A BRIEF COMMUNICATION

Serum Leptin in Nonpregnant and Pregnant Women and in Old and New World Nonhuman Primates

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Leptin is a hormone that is produced during mammalian pregnancy in the placental trophoblast and other tissues, including! fetal and maternal adipocytes. Synthesis of the polypeptide and the presence of its specific receptors throughout the human maternal fetoplacental unit suggest direct effects on conceptus growth and development. However, both the physiologic roles of leptin and the mechanisms regulating leptin synthesis in human pregnancy differ from those in laboratory and domestic species, necessitating the development of nonhuman primate research models. Therefore, we compared serum leptin concentrations in nonpregnant and pregnant women with those in both old world nonhuman primates (i.e., baboon, rhesus monkey, cynomolgus monkey) and new world nonhuman primates (i.e., squirrel monkey, titi monkey). As expected, maternal leptin levels were elevated in human and baboon pregnancies ($P < 0.05$ and $P < 0.001$, respectively). Levels in both species of old world monkeys were also greatly enhanced ($P < 0.001$). Although maternal serum concentrations were slightly elevated compared to nonpregnant levels in both species of new world monkeys, overall concentrations were dramatically lower than for either old world primates or humans. Results provide comparisons of serum leptin concentrations in pregnant and nonpregnant humans and baboons with those in both old and new world monkeys and further characterize these nonhuman primates as models for the investigation of leptin dynamics in pregnancy. Exp Biol Med 230:251–254, 2005

Key words: leptin; pregnancy; old world primates; new world primates

The discovery of leptin, a 16 kDa polypeptide produced predominately in adipose tissue, stimulated a wealth of research concerning its interactions with mechanisms regulating obesity (1). Although physiologic roles for leptin include the hypothalamic regulation of satiety and energy expenditure, direct associations with reproductive function have also been described (2, 3). In addition to its synthesis in adipose tissue, leptin is also produced by the placental trophoblast, a circumstance that has led to a number of recent studies that research the regulation of this hormone and its physiologic role(s) in mammalian pregnancy (3–6).

Maternal serum leptin levels are enhanced during pregnancy when compared to those in nonpregnant women (7, 8), and we have reported that concentrations are elevated to an even greater extent over nonpregnant levels in the pregnant baboon—as much as 25-fold greater in late pregnancy (9). Therefore, we have characterized the baboon

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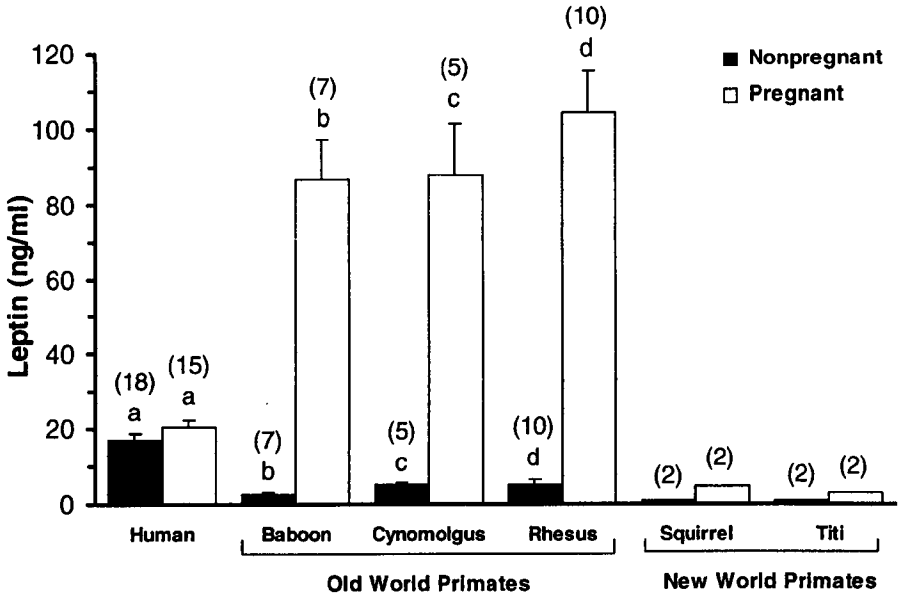

