Longitudinal Study of Plasma Lipoproteins and Hormones During Pregnancy in Normal and Diabetic Women

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Plasma lipoproteins were studied longitudinally at the 1st, 2nd, and 3rd trimester of gestation and at postpartum and postlactation in 12 age-matched PGDM women, 9 GDM women, and 12 healthy control subjects. FPG and HbA_{1c} were higher in every case in PGDM women than in control subjects, whereas in GDM patients, glucose was augmented only after parturition. FFA and β-hydroxybutyrate levels were higher in both PGDM and GDM patients than in control subjects during gestation but not after parturition. Total TGs and VLDL, LDL, and HDL TGs increased with gestational time in the three groups and declined at postpartum, and although total cholesterol and VLDL, LDL, and HDL cholesterol followed a similar trend, their rise was less pronounced, and the decline after parturition was slower than that of the TGs in the three groups, with no difference among them. The VLDL TG/cholesterol ratio declined in the three groups at the 3rd gestational trimester, whereas in both LDL and HDL, the TG/cholesterol ratio, but not the cholesterol/phospholipid ratio, increased during gestation in the three groups, indicating a specific enrichment of TGs in these particles. The increase in apoA-I and apoB with gestation was parallel to the respective changes in HDL and LDL cholesterol and, again, no difference was observed between the three groups. Plasma levels of β-estradiol, progesterone, and prolactin increased sharply with gestation and declined at postpartum in the three groups, but absolute values of β-estradiol and prolactin, at the three trimesters of gestation, were lower in PGDM patients, but progesterone levels were lower than controls in GDM women only at the 3rd trimester. The logarithm for each of these hormones correlated linearly with VLDL, LDL, and HDL TGs, and the highest correlation coefficient value corresponded to the regression between β-estradiol and HDL TGs. Because estrogens are known to increase VLDL production, decrease hepatic lipase activity, and increase HDL TG levels, we propose that the decreased estradiol levels in our diabetic patients impede an exaggerated rise of circulating lipoproteins above the normal range. We also propose that the development or lack of development of a dyslipidemic condition in diabetic pregnancy depends on the balance between the metabolic control and the level of sex hormones.
***Diabetes* 41:1651–59, 1992**

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Received for publication 20 March 1992 and accepted in revised form 23 July 1992.

PGDM, pregestational diabetes mellitus; GDM, gestational diabetes mellitus; FPG, fasting plasma glucose; FFA, free fatty acid; TG, triglyceride; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; apo, apolipoprotein; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; OGTT, oral glucose tolerance test; BMI, body mass index; $_2$-EDTA, ethylenediamine tetraacetic acid, disodium salt; CV, coefficient of variation; HPLC, high-performance liquid chromatography; ANOVA, analysis of variance; NS, not significant.

Hyperlipidemia is a common feature in normal pregnancy and consists primarily of TGs, with smaller rises in phospholipids and cholesterol (1,2). More recently, it has been shown clearly that increments in plasma TGs during late gestation are found in all the lipoprotein fractions, whereas the changes in cholesterol content are more moderate (3–6). Diabetes also is generally associated with disturbances in lipoprotein metabolism with a tendency toward hypertriglyceridemia rather than hypercholesterolemia (7–9). Summatory or synergistic effects therefore would be expected when both diabetes and pregnancy are associated. Exaggerated increments in plasma TGs rather than cholesterol have been found in diabetic pregnancy (10–12), although the effect varies depending on the lipoprotein fraction considered and the type of diabetes, and conditions do exist wherein diabetic pregnancy seems to be associated with normal TG levels (13–15)

and even decreased cholesterol levels (11). The reason for this variation is not known. Because it is known that most of the lipoprotein changes that occur during gestation may be correlated with plasma levels of gestational hormones (5,16), and that estrogen effects on lipoprotein metabolism mimic many of the changes found in gestation (6,17), it has been proposed that all of these parameters are interrelated during normal gestation (6).

Important interspecies differences have been observed in the metabolism of lipoproteins and even in the degree and direction of the response to pregnancy insofar as lipoprotein levels are concerned. In the rhesus monkey, plasma TGs decline and then rise, but total cholesterol, LDL cholesterol, and HDL cholesterol fall throughout gestation (18–20), whereas in the rat, HDL cholesterol does not increase, and LDL cholesterol increases slightly, although its levels remain much lower than in humans (21,22). These interspecies differences force us to circumscribe the studies on the pathophysiology of lipoprotein metabolism in diabetic pregnancy to women. Thus, we conducted a longitudinal study on the level of major plasma lipoproteins, β-estradiol, progesterone, and prolactin levels in normal women and in women with PGDM or from GDM.

RESEARCH DESIGN AND METHODS

The participants in this study were 33 pregnant women: 12 were known PGDM patients, 9 were GDM patients, and 12 were healthy women who were considered control subjects. Under the criterium of basal plasma C-peptide levels, 10 of the PGDM patients were considered to have IDDM (C-peptide <0.2 nM) and 2 were considered to have NIDDM. All of these PGDM patients received insulin treatment before and during pregnancy and had a previous history of diabetes ranging from 4 to 19 yr. GDM patients were diagnosed at the 1st gestational trimester from those who had previous clinical and obstetric histories suggesting diabetes (i.e., familiar antecedents, advanced maternal age, macrosomia) and on the basis of a glucose challenge test (50 g) and an OGTT (100 g), following the criterium of the 2nd International Workshop Conference on Gestational Diabetes Mellitus (23). They all presented normal glucose tolerance at postpartum. All GDM patients except 2 required insulin therapy during gestation, but none required insulin therapy before or after gestation. All of the studied subjects lactated postpartum.

