Method-Dependent Increase in Lipoprotein(a) in Insulin-Dependent Diabetes Mellitus During Pregnancy

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The current prevalent view is that plasma lipoprotein(a) [Lp(a)] concentrations are under strong genetic control. Most dietary and drug interventions seem to have little or no effect on plasma Lp(a) levels. However, evidence for a possible regulatory role of hormones is accumulating, for instance, fluctuations of Lp(a) levels during pregnancy have been reported. Also, in insulin-dependent diabetes mellitus (IDDM) patients, elevated Lp(a) levels have been reported. In the present longitudinal study, plasma lipid concentrations, including Lp(a), were determined in IDDM women before pregnancy, during pregnancy, and 3 months postpartum. In our study population, Lp(a) concentration was not significantly correlated with either hemoglobin A_{1c} (HbA_{1c}) levels or apolipoprotein(a) [apo(a)] phenotype. Changes in other lipid parameters observed during pregnancy in our IDDM population were similar to those reported during normal pregnancy. Lp(a) concentrations were quantified using two different immunochemical methods that possess different sensitivities and specificities: an immunoradiometric assay (IRMA) using two different anti-apo(a) antibodies, and an enzyme-linked immunosorbent assay (ELISA) using an anti-apo(a) and an anti–apo B antibody. Median prepregnancy Lp(a) concentrations were 118 mg/L (range, 15 to 672) as determined with the IRMA and 107 mg/L (range, 21 to 451) as determined with the ELISA. Women with IDDM showed, in general, no significant change in Lp(a) concentration during pregnancy when it was assayed with the IRMA, although a tendency to increased values was observed. When Lp(a) concentrations were determined with the ELISA, a strong and significant increase in Lp(a) from weeks 17 to 24 of pregnancy onward was found. The latter results confirm the prevalent view that during pregnancy Lp(a) levels are increased. However, the present results and those of others on Lp(a) in normal pregnancy strongly emphasize the importance of method selection when determining Lp(a) concentrations.

IT HAS BEEN KNOWN for a long time that patients with increased plasma concentrations of low-density lipoprotein (LDL) cholesterol have an increased risk of premature cardiovascular disease. Another potentially atherogenic lipoprotein is lipoprotein(a) [Lp(a)]. Increased levels of Lp(a), which is a hybrid of LDL and a plasminogen-like glycoprotein, apolipoprotein(a) [apo(a)], represent an independent risk factor not only for coronary artery disease but also for cerebrovascular disease.[1-3] The current opinion is that circulating concentrations of Lp(a) are relatively constant in a single individual but vary widely between subjects, ranging from less than 10 to greater than 1,000 mg/L. In the white population, plasma Lp(a) distribution is highly skewed toward normal levels (<300 mg/L)[4,5]; in the black population, a gaussian distribution is observed, reflecting median plasma levels that are significantly higher than those found in white populations.[6] Differences are determined largely by genetic factors, with 69% of the variability in plasma Lp(a) levels being a result of size polymorphism of apo(a),[7] which in turn arises from the number of repeated kringle-4 encoding sequences in the apo(a) gene.[8] Other sequences at the apo(a) locus account for an additional 22%.[7] The apo(a) gene may therefore account for greater than 90% of the variance in plasma levels.

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Submitted December 31, 1994; accepted March 24, 1995.

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0026-0495/95/4412-0016$03.00/0

In contrast to most other risk factors, plasma levels of Lp(a) are insensitive to most dietary manipulations.[9-11] Also, traditional LDL-lowering drugs, which are based on upregulating LDL receptor levels, are not successful in decreasing Lp(a) levels.[12-14] Thus far, the only agents that have been able to reduce Lp(a) levels are niacin, neomycin,[15] and certain steroids.[16-18] Several groups have reported on the influence of sex hormones on plasma Lp(a) levels, but the mechanism(s) by which hormones affect plasma Lp(a) levels is still not understood. Lp(a) levels are reported to vary within the normal female menstrual cycle.[19] Studies in postmenopausal women with estrogen[20] or estrogen plus progesterone replacement therapy[20,21] show a 20% to 50% decrease in Lp(a) levels, especially in subjects with high basal concentrations. Also, during pregnancy, fluctuations in Lp(a) levels have been reported.[22] Plasma Lp(a) levels increased steadily during the first trimester of pregnancy and reached a maximum in the middle of the second trimester; then plasma levels decreased, reaching a basal value at the time of birth. In another study,[23] Lp(a) concentrations increased until the end of pregnancy, when a maximum was reached, suggesting modulation by endogenous hormones.

