body weight

In humans, cerebrospinal fluid (CSF) and plasma leptin concentrations correlate in a nonlinear manner, with their ratio being lower in persons with higher plasma leptin levels or body mass index (BMI) (weight kg/ length m^2) (Caro et al 1996, Schawartz et al 1996). Leptin enters the brain by a saturable system (Caro et al 1996, Schwartz et al 1996, Banks et al 1996) independent of insulin (Banks et al 1996). The OB-R-mediated transport of leptin into the central nervous system through the blood-brain barrier takes place in the choroid plexus (Tartaglia et al 1995) and in brain microvessels (Golden et al 1997, Bjorbaek et al 1998). The most abundant OB-R isoform in both locations is the short isoform, OB-Ra (Boado et al 1998, Bjorbaek et al 1998). It has been postulated that leptin can also enter the central nervous system independently of leptin receptors (Wu-Peng et al 1997). The saturable mechanism mediating CSF leptin transport, and reduced efficiency of brain leptin delivery in hyperleptinemic obese patients, may provide a mechanism for the leptin resistance observed in such individuals (Schwartz et al 1996). However, the basis for leptin resistance in humans is unknown, and data from animal studies indicate that this condition is likely to be heterogenous (Friedman et al 1998).

Leptin receptors have been found in several hypothalamic nuclei (Mercer et al 1996, Fei et al 1997). Each of these nuclei is important in regulating body weight by expressing one or more neuropeptides and neurotransmitters that regulate food intake and/or body weight (Spiegelman et al 1996). Of the hypothalamic nuclei, the lateral modulates activity of the parasympathetic and ventromedial sympathetic nervous systems (Friedman et al 1998). Neuropeptide Y is the most potent orexigenic (appetite-increasing) agent when administered intrathecally. In other respects, also, its functions are opposite to those of leptin: decreased energy expenditure and increased lipogenesis (Woods et al 1998) Neuropeptide Y mRNA is increased in ob/ob mice and decreases after leptin treatment (Stephens et al 1995).

Leptin may stimulate the action of the anorexogenic (appetite-decreasing) agents and antagonize the orexigenic effects of others. Thus, during starvation, leptin levels fall, and activate a behavioral and metabolic response that is adaptive when food is unavailable. Weight gain increases plasma leptin concentration and elicts a different response, leading to a state of negative energy balance. It is not known whether the same or different neurons respond to increasing and decreasing leptin levels (Friedman at al 1998).

Moreover, different thresholds exist for several of leptin's actions (Ioffe et al 1998). Recent animal studies suggest, however, that the dose-dependent effects of leptin on hypothalamic target neurons at the level of mRNA expression are variable, with some neurons, like neuropeptide Y, responding across a broad dose-range, and others showing a limited response within the low range. This further suggests that the central targets of leptin that mediate the transition from starvation to the fed state may be distinct from those that mediate the response to overfeeding and obesity (Ahima et al 1999).

2.3.1 Leptin and interactions between glucose and insulin metabolism

In humans, plasma insulin does not acutely regulate leptin production in healthy individuals or in patients with insulin-dependent diabetes mellitus (IDDM) or non-insulin-dependent diabetes mellitus (NIDDM) (Malmström et al 1996, Tuominen et al 1997, Vidal et al 1996). However, after four hours of supraphysiological hyperinsulinemia, leptin concentrations increase (Utriainen et al 1996). During prolonged exposure to insulin in vitro (abdominal adipocytes), and in vivo, increased leptin production can also be demonstrated in both healthy individuals and in NIDDM patients (Kolaczynski et al 1996a, Malmström et al 1996), whereas hyperglycemia or high plasma free fatty acid (FFA) levels have no effect on leptin release (Boden et al 1997). The circadian rhythm of leptin correlates inversely with the 24-h cycle of insulin sensitivity (Boden et al 1997). Chronic hyperinsulinemia in insulin-resistant men has been found to be associated with higher plasma leptin levels independent of body fat mass (Segal et al 1996), whereas this association has not been found in postmenopausal women (Larsson et al 1996). Insulin resistance is defined as an impaired ability of insulin to stimulate the uptake and disposal of glucose by muscle (Reaven et al 1996).

In most studies leptin has been shown to suppress insulin secretion from human pancreatic beta-cells (Seuffert et al 1999). Conversely, stimulatory effects of insulin have been demonstrated on leptin production taking place in adipocytes (Seufert et al 1999). During fasting, leptin concentrations decrease. However, if insulin and glucose levels are maintained at basal levels, no change occurs in leptin levels during fasting, and hyperketonemia does not affect leptin concentrations (Boden et al 1996, Kolaczynski et al 1996b). On the other hand, short-term overfeeding is associated with moderate elevation in circulating leptin levels, and long-term overfeeding, resulting in weight gain, causes a

rise in leptin concentrations paralleling the increase in percentage of body fat (Kolaczynski et al 1996c).

In rat adipocytes, leptin mRNA levels rise after food intake, and after insulin injection (Saladin et al 1995). However, the increased leptin secretion over 96 hours caused by insulin is more closely related to the amount of glucose taken up by the adipocytes than to insulin concentration per se, suggesting a role for glucose transport or metabolism, or both, in regulating leptin secretion (Mueller et al 1998). The insulin-stimulated release of leptin from adipocytes is blocked by norepinephrine or by a selective beta 3-adrenergic receptor agonist, suggesting that the beta 3-adrenergic receptor plays a central role in regulating the release of leptin from the adipocyte (Gettys et al 1996).

Leptin also affects the intrahepatic glucose fluxes: in mice, the administration of leptin after feeding results in marked suppression of glycogenolysis, whereas the percentage contribution of gluconeogenesis to hepatic glucose production increases (Rosetti et al 1997). That similar effects, with much lower doses of leptin, can be achieved with intracerebroventricular leptin admistration, suggests that regulation of hepatic glucose fluxes may be mediated via its central receptors (Liu et al 1998).

In addition, leptin has differential, tissue-specific effects on glucose and oxygen utilization. Leptin increases glucose uptake and utilization in brown adipose tissue and muscle, but decreases these in white adipose tissue. These differences are at least partly due to the enhancing effect of leptin on the expression of glucose transporter protein-4 and mRNA in brown adipose tissue, whereas leptin decreases their expression in white adipose tissue.

Leptin also increases the oxygen consumption in brown adipose tissue (Wang et al 1999). This increase may also result from the stimulatory effect of leptin on the levels of uncoupling proteins or by preventing their fall in brown adipose tissue from occurring at times of reduced energy intake. These effects are suggested to be mediated via hypothalamic leptin receptors, because the effects of i.v. and intracerebroventricularly administered leptin are qualitatively similar (Rouru et al 1999). These mechanisms may be important in leptin’s participation in the regulation of body weight and energy expenditure.

2.3.2 Leptin and interactions between other hormones

The ob/ob mutant mice, in addition to being obese, are hyperinsulinemic and hypercorticosteronic (Garthwaite et al 1980). A similar phenotype is observed in db/db mice or fa/fa rats suffering from mutations in the leptin receptor (Chen et al 1996, Chua et al 1996). Chronic leptin replacement in ob/ob mice, but not in db/db mice, corrects this hypercorticosteronemia (Stephens et al 1995). In animal studies, mainly mouse and rat, leptin, glucocorticoids, and the hypothalamus-pituitary-adrenal (HPA) axis have recently been found to form a bi-directional circuit between HPA axis function and adipose tissue metabolism (Heiman et al 1997, Spinedi et al 1997).

In ob/ob rat adipocytes, administration of glucocorticoids increases both leptin mRNA levels and secreted leptin levels in vitro, and, in these animals, beta-adrenergic agonists which increase intracellular cyclic AMP (cAMP) directly decrease leptin mRNA expression (Slieker et al 1996). Either the lack of circulating glucocorticoid or the increased plasma adrenocorticotrophic hormone (ACTH) concentrations, or both, are responsible for decreasing leptin output, whereas decreased plasma ACTH concentrations allow an increase in leptin secretion into the circulation (Spinedi at al 1998). In hypothalamic cells in the rat, leptin inhibits secretion of corticotropin-releasing hormone (CRH) in a dose-dependent manner. However, leptin does not alter secretion of ACTH from rat pituitary cells. Leptin's ability to inhibit CRH release is the likely explanation for its ability to inhibit activation of the HPA axis in response to stress (Heiman et al 1997). Interestingly, in bovine adrenocortical cells, leptin has also been demonstrated to inhibit adrenal steroidogenesis. This regulation has been suggested to take place at the transcriptional level (Bornstein et al 1997). In rat as well in human adrenocortical cells, leptin inhibits stimulated corticosterone secretion in a dose-dependent manner. These effects of leptin in adrenal cells are likely mediated by the long isoform of the leptin receptor (OB-R), because it is expressed in the adrenal tissue, and leptin has no inhibitory effect in adrenal glands obtained from db/db mice (Pralong et al 1998).

In humans, treatment with either dexamethasone or corticosteroids increases plasma leptin concentrations (Kolaczynski et al 1997, Wolthers et al 1998). In patients with Cushing's syndrome, leptin levels are elevated in comparison with those in healthy controls even after adjustment for body fat; after treatment they decline concomitantly with corticosterone concentrations (Masuzaki at al 1997). In contrast to these findings, Licinio found no change in pre- and post-operative leptin values in patients with Cushing's

disease, despite a 15-fold decline in ACTH levels. They also reported that rapid fluctuations in plasma leptin levels are inversely related to pituitary-adrenal function (Licinio et al 1997). In human adult survivors of acute sepsis, both leptin and cortisol levels are significantly higher than in controls (Bornstein et al 1998). Thus, there exists some discrepancy as to the effects of glucocorticoids on leptin concentrations in humans (Torpy et al 1998).

Thyroid hormone status influences leptin plasma concentration. During hypothyroidism, decreased leptin levels appear, whereas during hyperthyroidism, leptin levels remain unchanged (Valcavi et al 1997). During protracted critical illness, treatment with TSH-releasing hormone (TRH) - which usually elicits a rise in thyroid hormones does not affect circulating leptin levels (Van den Berghe et al 1998).

Although in bovine adipose tissue, GH administration alone does not affect leptin gene expression, GH, in combination with high concentrations of dexamethasone or insulin, or both, attenuates the ability of insulin or dexamethasone to stimulate leptin expression in vitro. In vivo, in castrated male cattle, however, GH treatment increases adipose tissue leptin and insulin-like growth factor I (IGF-I) mRNA concentrations (Houseknecht et al 2000).

In healthy humans, GH administration has no effect on serum leptin concentrations (Wolthers et al 1998). In GH deficiency, serum leptin is elevated, and it is lowered by GH substitution (Nyström et al 1997).

The sexual diphormism in leptin concentrations observed in humans and rodents is likely due to differences in concentrations of testosterone and other sex hormones. Testosterone has been shown to downregulate leptin production at both the protein and mRNA levels in human adipocytes (Wabitsch et al 1997). Male patients with idiopathic hypogonadotropic hypogonadism and Klinefelter's syndrome have significantly higher leptin levels than do normal men. During testosterone or gonadotopin treatment for three months leptin levels fail to change significantly (Ozata et al 1998). In adult males, leptin correlates negatively with testosterone and positively with estradiol, and also positively in adult females with fasting plasma estradiol, independently of age, amount of body fat, and waist/hip ratio (Paolisso et al 1998). Administration of testosterone to healthy young men suppresses serum leptin levels significantly (Luukkaa et al 1998). However, in some studies, such correlations of testosterone with leptin levels have become non-significant

after adjustment for BMI (Haffner et al 1997). In male-to-female transsexuals, introduction of anti-androgen and estradiol treatment leads to significant increase in serum leptin concentrations. Conversely, testosterone treatment of females reduces leptin levels. These results indicate that in the regulation of serum leptin levels sex steroid hormones play an important role (Elbers et al 1997).