Anthropometric and laboratory characteristics of these patients are summarized in Tables 1 and 2 respectively. The subjects' ages, BMI (in kg/m^2) before pregnancy, and body weight increases with gestation were quite similar among the three groups (Table 1). In normal women, FPG levels were lower at the 3rd trimester than at any other gestational trimester or at postpartum (Table 2). In PGDM patients, plasma glucose levels were always higher than in normal subjects, and values attained the highest level after the end of the lactational period (postlactation). In GDM patients, FPG levels during gestation were similar to those in normal subjects, but after parturition they increased to significantly higher levels

TABLE 1
Age and BMI before pregnancy and total weight increment during pregnancy in normal and diabetic women

	Age (yr)	BMI (kg/m^2)	Weight increment (kg)
Normal control subjects	28.7 ± 1.3	22.37 ± 0.35	9.50 ± 0.12
PGDM women	29.0 ± 1.3	22.20 ± 0.28	9.08 ± 0.10
GDM women	30.8 ± 2.1	23.27 ± 0.64	9.17 ± 0.14

Values are means ± SE. Statistical comparison between the groups was not significant ($P > 0.05$) for all parameters.

(Table 2). Plasma HbA_{1c} concentrations were measured during gestation. As shown in Table 2, in PGDM patients, HbA_{1c} concentrations were slightly but significantly higher than in normal subjects during gestation, whereas values in GDM patients did not differ from normal control subjects.

All women who had given informed consent were seen during the 1st (wk 9–10), 2nd (wk 21–23), and 3rd trimesters of gestation (wk 32–34), and at 2–4 wk after parturition (postpartum) and at postlactation. Venous blood samples were obtained from seated subjects after a 12-h overnight fast—just before the first morning insulin in the case of the diabetic patients—in tubes containing 1.0 mg/ml of Na_2-EDTA. After centrifugation, an aliquot of plasma was frozen at −80°C for hormone, apo, and metabolite measurements, and another aliquot was subjected immediately to sequential ultracentrifugation in a Ti 50 Beckman rotor (Palo Alto, CA). VLDLs were floated at 45,000 rpm for 18 h at d = 1.006 g/ml. The infranatant was brought to d = 1.063 with solid KBr and subjected to ultracentrifugation at 47,000 rpm for 20 h for isolation of LDL; the last infranatant was brought to d = 1.21 with KBr and ultracentrifugated at 47,000 rpm for 44 h. Floating supernatants were recovered by tube slicing, and, after proper dilution, were used for triacylglycerol, cholesterol, and phospholipid measurements with a Hitachi 705 autoanalyzer (Boehringer Mannheim, Mannheim, Germany).

All hormones were measured using commercially available RIA kits following the protocols given by the manufacturers as follows: prolactin (kit Allegro PRL (Nichols Institute), 17-β-estradiol (Sorin Biomedica), progesterone (ICN, Irvine, CA). The interassay CV was <9% for all hormone determinations. ApoA-I and apoB were measured by immunonephelometry (ICS nephelometer, Beckman). FFAs were measured enzymatically using the commercial kit from Wako Chemicals. β-hydroxybutyrate also was measured enzymatically (24) in deproteinized plasma samples (25). Plasma glucose was measured enzymatically with a Hitachi 737 autoanalyzer (Boehringer Mannheim), and HbA_{1c} was measured by HPLC using a Daiichi HA-8110 autoanalyzer (Tokyo, Japan) (26).

An aliquot of VLDL was delipidated with ether-acetone (1:1) washings, and the apo were separated by isoelectrofocusing at pH range 4–6.5 (27). After staining with Coomassie G-250, the gel was subjected to densitomet-

TABLE 2
FPG and HbA_{1c} in normal and diabetic women during pregnancy, postpartum, and postlactation

	FPG (mM)	HbA_{1c} (%)
Normal control subjects		
1st trimester	4.44 ± 1.11 (ab)	4.50 ± 0.36 (a)
2nd trimester	4.65 ± 0.14 (ab)	4.30 ± 0.43 (a)
3rd trimester	4.40 ± 0.13 (a)	4.40 ± 0.27 (a)
Postpartum	4.86 ± 0.09 (b)	
Postlactation	4.86 ± 0.18 (b)	
PGDM women		
1st trimester	8.84 ± 1.24 (a)*	6.43 ± 0.26 (a)†
2nd trimester	8.77 ± 0.67 (a)†	5.54 ± 0.20 (b)‡
3rd trimester	8.70 ± 1.07 (ab)†	5.52 ± 0.17 (b)*
Postpartum	8.39 ± 1.24 (a)†	
Postlactation	15.04 ± 1.12 (b)†	
GDM women		
1st trimester	5.01 ± 0.48 (ab)	5.37 ± 0.37 (a)
2nd trimester	4.91 ± 0.27 (a)	4.83 ± 0.18 (a)
3rd trimester	4.63 ± 0.23 (a)	4.90 ± 0.18 (a)
Postpartum	5.78 ± 0.24 (b)*	
Postlactation	5.58 ± 0.24 (ab)‡	

Values are means ± SE. Letters in parenthesis correspond to statistical comparison by Student's *t* dependent test between the groups at each of the gestational or postgestational stages studied: the same letter within one parameter means no statistical difference between the groups, whereas different letters indicate significant differences between the corresponding groups ($P \leq 0.05$). Statistical comparisons of PGDM or GDM women versus control subjects were calculated by Student's *t* independent test.
*$P < 0.01$.
†$P < 0.001$.
‡$P < 0.05$.

ric analysis, and the peak areas of the isoforms of apoE, apoC-II, and apoC-III were added together. The value for each apo included all the corresponding isoforms and was expressed as a percent of the total summed area.