Insulin-dependent diabetes mellitus (IDDM) has also been shown to influence Lp(a) levels. On the basis of various, often contradictory studies, Haffner[24] summarized the role of Lp(a) in IDDM. It was concluded that in IDDM, Lp(a) concentrations are probably elevated and probably related to metabolic control of glucose concentrations. Furthermore, Lp(a) concentrations are increased when microalbuminuria is present. This is in contrast to non–insulin-dependent diabetes mellitus, in which Lp(a) concentrations are not elevated and do not change with metabolic control.[24]

When both IDDM and pregnancy coexist, possible addi-

tional or even synergistic effects may be expected. This led us to investigate plasma Lp(a) levels in pregnant women with IDDM.

SUBJECTS AND METHODS

Subjects

The participants in this study were 15 women with IDDM. According to the White classification, six were class B, six class C, two class D, and one class FR. Two patients (one class B and one class C) developed mild microalbuminuria (35.9 and 39.7 μg/min, respectively) during the third trimester; the remainder of the patients had levels in the normal range (< 15 μg/min) throughout their pregnancy. All 15 women received insulin treatment before and during pregnancy. The mean age of the women was 29 years (range, 26 to 36). Their mean body mass index before pregnancy was 22.6 ± 3.7 kg/m^2 (range, 18.3 to 29.4). The subjects were longitudinally studied, and plasma samples were collected before, every fortnight during, and 3 months after pregnancy. All samples were collected at the Department of Internal Medicine, University Hospital Groningen, and were analyzed at the Department of Clinical Chemistry, University Hospital Utrecht, The Netherlands.

Lp(a) Quantification

Quantification of Lp(a) in plasma was performed using two commercially available immunochemical methods. A sandwich immunoradiometric assay (IRMA) specific for apo(a) was obtained from Pharmacia (Uppsala, Sweden). In an one-step incubation, Lp(a) reacts with a monoclonal anti-apo(a) capturing antibody coated to Sepharose beads and a second, ^{125}I-labeled, monoclonal anti-apo(a) detecting antibody. The formed antibody-antigen complex is separated from excess tracer by centrifugation and aspiration. Radioactivity in the pellet is directly proportional to the concentration of apo(a) in the sample. Results are expressed in units per liter; these units are converted to milligrams per liter by multiplying with a factor 0.7 given by the manufacturer. The IRMA has a coefficient of variation of less than 5%.

The Apo-Tek Lp(a) enzyme-linked immunosorbent assay (ELISA) was obtained from Organon Teknika (Rockville, MD). This two-step kit uses a monoclonal capturing antibody directed against apo(a), which is coated to microplate wells. After incubation with a patient's EDTA-plasma, unbound antigen is washed away. The immune complex is further incubated with a polyclonal peroxidase-conjugated anti–apo B detecting antibody. After washing, the plate is incubated with the chromogenic substrate, tetramethylbenzidine and hydrogen peroxide. After stopping color development with sulfuric acid, plates were read using a Bio-Rad (Richmond, CA) microplate reader (3550-UV) at 450 nm. The absorbance is directly proportional to the concentration of Lp(a). This method was shown to quantify Lp(a) on a molar basis independently of apo(a) isoform size.

Phenotyping of Apo(a)

Apo(a) phenotype was determined by the method reported by Kamboh et al,[25] with only minor modifications. In short, reduced plasma samples were applied to a 1.5% sodium dodecyl sulfate–agarose gel and electrophoresed at 70V for 16 hours at 4°C using a submarine gel unit and power supply (Bio-Rad). After electrophoresis, proteins were transferred to nitrocellulose membrane (Bio-Rad) by semidry blotting for 3 hours at 240 mA in a semidry blotting apparatus (Bio-Rad). The nitrocellulose was blocked with 5% nonfat dry milk (Nutricia, Zoetermeer, The Netherlands) in H_2O. Apo(a) was detected on the blot with a rabbit polyclonal antibody against human Lp(a) (Behringwerke, Marburg, Germany) as the first antibody and a goat antirabbit IgG conjugated with alkaline phosphatase (Pierce, Rockford, IL) as the second antibody. Both antibodies were diluted 1:1,000 in 10 mmol/L Tris hydrochloride buffer (pH 7.4) containing 0.15 mmol/L NaCl, 2 mmol/L EDTA, and 1% nonfat dry milk. Bound alkaline phosphatase was visualized using a combination of BCIP (5-bromo-4-chloro-3′-indolylphosphate *p*-toluidine salt) and NBT (Nitro Blue Tetrazolium chloride) substrate (Pierce). Standards (Immuno, Vienna, Austria) with known phenotypes were applied on each gel. The nomenclature described by Utermann et al[26] was used.

