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Leptin Production and Release in the Dually *in Vitro* Perfused Human Placenta*

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ABSTRACT

There is clear evidence that the placenta produces leptin. However, it is still unclear to what extent leptin is released into the maternal and the fetal circulation. The aim of our study was to determine placental leptin release rates into these 2 compartments. In 10 term placentas, using dual *in vitro* perfusion of an isolated cotyledon, concentrations of leptin, hCG, and human placental lactogen (hPL) were determined in perfusates and in the tissue before and after perfusion. With perfusions lasting 270–840 min, total leptin production was 225 pg/g·min [median; interquartile range (IQR), 76–334 pg/g·min]. The release into the fetal circulation was very low (median, 2.5; IQR, 1.1–5.9 pg/g·min) compared with the release into the maternal circulation (median, 203; IQR, 79–373 pg/g·min) corresponding to 1.6% and 98.4% of net release. Only 0.05% of hPL and hCG were released into the fetal circulation and 99.95% into the maternal circulation, confirming previous results. Release into the fetal circulation correlated significantly with release into the maternal circulation for leptin (r = 0.648; $P < 0.05$) and hPL (r = 0.721; $P < 0.05$). Furthermore, release of leptin into the fetal circulation was positively correlated with release of fetal hCG (r = 0.661; $P < 0.05$).

Most of the leptin produced by the placenta is released into the maternal circulation, but compared with other placental hormones (hCG and hPL), a considerably higher proportion of leptin is released into the fetal circulation. These findings may at least partially explain the marked increase in maternal serum leptin levels in pregnancy. The rapid postnatal decrease in leptin levels in both the mother and the neonate is also consistent with the concept of placental origin. (*J Clin Endocrinol Metab* **85:** 4298–4301, 2000)

LEPTIN PLAYS A key role in the weight control mechanisms by signaling information on total body energy stores to the central nervous system (CNS) (1, 2). It is thought to influence food intake by altering the balance of neuropeptide production (neuropeptide Y, agouti-related protein, and melanocortin) in the arcuate nucleus, whose axons project to a number of areas involved in food intake regulation (3). In men and nonpregnant women leptin is predominantly produced by adipose tissue, and its levels in the circulation are largely determined by the fat mass (1, 4, 5). In pregnancy, maternal plasma leptin levels rise markedly 2- to 4-fold compared with those in nonpregnant women (6–10), and they decline sharply after delivery (11–13). Three factors may contribute to these changes: 1) fat mass increases during pregnancy by about 15% (14), which, however, cannot fully explain the up to 4-fold leptin rise; 2) hCG is produced in large amounts during pregnancy, and *in vitro* studies have shown that hCG stimulates leptin production in adipose tissue (6); and 3) *in vitro* experiments using trophoblast cultures have shown significant leptin production in these cells (15, 16).

In the fetus, in addition to adipose tissue, the placenta seems to be a source of leptin production. This can be concluded from the following findings: 1) leptin levels are higher in the umbilical vein (mean, 12.9 ng/mL) than in the artery (mean, 9.8 ng/mL) (17); and 2) neonatal leptin levels decrease sharply after birth (mean, 3.0 ng/mL) (17) without any significant postnatal loss of body fat mass (11, 13, 18–20). Although clear evidence for placental leptin production has been derived from *in vitro* trophoblast cultures (16, 21), only the dual perfusion system can address the question of relative release of a placental product into the maternal and fetal circulation. This model has already successfully been used in previous studies to investigate the placental release of hCG and human placental lactogen (hPL), which are important hormones for successful pregnancy and are released almost completely into the maternal circulation (22). It was, therefore, the aim of the current investigation to study leptin release compared with that of hCG and hPL as known markers of placental hormone release (22), using the well established model (23–26) of dual closed loop perfusion in an isolated cotyledon of a term placenta.