Statistical analyses. Results are given as means ± SE. Data were analyzed using the Statgraphics 5.0 program by independent Student's *t* test, by ANOVA of one factor, and multiple range analysis following the *t* of Schaeffe. Simple regressions were analyzed by means of Pearson's *r* correlation coefficient for lineal ($y = a + bx$), semilogarithmic ($y = a + b.\log x$), and multiplicative ($y = ax^b$) variations.

RESULTS

As shown in Table 3, plasma levels of FFAs and β-hydroxybutyrate did not change with gestation in normal control women. In both PGDM and GDM women, these two parameters also were kept stable through gestation, but values in both groups were significantly higher than in normal control subjects during all trimesters of gestation, although not at postpartum. Also, as shown in Table 3, plasma TG levels progressively increased with gestation among the groups and began to fall rapidly after parturition, attaining values at 2–4 wk of postpartum that are not different from those in nonpregnant and nonlactating conditions (postlactation). Plasma cholesterol levels also rose progressively with gestational time among the three groups—but differently from TGs—and values remained elevated at 2–4 wk postpartum (Table 3). No difference in TGs or cholesterol levels was detected between any of the diabetic women and control subjects at any of the time points studied.

Table 4 summarizes the levels of lipidic moieties in the three major lipoprotein fractions at various points during gestation and postpartum in the three groups. Both VLDL TGs and VLDL cholesterol increased progressively with gestation among the groups, attaining their highest level at the 3rd trimester and fell at 2–4 wk postpartum when they reached nonpregnant levels. LDL TG and LDL cholesterol levels also increased with gestational time in all three groups. Although LDL TGs showed a clear decline at postpartum, LDL cholesterol values remained elevated at 2–4 wk postpartum and did not reach the values found at the 1st gestational trimester until postlactation, with no differences among the groups. The changes in LDL phospholipids were similar to those in LDL cholesterol, increasing progressively as gestational time advanced and declining slowly after parturition. As shown in Table 4, HDL TGs progressively increased in the three groups with gestational time and returned to values found at the 1st trimester just after parturition. However, HDL cholesterol levels increased from the 1st to the 2nd gestational trimester, but declined slightly at the 3rd trimester, and this tendency was maintained until postlactation, with no difference among the three groups. Practically no change in HDL phospholipids was observed during gestation in the three groups (Table 4).

Nonparallel changes in TG and cholesterol concentrations forced us to calculate their respective ratios in the different lipoprotein fractions. As shown in Table 5, the TG/cholesterol ratio in VLDL decreased slightly at the 3rd trimester and recovered at postpartum in the three groups with no significant differences among them. In contrast, the LDL TG/cholesterol ratio increased progressively as gestational time advanced, but it decreased at postpartum to the same values as at postlactation; here

TABLE 3
Plasma lipidic components in normal and diabetic women during pregnancy, postpartum, and postlactation

	FFA (μM)	β-OH-butyrate (μM)	TG (mM)	Cholesterol (mM)
Normal control subjects				
1st trimester	353.1 ± 49.1 (a)	74.8 ± 36.6 (a)	0.68 ± 0.10 (a)	4.48 ± 0.18 (a)
2nd trimester	328.2 ± 35.4 (a)	76.4 ± 11.3 (a)	1.17 ± 0.10 (b)	6.02 ± 0.21 (b)
3rd trimester	314.1 ± 33.9 (a)	110.6 ± 43.6 (a)	2.03 ± 0.26 (c)	6.69 ± 0.36 (c)
Postpartum	355.5 ± 44.1 (a)	84.1 ± 33.6 (a)	0.95 ± 0.18 (ab)	5.86 ± 0.33 (b)
Postlactation	319.6 ± 36.0 (a)	78.1 ± 21.8 (a)	0.62 ± 0.06 (a)	4.81 ± 0.35 (a)
PGDM women				
1st trimester	599.8 ± 83.2 (a)*	455.3 ± 114.5 (a)†	0.58 ± 0.05 (a)	4.18 ± 0.16 (a)
2nd trimester	483.8 ± 62.2 (a)*	378.1 ± 115.3 (a)*	1.22 ± 0.10 (b)	5.81 ± 0.36 (b)
3rd trimester	545.1 ± 54.8 (a)†	366.5 ± 95.5 (a)*	1.92 ± 0.20 (c)	6.21 ± 0.45 (b)
Postpartum	554.6 ± 120.5 (a)	313.3 ± 126.6 (a)	0.85 ± 0.08 (d)	6.09 ± 0.40 (b)
Postlactation	457.1 ± 107.3 (a)	566.8 ± 204.9 (a)	0.68 ± 0.09 (acd)	4.82 ± 0.20 (ab)
GDM women				
1st trimester	642.4 ± 63.9 (a)‡	444.9 ± 97.9 (a)‡	0.87 ± 0.10 (a)	4.67 ± 0.37 (a)
2nd trimester	474.6 ± 38.5 (bd)*	370.3 ± 116.8 (ab)†	1.54 ± 0.22 (b)	5.90 ± 0.35 (ab)
3rd trimester	499.6 ± 36.3 (ab)†	348.3 ± 101.3 (ab)*	2.06 ± 0.23 (c)	6.30 ± 0.33 (b)
Postpartum	319.7 ± 30.9 (c)	50.0 ± 7.3 (c)	1.05 ± 0.15 (ab)	6.07 ± 0.35 (ab)
Postlactation	371.9 ± 23.8 (d)	49.5 ± 11.6 (abc)	0.99 ± 0.23 (abc)	5.46 ± 1.39 (ab)