Other Methods

Total cholesterol level was measured enzymatically on a Hitachi 717 analyzer (CHOD-PAP; Boehringer Mannheim, Almere, The Netherlands). High-density lipoprotein (HDL) cholesterol level was measured enzymatically on a Kodak Ektachem 700 analyzer (Eastman Kodak, Rochester, NY) after precipitation of all apo B–containing lipoproteins with phosphotungstic acid and magnesium chloride. LDL cholesterol level was calculated according to the method reported by Friedewald et al.[27] Triglycerides were analyzed using an enzymatic colorimetric method with reagents from Boehringer Mannheim. Apos A-1 and B-100 were determined using a routine nephelometric method (Behring). Hemoglobin A_{1c} (HbA_{1c}) level was measured by affinity chromatography.[28]

Statistical Analysis

The mean ± SD and median values were used to describe group characteristics and blood measurements. The nonparametric Wilcoxon's signed-rank test was used to test for differences. The relationship between HbA_{1c} and apo(a) phenotype with Lp(a) concentrations was evaluated by linear regression analysis. A two-tailed *P* value less than .05 was considered significant.

RESULTS

HbA_{1c}

All women were on strict metabolic control of glucose levels. Increases or decreases in glucose concentrations were corrected by insulin regimen adjustments. The good metabolic control is reflected in the mean HbA_{1c} values of these women during pregnancy (Table 1). In the study population, no significant correlation was observed between HbA_{1c} level and Lp(a) concentration.

Lp(a)

Lp(a) concentrations were determined by two different immunochemical methods. As in the normal population, prepregnancy

Table 1. HbA_{1c} Concentrations (mean ± SD) During Pregnancy in IDDM Women

Gestational Age	HbA_{1c} (%)
Prepregnancy	6.6 ± 1.3
Weeks 0-8	6.2 ± 1.1
Weeks 9-16	5.8 ± 1.1*
Weeks 17-24	5.7 ± 0.8†
Weeks 24-32	5.7 ± 0.7†
Weeks 33-40	5.6 ± 0.6†
Postpartum	6.4 ± 1.4

NOTE. Normal range for HbA_{1c} is 4% to 6.5%. Statistical comparison with prepregnancy concentrations was made by Wilcoxon's signed-rank test.

*$P < .05$.

†$P < .01$.

plasma Lp(a) concentration varied widely from subject to subject, ranging from 15 to 672 mg/L (median, 118) when determined with the IRMA and from 21 to 451 mg/L (median, 107) when determined with the ELISA. Because of differences in Lp(a) as measured by IRMA and ELISA, results of each method are shown separately (Figs 1 and 2, respectively). Furthermore, because of the large interindividual variation in baseline levels, the observed changes are presented as a percentage of prepregnancy values. Relative changes in plasma Lp(a) concentrations with gestational age are shown for each of 15 women separately. The results clearly show that considerable differences exist between Lp(a) levels as determined by IRMA or ELISA.

In general, with the IRMA method, no significant changes in Lp(a) concentrations were detected, although a small significant change in Lp(a) concentration was observed in weeks 25 to 32 ($P = .025$), and a strong tendency ($P = .05$) to increased Lp(a) concentrations in weeks 33 to 40 (Table 2). In individual cases, fluctuations in Lp(a) concentrations ranged from a 53% decrease to a 97% increase as compared with prepregnancy values. Three women did show marked and consistent increases in Lp(a) concentration until the end of their pregnancies. Lp(a) levels in these three women increased in weeks 33 to 40 to 350%, 420%, and 664% of the respective prepregnancy Lp(a) concentrations (Fig 1).