Materials and Methods

Dual in vitro closed loop perfusion of the placenta (23, 27)

Dual *in vitro* closed loop perfusion of an isolated cotyledon was performed in 10 placentas using a previously described model (23, 27). The placentas were obtained from uncomplicated term pregnancies after either vaginal or cesarean delivery and were placed in a bath of physiological saline within 15 min. To perfuse the fetal side, the chorionic artery and vein of a suitable cotyledon were cannulated, and subsequently the cotyledon was fixed in the perfusion chamber. To perfuse the maternal side, 3 blunt metal cannulas were introduced into the intervillous space by penetration of the decidual plate. Fetal and maternal cannulas were connected to separate perfusion circuits. The com-

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position of the perfusate was previously described (25, 26): tissue culture medium NCTC-135 diluted by Earle's solution (2:1) with addition of glucose (2 g/L), dextran 40 (10 g/L), heparin (2500 IU/L), and clamoxyl (250 mg/L). Two gas exchange devices (Mera Silox-S 0.3, Senko Medical Instruments, Tokyo, Japan) were used; the fetal perfusate was equilibrated with 95% N_2 and 5% CO_2, whereas an atmospheric gas mixture with 5% CO_2 was used for the maternal side. Flow rates were 12 mL/min in the maternal and 4–6 mL/min in the fetal circuit.

The experiment started after a prophase of 30 min with open perfusion to remove all blood from the intervillous space and the villous vascular compartment. After the prophase, perfusion was continued with closed circuits on both the maternal and the fetal side with equal starting volumes of 150 mL in each circuit. After 2 h the medium was completely exchanged on the maternal and fetal sides. The duration of the second phase after medium exchange varied between 2 and 6 h. The experiment was terminated whenever the loss of perfusate from the fetal into the maternal compartment exceeded 4 mL/h. In five experiments a second exchange of medium was performed after 6 h, and the experiment was extended for another 3–6 h (phase 3). The production rates of the various hormones were calculated separately for the three experimental phases.

To monitor tissue energy metabolism, glucose consumption and lactate production were measured intermittently throughout the experiment (27).

Measurement of hormone concentrations

Concentrations of leptin, hCG, and hPL were measured in all perfusate samples taken from the maternal and fetal circulations. To control for tissue release during perfusion, the tissue contents of leptin, hCG, and hPL were measured before and after the experiment. A specimen was obtained from unperfused tissue before (t_0) and from perfused tissue at the end of the experiment (t_1). For the determination of tissue hormone content, 1 g (wet weight) of tissue was homogenized after addition of 3 mL Earle's buffer using a Polytron (Brinkmann Instruments, Inc., Westbury, NY) for 2–3 min. After centrifugation at 1400 × *g* for 10 min, the supernatant was removed and stored at −20 C until analysis (28). Leptin levels were measured in duplicate using a commercially available RIA (Mediagnost, Tubingen, Germany) (1). hPL and hCG levels were measured by enzyme-linked immunosorbent assay as previously described (26).

Calculations and statistics

The following definitions were used: release into the fetal circulation (Rf), (final hormone concentration in the fetal circulation) × (volume of fetal perfusate); release into the maternal circulation (Rm), (final hormone concentration in maternal circulation) × (volume of maternal perfusate); total release (TR) = Rf + Rm; tissue accumulation (TA), (tissue hormone concentration at t_1 − tissue hormone concentration at t_0) × cotyledon weight; and total production (TP) = TR + TA. Rf, Rm, TR, and TA were normalized for tissue weight and duration of the perfusion. For comparative purposes, total production normalized for tissue weight and duration of perfusion was expressed as a percentage of the initial tissue concentration: total production of the whole placenta = (TP/cotyledon weight) × placental weight. To estimate the placental contribution to total maternal leptin production our results were compared with the only reference data published to date about total *in vivo* leptin production rates (797 ± 283 ng/min; data established in healthy male adults) (29).

Data are presented as the median and interquartile range (IQR). Correlation analysis was performed using Spearman's ρ. Differences were considered significant if $P < 0.05$. Statistical analyses were performed using SPSS 8.0 for Windows (SPSS, Inc., Chicago, IL).

Results

Clinical data are shown in Table 1. The median weight of the cotyledons studied was 16.0 g (range, 13.6–24.2 g), and the median perfusion time was 480 min (range, 270–840 min). Table 2 shows the results of total release expressed as release into the maternal and fetal circulations, tissue accumulation, and total production for leptin, hCG, and hPL. The total leptin production rate was 225 pg/g·min (IQR, 76–334 pg/g·min). The total production of leptin accounted for 345% of the initial tissue content, whereas hCG and hPL accounted for only 238% and 146%, respectively. Leptin release decreased slightly during the course of the experiment. This decrease, however, was statistically not significant. The leptin content of placental tissue declined moderately during perfusion (0.5% of the totally released leptin). Leptin release into the fetal circulation was 2.5 pg/g·min (IQR, 1.1–5.9 pg/g·min) and accounted for only 1.6% of the total release, whereas more than 98% of TR (median, 203; IQR, 79–373 pg/g·min) was on the maternal side (Fig. 1). The total release rates for hCG and hPL were similar to previously published values (26). The major part (99.94% and 99.95%, respectively) appeared in the maternal circulation. The fractional release of leptin into the fetal circulation was considerably larger than for the classical placental hormones, hCG and hPL (Fig. 1).