Values are means ± SE. Letters in parenthesis correspond to statistical comparison by Student's *t* or postgestational stages studied: the same letter within one parameter means no statistical difference between the groups, whereas different letters indicate significant differences between the corresponding groups ($P \leq 0.05$). Statistical comparisons of PGDM or GDM women versus control subjects were calculated by Student's *t* independent test.
*$P < 0.05$.
†$P < 0.01$.
‡$P < 0.001$.

again, no difference was noted among the three groups (Table 5). This change seems to be the result of a specific enrichment in TGs in the LDL particle because, also as shown in Table 5, the LDL cholesterol/phospholipid ratio remained stable throughout gestation in the three groups. A progressive and intense increase in the HDL TG/cholesterol ratio with gestation also was seen across the three groups, and values at postpartum and postlactation always were below those of any of the gestational times studied (Table 5). Here again, the HDL cholesterol/phospholipid ratio did not change at any of the time points studied in any of the groups, indicating that the compositional change with gestation in HDLs corresponded specifically to an enrichement in TGs.

To determine whether gestational variations in the lipoprotein lipidic composition also were followed by changes in the apo content, isoelectrophocusing separation was conducted in isolated VLDL proteins. The apoE content ranged between 16.6 and 24.7%, apoC-III content between 68.3 and 71.4%, and apoC-II between 5.7 and 15.1%, when the total optical density of these three apos was considered as 100%, but no difference could be found in these percentages in the course of gestation, nor postpartum, nor between the three groups (data not shown).

Table 6 summarizes the apoA-I and apoB plasma concentrations of the groups. Both apos increased with gestation and returned to postlactating values just after parturition, although the change was greater for apoB than apoA-I. No difference could be found in either of these parameters between the three groups.

To determine the relationship between these apo concentrations and the concentration of lipidic components in the lipoprotein fractions in all samples from the three groups and at various times of gestation and postpartum, linear regressions were determined when plotting all individual values. As shown in Table 7, although the plasma apoB concentration was correlated significantly with VLDL cholesterol, VLDL TGs, LDL cholesterol, and LDL TGs, the best fit was found for LDL cholesterol with a correlation coefficient of 0.8, indicating a tight relationship between these two parameters. ApoA-I correlated significantly with both HDL cholesterol and HDL TGs, although the fit was better for the former (Table 7).

Because most of the lipoprotein metabolism changes that occur during gestation are driven by sex hormone variations, the plasma levels of β-estradiol, progesterone, and prolactin also were determined. As shown in Table 8, plasma levels of β-estradiol increase intensely with gestation, attaining the highest value at the 3rd trimester, and sharply fell at postpartum; this trend was repeated in all three groups. Values of β-estradiol levels during gestation were, however, always lower in diabetic women than in normal women—the difference being significant between PGDM women and normal control subjects at the 1st and 2nd trimester, but not at the 3rd trimester, nor between GDM women and control subjects at any of the time points studied (Table 8).

Progesterone levels also increased progressively with gestation in the three groups, but declined sharply after parturition. Progesterone levels during gestation also were lower in GDM women than in control women, although the difference was significant only at the 3rd trimester (Table 8). Prolactin levels also increased progressively with gestation and declined at postpartum,

although unlike the other hormones, its values did not reach basal levels (e.g. those normally seen in nonpregnant women) until postlactation, and these sequential changes were similar among the three groups (Table 8). Prolactin levels were, however, lower in diabetic women than in control women, although the difference was statistically significant at the trimesters of gestation in the PGDM women but not in the GDM women (Table 8).

Because some of the sex hormone changes in the course of gestation paralleled the changes in plasma lipoprotein TG levels, the semilogarithmic linear correlations between these parameters in all samples studied were calculated individually. As shown in Table 9, the correlation was significant for the three hormones studied (prolactin, estradiol, and progesterone) versus VLDL, LDL, and HDL TGs. The highest linear correlation coefficient value was found for the three hormones, however, when they were plotted against HDL TGs rather than when they were compared with the other lipoproteins, and the greatest r value was noted for HDL TGs versus estradiol ($r = 0.748$).

DISCUSSION

In addition to demonstrating the longitudinal changes of lipids, lipoproteins, apos, and three pregnancy-related hormones at various stages of gestation in healthy women, this study also describes the effect of both PGDM and GDM on these parameters. In agreement with previous studies (3–6,28), it becomes apparent that pregnancy causes increments in both plasma TGs and cholesterol that correspond to increments in all lipoprotein fractions. The increase in plasma TGs with gestational time is greater and the return to normalcy after parturition faster than in cholesterol. Although it would be expected that this change would correspond mainly to VLDLs, which are the main TG carrier lipoproteins under normal fasting conditions (29), the increase in TG content during late gestation appeared especially striking in both LDL and HDL, the particles of which became proportionally enriched in TGs compared with any of the other components. Although a proportional enrichment in TGs in both LDL and HDL at late gestation have been observed by other researchers (3,4,6), the physiological significance of this phenomenon has not been emphasized.