In general, with the ELISA method, a significant increase in mean Lp(a) concentration was detected from weeks 17 to 24 of pregnancy until postpartum. Mean Lp(a) levels increased from 129 ± 111 mg/L prepregnancy to 254 ± 176 mg/L in weeks 33 to 40 of pregnancy (Table 2). In contrast to Lp(a) levels measured with the IRMA method, almost all patients showed an increase in Lp(a) when the ELISA method was used for quantification.

Apo(a) Phenotype

Apo(a) phenotypes determined in the diabetic women as classified according to the nomenclature of Utermann et al[26] are shown in Table 3. No significant correlation was observed between apo(a) phenotype and change in plasma Lp(a) concentration determined by either the IRMA or ELISA method.

Plasma Lipids and Other Lipoproteins

Serum total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides are presented in Table 4. Before pregnancy, all women were normolipidemic; during pregnancy, most lipid levels changed significantly. After an initial decrease in the first 8 weeks of pregnancy, both total cholesterol and triglyceride levels increased from, respectively, 5.2 ± 1.3 and 1.01 ± 0.25 to 8.0 ± 2.3 and 4.00 ± 2.24 mmol/L in weeks 33 to 40 of pregnancy. Changes in total cholesterol levels were caused by fluctuations in LDL, as well as HDL, concentrations. In general, changes in LDL and HDL are reflected in apo B-100 and apo A-1 levels in these subjects. Apo B-100 and apo A-1 were, respectively, 0.48 ± 0.14 g/L (reference range, 0.4 to 0.8) and 1.45 ± 0.29 g/L (reference range, 1.2 to 1.9) before pregnancy and reached maxima of, respectively, 0.92 ± 0.31 g/L ($P < .001$) and 1.80 ± 0.25 g/L ($P < .01$) in weeks 33 to 40. Three months after delivery, all lipid levels had returned to prepregnancy values.

DISCUSSION

Hyperlipidemia is a common feature in pregnancy and consists primarily of increases in triglycerides and cholesterol. Another cause of secondary hyperlipidemia is diabetes mellitus. This prompted us to investigate various parameters of lipid metabolism in pregnant women with IDDM. Special interest was focused on the course of Lp(a) concentrations in these patients.

Knopp et al[29] presented reference values for lipids and apolipoproteins at 36 weeks' gestation based on a prospective epidemiologic survey. Compared with levels in nonpregnant women, median plasma triglyceride concentrations were increased 3.6-fold in normal pregnancy. This increase is on the same order of magnitude as the almost fourfold increase in triglycerides measured in our population. Also, increases in other lipid parameters observed in our study

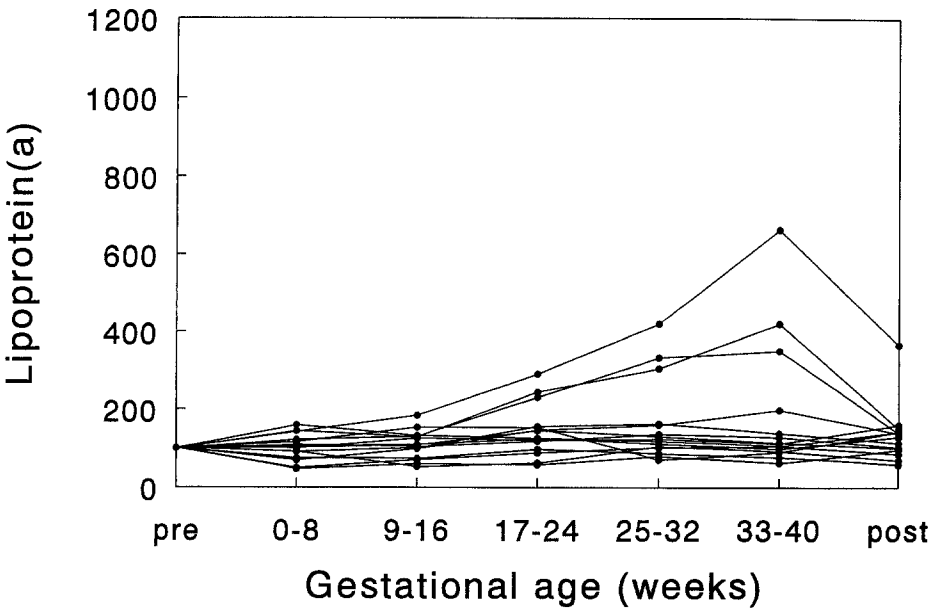

Fig 1. Lp(a) levels during pregnancy for each of the IDDM women separately as measured by IRMA. Values are expressed as a percentage of prepregnancy concentrations.