2.3.3 Leptin during childhood and puberty

Levels of circulating soluble leptin receptor are low at birth, high in prepubertal children; they fall throughout puberty, and remain stable during adult life. The highest levels of soluble leptin receptors occur during the years when the pituitary gonadal axis is quiescent. Thus, these changes in concentrations of soluble leptin receptor could explain how leptin regulates puberty (Quinton et al 1999).

Prepubertal girls have higher plasma leptin concentrations than boys independent of adiposity, according to some (Hassink et al 1996, Garcia-Mayor et al 1997), but not all studies (Arslanian et al 1998). BMI correlates strongly with serum leptin concentrations (Hassink et al 1996, Nagy et al 1997). However, in prepubertal children this gender difference in leptin concentrations seems to disappear when fat distribution is taken into account (intra-abdominal vs. subcutaneous abdominal) (Nagy et al 1997). In children as in adults, subcutanous fat correlates much more strongly than does visceral fat with serum leptin concentrations (Caprio et al 1996). No gender difference exists in CSF leptin levels, but the CSF/plasma leptin ratio is lower in girls than in boys. CSF/plasma ratios for lean children are higher than those for obese children (Wiedenhoft et al 1999). No effect of ethnicity on leptin concentrations has been evident (Hassink et al 1996, Nagy et al 1997).

In healthy mice, leptin treatment results in earlier maturation of the reproductive tract, leading to earlier reproduction (Chehab et al 1997). In the ob/ob mouse, puberty does not progress until exogenous leptin is administered (Chehab et al 1996). An analogous pattern seems to exist in the few known leptin-deficent human patients: a 22-year-old adult male showed clinical features of hypogonadism and had not yet entered puberty, and a 34-year-old woman showed primary amenorrhoea (Strobel et al 1998).

A 9-year-old prepubertal girl with congenital leptin deficiency was treated with daily subcutaneous recombinant methionyl leptin injections. Her bone age before treatment was 12.5 years, height 140 cm, and weight 94.4 kilograms. After one week of treatment her

marked hyperphagia normalized. The patient lost weight within two weeks after the initiation of leptin treatment; 95% of her weight loss was accounted for body fat. At the beginning of the treatment she was normoglycemic but had high plasma insulin while fasting. Cholesterol and triglyceride concentrations as well as her thyroid, adrenal, and somatotrophic function, as indicated by insulin-like growth factor I, and basal metabolic rate when expressed per unit of lean mass remained normal throughout the one-year treatment. At baseline the patient's serum concentrations of estradiol, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were consistent with her prepubertal status. However, after 12 months of leptin treatment, her noctural pattern of gonadotropin secretion was pulsatile, which is consistent with early puberty. Whether adequate serum leptin concentrations are required for normal pubertal development or, alternatively, whether leptin plays an active role in the initiation of puberty is unknown (Farooqi et al 1999).

In normal girls from 5 to 15 years of age, and in boys until the age of 10 years, leptin concentrations increase in parallel with body weight. In girls, onset of puberty is marked by an increase in leptin that is first followed by an increase in FSH, and later by increases in LH and estradiol. A similar pattern occurs in boys, despite the fact that leptin concentration drops after 10 years of age when testosterone rises (Garcia-Mayor et al 1997, Mantzoros et al 1997). The analysis of changes in leptin concentrations according to pubertal stages in girls showed steadily increasing leptin concentrations from Tanner stage 1 (=prepubertal) to 5 (=adult), whereas in boys, leptin levels were highest at Tanner stage 2, and declined thereafter (Blum et al 1997, Wabitsch et al 1997, Carlsson et al 1997). In boys, testosterone accounted for 10% and BMI for 52% of the variation in leptin (Blum et al 1997). Moreover, at Tanner stages G3-G5, leptin is related negatively to testicular volume (Clayton et al 1997).

2.4 Leptin concentrations during human pregnancy and the perinatal period

2.4.1 Leptin plasma concentrations during human pregnancy: in normal, diabetic, and pre-eclamptic women

Maternal serum leptin concentrations increase progressively up to 2-to-3-fold during the first two trimesters, followed by a slight decline thereafter. Until the second trimester, maternal BMI and weight correlate with serum leptin levels (Hardie et al 1997, Tam et al 1998, Tamura et al 1998). Maternal serum leptin levels do not correlate with infants's birth weight (Schubring et al 1997, Tam et al 1998, Tamura et al 1998). A correlation between maternal and cord plasma leptin levels is shown in some (Tamura et al 1998, Varvarigou et al 1999), but not all studies (Schubring et al 1997, Hassink et al 1997, Lepercq et al 1997, Helland et al 1998, McCarthy et al 1999). The lack of correlation between maternal and cord leptin concentrations in normal pregnancy, as observed in most studies, is consistent with a noncommunicating, 2-compartment model of fetoplacental leptin regulation (McCarthy et al 1999). Gender of the fetus may modify the increase in maternal leptin levels from 18 to 35 weeks of pregnancy: increase in leptin levels was not significant in women carrying male fetuses (Helland et al 1998).

No significant difference exists between type I diabetes mellitus (DM) pregnant mothers and healthy mothers in total, and in free and bound leptin levels (Lepercq et al 1997, Stock et al 1998, Lewandowski et al 1999). However, type I DM mothers have significantly higher soluble leptin receptor levels. This may implicate the development of the leptin resistance seen in type I DM mothers during pregnancy (Lewandowski et al 1999).

During pre-eclampsia, increased leptin concentrations occur in maternal plasma (Mise et al 1998, McCarthy et al 1999, Kokot et al 1999, Sattar et al 1998, Laivuori et al 2000). Maternal and cord plasma leptin concentrations correlate during pre-eclampsia; increased delivery of leptin from the placenta to the mother may result in increased maternal free fatty acids and glucose for the fetus and thus partially compensate for the nutritional deprivation caused by this reduction in placental perfusion (McCarthy et al 1999). During normotensive and pre-eclamptic pregnancy, circulating leptin levels correlate with fasting insulin levels. However, during the puerperal period, circulating leptin concentrations and insulin sensitivity correlate only in women with prior pre-eclampsia. Therefore, hyperleptinemia observed in pre-eclamptic women may be part of the insulin-resistance

syndrome. During pregnancy, placental leptin production may thus interfere with the correlation between leptin and insulin (Laivuori et al 2000). It has also been postulated that during pre-eclampsia, hypoxic conditions in the placenta augment trophoblast cells' production of increased amounts of leptin (Mise et al 1998).

2.4.2 The placenta as a source of leptin

The placenta has been shown to produce leptin (Masuzaki et al 1997). Accordingly, leptin mRNA has been found in placental tissue (Hassink et al 1997, Lepercq et al 1998, Mise et al 1998). In the placentas of diabetic mothers requiring insulin therapy are higher levels of leptin mRNA than for nondiabetic women (Lepercq et al 1998). In the mouse and human placenta, OB-R mRNA has been detected in trophoblasts (Henson et al 1998, Yamaguchi et al 1998); during pregnancy its concentrations increase, and cAMP can reduce its expression (Yamaguchi et al 1998). In human pregnancies, both in early gestation, at 7 to 14 weeks, and at term, placental leptin and OB-R transcipts have been identified in trophoblasts. No changes are apparent for OB-R mRNA concentrations, but leptin mRNA concentrations are significantly lower at term than in early pregnancy (Henson et al 1998).

A higher leptin concentration in umbilical vein than in umbilical artery plasma suggests that the placenta may be a site for leptin production (Yura et al 1998). This phenomenon has not, however, been found in all studies (Marchini et al 1998, Ertl et al 1999), and the opposite finding has been presented by Schubring et al 1997. However, a repeated finding in most studies is a significant correlation between placental weight and cord plasma leptin concentration (Koistinen et al 1997, Gomez et al 1999, Varvarigou et al 1999), with few exceptions (Yura et al 1998). A rapid postpartal decline in leptin plasma concentrations of the mother and the newborn infant further supports the theory of the placenta as a source of leptin (Mise et al 1998, McCarthy et al 1998, Helland et al 1999, Yura et al 1998, Matsuda et al 1999, Harigaya et al 1999, Ertl et al 1999).

2.4.3 Effect of maternal diabetes mellitus, pre-eclampsia, type of birth, and maternal smoking on leptin concentrations in cord plasma

Newborn infants of diabetic mothers have higher cord plasma leptin concentrations than do infants of nondiabetic mothers. In addition, cord plasma leptin and the glycated

hemoglobin A fraction (HbA1C) of the infant have been shown to correlate significantly (Shekhawat et al 1998). In a study by Persson et al (1999) infants both of mothers with type I DM and with gestational diabetes mellitus (GDM) had 3- to 4-fold higher cord leptin concentrations than did controls, although the HbA1C level fell within the normal range in type I DM and healthy mothers. Maffei et al (1998) found that infants of type I DM mothers had higher cord plasma leptin, C-peptide, and insulin concentrations than did infants of healthy or GDM mothers; the mothers with type I DM had higher HbA1C than did control or GDM mothers. Gross et al (1998) found, as well, that infants of diabetic mothers had higher cord plasma leptin concentrations than those of nondiabetic mothers. This group of diabetic mothers comprised both type I DM, GDM, and insulin-treated GDM mothers. However, these groups were not compared with one another, due to the small number of patients in each group. Lepercq et al (1998) found in diabetic (both type I DM and GDM) mothers higher insulin levels than in control mothers, and the infants of these diabetic mothers also showed higher cord plasma concentrations of insulin and leptin, but not of glucose.

During pre-eclampsia, increased leptin concentrations in cord blood are observed in some (Kokot et al 1999), but not in other studies (McCarthy et al 1999). Thus far, this phenomenon has received scant attention.

Vaginal delivery or delivery by Cesarean section has been not found to affect cord leptin concentrations in term infants (Marchini et al 1998, Tarquini et al 1999). Maternal smoking has been attributed to elevated cord plasma leptin concentrations in some (Mantzoros et al 1997), but not in all studies (Helland et al 1998).

2.4.4 Leptin and fetal development

The leptin hormone-deficient ob/ob mice are sterile; their fertility, however, is restored by administration of leptin (Chebab et al 1996). In such mice, despite withdrawal of leptin replacement therapy at a very early stage of gestation, 0.5 days postcoitally, pregnancy continues to term, and pups are delivered normally without signs of deformities (Mounzih et al 1998).

Leptin can be detected in human and mice oocytes, and in embryos at the preimplantation stage. At the morula stage, leptin-containing cells are distributed in the outer portion which later forms the trophoblast (Antczak et al 1997). Leptin hormone is present in the yolk-sac

fluid, and stimulates the proliferation of yolk-sac and fetal liver cells in a dose-dependent fashion (Cioffi et al 1996, Mikhail et al 1997). Leptin directly stimulates hematopoietic precursors: The hormone alone can increase the number of macrophage and granulocyte colonies, and leptin and erythropoietin act synergistically to increase erythroid development. These results suggest that leptin may have an important unanticipated role in the development of the hematopoietic and immune systems (Mikhail et al 1997). In addition, in the murine fetal liver, OB-R expression is significant at the time that the liver is the major hematopoietic organ. Additionally, in both mice and human beings, OB-R is expressed in the fetal liver, and at significant levels in many myeloid, and at least in some lymphoid, cell lines (Cioffi et al 1996).

OB-R is expressed in human vasculature and in primary cultures of human endothelial cells. In vivo and in vitro assays have revealed that leptin has angiogenic activity. Leptin may thus spur blood-vessel growth in the maturing egg and early embryo (Sierra-Honigman et al 1998, Barinaga et al 1998).