The increased VLDL synthesis induced by high estrogen levels may be responsible for the increase in circulating VLDLs during pregnancy because on the one hand, it has been shown that endogenous production of TG-rich lipoproteins is enhanced in pregnancy (30,31) and, on the other, that high estrogen levels have been shown to increase VLDL production (32). The positive correlation found between the VLDL TG level, β-estradiol levels, and prolactin levels agrees with this possibility, although the correlation of prolactin is more likely to be secondary to the changes in plasma estrogen because prolactin secretion is stimulated by estrogen (33).

A decreased removal of VLDL TG from plasma caused by decreased lipoprotein lipase activity also could mediate a progressive increment in the plasma level of these

TABLE 4
Plasma lipoprotein lipids in normal and diabetic women during pregnancy, postpartum, and postlactation

	VLDL TG (mM)	VLDL cholesterol (mM)	LDL TG (mM)	LDL cholesterol (mM)	LDL phospholipid (mM)	HDL TG (mM)	HDL cholesterol (mM)	HDL phospholipid (mM)
Normal control subjects								
1st trimester	0.25 ± 0.09 (a)	0.10 ± 0.03 (a)	0.21 ± 0.02 (a)	2.82 ± 0.18 (a)	0.71 ± 0.06 (a)	0.16 ± 0.01 (a)	1.55 ± 0.13 (acd)	1.49 ± 0.10 (ad)
2nd trimester	0.36 ± 0.06 (ab)	0.15 ± 0.03 (ac)	0.43 ± 0.05 (b)	4.11 ± 0.15 (b)	1.09 ± 0.09 (b)	0.27 ± 0.03 (b)	2.00 ± 0.20 (b)	1.87 ± 0.14 (b)
3rd trimester	0.80 ± 0.20 (b)	0.36 ± 0.09 (b)	0.74 ± 0.09 (c)	4.57 ± 0.33 (c)	1.35 ± 0.09 (c)	0.34 ± 0.02 (b)	1.76 ± 0.13 (ab)	1.72 ± 0.10 (bc)
Postpartum	0.43 ± 0.14 (a)	0.17 ± 0.06 (a)	0.32 ± 0.02 (d)	4.20 ± 0.31 (bc)	1.27 ± 0.06 (c)	0.14 ± 0.01 (a)	1.48 ± 0.08 (c)	1.55 ± 0.07 (cd)
Postlactation	0.28 ± 0.06 (a)	0.14 ± 0.02 (c)	0.19 ± 0.02 (a)	3.17 ± 0.28 (a)	0.82 ± 0.10 (a)	0.12 ± 0.01 (a)	1.28 ± 0.07 (d)	1.31 ± 0.06 (a)
PGDM women								
1st trimester	0.18 ± 0.03 (a)	0.08 ± 0.01 (a)	0.19 ± 0.02 (a)	2.53 ± 0.16 (a)	0.67 ± 0.05 (a)	0.15 ± 0.01 (a)	1.57 ± 0.09 (a)	1.48 ± 0.09 (a)
2nd trimester	0.40 ± 0.05 (b)	0.14 ± 0.02 (b)	0.48 ± 0.03 (b)	3.74 ± 0.29 (b)	1.12 ± 0.10 (b)	0.29 ± 0.03 (b)	1.93 ± 0.12 (b)	1.84 ± 0.10 (b)
3rd trimester	0.91 ± 0.14 (c)	0.46 ± 0.08 (c)	0.62 ± 0.06 (c)	4.01 ± 0.36 (bc)	1.23 ± 0.12 (c)	0.32 ± 0.03 (b)	0.77 ± 0.12 (c)	1.70 ± 0.10 (bc)
Postpartum	0.33 ± 0.06 (b)	0.13 ± 0.02 (ab)	0.34 ± 0.03 (d)	4.50 ± 0.36 (c)	1.26 ± 0.10 (b)	0.14 ± 0.02 (a)	1.46 ± 0.12 (ac)	1.50 ± 0.09 (ac)
Postlactation	0.33 ± 0.10 (abc)	0.13 ± 0.04 (abc)	0.22 ± 0.04 (abcd)	3.29 ± 0.22 (abc)	0.87 ± 0.09 (ab)	0.09 ± 0.02 (a)	1.40 ± 0.11 (a)	2.58 ± 0.08 (a)
GDM women								
1st trimester	0.44 ± 0.05 (a)	0.14 ± 0.03 (ab)	0.31 ± 0.04 (ac)	3.07 ± 0.26 (a)	0.89 ± 0.09 (a)	0.17 ± 0.04 (a)	1.46 ± 0.21 (a)	1.43 ± 0.20 (a)
2nd trimester	0.65 ± 0.14 (b)	0.28 ± 0.08 (a)	0.51 ± 0.04 (b)	4.07 ± 0.28 (ab)	1.15 ± 0.08 (a)	0.28 ± 0.04 (b)	1.55 ± 0.13 (a)	1.55 ± 0.11 (a)
3rd trimester	0.84 ± 0.17 (ab)	0.48 ± 0.10 (b)	0.64 ± 0.08 (ab)	4.29 ± 0.33 (ab)	1.15 ± 0.10 (a)	0.36 ± 0.04 (c)	1.52 ± 0.16 (a)	1.51 ± 0.12 (a)
Postpartum	0.59 ± 0.13 (ab)	0.21 ± 0.05 (a)	0.27 ± 0.03 (c)	4.37 ± 0.35 (b)	1.18 ± 0.12 (a)	0.12 ± 0.01 (a)	1.48 ± 0.16 (a)	1.47 ± 0.12 (a)
Postlactation	0.56 ± 0.18 (ab)	0.21 ± 0.07 (ab)	0.26 ± 0.06 (abc)	4.04 ± 1.27 (ab)	1.27 ± 0.06 (a)	0.12 ± 0.02 (abc)	1.20 ± 0.05 (a)	1.27 ± 0.12 (a)

