Pre-β-HDL stimulates placental lactogen release from human trophoblasts

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Departments of 1Pediatrics and 2Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229; and 3Atherosclerosis Research Unit, Department of Medicine, University of Alabama, Birmingham, Alabama 35294

Handwerger, Stuart, Geeta Datta, Brian Richardson, Carrie M. Schmidt, Tariq Siddiqi, Lisa Turzai, and G. M. Anantharamaiah. Pre-β-HDL stimulates placental lactogen release from human trophoblast cells. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E384–E389, 1999.—To examine whether pre-β-high-density lipoprotein (HDL) may be involved in regulation of human placental lactogen (hPL) release, pre-β-HDL was isolated from term pregnancy serum, and the effect of purified pre-β-HDL on hPL release from trophoblast cells was examined after 1 h of exposure. Pre-β-HDL stimulated a dose-dependent increase in hPL release with half-maximal stimulation at a dose of 300–400 μg/ml, which is within the normal physiological range during pregnancy. Analysis of pre-β-HDL and α-HDL in serum from pregnant women at different stages of gestation (determined by Western blot analysis) indicated that the pre-β-HDL-to-α-HDL ratio increased linearly after the 10th week of gestation (r = 0.88, P < 0.001), reaching a maximum sixfold greater than that of nonpregnant women. The increase in serum pre-β-HDL during pregnancy paralleled that of plasma hPL concentrations (r = 0.93, P < 0.001). Two-dimensional electrophoresis indicated that the increase in pre-β-HDL was due primarily to an increase in pre-β-3-HDL and pre-β-2-HDL, two of the three forms of pre-β-HDL present in blood. These results suggest a role for pre-β-HDL in the regulation of hPL expression during pregnancy.

HUMAN PLACENTAL LACTOGEN (hPL) is a protein hormone expressed by the syncytiotrophoblast cells of the placenta that has striking homologies in its amino acid sequence and biological properties to growth hormone and prolactin (see Ref. 10 for review). However, the factors that regulate hPL expression are different from those for growth hormone and prolactin (11). For example, growth hormone-releasing hormone and somatostatin, which regulate growth hormone release, and thyrotropin-releasing hormone and dopamine, which regulate prolactin release, have no effect on the release of hPL.

Recent studies from our laboratory strongly suggest that high-density lipoproteins with α-mobility on agarose gel electrophoresis (α-HDL) have a physiological role in the regulation of the secretion of hPL (10). In vitro studies, HDL isolated from serum by density gradient ultracentrifugation (α-HDL) stimulated a dose-dependent increase in hPL release from placental explants and trophoblast cells at concentrations within the normal physiological range present in serum during pregnancy (14). In vivo experiments, the acute intravenous infusion of α-HDL into pregnant ewes stimulated an increase in serum placental lactogen concentrations (9).

The stimulatory effect of α-HDL on hPL release was not due to a generalized effect on the placenta, because the release of chorionic gonadotropin was unaffected (14). Furthermore, α-HDL had no effect on the release of luteinizing hormone and follicle-stimulating hormone from rat pituitary cells and the release of growth hormone and prolactin from human pituitary cells in culture (15). Subsequent investigations indicated that the effect of α-HDL is due to the apolipoprotein constituents of α-HDL rather than the lipid constituents (14). Delipidation of α-HDL did not diminish stimulatory activity, and purified delipidated apolipoproteins stimulated hPL release. Because apolipoprotein (apo) A-I constitutes ∼95% of the total apolipoproteins in α-HDL, almost all of the activity can be attributed to apoA-I. Later experiments showed that apoA-I also stimulates the synthesis of hPL by stimulating gene transcription (12).

HDL in serum can be separated into two subfractions with α- and pre-β-electrophoretic mobilities that differ in both composition and structure (4, 17, 33). α-HDL, which is the more abundant form of HDL, is composed of ∼50% lipid and 50% protein (see Ref. 8 for review). Pre-β-HDL, which is a less negatively charged form of HDL, contains much less lipid. Pre-β-HDL particles are generated, at least in part, by the incubation of α-HDL with cholesteryl ester transfer protein and either very low density lipoproteins or low-density lipoproteins (3, 16, 22). Numerous studies indicate that α-HDL and pre-β-HDL particles play key roles in reverse cholesterol transport (see Refs. 7, 16, and 34 for review).

α-HDL in serum is a heterogeneous population of lipoprotein particles that can be divided into two major density classes, HDL2 and HDL3, in which HDL2 particles are larger and more buoyant than HDL3 particles (8). HDL2 particles can be further subdivided into two subclasses by nondenaturing gradient gel electrophoresis, and HDL3 particles can be further subdivided into three subclasses.