During murine fetal development, high levels of the leptin gene, leptin-receptor, and receptor variants have been observed in the placenta, fetal cartilage/bone, and hair follicles. Receptor expression has also been detected in the lung, leptomeninges and choroid plexus of the fetal brain. Leptin, leptin-receptor, and receptor variant mRNAs are expressed more densely in the placenta than in fetal tissues. The expression of leptin-receptor mRNA and leptin protein in the same tissue, but in different cell populations, indicates that in the fetus, leptin may function in an autocrine or paracrine manner (Hoggard et al 1997a).

2.4.5 Development of fetal adipose tissue

Adipose tissue clearly differentiates around a rich bed of capillaries, and for adipose tissue to develop, there must be a sluggish blood supply. Capillary endothelium itself may give rise to adipocytes. However, it is not yet certain from which cells adipocytes develop, although they temporarily exhibit structural similarities to fibroblasts and endothelial cells. In the embryo, the precursor cells of brown and white adipocytes appear to acquire their distinctive cytogenic properties at a very early stage. In vivo and in vitro observations suggest that the brown adipose precursor cell is morphogenetically distinct from the white

adipose precursor cell on the one hand, and from the fibroblasts and endothelial cells on the other (Ryan 1992, Nnodim 1987).

In early fetal development, the rate of fetal fat synthesis is very low and is probably limited to synthesis of structural lipids of cellular membranes and other structures (Widdowson et al 1951). Given that lipid metabolism is perfectly developed in early pregnancy, fetal lipid composition remains unchanged from 6 to 32 weeks of gestation (Carrera 1998).

During the second half of gestation, fetal fat synthesis and storage rates increase exponentially (Carrera 1998). In relation to fat distribution, an increase in body fat of 10 to 80 g occurs between day 200 and birth, whereas subcutaneous fat shows an exponential increase, from 20 to 350 g during the same period. Subcutaneous fat-wasting characterizes fetal growth retardation, and, starting from day 200 and coinciding with an increase in fat synthesis, a marked increase in energy of fat origin appears (100 kcal/day) (Carrera 1998).

Brown adipose tissue in fetuses at 25 to 27 weeks of gestation is fairly well differentiated and thermogenetically active (Zancanaro et al 1995). In most small for gestational age (SGA; birth weight < -2 SD) infants the development of brown fat corresponds to that of AGA infants of the same postconceptional age (Moragas et al 1983).

2.4.6 Leptin and fetal growth

Leptin is present in human cord blood as early as at 18 weeks of gestation (Jaquet et al 1998), and leptin concentrations increase progressively throughout gestation from 1.30 $\pm$ 0.53 ng/ml at 30 weeks of gestation to 7.98 $\pm$ 4.96 ng/ml at term (Gomez et al 1999). After 34 to 35 weeks of gestation, a rapid increase in cord plasma leptin concentration is evident (Jaquet et al 1998, Harigaya et al 1997, Matsuda et al 1999), and gestational age (GA) has been shown to correlate with cord plasma leptin concentrations from that point onward (Matsuda et al 1997, Jaquet et al 1998). Leptin concentrations in preterm and term infants increase concomitantly with birth weight (Shekhawat et al 1998, Jaquet et al 1998, Sivan et al 1997, Schubring et al 1997, Hassink et al 1997, Koistinen et al 1997, Harigaya et al 1997, Ertl et al 1999, Gomez et al 1999). Genetic inheritance may also influence cord plasma leptin concentrations, because it has been demonstrated that cord plasma leptin concentration is elevated in the presence of a family history of obesity on the paternal side, but not on the maternal side; also in the same study, higher leptin

concentrations were observed in those infants born in spring and summer than in fall, and higher in infants born before noon (Tarquini et al 1999).

In term infants, type of fetal growth has been shown to influence cord plasma leptin concentrations: large for gestational age (LGA; birth weight > 2 SD) infants have higher leptin concentrations than appropriate for gestational age (AGA) or SGA infants (Sivan et al 1997, Koistinen et al 1997, Marchini et al 1998, Jaquet et al 1998, Harigaya et al 1997, Cinaz et al 1999, Varvarigou et al 1999). Moreover, fetal growth expressed as relative birth weight (weight SD) has been shown to correlate with cord plasma leptin concentration (Matsuda et al 1997).

The amount of adipose tissue of an infant has been estimated in different studies by different indirect indices such as weight/length ratio (Matsuda et al 1999), body mass index (BMI) (kg/m^2) (Hassink et al 1997, Jaquet et al 1998, Marchini et al 1998, Gomez et al 1999, Ertl et al 1999), ponderal index (kg/m^3) (Harigaya et al 1997, Lepercq et al 1999, Tarquini et al 1999, Varvarigou et al 1999, Ong et al 1999), or Kaup index ($g/cm^2 x10$) (Matsuda et al 1997). Cord blood leptin concentrations have correlated with these parameters in most studies, but not in all (Tarquini et al 1999).

The ponderal index has been shown to differentiate fetal overgrowth into symmetric and asymmetric subtypes, and show that infants with the asymmetric type of overgrowth have the higher cord plasma insulin and leptin concentrations (Lepercq et al 1999). In addition, fetal fat mass has been approximated by measurements of triceps skinfold thickness or midarm circumference (Hassink et al 1997), or by subscapular, biceps, and triceps skinfold thickness (Schubring et al 1999). These, too, have correlated with cord plasma leptin concentrations, and showed that skinfold thickness and weight account for approximately 35 to 70% of the variation in cord plasma leptin levels (Hassink et al 1997).

2.4.7 Hormonal and metabolic adaptation of the infant to extrauterine life

Fetal adaptation from intrauterine to extrauterine life requires hormonal and metabolic changes to take place prior to birth, at birth, and within the first days of extrauterine life.

Plasma levels of CRH are markedly elevated during the perinatal period, resulting in a surge of ACTH secretion (Winter 1992). Part of the increase in CRH secretion is due to placental production of CRH into the fetoplacental circulation, especially during pre-

eclampsia (Laatikainen et al 1991). At birth, this elicits an immediate response in fetal adrenal secretion of cortisol. Once the neonate is separated from the placenta, considerable reduction in the metabolic clearance of cortisol occurs, and therefore a marked increase in adrenal reserve capacity to maintain circulating cortisol levels (Winter 1992).

One of the main metabolic significances of cortisol during the neonatal period is the maintenance of blood glucose levels through stimulation of hepatic gluconeogenesis, reduction in extrahepatic protein synthesis, and inhibition of insulin secretion. After birth, plasma ACTH levels fall rapidly (Winter 1992).

Production of active thyroid hormones is markedly increased in association with the events of birth. During the first hours after birth, an abrupt 3- to 6-fold increase appears in serum thyroid-stimulating hormone (TSH) concentration. The thyroid gland is adrenergically innervated, and the postnatal increase in serum catecholamine concentrations, as well as the cold-stimulated TSH surge may augment these changes in the T4/T3 ratio of secreted thyroid hormones. The metabolic significance of the neonatal thyroid hormone surge is not entirely clear, since neonates with congenital thyroid agenesis do not usually exhibit impaired environmental adaptation (Polk et al 1992).

The adaptation of the newborn to thermally cooler surroundings requires heat production from nonshivering thermogenesis. This takes place in brown adipose tissue, with the heat production governed by signals from the hypothalamus. These signals are relayed via the sympathetic nervous system and transmitted to the cell as a norepinephrine stimulus (Nedergaard et al 1992).

In the human neonate the basal metabolic rate increases within the first 2 postnatal days (Hill et al 1965). The weight loss observed during the same time can be explained mostly by physiologic fluid loss (Maclaurin et al 1966).

Before birth, about 80% of the energy expended is derived from carbohydrate oxidation. At birth, the fetus becomes dependent on an external supply of energy and nutritients. During the first hours of life the amount of energy received is low even when oral food is supplied quickly. During the first day of life, fat provides between 60 and 70% of energy expenditure as a result of active lipolysis (Putet 1992).

Fetal glucose utilization during intrauterine life closely matches umbilical uptake. The majority of the transplacentally derived glucose is oxidized directly, with the remainder

directed into hepatic glycogen deposition. At birth, plasma glucose concentrations decrease to half before 2 to 4 hours of age. This decrease in glucose is even greater in preterm or SGA infants. At 2 to 3 days of age, plasma glucose concentrations rise to the normal range. Plasma glucose concentrations are maintained initially by hepatic glucogenolysis and stimulation of gluconeogenesis, integration of which events is mediated in great part through catecholamine secretion (Padbury et al 1992).

In male, but not in female infants there occurs a postnatal rise in testosterone concentrations, whereas for concentrations in the early postnatal period, no gender difference exists in serum estradiol (Winter et al 1976, Sizonenko et al 1978).

2.4.8 Postnatal changes in leptin concentrations

At 6 hours of age, the concentration of circulating leptin in the human newborn infant is of a similar magnitude to that in cord plasma, but by 2 days, a significant drop in the leptin plasma concentrations has taken place, and leptin levels remain at this level until one week of age. Interestingly, the differences observed between AGA, SGA, and LGA infants in cord plasma disappear by 48 hours (Harigaya et al 1997). At 16 hours of age the concentration of leptin in the newborn infant is considerably lower than in cord blood, and remains so at 3 days (Marchini et al 1998). Other studies, too, report a significant difference between cord plasma and postnatal leptin concentrations (Yura et al 1998, Matsuda et al 1999, Harigaya et al 1999, Helland et al 1998, Ertl et al 1999). The plasma leptin levels at 4 and 14 weeks of age are lower than in umbilical cord plasma (Helland et al 1998).

This reduction in leptin concentration after birth is possibly a result of cessation of the contribution of placental leptin (Harigaya et al 1997).

A low leptin concentration shortly after birth may be beneficial to the newborn to enhance food intake (Harigaya et al 1997, Marchini et al 1998, Yura et al 1998), as an indicator of which is a negative correlation between cord leptin and weight gain from birth to 4 months (Ong et al 1999). However, somewhat confusingly, a positive correlation has been found between an infant's weight gain and the magnitude of leptin rise that occurs between 3 to 6 and 30 days of age (Harigaya et al 1999).

The drop in the infant's body temperature after birth may also contribute to the initial decrease in leptin production (Trayhurn et al 1995). Fasting and cold-exposure are accompanied by an increase lipolysis and in levels of free fatty acids. The latter cause a concentration-dependent inhibition of leptin mRNA levels in cultured mouse adipocytes (Rentsch et al 1996).

The initially high leptin concentrations may be an important factor in regulation of the non-shivering thermogenesis in brown adipose tissue: Leptin regulates the function of uncoupling protein-3 regulated also by thermogenic stimuli, thyroid hormone, and beta3-adrenergic agonists (Gong et al 1997). Leptin seems to be necessary for this postnatal recruitment process in brown adipose tissue to start thermogenesis, since in the ob/ob mouse this process is blunted (Goodbody et al 1982).

The gender difference in leptin concentrations in cord blood at birth is reported in some (Helland et al 1998, Jaquet et al 1998, Matsuda et al 1997, Gomez et al 1999, Maffeis et al 1999, Matsuda et al 1999, Ong et al 1999), but not all studies (Schubring et al 1997, Koistinen et al 1997, Harigaya et al 1997, Tamura et al 1997, Tarquini et al 1999, Harigaya et al 1999, Yura et al 1998, Marchini et al 1998, Shekhawat et al 1998). In studies observing postnatal changes in leptin concentration, no mention exists of possible later development of a gender difference in leptin concentration, in the case that no difference had existed in cord blood (Harigaya et al 1997, Marchini et al 1998, Yura et al 1998). The gender difference could be a result of the postnatal testosterone surge, because testosterone has been shown to downregulate production of leptin at both protein and mRNA levels in human adipocytes (Wabitsch et al 1997). An inverse relationship has been demonstrated between cord blood leptin and testosterone (Ertl et al 1999). However, no gender difference in testosterone or estradiol concentrations has been found concurrent with a gender difference in leptin concentrations in cord plasma (Matsuda et al 1997, Maffeis et al 1999). A confounding factor may be fetal size: Maffei et al (1998) found lower testosterone concentrations in cord blood of AGA infants than in LGA infants.