Values are means ± SE. Letters in parenthesis correspond to statistical comparison by Student's t dependent test between the groups at each of the gestational or postgestational stages studied; the same letter within one parameter means no statistical difference between the groups, whereas different letters indicate significant differences between the corresponding groups ($P \leq 0.05$). Statistical comparisons of PGDM or GDM women versus control subjects were calculated by Student's t independent test and were NS ($P > 0.05$) in all cases.

TABLE 5
Plasma lipoprotein TG/cholesterol and cholesterol/phospholipid ratios in normal and diabetic women during pregnancy, postpartum, and postlactation

	VLDL TG/ cholesterol	LDL TG/ cholesterol	LDL cholesterol/ phospholipid	HDL TG/ cholesterol	HDL cholesterol/ phospholipid
Normal control subjects					
1st trimester	2.49 ± 0.13 (ab)	0.10 ± 0.00 (a)	3.90 ± 0.08 (ab)	0.09 ± 0.01 (a)	1.47 ± 0.02 (a)
2nd trimester	2.44 ± 0.22 (abc)	0.13 ± 0.01 (b)	3.72 ± 0.05 (a)	0.14 ± 0.01 (b)	1.42 ± 0.02 (a)
3rd trimester	2.23 ± 0.22 (ac)	0.18 ± 0.01 (c)	3.77 ± 0.08 (a)	0.20 ± 0.02 (c)	1.40 ± 0.02 (a)
Postpartum	2.97 ± 0.35 (b)	0.09 ± 0.00 (a)	3.93 ± 0.13 (ab)	0.07 ± 0.01 (d)	1.42 ± 0.26 (a)
Postlactation	2.01 ± 0.17 (c)	0.07 ± 0.00 (a)	4.03 ± 0.05 (b)	0.06 ± 0.01 (d)	1.55 ± 0.05 (a)
PGDM women					
1st trimester	2.49 ± 0.22 (ab)	0.09 ± 0.00 (a)	3.96 ± 0.08 (a)	0.07 ± 0.00 (a)	1.65 ± 0.05 (a)*
2nd trimester	2.92 ± 0.22 (a)	0.15 ± 0.00 (b)	3.80 ± 0.02 (ab)	0.13 ± 0.01 (b)	1.47 ± 0.02 (b)
3rd trimester	2.14 ± 0.13 (b)	0.18 ± 0.00 (c)	3.70 ± 0.05 (b)	0.17 ± 0.01 (c)	1.40 ± 0.02 (b)
Postpartum	2.57 ± 0.17 (ab)	0.09 ± 0.00 (a)	4.03 ± 0.10 (a)	0.06 ± 0.01 (d)	1.47 ± 0.02 (ab)
Postlactation	2.57 ± 0.17 (ab)	0.08 ± 0.01 (a)	4.14 ± 0.23 (ab)	0.04 ± 0.00 (abcd)	1.55 ± 0.02 (b)
GDM women					
1st trimester	2.71 ± 0.30 (a)	0.11 ± 0.01 (a)	3.80 ± 0.08 (ab)	0.08 ± 0.02 (ac)	1.50 ± 0.05 (a)
2nd trimester	2.44 ± 0.22 (a)	0.15 ± 0.01 (b)	3.70 ± 0.02 (ab)	0.15 ± 0.02 (b)	1.47 ± 0.02 (a)
3rd trimester	1.79 ± 0.13 (b)	0.18 ± 0.01 (c)	3.83 ± 0.10 (a)	0.19 ± 0.02 (bc)	1.50 ± 0.05 (a)
Postpartum	2.79 ± 0.13 (a)	0.07 ± 0.01 (d)	3.90 ± 0.08 (ab)	0.06 ± 0.01 (a)	1.52 ± 0.08 (a)
Postlactation	2.62 ± 0.22 (ab)	0.07 ± 0.01 (abcd)	3.98 ± 0.02 (b)	0.10 ± 0.02 (abc)	1.50 ± 0.02 (a)

Values are means ± SE. Letters in parenthesis correspond to statistical comparison by Student's *t* dependent test between the groups at each of the gestational or postgestational stages studied: the same letter within one parameter means no statistical difference between the groups, whereas different letters indicate significant differences between the corresponding groups ($P \leq 0.05$). Statistical comparisons of PGDM or GDM women versus control subjects were calculated by Student's *t* independent test. *$P < 0.01$.

particles. However, we recently observed that, once corrected by endogenous substrate, the change in post-heparin LPL during gestation occurs after the rise of plasma VLDL TGs and is much smaller than previously thought (34); therefore, it could not justify such intense increment in VLDL TGs.