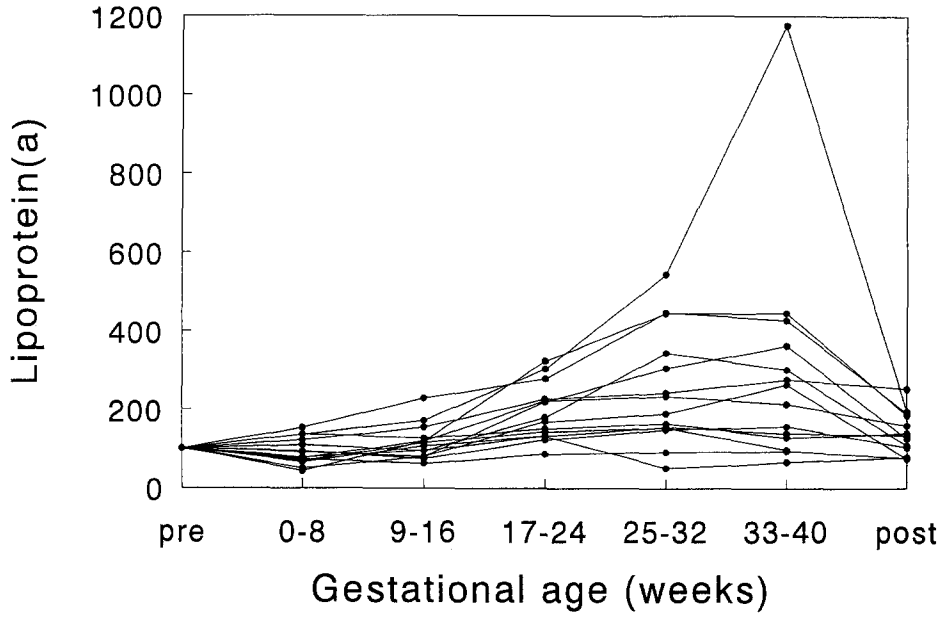

Fig 2. Lp(a) levels during pregnancy for each of the IDDM women separately as measured by ELISA. Values are expressed as a percentage of prepregnancy concentrations.

are similar to reference values reported in normal pregnancy by both Knopp et al[29] and Piechota and Staszewski.[30] The increase in LDL cholesterol, for instance, was 39% in our study, versus 49% and 36% in the studies by, respectively, Knopp et al[29] and Piechota and Staszewski.[30] The changes during pregnancy observed in our study support the results of an earlier study by Hollingsworth and Grundy[31] indicating that pregnant women with IDDM are not different from normal pregnant women with respect to pregnancy-associated changes in lipid parameters. The changes observed are also similar to those in diabetic pregnancy as reported by others.[32-35] However, none of these studies actually measured the effect of pregnancy in IDDM on Lp(a) levels. In the present study, various lipid parameters, including Lp(a), were measured in IDDM women before pregnancy, at various time points during pregnancy, and postpartum. Because of large interindividual differences in Lp(a) concentrations in the normal human population, subjects in the present study were evaluated longitudinally.

For Lp(a) quantification, two different immunochemical methods were used in parallel. The results obtained with these two methods showed some remarkable differences. The IRMA showed that during pregnancy, in general, most Lp(a) concentrations did not change, although, due to a sharp increase in three individuals, a tendency to increased levels was noted. The ELISA, on the other hand, showed that Lp(a) levels were highly significantly increased from weeks 17 to 24 of pregnancy until childbirth. These results point to a large difference in sensitivity and specificity between the two methods used. Although an exact comparison between these two methods is beyond the scope of the present report, some considerations are in order. Since no internationally recognized Lp(a) standard exists, assay format selection is important in Lp(a) quantification, as recently pointed out by Taddei-Peters et al[36] and Leus et al.[37] Especially the latter study, performed in our laboratory, prompted us to use the ELISA with the IRMA in quantifying Lp(a). Although the IRMA is a sensitive and specific method, the ELISA is superior, especially since it uses a sandwich technique with both an anti–apo B and an anti-apo(a) antibody, whereas the IRMA uses two anti-apo(a) antibodies. Furthermore, the ELISA has been shown to be less sensitive to Lp(a) phenotype variations.[37] The present results demonstrate clearly the importance of selecting the right method for quantifying Lp(a), since the IRMA method suggests no or hardly any effect and the