Two-dimensional electrophoresis has shown that pre-β-HDL consists of at least three subgroups of particles, pre-β1-HDL, pre-β2-HDL, and pre-β3-HDL (see Ref. 9 for review). Pre-β1-HDL (60–75 kDa), which contains very small quantities of lipids, is a good acceptor of...
Because apoA-I is present in serum in both α-HDL and pre-β-HDL, the present study was performed to determine whether pre-β-HDL stimulates hPL release from primary cultures of normal trophoblast cells and whether serum pre-β-HDL concentrations increase in the maternal serum during gestation. The results suggest a physiological role for pre-β-HDL in the regulation of hPL release during pregnancy.

**MATERIALS AND METHODS**

Serum samples. Serum samples (5–10 ml) were collected from pregnant women attending the Obstetrics Clinic at the University of Cincinnati Hospital. The protocol to obtain the blood samples was approved by the Human Investigation Committees of the University of Cincinnati and the Children's Hospital Medical Center, and permission for each blood sample was obtained from the patient.

Isolation of pre-β-HDL. Pre-β-HDL was isolated from two pools of human serum by agarose (0.6%) gel electrophoresis carried out at 100 V for 4 h. Lot 1 consisted of serum from 15 pregnant women of 36- to 38-wk gestation, and lot 2 consisted of serum from 14 women of 32- to 36-wk gestation. A representative slice of the gel was transferred to a nitrocellulose membrane and subjected to Western blot analysis with a polyclonal antiserum to apoA-I as previously described from our laboratories (29). The areas of the gel corresponding to pre-β-HDL bands were then eluted overnight in PBS at 4°C. The purity of HDL subspecies was confirmed by agarose gel electrophoresis under the same conditions.

Trophoblast cell cultures. The protocol for obtaining placentas was approved by the Human Investigation Committees of the University of Cincinnati and the Children's Hospital Medical Center. Third trimester placentas were obtained from women with normal pregnancies and deliveries, and cytotrophoblast cells were isolated by enzymatic disaggregation and Percoll gradient fractionation and were cultured essentially as described previously (28).

After isolation, the cells were washed in serum-free RPMI-1640 with penicillin (25 U/ml) and streptomycin (25 µg/ml), resuspended in 10% human pregnancy serum (second trimester) in RPMI-1640, counted, and plated at a density of 1 × 10^6/well in 24-well plates (1 ml medium/well). After 8 days in culture in a humidified atmosphere of 5% CO₂-95% air, the cells were washed extensively with RPMI-1640 (28). The cells were then exposed for 0.5 h to medium containing 2% human pregnancy serum with pre-β-HDL at the indicated concentrations. Control cells were exposed to medium containing 2% human pregnancy serum alone. The medium removed from the cells was stored at −20°C before assay. The amounts of hPL in the media and cell homogenates were measured by homologous radioimmunoassays with reagents provided by
the National Pituitary Program. Statistical differences between sample means were calculated by analysis of variance followed by planned orthogonal contrasts or by the Newman-Keuls test, depending on the experimental design of each individual experiment.

Determination of serum pre-β-HDL. The amount of pre-β-HDL in serum was determined by agarose gel electrophoresis. Agarose gel electrophoresis was carried out as described in isolation of pre-β-HDL. After transfer to a nitrocellulose membrane, the apoA-I-containing particles were identified with a biotinylated goat polyclonal antibody to purified human apoA-I kindly provided by Dr. Roger Lallone, Brookwood Biomedical Center, Birmingham, AL. The presence of α-HDL and pre-β-HDL particles was detected with streptavidin alkaline phosphatase. Quantitation was carried out with the Image Quant software. The ratio of pre-β-HDL to α-HDL was calculated. Actual amounts of apoA-I in the particles were estimated with 5 µg of apoA-I as standard.

In selected instances, two-dimensional electrophoresis of plasma samples was performed essentially as described by Asztalos et al. (2). Agarose gel electrophoresis of plasma (2 µl) was performed in one dimension on a 0.7% agarose gel in Tris-tricine buffer (25 mM, pH 8.6), and the lipoproteins were separated by charge. One lane was cut and run in the second dimension on a 4–20% polyacrylamide gel (1.5 mm thick, obtained from Novex) along with a high-molecular-weight marker. The gel was run under native conditions (Tris-glycine buffer, pH 8.3). After the run, the gels were blotted onto nitrocellulose and apoA-I-containing particles were identified with the biotynlated polyclonal antiserum to apoA-I and streptavidin conjugated to alkaline phosphatase.