Elevations in LDL concentration at mid and late pregnancy may initially be a secondary consequence of enhanced conversion of VLDL because of its abundance, but its specific enrichment in TGs would require another explanation. First, the presence of some remnant particle (IDL) cannot be ruled out because the density range used for LDL isolation was 1.006–1.063, and a two- to threefold TG elevation in the density 1.019-1.063 corresponding to an IDL-rich fraction has been observed previously in healthy women at late gestation (35). Second, an eventual increase in the interchange of neutral lipids between lipoproteins could contribute to the increase of TGs in LDL, but the activity of cholesteryl ester transfer protein has not yet been determined in human pregnancy. Third, the accumulation of TGs in LDL may be a consequence of, or related to, a decrease in hepatic lipase activity because a similar accumulation of TGs in LDL and HDL but not in VLDL particles has been observed previously in patients suffering from hepatic lipase deficiency (36), and it is known that LDL TGs are substrates for this enzyme (37). In addition, pregnant women are known to exhibit low hepatic lipase activity (16), and this may even be responsible for a reduction in the conversion of HDL_2 TGs into HDL_3, leading to an accumulation of the former. It is currently known that HDL_2 rather than HDL_3 is most responsible for the increment in HDL during gestation (3,6). An exaggerated accumulation of TGs in HDL would facilitate the neutral lipid transfer protein-mediated transfer of TGs to LDL and, therefore, could be responsible for the accumulation of TGs in these particles.

TABLE 6
Plasma A-I and B apoproteins in normal and diabetic women during pregnancy, postpartum, and postlactation

	Apo A-I (g/L)	Apo B (g/L)
Normal control subjects		
1st trimester	1.31 ± 0.08 (a)	0.62 ± 0.04 (a)
2nd trimester	1.54 ± 0.12 (ab)	0.87 ± 0.03 (bc)
3rd trimester	1.59 ± 0.12 (b)	1.07 ± 0.10 (c)
Postpartum	1.38 ± 0.07 (a)	0.82 ± 0.06 (b)
Postlactation	1.40 ± 0.07 (ab)	0.63 ± 0.05 (a)
PGDM women		
1st trimester	1.44 ± 0.13 (a)	0.53 ± 0.03 (a)
2nd trimester	1.83 ± 0.17 (b)	0.86 ± 0.07 (b)
3rd trimester	1.80 ± 0.14 (b)	0.97 ± 0.10 (b)
Postpartum	1.43 ± 0.10 (ab)	0.91 ± 0.10 (b)
Postlactation	1.29 ± 0.05 (ab)	0.62 ± 0.04 (ab)
GDM women		
1st trimester	1.52 ± 0.18 (abc)	0.70 ± 0.08 (a)
2nd trimester	1.62 ± 0.14 (a)	0.97 ± 0.06 (b)
3rd trimester	1.62 ± 0.13 (abc)	1.20 ± 0.11 (c)
Postpartum	1.38 ± 0.09 (bc)	0.91 ± 0.08 (b)
Postlactation	1.34 ± 0.09 (c)	0.84 ± 0.22 (abc)

Values are means ± SE. Letters in parenthesis correspond to statistical comparison by Student's *t* dependent test between the groups at each of the gestational or postgestational stages studied: the same letter within one parameter means no statistical difference between the groups, whereas different letters indicate significant differences between the corresponding groups ($p \leq 0.05$). Statistical comparisons of PGDM or GDM women versus control subjects were calculated by Student's *t* independent test and were NS ($P > 0.05$) in all cases.

TABLE 7
Correlations between plasma apoA-I and apoB versus lipoprotein TG and cholesterol content in women during pregnancy, postpartum, and postlactation

	Plasma APO A-I			Plasma APO B		
	n	*r*	*P*	*n*	*r*	*P*
VLDL TG				136	0.517	0.0000
VLDL cholesterol				137	0.524	0.0000
LDL TG				135	0.649	0.0000
LDL cholesterol				136	0.807	0.0000
HDL TG	135	0.466	0.0000			
HDL cholesterol	136	0.690	0.0000			

Lineal regression analysis formula, $Y = a + bX$.

The initial factor that may drive all these changes in lipoprotein levels during gestation could be the exaggerated increment of estrogens because these hormones are known to inhibit hepatic lipase activity (38,39), and an inverse correlation between HDL_2 and hepatic lipase activity (40) and even between TGs and hepatic lipase activity has been observed after estrogen treatment of postmenopausal women (41). In support of this hypothesis, we found that HDL TGs and β-estradiol levels show the highest correlation coefficient of all the hormonal-lipoprotein combinations studied ($r = 0.748$). The correlation observed between estradiol and LDL TG also may be explained by the decrease in hepatic lipase activity produced by estrogens because this enzyme has been implicated in the hydrolysis of LDL TGs (37). Further experimental support is required to test these possibilities.