Figure 1. Leptin concentrations in pregnant and nonpregnant women, old world primates (mean ± SEM), and new world primates (mean). Common lowercase letters indicate significant differences (a, $P < 0.05$; b, c, d, $P < 0.001$). Number in parentheses (*n*) represents the number of individuals from each of which a single sample was obtained.

as an animal model for the study of leptin in pregnancy (9–16). Because the baboon is the only nonhuman primate investigated thus far with respect to leptin in pregnancy, it may be efficacious to determine levels in other nonhuman primates so that they can be compared to both the human and baboon. Therefore, in the current study we investigated two other species of old world primates, rhesus and cynomolgus monkeys, as well as two species of new world primates, squirrel and titi monkeys.

Materials and Methods

Subjects. Samples were obtained from normally cycling women of reproductive age without regard to stage of the menstrual cycle ($n = 18$). In addition, samples were collected from other women during the third trimester of normal singleton pregnancies ($n = 15$). No women participating in the study were obese as the calculated Body Mass Index (BMI) was <30 for all subjects, and generally was <27. Diets were uncontrolled and conformed to the typical high-fat American diet. Studies were approved by the Institutional Review Board of the Texas Tech University Health Sciences Center. Nonhuman primates studied included three species of old world monkeys: the baboon (*Papio* sp; 7 nonpregnant and 17 pregnant), rhesus monkey (*Macaca mulatta;* 10 nonpregnant and 10 pregnant), and cynomolgus monkey (*Macaca fascicularis;* 5 nonpregnant and 5 pregnant). Two species of new world monkeys were also studied: the squirrel monkey (*Saimiri sciureus;* 2 nonpregnant and 2 pregnant) and the titi monkey (*Callicebus moloch;* 2 nonpregnant and 2 pregnant). As with the human subjects, blood samples were collected from all nonpregnant, nonhuman primates without regard to the stage of the menstrual cycle. Samples from pregnant individuals were obtained in late gestation, during a period equivalent to the last trimester of human pregnancy (as determined by menstrual history and/or observed matings). In the baboons, squirrel monkeys, and titi monkeys, blood was collected under ketamine HCl anesthesia (Ketaset, 10–15 mg/kg body weight; Fort Dodge Pharmaceuticals, Fort Dodge, IA). In rhesus and cynomolgus monkeys, animals were awake and had been trained to extend an arm for blood draws from the cephalic vein. A single blood sample was obtained from each nonhuman primate involved in the study. All nonhuman primates were fed standard primate laboratory chow (<5% fat) with fruit, vegetables, and vitamin supplements. All were maintained in accordance with United States Department of Agriculture regulations and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Protocols were approved by the Institutional Animal Care and Use Committees of the California or Tulane National Primate Research Centers.

Assays. Leptin was assayed by specific radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX). In an earlier study, we had validated this assay for the baboon (11). Subsequently, the assay was validated for all other species of nonhuman primates investigated in the current study, using serial dilutions that were examined for linearity and parallelism with the human standard curve.

Statistics. Student's *t* test was used for statistical comparisons within the same species.

Results

Serum leptin concentrations for all humans and nonhuman primates are presented in Figure 1. In women, serum leptin levels (mean ± SEM) were 19.4% greater in the third trimester of pregnancy (20.3 ± 2.1 ng/ml; $P <$