RESULTS

Effect of pre-β-HDL on hPL release from trophoblast cells. Exposure of trophoblast cells to pre-β-HDL for 0.5 h resulted in a dose-dependent stimulation of hPL release. In the experiments depicted in Fig. 1, the maximal amounts of hPL released by primary cultures of normal trophoblast cells exposed to pre-β-HDL lot 1 and pre-β-HDL lot 2 were 8.2- and 4.3-fold greater than those released by cells exposed to control medium alone (P < 0.01 in each instance). Half-maximal stimulation of hPL release by each lot of pre-β-HDL occurred at an HDL concentration of ~150–350 µg/ml. The magnitude of stimulation of hPL release by pre-β-HDL was comparable to that previously reported for α-HDL (14). The amounts of chorionic gonadotropin released by the trophoblast cells exposed to the two lots of pre-β-HDL were not statistically different from those released by control cells (data not shown).

Pre-β-HDL levels during pregnancy. Because pre-β-HDL stimulates hPL release, experiments were performed to determine whether serum pre-β-HDL concentrations increase during pregnancy. Serum pre-β-HDL concentrations were determined by Western blot analysis, with a polyclonal antiserum to apoA-I, after one-dimensional agarose gel electrophoresis. As shown in Fig. 2, analysis of serum from nonpregnant women and pregnant women from each trimester of pregnancy revealed a progressive increase in pre-β-HDL and α-HDL levels during pregnancy. Pre-β-HDL in the nonpregnant women and in the pregnant women in the first 20 wk of gestation consisted of a single band. However, pre-β-HDL in the pregnant women after week 20 of gestation consisted of two distinct bands. Densitometric analysis of pre-β-HDL bands from 22 pregnant women revealed a linear increase in serum pre-β-HDL concentrations from week 10 of gestation until term (r = 0.88, P < 0.001; Fig. 3). Although α-HDL concentrations also increased during pregnancy, the increase in pre-β-HDL concentrations was not due to a generalized increase in HDL. The pre-β-HDL-to-α-HDL ratio from weeks 10 to 35 increased by sixfold (0.05 at 10 wk to 0.30 at 35 wk; Fig. 4).

Because one-dimensional agarose gels indicated that serum from term pregnant women contained two pre-β-HDL bands, two-dimensional gels were performed to resolve the bands into pre-β-HDL subclasses. As shown in Fig. 5, pre-β-HDL has two bands (bands a and b). In the nonpregnant women, pre-β1-HDL band b is more intense than pre-β2-HDL band a, whereas in pregnant women, pre-β1-HDL band a is more intense than pre-β2-HDL band b. The amounts of pre-β2-HDL (band c) in the serum of the pregnant women (39 wk) increases from 0.6 µg/ml in the nonpregnant women to 17 µg/ml in the pregnant women. The increase in pre-β2-HDL is more intense than the increase in pre-β1-HDL (band a) or α-HDL (band c).

Fig. 3. Pre-β-HDL levels during pregnancy. Pre-β-HDL levels in 22 pregnant women from 10 to 40 wk of gestation were determined by Western blot analysis after agarose electrophoresis, as described in MATERIALS AND METHODS. Amount of pre-β-HDL in each sample was determined by densitometry.
DISCUSSION

During pregnancy, significant changes occur in serum HDL concentrations. HDL cholesterol increases by 30–40%, and total apoA-I concentrations increase by ~48% [130.4 ± 19.1 (SD) mg/dl at 8 wk of gestation to 193.0 ± 32.3 mg/dl at 38 wk] (6). Time-series analysis of hormone and lipid concentrations during gestation reveals that plasma total cholesterol concentrations correlate with plasma hPL, estradiol, and progesterone concentrations throughout the whole period of gestation. Analysis of linear correlation between lipoprotein and hormone concentrations at different weeks of gestation reveals that apoA-I and HDL cholesterol correlate well with plasma hPL, estradiol, and progesterone concentrations (6). In addition, the ratio of HDL₃ to HDL₂ in serum changes, with HDL₃ being the predominant α-HDL subclass in nonpregnant women and HDL₂ being the predominant subclass during pregnancy (31). The results of the present study indicate that pre-β-HDL concentrations also increase during pregnancy, with the greatest increase occurring in pre-β₁-HDL concentrations. The ratio of pre-β-HDL to α-HDL, as determined by Western blot analysis, increased by approximately sixfold from the 10th to the 38th week of gestation. Because the amount of pre-β-HDL in nonpregnant women has been reported to be ~60 ± 30 (SD) µg/ml (24), our results indicate that pre-β-HDL concentrations near term are in the range of 200–450 µg/ml.