Another aim of this study was to determine how PGDM or GDM affect these parameters, but we were unable to find any difference between these women and normal control women in any of the lipoprotein parameters studied. This agrees with some previous findings (13,15) although not with others, even from the same researchers (14,15). Although the lack of a hyperlipidemic condition in our GDM and PGDM patients could be interpreted as a result of the good metabolic control of our patients, shown by their near-normal HbA_{1c} levels, the glucose plasma levels were augmented, and both FFAs and ketone bodies were higher in the two diabetic groups during all gestational trimesters than in the control subjects. This indicates that adipose tissue lipolytic activity and liver consumption of released FFAs for ketogenesis are augmented in these patients. Because, under the fasting conditions, liver production of TGs is highly dependent on FFAs arriving from circulation (42), it would be expected that in these diabetic patients VLDL synthesis and lipoprotein alterations would be exaggerated during pregnancy. Hyperlipidemia occurring during ges-

TABLE 8
Plasma estradiol, progesterone, and prolactin in normal and diabetic women during pregnancy, postpartum, and postlactation

	β-Estradiol (nM)	Progesterone (nM)	Prolactin (μg/L)
Normal control subjects			
1st trimester	9.23 ± 1.79 (a)	100.17 ± 15.42 (a)	43.80 ± 8.35 (a)
2nd trimester	38.45 ± 5.14 (b)	185.55 ± 21.15 (b)	118.00 ± 7.69 (b)
3rd trimester	66.60 ± 8.26 (c)	453.56 ± 26.20 (c)	141.33 ± 4.55 (c)
Postpartum	0.13 ± 0.02 (d)	0.83 ± 0.16 (d)	62.54 ± 13.16 (d)
Postlactation	0.18 ± 0.07 (d)	6.36 ± 5.72 (ad)	6.12 ± 0.83 (e)
PGDM women			
1st trimester	3.60 ± 0.71 (a)*	78.00 ± 5.72 (a)	24.25 ± 3.60 (a)†
2nd trimester	25.46 ± 3.17 (b)†	194.84 ± 19.27 (b)	87.67 ± 8.66 (b)†
3rd trimester	52.65 ± 6.14 (c)	445.01 ± 74.89 (c)	103.00 ± 9.52 (c)*
Postpartum	0.30 ± 0.09 (d)	1.49 ± 0.44 (d)	37.40 ± 11.45 (a)
Postlactation	0.23 ± 0.07 (ad)	2.77 ± 1.30 (d)	6.00 ± 0.70 (d)
GDM women			
1st trimester	5.22 ± 1.46 (a)	93.94 ± 14.18 (ad)	34.20 ± 9.99 (ac)
2nd trimester	29.40 ± 6.42 (a)	145.36 ± 21.65 (a)	99.55 ± 18.03 (ab)
3rd trimester	50.05 ± 5.75 (b)	348.91 ± 35.68 (b)†	132.44 ± 11.37 (b)
Postpartum	0.19 ± 0.05 (c)	1.75 ± 0.79 (c)	41.22 ± 10.71 (c)
Postlactation	0.22 ± 0.05 (ab)	1.94 ± 1.11 (cd)	7.33 ± 1.85 (a)

Values are means ± SE. Letters in parenthesis correspond to statistical comparison by Student's *t* dependent test between the groups at each of the gestational or postgestational stages studied: the same letter within one parameter means no statistical difference between the groups, whereas different letters indicate significant differences between the corresponding groups ($P \leq 0.05$). Statistical comparisons between PGDM or GDM women versus control subjects were calculated by Student's *t* independent test.
*$P < 0.01$.
†$P < 0.05$.

TABLE 9
Lineal semilogaritmic regressions between plasma lipoprotein TG content and hormones in women during pregnancy, postpartum, and postlactation

	log Estradiol	log Progesterone	log Prolactin
VLDL TG			
n	132	133	135
r	0.273	0.202	0.254
p	0.0016	0.0195	0.0030
LDL TG			
n	131	132	134
r	0.545	0.460	0.516
p	0.0000	0.0000	0.0000
HDL TG			
n	128	128	130
r	0.748	0.678	0.615
p	0.0000	0.0000	0.0000

Lineal semilogaritmic regression analysis formula, $Y = a + b(\log X)$.

tation under normal conditions seems to be driven primarily by the increases in circulating steroid hormone (as stated previously). Therefore, the decreased level of these hormones in diabetic women during gestation may have restrained the development of an overtly hyperlipidemic condition in our diabetic pregnant patients.

Other authors also have reported decreased plasma estradiol and prolactin levels in pregnant diabetic women who had wide glycemic excursions (43,44) that mainly corresponded to the first trimester of gestation. In a recent study, decreased plasma cholesterol and TG levels have even been found in early pregnant diabetic women with enhanced HbA_{1c} values (45), and although hormonal levels have not been analyzed, they also would be expected to be decreased. In this way, a balance between the degree of metabolic control and hormonal dysfunction may determine the development or lack of development of dyslipidemia in diabetic pregnant women, and this could explain the variety of findings reported under similar conditions. We therefore propose that the decreased estradiol levels in our diabetic patients impede an exaggerated rise of circulating lipoproteins above the normal range. Because the degree of metabolic control may affect both the dyslipidemic condition of the diabetic patient (12,15,46) and the level of sex hormones during pregnancy (43,44), we propose that the development or lack of development of exaggerated hyperlipidemia in diabetic pregnancy depends on the balance between these two conditions.

ACKNOWLEDGMENTS

This study was conducted with a grant from the Fundación Ramón Areces, Madrid, Spain.

We thank Antonia Arbiell and Genoveva Alfayate for technical and secretarial assitance; Drs. Trinidad Vela, Cristina Grande, and José Maria Olmos for performing some of the analyses; Drs. Mercedes Jañez, Maria Luisa de la Morena, and Jaime Pillado for help in the selection of the patients; and Carol F. Warren for editorial help.

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