In newborn infants, plasma leptin levels are lower during fasting and increase after breast feeding (Cinaz et al 1999). In human milk, leptin levels correlate with the mothers' serum concentrations but are one magnitude of order lower. In nursing rats, leptin is transferred via milk to the stomach and then into the circulation of the infant rat (Casabiell et al 1997).

2.5.1 Erythropoietin as an indicator of fetal hypoxia

Erythropoietin (EPO) is produced in the fetal liver, and near term also in the kidney (Zanjani et al 1989). It regulates the synthesis of bone marrow progenitor cells and erythrocytes (Zanjani et al 1989), with tissue hypoxia being the main stimulator of erythropoietin production (Zanjani et al 1989, Widness et al 1986). EPO has also been shown to be produced by placental trophoblast cells. Whether EPO expression in the placenta is regulated by hypoxia, and the proportion of placental EPO of all EPO in feto-placental circulation are thus far unknown (Conrad et al 1996). Since EPO is not stored, plasma EPO levels are an indicator of rate of EPO synthesis. In response to moderate to severe tissue hypoxia, a statistically significant increase in erythropoietin concentrations can be measured within 2 to 4 hours (Widness et al 1986). Increased fetal plasma, amniotic fluid, and cord plasma EPO concentrations are evident in pregnancies complicated by pre-eclampsia, intrauterine growth restriction, and maternal diabetes (Teramo et al 1987, Widness et al 1981, Mamapopulos et al 1994). Both in normal and in abnormal pregnancies, fetal plasma EPO concentrations correlate well with amniotic fluid EPO levels before labor (Teramo et al 1987).

3 OBJECTIVES OF THE STUDY

The aims of these studies were to examine any possible changes in leptin concentrations taking place during postnatal adaptation. We also studied the effect of maternal diabetes and pre-eclampsia on fetal and neonatal leptin concentrations, and whether fetal leptin concentrations are affected by the feto-placental hypoxia which often complicates these pregnancies.

The specific aims were:

(I) To study three aspects of leptin metabolism during the early postnatal period. First, we determined whether plasma leptin levels change when the nutrition of the newborn is transferred from the fetoplacental unit to periodic enteral feeding. Second, we studied whether plasma leptin concentration in newborn infants is associated with adipose tissue thickness as determined by ultrasound. Third, we examined the possible development of a gender difference in leptin levels during the first 3 postnatal days.

(II) To examine whether leptin concentrations are associated with gestational age and birthweight in infants born before 32 weeks of gestation, especially whether maternal pre-eclampsia and fetal growth restriction results in altered leptin levels in preterm infants.

(III) To learn whether leptin concentrations of fetuses of diabetic mothers are associated with fetal hypoxia, as indicated by fetal erythropoietin levels.

(IV) To discover to what extent leptin circulates in free and bound form in newborn infants, and whether maternal gestational diabets mellitus affects these variables at birth and during postnatal adaptation.

4 PATIENTS AND METHODS

Patients and study designs (I, II, III, IV)

4.1.1 Changes in leptin concentration during the early postnatal period (I)

We studied 38 healthy AGA newborn infants (20 male, 18 female; gestational age 39.7±1.3 weeks (mean±SD), range 36.3 - 41.9 weeks) born in the Helsinki City Maternity Hospital. Birth weight was 3470±552 g (range 2470 - 4630 g). Relative birth weight as determined by reference to a Finnish newborn population of 74 766 singletons born from 1978 to 1982 (Pihkala et al 1989) was -0.26±1.1 SD, range -2.0 - +2.0 SD, (Table Ia).

Table I a. Demographic data and plasma testosterone concentrations for the newborns.

	Males (n=20)		Females (n=18)	
	At birth	At 3 days' age	At birth	At 3 days' age
Gestational age (weeks)	39.9 ± 1.3	-	39.4 ± 1.2	-
Weight (g)	3675 ± 509[a]	3469 ± 497[b,c]	3243 ± 519	3047± 474[c]
Length (cm)	52 ± 2[b]	-	49 ± 2	-
Relative birth weight (SD)	0.0 ± 1.0	-	-0.6 ± 1.1	-
BMI (kg/m^2)	13.8 ± 1.2	13.0 ± 1.1[c]	13.5 ± 1.2	12.7± 1.1[c]
Arm circumference (cm)	-	11.6 ± 0.8[a]	-	11.0 ± 0.8
Subcutaneous fat (mm)	-	4.5 ± 1.3	-	4.9 ± 1.1
Testosterone (nmol/L)	5.8 ± 1.7	2.4 ± 0.9[c,d]	6.3 ± 3.1	1.4 ± 0.7[c]

[a] = P<0.05 vs females, [b] = P<0.01 vs females, [c] = P<0.001 vs at birth, [d] = P<0.001 vs females

Placental weight ranged from 370 to 850 g. Two infants were delivered by Cesarean section. Two mothers had gestational diabetes treated with diet only. BMI ranged from 11.2 to 16.1 kg/m^2 in the newborns, and from 15.8 to 43.8 kg/m^2 in the mothers.

A mixed blood sample was obtained from the umbilical cord at birth, and a venous blood sample was taken when the infant was 3 days old (mean age 73±9 h, range 60 - 100 h). Both samples were taken into tubes containing EDTA; these tubes were centrifuged at 3500 rpm for 10 minutes, and plasma was frozen and stored at -20°C until analysis. Birth weight and length were recorded at birth. At the age of 3 days, the weight was recorded, and the circumference of the proximal third of the left arm measured with a soft metric measuring tape. The thickness of subcutaneous adipose tissue at the same site was measured three times with a 7 MHz linear ultrasound transducer (Acuson 128, Mountain View, CA, USA).

4.1.2 Leptin in preterm infants (II)

We studied 74 preterm infants born in consecutive preterm deliveries in the Department of Obstetrics and Gynaecology of Helsinki University Central Hospital at GA 24.1 to 32 weeks and birth weight 385 to 2100 g (Table II a). The upper limit of GA was chosen to minimize the effect of accumulating fetal fat mass as a source of leptin (Carrera et al 1998). GA was determined by ultrasound during the first trimester. Relative birth weight (weight SD) was determined by reference to a Finnish newborn population of 74 766 singeltons born from 1978 to 1982 (Pihkala et al 1989). Of these infants, 14 were born to mothers with established proteinuric pre-eclampsia (Table II b). Intrauterine growth retardation (IUGR, weight < - 2 SD) affected ten infants (Table II c); of these IUGR infants, five were born to pre-eclamptic mothers and two infants each were from two triplet pregnancies without pre-eclampsia. Four pairs of twins and six infants from triplet pregnancies were included in the study. In 59 cases the mother recieved antenatal treatment with corticosteroids as two doses of 12 mg betamethasone at a 12-hour interval more than 12 hours before delivery (mean 4 days 7 hours, SD 3 days 17 hours, range 12 hours - 16 days), (Table II d). BMI was determined as weight(kg)/ length(m)^2. Of the infants, 32 were delivered vaginally and 42 by Cesarean section. Twelve mothers smoked at least five cigarettes a day. Infants of diabetic mothers and infants with malformations were excluded. Blood samples from the umbilical vein were taken at birth into EDTA tubes. The tubes were centrifuged at 1000 x g for 5 minutes, and plasma was frozen and stored at -20°C until analysis.

Table II a. Patient data.

N	74
Male/Female	37/37
Gestational age (weeks) (SD)	28.7 (2.4)
Weight (g) (SD)	1180 (396)
Length (cm) (SD)	37.5 (4.0)
BMI (kg/m^2) (SD)	8.5 (1.3)
Placenta (g) (SD)	451 (180)

Table II b. Infants with and without maternal pre-eclampsia

	pre-eclampsia	no pre-eclampsia
N	14	60
Male/Female	10/4	27/33
Gestational age (weeks) (SD)	29.4 (1.5)	28.5 (2.5)
Weight (g) (SD)	1048 (221)	1211 (421)
Length (cm) (SD)	36.5 (2.5)	37.5 (3.9)
BMI (kg/m^2) (SD)	7.7 (0.4)[b]	8.6 (1.4)
Placenta (g) (SD)	322 (118)[b]	481 (179)

[b] p<0.05 vs. infants without maternal pre-eclampsia

Table II c. Infants with and without IUGR

	IUGR	no IUGR
N	10	64
Male/Female	5/5	32/32
Gestational age (weeks) (SD)	29.6 (2.6)	28.5 (2.3)
Weight (g) (SD)	940 (367)[c]	1218 (389)
Length (cm) (SD)	36.0 (4.0)	37.5 (3.7)
BMI (kg/m^2) (SD)	7.2 (0.7)[c]	8.6 (1.3)
Placenta (g) (SD)	358 (157)	465 (180)

[c] p<0.05 vs. infants without IUGR

Table II d. Infants with and without exposure to antenatal betamethasone

	betamethasone	no betamethasone
N	59	15
Male/Female	29/30	8/7
Gestational age (weeks) (SD)	29.1 (2.1)[d]	27.0 (2.7)
Weight (g) (SD)	1211 (379)	1060 (450)
Length (cm) (SD)	38.0 (3.5)[d]	35.0 (4.0)
BMI (kg/m^2) (SD)	8.7 (1.2)[d]	7.7 (1.4)
Placenta (g) (SD)	456 (191)	429 (130)

[d] p<0.05 vs infants without exposure to antenatal steroids

4.1.3 Increased leptin in fetal hypoxia (III)

We measured leptin and erythropoietin concentrations in cord vein plasma and in amniotic fluid samples from 25 singleton fetuses of mothers with Type I diabetes mellitus (DM), and of these 7 had additional proteinuric pre-eclampsia. Mothers' glycemic control was evaluated by the glycated hemoglobin-A1C fraction (HbA1C) every 2 to 4 weeks throughout pregnancy. During the last month of pregnancy the mean HbA1C (SD) was 6.8% (1.2%), (range 4.6 to 9.8%). All infants of this study were delivered at Helsinki Unversity Central hospital by elective Cesarean section, the indications for which were complicated maternal DM, contracted pelvis, previous Cesarean section, or fetal macrosomia. Gestational age corrected by ultrasound examination, ranged from 35.3 to 39.3 weeks, and birth weight from 2690 to 5430 g. The relative birth weight, as determined by reference to a series of 74 766 Finnish singleton newborns (Pihkala et al 1989), ranged from -1.1 to 5.6 SD (Table III a).

Table III a. Patient data

	mean (SD)	range
Males / Females (n)	14/11	
Gestational age (weeks)	37.2 (1.0)	35.3 - 39.3
Birth weight (g)	3962 (762)	2690 – 5430
Relative birth weight (SD)	2.0 (1.8)	-1.1 - 5.6
Length (cm)	49.6 (2.6)	44.5 - 53.5
BMI (kg/m^2)	15.9 (1.9)	12.2 – 19.7
Head circumference (cm)	35.1 (1.5)	31.5 – 38.0
Apgar score	9 (1)	7 –10
Cord artery Hb (g/l)	154 (16)	120 – 183
Cord artery pO_2 (kPa)	2.2 (0.4)	1.1 - 2.9
Cord artery pH	7.24 (0.05)	7.15 - 7.35

In normal pregnancies we have previously found a median amniotic fluid EPO of 7.5mU/ml (Teramo et al 1987). In our hospital three times this median, ie., amniotic fluid EPO 22.5 mU/ml, is considered the lower limit of significant fetal hypoxia. Based on this limit, patients were divided into two groups: hypoxic and non-hypoxic (Table III b).