Several lines of evidence suggest a physiological role for pre-β-HDL in the regulation of hPL secretion during pregnancy. Pre-β-HDL, like α-HDL, stimulates hPL at concentrations within the normal physiological range for pregnancy. Half-maximal stimulation of hPL release by pre-β-HDL occurs at a concentration of 150–350 µg/ml. In addition, pre-β-HDL levels increase during pregnancy with a pattern nearly identical to those for hPL. Because the relative increase in serum pre-β-HDL concentrations during pregnancy is much greater than the relative increase in α-HDL concentrations, pre-β-HDL, mainly in the form of pre-β₁-HDL

sample were greater than in the nonpregnant women plasma sample. Pre-β₁-HDL, which is present in serum in only trace amounts, was not detected in either group of women. Serum from the nonpregnant women contained both HDL₁ (in the range of 12.2–8.8 nm stokes diameter) and HDL₃ (in the range of 8.8–7.2 nm stokes diameter) subspecies (25), whereas serum from a woman at 39 wk of gestation contained a major HDL₂ band and some other minor subspecies. A significant decrease in the HDL₃ band was apparent. A similar decrease in HDL₃ during pregnancy has been reported previously (31).

The pattern of pre-β-HDL concentrations during pregnancy paralleled almost exactly the pattern of serum placental lactogen concentrations over the same time interval (Fig. 6). The r value for the regression line of placental lactogen vs. pre-β-HDL was 0.93, and the P value was 0.002.
(Fig. 5, band a), may also be important in the regulation of hPL release during pregnancy.

Previous studies from our laboratories have examined the molecular and cellular mechanisms involved in apoA-I-mediated hPL release. Both the cAMP and phosphoinositide hydrolysis signal transduction pathways were shown to be involved in apoA-I-mediated hPL release (35, 36). However, the mechanisms by which apoA-I acts at the level of the plasma membrane to activate adenylyl cyclase and phospholipase C are unknown. Although trophoblast cells have high-affinity binding sites for HDL, these binding sites are not important for HDL action (19). Studies with synthetic amphipathic peptides that mimic the action of apoA-I on hPL release strongly suggest, however, that binding of apoA-I to membrane phospholipids that results in a change in the conformation of apoA-I is critical for apoA-I action (18).

As indicated earlier, HDL stimulates hPL synthesis by inducing gene expression (12). However, the cis-acting elements on the hPL promoter and the transcription factors involved in apoA-I-induced hPL gene expression are poorly understood. Preliminary studies from our laboratory suggest that the action of apoA-I on the hPL promoter is mediated, at least in part, by two regions of the promoter-containing composite nuclear hormone receptor response elements (32). At present, the hormonal and other factors that mediate the increase in pre-β-HDL concentrations during pregnancy are unknown. Studies by several groups strongly suggest that the changes in α-HDL concentrations and the relative distribution of the α-HDL subclasses are due to the increase in estrogen concentrations (6, 23). Although it is not known whether the increase in the concentration of apoA-I is due to an increased synthesis or decreased clearance of apoA-I, a study in Hep G2 cells (1) suggests that the effect of estrogen is due to an increase in apoA-I gene expression. Because the increase in plasma pre-β-HDL and the increase in estrogen concentrations during pregnancy have a similar pattern, it is possible that the changes in pre-β-HDL concentrations and the distribution of the pre-β-HDL subclasses during pregnancy may also be due to estrogen.

Aberrations in hPL secretion have been detected in several pathological conditions of pregnancy associated with intrauterine growth retardation, including pre-eclampsia, pregnancy-induced hypertension, and diabetes (see Ref. 12 for review). Although it is probable that many factors contribute to the abnormal hPL concentrations observed in these conditions of pregnancy, several studies suggest that abnormal apoA-I concentrations may contribute to the aberrations in hPL concentrations. In an earlier study, Rosing and et al. (30) noted decreased serum apoA-I concentrations in pre-eclampsia. Kaaja et al. (20) also noted decreased apoA-I concentrations in women with pregnancy-induced hypertension, and Knopp et al. (21) noted lower than normal apoA-I concentrations in pregnant women with insulin-dependent diabetes mellitus. In the women with diabetes, apoA-I concentration did not increase from 12 to 28 wk of gestation, whereas apoA-I concentrations in normal women increased by 20%. However, studies have not as yet been performed to determine whether the decrease in hPL concentrations in the pathological conditions of pregnancy is associated with a decrease in pre-β-HDL concentrations.

Studies over the past few years indicate that apoA-I has a number of biological actions other than stimulating hPL release, including stimulation of endothelial cell proliferation (5) and endothelin-1 production by renal cells (27), and that it decreases neutrophil degranulation and superoxide production (3). Because pre-β-HDL stimulates hPL release, it is possible that pre-β-HDL is important in mediating the other biological actions attributed to apoA-I.

In conclusion, the results of this study indicate that physiological concentrations of pre-β-HDL stimulate the release of hPL from trophoblast cells. Because maternal serum pre-β-HDL concentrations increase markedly during pregnancy with a pattern that closely parallels that of placental lactogen, these results suggest that pre-β-HDL may also be important for regulation of hPL release during pregnancy.

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