TABLE 1. Clinical data of the 10 newborn-mother pairs whose placentas were studied

Parameter	Median	IQR
Wt gain during pregnancy (kg)	11.7	11.1–16.5
Maternal ht (cm)	169	165–172
Maternal wt at birth (kg)	81	68–85
Gestational age (weeks)	39	38–40
Birth wt (g)	3190	2970–3270
Birth length (cm)	48	47–49
Placental wt (g)	560	485–630

IQR, Interquartile range.

Total leptin release of the whole placenta was calculated to be 115 ng/min (IQR, 42–179 ng/min), which corresponds to 14% of the average leptin production in normal weight adults (29).

The release into the fetal circulation correlated significantly with the release into the maternal circulation for leptin (r = 0.648; $P < 0.05$) and hPL (r = 0.721; $P < 0.05$). Furthermore, the release of leptin into the fetal circulation was positively correlated with the release of hCG (r = 0.661; $P < 0.05$). No further correlations were detected between all parameters measured, including maternal or fetal weight or body mass index (BMI).

Discussion

Using the model of the dual closed loop perfusion, we were able to assess leptin production by the intact term placenta. In this system the relative release of placental hormones into the maternal and fetal circulations can be studied separately as previously shown for other placental proteohormones (22). The quantitative data obtained in these experiments support the qualitative results of *in vitro* studies investigating leptin messenger ribonucleic acid expression in trophoblast cultures (21).

Serum leptin levels rise during pregnancy 2- to 4-fold (6–10, 12), and the question remains of what the causes of this increase are. In our view the increased leptin production during pregnancy compared with that in nonpregnant women is based on three sources: 1) increase in body fat, 2) increase in extra body fat production by the placenta, and

TABLE 2. Placental hormone production and release (median, IQR) as measured in the dually perfused isolated cotyledon

Parameters	Leptin (pg)	hPL (ng)	hCG (mU)
Total production (. . . /g · min)	225	1124	52
	76; 334	582; 1627	36; 116
% of initial tissue content	345	146	238
	304; 735	69; 228	86; 514
Tissue accumulation (% of total production)	−0.5	1	2.4
Total release (. . . /g · min)	205	963	68
	80; 376	814; 1318	45; 103
Release into the fetal circulation (. . . /g · min)	2.5 (1.6%)[a]	0.5 (0.05%)[a]	0.04 (0.06%)[a]
	1.1; 5.9	0.3; 0.8	0.02; 0.3
Release into the maternal circulation (. . . /g · min)	203 (98.4%)[a]	963 (99.95%)[a]	67 (99.94%)[a]
	79; 373	813; 1317	45; 104
Total production of the whole placenta (. . . 10^3/min)	115	477	37
	42; 179	313; 1009	20; 63

[a] Percent of total release.

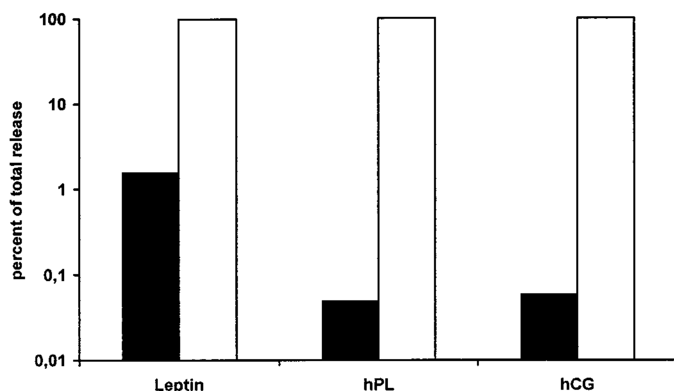

FIG. 1. Maternal and fetal release of leptin, hPL, and hCG as a percentage of total release (n = 10; □, maternal; ■, fetal).