Table III b. Data on hypoxic (amniotic fluid EPO>22.5 mU/ml) and non-hypoxic (amniotic fluid EPO <22.5 mU/ml) infants

	hypoxic infants (n=9)	non-hypoxic infants (n= 16)
	mean (SD)	mean (SD)
Gestational age (weeks)	36.5 (0.8)	37.6 (1.0)[a]
Birth weight (g)	3917 (797)	3987 (766)
Relative birth weight (SD)	2.5 (2.0)	1.7 (1.7)
Length (cm)	49.1 (2.7)	50.0 (2.6)
BMI (kg/m^2)	16.1 (2.2)	15.9 (1.9)
Head circumference (cm)	34.5 (1.5)	35.5 (1.5)
Apgar score	9 (0)	9 (1)
Maternal pre-eclampsia (n)	3	4
Maternal HbA1C (%)	7.4 (1.2)	6.5 (1.1)
Cord artery pO_2 (kPa)	1.9 (0.5)	2.3 (0.3)[a]
Cord artery Hb (g/l)	157 (13)	158 (12)
Cord artery pH	7.21 (0.06)	7.25 (0.04)

[a]=$p<0.05$, hypoxic vs. non-hypoxic infants

Blood samples from the umbilical vein were taken at birth into EDTA tubes, which were centrifuged at 1000 x g for 5 minutes; plasma was frozen and stored at -20°C until analysis. Amniotic fluid samples were obtained by amniocentesis performed within 3 days prior to the operation for the determination of fetal lung maturity, or at Cesarean section. Amniotic fluid samples were drawn into EDTA tubes and stored at -20°C.

4.1.4 Free and bound leptin (IV)

We studied 13 infants of normal mothers and 13 infants of mothers with gestational diabetes mellitus (GDM) born in Helsinki City Maternity Hospital. Mean gestational age (GA), corrected by ultrasound examination, was 40.1 ± 1.4 weeks, and birth weight was 3695 ± 534 g. Relative birth weight (weight SD) was determined by reference to a Finnish newborn population of 74 766 singeltons born from 1978 to 1982 (Pihkala et al 1989). The weight, length, and head circumference were measured at birth, and weight was measured when the control blood sample was obtained at 3 days of age. BMI was calculated using birth length both for BMI at birth and for BMI at 3 days of age. None of the infants presented clinical signs of hypoglycemia at 3 days. They appeared healthy at birth and at 3 days. No difference existed in clinical parameters between these two groups (Table IV a).

GDM was diagnosed after a 75-g oral glucose tolerance test according to recommendations by the Finnish committee on diagnosis and treatment of GDM (Hyvönen 1991, Teramo et al 1993). None of the mothers had pre-eclampsia. In our series, GDM was treated with diet only, and none of the mothers received treatment with insulin.

Blood samples were drawn at birth from the umbilical vein; at the postpartum age of 3 days (mean 62 ± 12 h, range 40-87h) a sample was taken from each infant from a superficial vein into an EDTA tube. The tubes were centrifuged at 2000 x g for 10 minutes, and plasma was stored at -20°C until analysis.

Table IV a. Patient data

	All infants	Infants of GDM mothers	Infants of healthy mothers
Male/Female	12/14	6/7	6/7
Gestational age (weeks)	40.2 ± 1.4	40.2 ± 1.4	40.3 ±1.4
Range	36.9 – 42.4	36.9 - 41.6	37.1 - 42.4
Birth weight (g)	3693 ± 549	3757 ± 614	3630 ± 491
Range	2570 – 4740	2570 – 4500	2760 – 4740
Birth weight (SD)	0.2 ± 1.0	0.3 ± 1.0	0.0 ± 0.9
Range	-2.0 - 2.0	-2.0 - 1.9	-2.0 - 2.0
Birth length (cm)	50.6 ± 2.0	50.3 ± 2.3	51.0 ± 2.4
Range	45.0 – 56.0	45.0 - 53.0	47.0 - 56.0
BMI at birth	14.3 ± 1.2	14.7 ± 1.4	13.9 ± 0.7
Range	11.8 - 17.1	11.8 - 17.1	12.5 - 15.1
Weight at 3 d of age (g)	3479 ± 530[a]	3543 ± 565[a]	3415 ± 506[a]
Range	2545 – 4600	2545 – 4245	2580 – 4600
BMI at 3 d of age	13.5 ± 1.2[a]	13.9 ± 1.3[a]	13.1 ± 1.0[a]
Range	11.7 – 16.3	11.7 - 16.3	11.7 - 14.7
Weight loss by 3 d age (g)	214 ± 87	214 ± 94	215 ± 83
Range	25 – 360	25 + 320	100 – 360
Weight of the placenta (g)	636 ± 121	635 ± 135	637 ± 111
Range	360 – 880	360 – 850	390 – 880

[a]=p<0.001 vs. at birth

4.2 Ethics (I, II, III, IV)

All studies were conducted in accordance with the Declaration of Helsinki. Studies I and IV were approved by the Ethics Committee of the Helsinki City Hospitals. Written informed consent of the parents was obtained before participation in the study. Studies II and III were approved by the Ethics Committee of the Department of Obstetrics and Gynecology of the Helsinki University Central Hospital.

4.3 Methods (I, II, III, IV)

4.3.1 Blood and amniotic fluid samples (I, II, III, IV)

Samples of mixed (I) or venous (II - IV) cord blood were drawn at birth into EDTA tubes (I-IV). At a postpartum age of 3 days (mean 73± 9, range 60-100h, Study I) or mean 62 h ± 12 h, range 40-87 h, Study IV, a sample was taken from a superficial vein. Blood was collected into EDTA tubes and spun for 5 (II, III), or 10 (I, IV) minutes at 3500 (I), 1000 (II,III), or at 2000 *g* (IV). Plasma was stored at -20^{o}C until analysis (I-IV). Amniotic fluid samples were drawn into EDTA tubes and stored at -20^{o}C (III).

4.3.2 Anthropometric data (I, II, III, IV)

Birth weight and length were recorded at birth. At the age of 3 days, weight was recorded (I and IV), and the circumference of the proximal third of the left arm was measured with a soft metric tape (I).

4.3.3 Measurement of subcutaneous tissue (I)

The thickness of subcutaneous adipose tissue at the same site was measured three times with a 7 MHz linear ultrasound transducer (Acuson 128). The coefficient of variation of these three measurements was 6.0%. This method has been validated previously using computer tomography as a reference standard (Koskelo et al 1991).

4.3.4 Assay of total leptin (I, II, III, IV)

Total leptin was determined by radioimmunoassay in all studies, I-IV (Linco Research, St. Charles, MO, USA), (Ma et al 1996). The detection limit of this assay is 0.26 μg/L in our laboratory as determined by calculating two standard deviations (mean of 13 assays) from the zero reference point. The intra-assay and interassay coefficients of variation at low concentration (I, II, IV) (2.8 ± 0.2 μg/L) are 4.7% (I, II), and 6.1% (IV), and 2.6% (I, II), and 3.0% (IV), respectively, and at medium concentration (I, II, 15.6 μg/L), (IV, 19.6 ± 1.4 μg/L) 3.8% (I, II), 6.7% (IV), and 2.2% (I, II, IV), respectively.

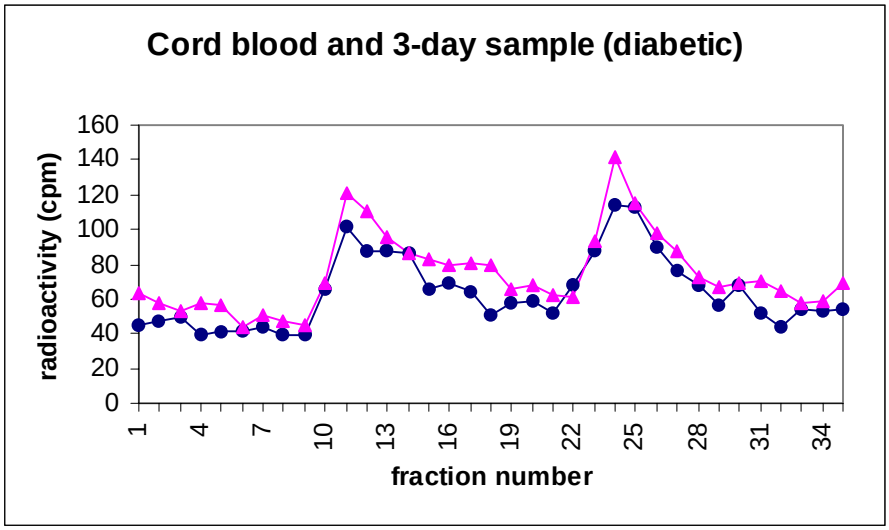
4.3.5 Assay of free leptin (IV)

150 µL of plasma sample was incubated with 150 µL of [^{125}I]leptin (standard amount) at room temperature at least overnight. Samples were then diluted 1:10 with eluting buffer (0.1 mol/L sodium phosphate buffer, pH 7.2), and filtered through a Millex-HV 0.45 µm filter (Millipore). Each cord plasma sample and 3-day sample was treated and eluted in parallel during the same day.

HPLC analysis was performed by LKB equipment using a 2150 HPLC pump, 2152 controller, and 2212 Helirac (LKB Bromma, Sweden). The column was an Ultropac Column, TSK G 3000 SW, 7.5X300mm (LKB) equipped with a sample injector with a 100 µL loop. Elution was performed with degassed 0.1 mol/L sodium phosphate buffer (pH 7.2). Flow-rate was constantly 1.0 mL/min and fraction volume was 1.0 mL. Elution time was 50 min, so given as 50 fractions per sample. Absorbance at 275 nm was monitored with a 2151 Variable wavelength monitor (LKB). The radioactivity of the samples was measured with 1282 Compugamma CS (LKB Wallac, Turku, Finland). In the elution profile the first peaks shown are bound leptin, and the last peaks represent free leptin (Figure IV). Peak areas were then estimated from the elution profiles.

Figure IV. The first peaks of the elution profile show bound leptin and the last peaks represent free leptin.

● = cord plasma; Δ = 3-day sample



Reproducibility of HPLC was performed using [^{125}I]leptin in 1% BSA-phophate buffer. Variation was 12%. Relative amounts of bound and free leptin were calculated from the total leptin concentration assayed by RIA for each sample.

4.3.6 Assay of testosterone (I)

Total plasma testosterone concentration was determined with RIA (Orion Diagnostica, Turku, Finland).

4.3.7 Assay of erythropoietin (III)

EPO was determined by radioimmunoassay (EPO-Trac®, Incstar, Stillwater, MN, USA; Garcia et al 1982).

4.3.8 Statistical methods (I, II, III, IV)

Leptin (I-IV) and EPO (III) concentrations were logarithmically transformed to normalize distribution when appropriate. Correlation coefficients were calculated with Spearman's test (I). Analysis of covariance was used to adjust leptin levels for possible confounders(I). Simple and multiple regression analyses were used (II, III, IV). Patient data are mean, SD, and range (I, III, IV). Results are mean and SD (I, II), and as median and interquartile range (II); median and range (III); median $\pm$ SEM, and range (IV). Wilcoxon’s rank sum test (I) or the paired T-test (II, IV) served for comparison of paired items. Paired items such as pre-eclampsia, antenatal steroids, and smoking were categorized as either no=0 or yes=1 (II). Comparison between groups was done with the Mann-Whitney U-test (III, IV), with a value of $p < 0.05$ considered statistically significant (I, II, III, IV). Calculations were done with either the Systat statistical package (Systat, Evanston, IL, USA), (I) or StatView 4.1 (Abacus Concepts INC., Berkeley, CA, USA), (II, III, IV).