0.05) than nonpregnant levels (17.0 ± 1.6 ng/ml; $P < 0.05$). In all three species of old world primates (i.e., baboons, cynomolgus monkeys, rhesus monkeys), a dramatic elevation was observed in the final third of pregnancy ($P < 0.001$) compared with levels in nonpregnant animals. Serum leptin concentrations in baboons were 86.9 ± 10.6 ng/ml during pregnancy, while nonpregnant concentrations were 2.7 ± 0.2 ng/ml. In cynomolgus monkeys, levels were 88.0 ± 13.7 in pregnant monkeys and 5.1 ± 0.5 ng/ml in nonpregnant monkeys. Similarly, in rhesus monkeys, concentrations were 104.9 ± 10.8 in pregnant monkeys and 5.4 ± 1.0 ng/ml in nonpregnant monkeys. In both species of new world monkeys, leptin levels in both pregnant and nonpregnant animals were comparatively low (<5.0 ng/ml). Mean levels in nonpregnant squirrel monkeys (1.1 ng/ml) and titi monkeys (1.1 ng/ml) were increased in pregnancy to 4.9 ng/ml and 2.9 ng/ml, respectively.

Discussion

Since the initial characterization of leptin (1), numerous reports have described the increase in maternal serum levels during pregnancy in humans (12–14) and laboratory and domestic animals (4, 5, 14). Although significant differences in the dynamics of leptin exist with respect to pregnancy in these species as compared to pregnancy in humans and nonhuman primates, the baboon is the only nonhuman primate model that has been developed to study the regulation and function of leptin in pregnancy (12–16). To this end, our studies demonstrated an increase in maternal serum leptin levels that is greater than the increase typical in women. Although, as in the human (6), the expression of placental leptin mRNA transcripts in the baboon (9) is greater in early pregnancy than at term, we have proposed that the placental trophoblast and the maternal adipose tissue each contribute to the peripheral pool of maternal leptin and are regulated by estrogen in a tissue-specific fashion (9, 11). In the interest of developing other nonhuman primate species as effective models, we investigated leptin levels in two additional species of old world primates and two species of new world primates in the present study. Both old world species and new world species were included because major differences in endocrinology between these groups have been reported, especially in regard to steroid hormone concentrations (17–22).

In women, the increase in leptin levels from nonpregnant to late pregnancy is relatively small. Levels in nonpregnant women are much greater than nonpregnant levels observed in any species of nonhuman primate in this study, perhaps due to a greater percentage body fat in women on the typical American diet. The comparatively low levels of leptin in nonpregnant, nonhuman primates may be due to the small amounts of adipose tissue in these animals, which are maintained on a very low-fat diet supplemented with fruit and vegetables. In the three examined species of old world primates, increases in serum leptin concentrations by late gestation were markedly elevated, sometimes as much as 25-fold greater than nonpregnant levels. Several factors might account for leptin increases during gestation, but results suggest that leptin levels increase much more dramatically than does adipose tissue. Therefore, we presume that increases in adipose tissue alone cannot account for the extent of the increases in leptin levels. Most likely, the hormonal milieu of pregnancy becomes increasingly more effective in stimulating leptin production from existing adipose tissue sources. Several reports have demonstrated that estrogen, particularly at high levels, may serve to stimulate leptin production from adipose tissue (23, 24), and we have previously proposed that the increasing levels of estrogen during primate pregnancy may serve this function (11, 14).

In the two species of new world primates examined, nonpregnant levels of leptin were slightly less than in old world primates, and the increase observed in late pregnancy was small (only 2- to 3-fold greater than nonpregnant values). Numerous studies have reported markedly elevated gonadal and adrenal steroid hormone levels in new world monkeys, and a form of steroid resistance has been associated with this increase (21, 22). Therefore, this resistance may limit the adipose tissue response to maternal serum steroid increases during pregnancy in new world monkeys. Clearly, further studies are needed to understand the basis for these differences in leptin regulation during pregnancy in new world and old world monkeys.

These results demonstrate elevated serum leptin levels in old world primates, adding the rhesus and cynomolgus monkeys to our earlier studies on the baboon. They constitute the first report of the finding that serum leptin levels are only minimally elevated in late pregnancy in new world primates, despite greater increases in estradiol reported for the squirrel monkey when compared to old world primates (25). Potential similarities in the physiologic roles of leptin, and changes in the rate of leptin biosynthesis with advancing pregnancy, may serve to introduce specific nonhuman primate species as models for studying the mechanisms regulating leptin dynamics in human pregnancy.

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