3) an unexplained part, because the impact of body fat accumulation and placental production cannot explain the total leptin production during pregnancy.

A significant rise of serum leptin is probably due to the accumulation of adipose tissue. Using the equation derived by Blum *et al.* to predict leptin concentration from body fat (4) and assuming an average increase in fat mass by 15% (14), it can be estimated that this increase in body fat causes a mean increase in leptin levels of 49–94% in normal adult women with BMI values between 18–30 kg/m^2. The equation expresses an exponential relation between BMI and serum leptin and is derived from a best-fit regression curve obtained from data for 587 healthy nonpregnant women (4).

In pregnancy, placental leptin production should also be taken into consideration. Based on our *in vitro* results it can be calculated that placental leptin release into the maternal circulation accounts for 14% of the leptin production in normal adult women. It appears, therefore, that neither fat accumulation nor placental leptin production alone nor the sum of both can fully explain the marked rise in leptin levels in pregnancy or the prompt decrease immediately after delivery. It cannot be ruled out that the calculation based on the *in vitro* production is an underestimate of the *in vivo* performance of the placenta.

Additional mechanisms may be involved. 1) Increased food intake, which is known to stimulate leptin levels (30), may play a role. 2) A stimulatory effect of hCG on the production of leptin was shown in adipocyte culture (6). 3) The release of the soluble leptin receptor by placental membrane shedding and subsequent binding of free leptin to this receptor may impair the bioactivity of leptin and protect leptin from degradation or excretion (31, 32). Indeed, free leptin seems not to be elevated in pregnancy, in contrast to bound leptin (33).

Placental leptin is also released into the fetal circulation. Although reasonable estimates of fetal fat mass are available (34, 35), leptin production rates by fetal adipose tissue are unknown. Therefore, it remains unclear to what extent fetal leptin originates from fetal adipose tissue or from the placenta. The placental leptin release into the fetal circulation found in the present study may explain the increased levels described in the umbilical venous compared with those in arterial blood (18, 20, 36).

The proportion of total placental leptin released into the fetal circulation was higher than the proportions of the classical placental hormones such as hCG and hPL. This may be due to differences in diffusion as a reflection of the differences in molecular weight (hCG, 39 kDa; hPL, 22 kDa; leptin, 16 kDa) or it may be a result of active transport with different rates of secretion. The impact of molecular weight on the fetal-maternal release ratio is supported by the fact that the relative amount of placental CRH (4.5 kDa) released into the fetal circulation is even higher and reaches 10% of the total production (27, 37, 38). Another reason for higher leptin release into the fetal circulation compared with hCG and hPL could be the production of leptin in villous endothelial cells, which is not described for hCG or hPL (32, 39).

The elevated levels of circulating leptin during pregnancy seem to conflict with the classical role of leptin as a feedback signal for the CNS from body energy stores. According to this concept, increased leptin would not allow increased food intake, which, however, is well documented and has a decisive role in fetal and maternal weight gain. Recently, developed concepts favor leptin as a starvation signal; falling leptin levels should cause refeeding, whereas higher than normal levels are ignored by the CNS (40). In pregnancy, the physiological function of high leptin levels is not clear. In the maternal body it may have effects on thermogenesis and mobilization of energy stores rather than on regulation of maternal food intake. In the placenta it could act as a GH and factor for angiogenesis in an autocrine manner. The benefit of placental leptin to the fetus may be growth and angio-

genesis too, as leptin is considered an important new growth factor in intrauterine and neonatal development (15, 32, 41). Studies in normal and diabetic pregnancies indicate that fetal hyperleptinemia caused by hyperinsulinemia stimulates fetal growth (19, 42). Placental leptin may also function as an antiinflammatory factor, because it counteracts proinflammatory cytokines such as tumor necrosis factor-α (43). This effect may be important, as successful pregnancy is associated with down-regulation of intrauterine proinflammatory cytokines (44, 45).

In conclusion, it could be shown that, using the dual closed loop placenta perfusion, the placenta produces leptin in large amounts and that most leptin is released into the maternal circulation, and a smaller portion appears on the fetal side. The role of placental leptin production is unclear, but it may be hypothesized that it is involved in promoting fetal growth and development, placental autocrine regulation of growth, angiogenesis, and immunity and in maternal adaptation to the altered metabolism in pregnancy.

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