5. RESULTS (I, II, III, IV)

5.1 Changes in leptin concentration during the early postnatal period (I)

Demographic data for the newborns are given in Table I a. At birth, cord plasma leptin concentration was 9.7±5.2 μg/L with no gender difference between male (8.6±4.6 μg/L) and female (10.9±5.6 μg/L, P=0.198) infants (Figure I). In the analysis of covariance, there was no statistically significant gender difference in leptin levels when adjusted for infant BMI (P=0.06) or subcutaneous fat (P=0.368). A slight gender difference appeared in leptin levels when birth weight alone was used as the covariate (log leptin, adjusted least squares means 0.81±0.28 vs. 1.03±0.26, P=0.021 in male and female infants, respectively).

Plasma leptin decreased in male (to 1.8±0.4 μg/L, P<0.001) and female (to 2.3±0.8 μg/L, P<0.001) infants by the third postnatal day (Figure I). At this age, gender difference was statistically significant (P=0.01).

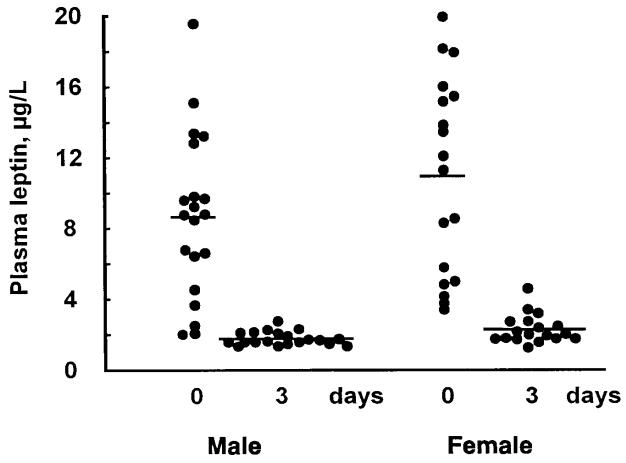


Figure I. Plasma leptin concentrations in cord blood and at 3 days of age in male (n=20) and female (n=18) newborn infants. At birth, plasma leptin levels were similar, but decreased both in males and females by the third postnatal day (P<0.001), with females having higher plasma leptin concentrations (P=0.01).

In male newborns, cord plasma leptin concentration correlated with the circumference of the arm but not with BMI, subcutaneous fat or birth weight (Table I b). In males at 3 days of age plasma leptin concentration correlated with none of these parameters (Table I b). In

female newborns, cord plasma leptin concentration correlated with BMI, subcutaneous fat, and circumference of the arm, but not with birth weight (Table I b). In 3-day-old females leptin concentration correlated with BMI, weight and arm circumference (Table I b). The decrease in leptin concentration in female newborns correlated with their BMI (r=0.63, P<0.01), subcutaneous fat (r=0.54, P<0.05), and arm circumference (r=0.72, P<0.01); and in male infants with arm circumference (r=0.50, P<0.05), but not with change in weight in either group. Neither cord plasma leptin nor plasma leptin at 3 days of age correlated with maternal BMI in male or female infants.

Table I b. Matrix of Spearman´s correlation coefficients in male (n=20) and female (n=18) newborns.

	Males		Females	
Leptin	At birth	3 days	At birth	3 days
BMI	0.430	-0.266	0.620[b]	0.470[a]
Subcutaneous fat	0.319	-0.049	0.535[a]	0.427
Birth weight	0.365	-0.063	0.406	0.462
Weight at 3 days	0.310	-0.083	0.457	0.487[a]
Arm circumference	0.481[a]	-0.028	0.722[b]	0.493[a]

[a]P<0.05, [b]P<0.01

5.2 Leptin in preterm infants (II)

Immunoreactive leptin was detectable in cord plasma samples from all preterm infants. Median leptin concentration was 1.01, interquartile range, 0.81-1.43 μg/L. A significant correlation existed between cord blood leptin and GA (r=0.336, p=0.0037), but not birth weight (r=0.155), relative birth weight (r=0.211), BMI (r=0.186), placental weight (r=-0.108), Apgar score (r=0.197), nor cord artery pH (r=-0.104).

Significantly higher leptin was found in infants of pre-eclamptic mothers (median 1.80; 1.11 - 2.08 vs. median 0.93; 0.79 - 1.29 μg/L; p=0.0007), in IUGR infants (median 1.80; 1.34 - 3.04 vs. median 0.93; 0.74 - 1.03 μg/L; p=0.0005), and in those exposed to antenatal steroids (median 1.18; 0.85 - 1.73 vs. median 0.76; 0.66 - 0.95 μg/L; p=0.02). Maternal smoking was not observed to affect cord blood leptin concentrations. Infants of

pre-eclamptic mothers had significantly smaller placentas than other infants [322 (SD 118) vs. 451 (SD 180) g; p<0.05].

When GA, presence of pre-eclampsia, and exposure to antenatal steroids were included as independent determinants of leptin concentration in multiple regression analysis, GA (partial r=0.257, p=0.02), and pre-eclampsia (partial r=0.32, p=0.004) were significantly and independently associated with leptin, whereas exposure to steroids remained non-significant (partial r=0.103, p=0.39).

When IUGR infants and infants born to pre-eclamptic mothers were excluded, simple regression analysis of the 55 remaining infants revealed significant correlations between cord leptin and GA (r=0.360, p=0.0069), BMI (r=0.424, p=0.0033), and birth weight (r=0.487, p=0.0002). In these 55 infants, cord plasma leptin concentration of those exposed to antenatal steroids (n=41) did not differ from those not exposed, (median 0.94; 0.81 - 1.30 vs. median 0.75; 0.65 - 0.86 μg/L, p=0.09).

5.3 Increased leptin in fetal hypoxia (III)

The cord plasma median concentration of leptin was 9.0 μg/l (range, 3.7 - 135.1μg/l, and that of EPO 26.2 mU/ml (range, 9.6 - 9263 mU/ml). In amniotic fluid, median concentration of leptin was 2.2 μg/l (range, 0.7 - 5.7 μg/l), and that of EPO 10.9 mU/ml (range, 1.0 - 1594 mU/ml).

The fetuses of the hypoxic group had significantly higher cord leptin concentrations (median 36.8; range, 12.5 - 135.1μg/l) than those in the non-hypoxic group (median 16.2; range, 3.7 - 52.2 μg/l), p=0.0066 (Figure III a). Fetuses of the hypoxic group had lower GA (p=0.016), and cord artery pO_2 (p=0.023) at birth (Table III b). Between groups, maternal HbA1C (p=0.1), Hb (p=0.5), and base excess (BE) (p=0.2) did not differ significantly, although cord blood pH showed a close to statistically significant difference (p=0.06).

In all fetuses, a significant correlation existed between cord plasma leptin and amniotic fluid EPO (r=0.727, p=0.0001), cord plasma EPO (r=0.644, p=0.0005, Figure III b), maternal HbA1C (r=0.612, p=0.0019), and relative birth weight (r=0.399, p=0.049); whereas negative correlations were found with cord artery pO_2 (r=-0.440, p=0.032) and pH (r=-0.414, p=0.040). In addition, significant correlations existed between amniotic fluid

EPO and cord plasma EPO (r=0.863, p=0.0001), maternal HbA1C (r=0.646, p=0.0009), cord artery pO_2 (r=-0.644. p=0.0007), and pH (r=-0.558, p=0.0038).

Figure III a. Box and whisker plot of cord plasma leptin concentrations of hypoxic (n=9) and non-hypoxic (n=16) fetuses at birth

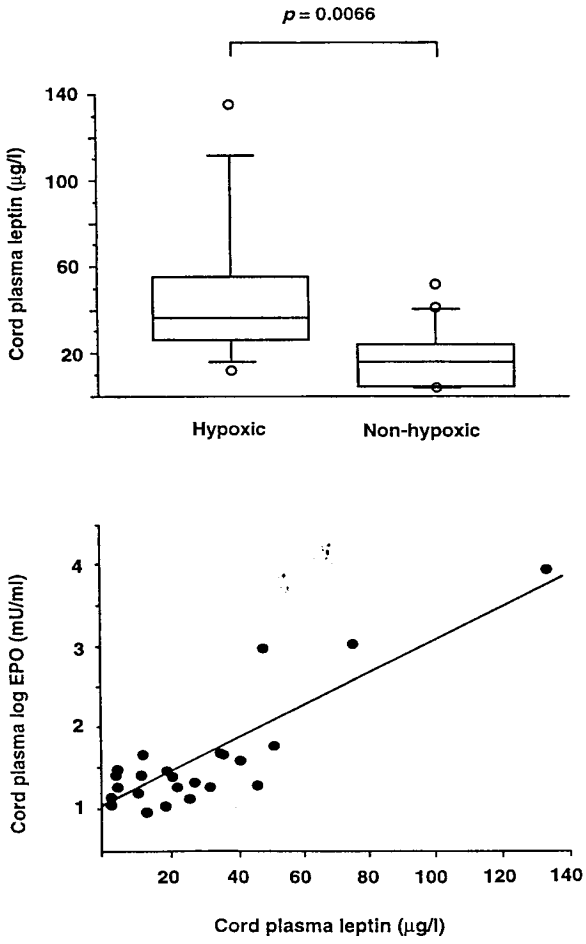


Figure III b. Correlation between umbilical cord EPO(log) and leptin

Neither cord plasma leptin, EPO, nor amniotic fluid EPO correlated with cord hemoglobin concentration at birth. Nor did amniotic fluid leptin correlate with clinical data or with these biochemical variables.

In multiple regression analysis with cord plasma leptin as the dependent variable, and amniotic fluid EPO, cord artery pO2, relative birth weight, maternal HbA1C, and GA as

independent variables, only EPO (partial r=0.558, p=0.031) remained significantly associated with cord plasma leptin.

5.4 Free and bound leptin (IV)

Infants of GDM mothers had higher concentrations of total leptin in cord plasma than did infants of healthy mothers ($p<0.05$). Likewise, the cord plasma concentrations of free and bound leptin, as well as the percentage of free leptin, were significantly higher in infants of GDM mothers (all $p<0.05$).

A significant decrease from birth to 3 days of age occurred in the concentrations of total, free, and bound leptin in infants of GDM mothers and infants of healthy mothers (Table IV b), but the percentage of free leptin remained stable from birth to 3 days of age in both groups of infants (Table IV b).

Table IV b. Plasma concentrations of total, free, and bound leptin, and percentage of free leptin at birth and at three days of age

Leptin concentration in cord plasma µg/L ± SEM, (range)	All infants	Infants of GDM mothers	Infants of healthy mothers
Total	13.2 ± 2.1 (2.4 - 43.8)	17.8 ± 3.3[a]	8.5 ± 1.8
Free	7.6 ± 1.3 (1.1 - 27.2)	10.7 ± 2.0[a]	4.4 ± 0.9
Bound	5.6 ± 0.8 (1.4 - 16.6)	7.2 ± 1.3[a]	4.1 ± 0.9
Percent of free	55.3 ± 1.3 (40.6 - 70.0)	58.7 ± 1.7[a]	51.8 ± 1.5
Leptin concentrations at three days of age µg/L ± SEM, (range)			
Total	1.8 ± 0.7[b] (0.6 - 3.7)	2.0 ± 0.2[b]	1.6 ± 0.2[c]
Free	1.0± 0.1[b] (0.5 - 2.0)	1.1 ± 0.1[b]	0.9 ± 0.1[b]
Bound	0.9 ± 0.1[b] (0.5 - 1.8)	0.9 ± 0.1[b]	0.9 ± 0.1[b]
Percent of free	55.4 ± 1.2 (44.5 - 67.7)	56.5 ± 1.6[a]	50.3 ± 1.2

[a]=$p<0.05$ vs. infants of healthy mothers, [b]=$p=0.0001$ vs. at birth, [c]=$p=0.0004$ vs. at birth

At 3 days, leptin concentrations in infants of GDM mothers and of healthy mothers were similar (Table IV b). Infants of GDM mothers tended to have somewhat higher concentrations of free leptin, but this difference did not reach statistical significance ($p=0.08$). However, the percentage of free leptin remained higher in infants of GDM mothers at 3 days ($p<0.05$, Table IV b).

In all infants, cord plasma concentrations of total, free, or bound leptin correlated with none of the clinical parameters presented in Table IV a (data not shown). At 3 days, a correlation existed between the percentages of free leptin and BMI ($r=0.487$, $p=0.0016$). In multiple regression analysis, with the percentage of free leptin at 3 days as the dependent variale, and GDM of the mother and BMI at 3 days of age as independent variables, the percentage of free leptin remained significantly dependent on the GDM of the mother (partial $r=0.432$, $p=0.028$), but not on BMI.

6 DISCUSSION (I, II, III, IV)

6.1 Changes in leptin concentration during the early postnatal period (I)

Our data confirm the results from previous studies that immunoreactive leptin is present in cord blood (Hassink et al 1997, Koistinen et al 1997, Sivan et al 1997, Schubring et al 1997, Matsuda et al 1997). Moreover, leptin concentrations in cord blood correlate well with the adiposity of the newborn as determined directly by measurement of the thickness of the subcutaneous fat by ultrasound. During intrauterine development, because the fetus is dependent on the maternal fuel and constituent supply, the presence of a satiety signal would seem rather paradoxical. The interleukin-6-type cytokine receptor-like structure and signaling capabilities of the leptin receptor (Bauman et al 1996), and the expression of messenger-RNA for leptin receptor isoforms in the lungs, liver, and kidneys of human fetuses (Cioffi et al 1996) raise the possibility that leptin may function as a regulator of growth during fetal development. Recent data suggesting the placenta as a source of leptin for the growing fetus (Hassink et al 1997, Masuzaki et al 1997) support the idea of leptin's having a physiologic function in the maturation process. At present, however, it is impossible to distinguish whether the leptin in cord blood is of fetal or placental origin, although there is a small, albeit significant, arterio-venous difference, with higher leptin concentrations in blood from the umbilical artery (Schubring et al 1997) suggesting that at least part of the leptin in the fetoplacental circulation is of fetal origin. This finding has recently been challenged (Yura et al 1998). Considering the same hormonal mileu shared by fetus and placenta, and the aforementioned discrepancies in arterio-venous differences in cord plasma leptin concentrations, it is doubtful whether the use of mixed cord plasma has had any significant impact on our results.

Leptin concentration decreased markedly during the first 3 postnatal days. The decrease in infant weight during the same period, which is mostly explained by physiologic fluid loss (Maclaurin 1966), is unlikely to explain the fall in leptin level, since we found no correlation between changes in weight and in leptin. The basal metabolic rate increases within the first 2 postnatal days in the human neonate (Hill et al 1965); growth and development in the neonatal period becomes dependent on an external energy and constituent supply while the nutrition of the newborn is being transferred from the fetoplacental unit to periodic enteral feeding. The postnatal decrease in leptin concentration may thus be a

physiological adaptation to these profound alterations in metabolic environment, and may serve to enhance the newborn's food intake. According to a recent study, the decrease in leptin concentrations occurs by 16 hours after birth, and leptin levels remain low until at least 3 days after delivery (Marchini et al 1998). The mechanisms behind the postnatal fall in leptin levels remain at present unclear; we cannot exclude the posibility that some of the decline in plasma leptin concentration reflects the removal of the placental source of leptin.

There is a well-established gender difference in leptin concentrations in adult humans (Rosenbaum et al 1996) and in prepubertal children (Hassink et al 1996, Caprio et al 1996), with females showing higher leptin concentrations per unit fat mass. However, data regarding any gender difference in leptin in newborn infants have been conflicting (Koistinen et al 1997, Schubring et al 1997, Matsuda et al 1997); a small gender difference in leptin concentrations was found when 100 newborn infants were studied (Hassink et al 1997). In our study, the unadjusted leptin levels did not statistically differ between male and female infants at birth. Recently, Helland et al. suggested that from 18 to 35 weeks of pregnancy, the gender of the infant modifies the increase in maternal leptin levels: This increase in leptin levels was not significant in women carrying male fetuses. Moreover, leptin levels were lower in male infants at birth, and the gender difference became more apparent when leptin/birth weight ratio was analyzed (Helland et al 1998). Similarily, in our study, female newborns had a higher plasma leptin/birth weight ratio than male newborns at birth. It is thus possible that the contribution of the placenta in leptin secretion (Masuzaki et al 1997) may in our study have interfered with the gender difference in cord plasma leptin levels.

By the third postnatal day, however, a clear gender difference in leptin concentrations had developed, with higher concentrations in female newborns. In our study, the thickness of the subcutaneous fat was similar in male and female newborns, and correlations between plasma leptin at 3 days and weight and arm circumference were evident in female but not in male newborns' suggesting that factors other than adiposity, such as the hormonal milieu, may interfere with the association between leptin and adiposity. No gender difference exists in serum estradiol concentrations during the early postnatal period, whereas a postnatal rise in serum testosterone concentrations occurs in male but not in female newborns (Winter et al 1976, Sizonenko 1978). Adult males show an inverse relationship between serum leptin and testosterone concentrations that is independent of

BMI (Nyström et al 1997). A negative correlation between serum leptin and testosterone concentrations is observed also in adult male IDDM patients (Tuominen et al 1997).

In our study, male newborns had higher plasma testosterone and lower leptin concentrations than female newborns at 3 days of age, a fact which raises the possibility that the gender difference in leptin levels may be a result of testosterone downregulating leptin secretion. Indeed, a recent study has shown this to be the case: testosterone downregulates leptin production both at the protein and at the mRNA level in human adipocytes (Wabitsch et al 1997). In leptin-deficient ob/ob mice, leptin treatment corrects the sterility attributed to insufficiency of hormones at the hypothalamic-pituitary level (Chehab et al 1996). Moreover, in mice, leptin treatment resulted in earlier maturation of the reproductive tract than in controls and also in earlier reproduction (Chehab et al 1997). In humans, an increase in serum leptin levels occurs in both girls and boys before the appearance of other reproductive hormones, suggesting that leptin may be an important signal triggering the start of puberty (Mantzoros et al 1997, Garcia-Mayor et al 1997). Thus, higher leptin levels in females shortly after birth may be physiologically important and relate to leptin´s role in reproduction. In conclusion, our data suggest that circulating leptin concentrations correlate with adiposity at birth in female but not in male newborns. Leptin concentration decreases markedly in both genders by the third postnatal day with a clear gender difference developing by that time, with higher leptin levels in females. The postnatal decrease in plasma leptin concentration may be a physiologically feasible adaptation to extrauterine life.

6.2 Leptin in preterm infants (II)

Our data demonstrate that in preterm infants maternal pre-eclampsia is associated with increased leptin concentration, and that pre-eclampsia is an independent determinant of leptin levels. Pre-eclampsia has recently been shown to increase maternal leptin levels, but no such association has yet been described in the fetus (Mise et al 1998). Reduced uteroplacental blood flow leading to fetoplacental hypoxia is important in the pathogenesis of IUGR, *eg,* in pre-eclampsia (Redman 1991). Mise et al (1998) postulated that the elevated maternal plasma leptin in pre-eclampsia is caused mostly by augmentation of the placental production of leptin in response to hypoxia (Mise et al 1998). However, our study showed no correlation between cord plasma leptin concentration and Apgar score or cord

artery pH. Similarily, in vitro data indicate that no significant increase in leptin secretion occurs until 72 hours of hypoxia (Mise et al 1998).

In our study, preterm infants with IUGR, with or without maternal pre-eclampsia, had higher cord leptin levels than did those experiencing normal growth. This is in contrast to observations in most but not all studies of full-term infants (Koistinen et al 1997, Harigaya et al 1997, Tamura et al 1998, Shekhawat et al 1998). Interestingly, in our study, when infants with IUGR and infants of pre-eclamptic mothers were excluded from the analysis, a correlation appeared between birth weight and leptin concentration similar to that observed in term infants. It is thus possible that pre-eclampsia, and IUGR and the concomitant increase in leptin concentration, may interfere with the association between leptin and birthweight. The overlap between groups of infants with IUGR and with maternal pre-eclampsia makes it difficult, however, to differentiate the effects of these two clinical conditions on leptin metabolism. Our finding of preterm IUGR infants' having higher leptin concentrations agrees with a finding by Shekhawat et al 1998, whereas no such relationship appeared in a study by Jaquet et al 1998. However, those studies did not examine the possible connection of pre-eclampsia with fetal leptin concentrations (Jaquet et al 1998, Shekhawat et al 1998). Moreover, in term infants, maturity and fetal fat mass may interfere with the effects of pre-eclampsia on fetal leptin.

In the preterm infants of this study, placental weight did not correlate with leptin concentration as it did in term infants (Koistinen et al 1997). In fact, infants of pre-eclamptic mothers had smaller placentas and increased leptin concentration. This finding may be explained by the theory that hypoxia augments placental production of leptin (Mise et al 1998).

Hypoxia and IUGR, the fetal hallmarks of pre-eclampsia, are associated with increased morbidity (Redman 1991, Kelly et al 1998). The roles of leptin in hematopoiesis, in fetal erythroid development, and in angiogenesis raise the possibility that relative hyperleptinemia is part of the fetal adaptation to hypoxia (Cioffi et al 1996, Sierra-Honigman et al 1998, Mikhail et al 1997, Umemoto et al 1997). In adults, plasma leptin levels are elevated in survivors of acute sepsis, and leptin has been called a stress-related hormone (Bornstein et al 1998). If this is true, leptin may play a role in the response to a severe stress state - such as pre-eclampsia - also in the fetus.

Shekhawat et al 1998 found use of antenatal steroids to be associated with increased cord plasma leptin levels in preterm infants. The mean GA in their study was 32 weeks, and infants of diabetic mothers were included. Unfortunately, no weight data were available on the infants exposed to antenatal steroids, making the interpretation of their results difficult. In our infants, we also found an association between the use of antenatal steroids and high cord plasma leptin. However, when IUGR infants and infants born to mothers with pre-eclampsia were excluded this correlation disappeared. Moreover, in multiple regression analysis, because antenatal exposure to steroids was not an independent determinant of leptin levels, it is therefore possible that the effect of antenatal steroids on leptin production is primarily dependent on maturity. Thus far, the effects of corticosteroids on leptin levels are still under dispute also in adults (Kolaczynski et al 1997, Torpy et al 1998).

We found that maturity, defined as GA, is, in preterm infants, a significant determinant of cord plasma leptin levels. This is in accordance with findings of studies on pre-term and term infants (Jaquet et al 1998, Koistinen et al 1997, Harigaya et al 1997, Tamura et al 1998, Shekhawat et al 1998), and may be related to the accumulation of adipose tissue in the fetus during late gestation (Carrera et al 1998). Given the presence of mRNA for leptin receptor isoforms in the lung, liver, kidneys, and hematopoietic cell lines of human fetuses, these data suggest that leptin may participate in the regulation of fetal growth and development (Cioffi et al 1996).

In conclusion, gestational age is an important determinant of cord plasma leptin levels in preterm infants. Leptin levels are increased in pre-eclampsia and IUGR, which may be part of fetal physiological adaptation to stress.

6.3 Increased leptin in fetal hypoxia (III)

Our study of diabetic pregnancies demonstrated that fetuses exposed to hypoxic conditions, as indicated by elevated amniotic fluid EPO concentrations, showed significantly higher cord plasma leptin concentrations. Moreover, in all fetuses, cord plasma leptin concentration strongly correlated with amniotic fluid and cord plasma EPO concentrations.

Our data are in agreement with the findings of Mise et al 1998, who demonstrated that hypoxia increases placental leptin production. They found in vitro that the rise in leptin

production in placental cells does not take place until after 72 hours of hypoxia. Because during hypoxic conditions a significant increase in fetal EPO concentrations can be observed within 2 to 4 hours (Widness et al 1986), the increased cord plasma leptin, together with the increased EPO concentrations in both amniotic fluid and cord plasma, probably reflect chronic or subchronic rather than acute fetoplacental hypoxia.

We found no correlation between amniotic fluid or cord plasma EPO and cord hemoglobin; this is in accordance with previous studies of infants of diabetic mothers (Teramo et al 1987, Widness et al 1990), whereas a negative correlation has been reported between EPO and Hb in normal and Rh-isoimmunized fetuses (Moya et al 1993). Evidence exists that in infants of diabetic mothers, erythropoiesis is qualitatively abnormal, resulting in a significant delay in the beginning of β–globin production (Perrine et al 1985), and in the fetus and in the preterm infant the response of erythropoiesis to EPO differs from that in the adult (Moya et al 1993, Brown et al 1984). These observations may also, in part, explain the lack of correlation between cord leptin and hemoglobin.

Both maternal glycemic control in diabetic pregnancies and pre-eclampsia affect cord plasma leptin concentrations (Shekhawat et al 1998, Mise et al 1998), with amniotic fluid and cord plasma EPO concentrations increased in such pregnancies (Teramo et al 1987, Widness et al 1981, Buescher et al 1998). However, neither the number of pre-eclamptic mothers nor maternal HbA1C significantly differed between the hypoxic and non-hypoxic infants. In addition, all infants of this study were delivered by elective Cesarean section, thus eliminating the possible hypoxia and increase in EPO concentration caused by labor (Widness et al 1984). Although a correlation existed between cord plasma leptin and cord blood pH and pO2, in multiple regression analysis only cord plasma EPO remained an independent and significant determinant of cord plasma leptin concentrations. This difference between the groups in cord plasma leptin concentrations may be primarily a result of fetal chronic or subchronic hypoxia.

Leptin has been shown to be involved in angiogenesis and shown to stimulate the proliferation of myelocytic and primitive hematopoietic progenitor cells (Sierra-Honigmann et al 1998, Umemoto et al 1997). In vitro data indicate that leptin acts synergistically with EPO to increase fetal erythroid development (Mikhail et al 1997). Therefore, along with concomitantly increased EPO concentrations, increased leptin levels may represent a cooperative and physiologically feasible adaptation mechanism to improve oxygen transportation capability during periods of decreased oxygen supply. These beneficial

effects from elevated leptin concentration in the fetus may not be limited to stimulation of erythroid development and angiogenesis: the cytokine-like properties of leptin receptors and the better survival of adult septic patients with higher leptin levels has led to researchers to consider leptin a stress-related hormone (Mikhail et al 1997, Sierra-Honigmann et al 1998, Umemoto et al 1997, Bornstein et al 1998). Leptin thus may improve fetal adaptation to such stressful conditions as chronic hypoxia.

We found a positive correlation between cord plasma leptin concentration and maternal HbA1C, a result suggesting that maternal glucose metabolism may affect leptin metabolism of the feto-placental unit. This phenomenon is supported by data showing that insulin increases leptin mRNA (Saladin et al 1995), and data indicating that leptin secretion in adipocytes is regulated by glucose metabolism (Mueller et al 1998). Mueller et al demonstrated that the increase in leptin secretion from adipocytes is primarily dependent on glucose, with the insulin effects' being secondary. In diabetic mothers, the expression of leptin mRNA and of protein in placental tissue is elevated (Lepercq et al 1998), implying that the correlation between cord plasma leptin and maternal HbA1C is an indicator of augmented leptin production in fetal adipocytes and in the placenta.

Human amniotic fluid cells have been shown to secrete leptin into the amniotic fluid (Masuzaki et al 1997). In our study, amniotic fluid leptin concentrations failed to correlate with any of the clinical or biochemical parameters, suggesting that secretion of leptin into the amniotic fluid is independent of these variables.

In conclusion, our study indicates that in Type I DM pregnancies, cord plasma leptin concentration correlates with severity of fetal hypoxia, as shown by the concentration of erythropoietin. This phenomenon may reflect one physiological response of the fetoplacental unit to chronic hypoxia.

6.4 Free and bound leptin (IV)

Although infants of GDM mothers and those of healthy mothers were similar in terms of characteristics, the former had higher total, free, and bound cord leptin concentrations.

These data therefore suggest that the differences in leptin metabolism between infants of GDM and healthy mothers are due to the effect of maternal glucose metabolism. Because infants of diabetic mothers have higher insulin concentrations in the cord plasma (Lepercq et al 1998, Persson et al 1999), this, or enhanced glucose metabolism, or both (Malmström et al 1996, Utriainen et al 1996, Mueller et al 1998) may have stimulated their leptin secretion in utero to a higher extent. It is also possible that some of the difference in leptin concentration between infants of GDM and healthy mothers is due to placental leptin production (Masuzaki et al 1997), because studies have demonstrated placental leptin protein content to be higher in insulin-treated diabetic than in healthy pregnant women (Lepercq et al 1998). This finding is in accordance with recent data demonstrating that cord plasma leptin concentrations are elevated to the same extent in infants of both GDM and type-1 DM mothers in comparison to concentrations in infants of healthy mothers (Persson et al 1999). They also concluded that the difference was explained mostly by enhanced placental leptin production.

In infants of GDM mothers, we observed an increase in free and bound leptin levels at birth suggesting that maternal GDM increases the concentrations of leptin-binding proteins in the fetoplacental circulation. This situation appears analogous to that in pregnant insulin-dependent diabetes mellitus women, who have higher levels of soluble leptin receptor (Lewandowski et al 1999). Taken together, these data show that insulin or maternal hyperglycemia, or both, may participate in fetal regulation of free leptin and leptin-binding protein levels.

In the infants of our study, the concentration of total leptin decreased significantly from birth to 3 days of age, confirming previous observations (Marchini et al 1998, Helland et al 1998, Yura et al 1998, Matsuda et al 1999, Harigaya et al 1999). Interestingly, our infants' decrease in free leptin concentration from birth to 3 days was of a magnitude similar to that in lean adults as a result of 24-hour fasting (Sinha et al 1996), suggesting that, during the early postnatal period when the infant adapts to extrauterine life, the decrease in concentration of total and of free leptin may be a physiological reaction to reduce the inhibitory action of leptin on food intake.

By 3 days, all differences in concentrations of total and bound leptin between infants of GDM mothers and healthy mothers disappeared. However, at 3 days the concentration of free leptin tended to be higher in infants of GDM mothers, although not statistically significantly. In these infants, at this stage, the percentage of free leptin remained, however, significantly higher. Although the number of infants studied is comparatively small, this result is intriguing because it suggests that abnormal glucose metabolism in the mothers with GDM may have long-term metabolic effects on their offspring. Indeed, it has been reported that the offspring of mothers with either GDM or type-1 DM are at increased risk for childhood obesity and for later development of disorders of their glucose metabolism (Silverman et al 1991, Pettitt et al 1991, Pettitt et al 1993). However, for the infants of diabetic mothers, further studies are warranted to elucidate to what extent altered leptin metabolism *in utero* and during the early postnatal period is predictive of further development of metabolic disorders. In addition, since cord blood leptin concentrations are negatively related to weight gain in human infants up to 4 months of age (Ong et al 1999), it would be interesting to study whether in these infants perinatal alterations in leptin metabolism influence postnatal growth.

This leptin-binding activity that is compatible with the affinity of the soluble leptin receptor is higher in prepubertal children than in infants and adults, and appears similar in infants and adults (Quinton et al 1999). In all infants of our study the percentage of free leptin was approximately 55%, comparable to that in lean adults (Sinha et al 1996). Free and bound leptin appear to behave as different compartments, and physiological alterations such as fasting affect the percentage of free leptin (Sinha et al 1996). Therefore, it is of interest to note that during the first postnatal days of our study, the percentage of free leptin remained relatively constant. In adults, percentage of free leptin is related to degree of obesity and BMI (Sinha et al 1996, Houseknecht et al 1996, Leonhardt et al 1999). Similarily, although a correlation did exist between percentage of free leptin and BMI at 3 days of age, as indicated by multiple regression analysis, this correlation appeared to be a function of maternal GDM, rather than of BMI.

In conclusion, infants of GDM mothers have higher cord plasma total, free, and bound leptin concentrations than do offspring of healthy mothers. By 3 days of age these leptin concentrations decrease in all infants, and the differences between infants of GDM and healthy mothers disappear. In contrast, during the early postnatal period the percentage of free leptin remains unaltered and also remains consistently higher in infants of GDM

mothers than in those of healthy mothers. These results seem to reflect the difference in fetoplacental leptin metabolism in GDM mothers at birth, and may be indicative of the difference persisting in these infants during their postnatal adaptation.

7 SUMMARY AND CONCLUSIONS

The aims of these studies were to examine changes in leptin concentrations taking place during postnatal adaptation. In particular we studied whether maternal diabetes and pre-eclampsia and the abnormal glucose metabolism and hypoxia associated with them influence leptin concentrations during the fetal and neonatal period.

The main findings and conclusions are:

In female, but not in male, infants, plasma leptin concentrations already correlate with adiposity at birth. A considerable drop in circulating leptin takes place from birth to 3 days of age in both female and male infants, and a gender difference in leptin plasma concentrations develops by this time. This rapid decrease in plasma leptin concentrations may be a result of the removal of placental leptin production, and the development of a gender difference in plasma leptin concentrations may reflect differences in hormonal mileu such as those caused by testosterone, on plasma leptin concentrations. The low leptin concentration during the postnatal period may be one part of a physiologically feasible adaptation mechanism.

The decline in leptin concentrations occurs both in infants of healthy mothers and in infants of mothers with GDM. However, concentrations of free, bound, and total leptin, and the percentage of free leptin are significantly higher in infants of mothers with GDM at birth. Moreover, in these infants, the finding that the percentage of free leptin remains higher suggests that maternal GDM may have long-term metabolic effects on offspring. Thus, this abnormal maternal glucose metabolism seems to affect leptin metabolism of the offspring not only in utero, but also during the period of postnatal adaptation. In addition, the positive correlation of maternal HbA1C with cord plasma leptin suggests that maternal glucose metabolism augments leptin metabolism of the feto-placental unit.

In preterm infants, gestational age is an important determinant of cord plasma leptin levels. Chronic or subchronic feto-placental asphyxia, as indicated either by elevated fetal erythropoietin levels in term fetuses or by fetal growth restriction in preterm fetuses of pre-eclamptic mothers, is associated with increased fetal leptin levels. This may be part of the physiological adaptation to stress such as hypoxia during the fetal period.

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