Table 2. Lp(a) Concentrations (mean ± SD) During Pregnancy as Measured by IRMA and ELISA

Gestational Age	Lp(a) (mg/L) IRMA	Lp(a) (mg/L) ELISA
Prepregnancy	179 ± 185	129 ± 111
Weeks 0-8	169 ± 185	109 ± 82
Weeks 9-16	181 ± 199	140 ± 124
Weeks 17-24	211 ± 222	215 ± 160‡
Weeks 24-32	217 ± 210*	239 ± 174†
Weeks 33-40	212 ± 190	254 ± 176†
Postpartum	221 ± 252	172 ± 176

NOTE. Statistical comparison with prepregnancy concentrations was made by Wilcoxon's signed-rank test.

*$P < .05$.

†$P < .01$.

‡$P < .005$.

Table 3. Apo(a) Phenotypes in Pregnant IDDM Women (N = 15)

Apo(a) Phenotype	Frequency
S4S2	1
S3	4
S3S2	3
S3S1	1
S2	1
S2S1	2
S1	3

Table 4. Lipid Levels in Pregnant IDDM Women

Gestational Age	Cholesterol (mmol/L)			Triglycerides (mmol/L)
	Total	LDL	HDL	
Prepregnancy	5.2 ± 1.3	3.54 ± 1.03	1.16 ± 0.32	1.01 ± 0.25
Weeks 0-8	4.4 ± 0.8†	2.72 ± 0.68†	1.19 ± 0.22	0.80 ± 0.17*
Weeks 9-16	5.0 ± 0.8	3.01 ± 0.68†	1.47 ± 0.27†	1.10 ± 0.30
Weeks 17-24	6.6 ± 1.0†	4.22 ± 0.81†	1.54 ± 0.38‡	1.73 ± 0.60‡
Weeks 25-32	7.4 ± 1.1‡	4.75 ± 0.75†	1.36 ± 0.40*	2.93 ± 1.54‡
Weeks 33-40	8.0 ± 2.3†	4.93 ± 1.52†	1.36 ± 0.68	4.00 ± 2.24‡
Postpartum	5.7 ± 1.2	3.98 ± 1.09	1.25 ± 0.26	1.22 ± 0.54

NOTE. Data are the mean ± SD (N = 15). Statistical comparison with prepregnancy concentrations was made by Wilcoxon's signed-rank test.

*$P < .05$.

†$P < .01$.

‡$P < .001$.

ELISA method points to a strong increase in Lp(a) during pregnancy. For the above-mentioned reasons, it is believed that the results obtained with the ELISA better reflect the true situation and that an increase in Lp(a) during pregnancy in IDDM is apparent.

The literature on the course of Lp(a) concentration during normal pregnancy does not show consensus. In a study by Zechner et al,[22] Lp(a) levels were reported to increase during the first trimester of pregnancy. In the 19th week, levels were on average 2.8 times higher than in the 8th week. Plasma Lp(a) decreased from the 19th week onward, reaching a basal value at the time of birth, despite the fact that apo B-100 and total cholesterol also increased significantly after week 19. In a cross-sectional study by Panteghini and Pagani,[23] median Lp(a) concentration increased until the end of pregnancy, when a maximum of 141 mg/L was reached (nonpregnant controls, 61 mg/L). In parallel, levels of plasma triglycerides, total cholesterol, and apo B-100 also increased. In yet another study, Knopp et al[29] reported that sinking prebetalipoprotein was less common in pregnant women than in women at 6 weeks postpartum and controls. Although not specific, a reduced prevalence of sinking prebetalipoprotein suggests reduced Lp(a) levels rather than increased Lp(a) levels during pregnancy. This discrepancy in results within normal pregnancy could primarily be due to methodological differences.

Differences in Lp(a) pattern and concentration as observed in normal pregnancy may result from either the method used for quantification of Lp(a) or the study design. Knopp et al[29] used sinking prebetalipoprotein as an indicator for the presence of Lp(a); although there is a close association between elevated Lp(a) levels and the presence of sinking prebetalipoprotein, the terms are not completely interchangeable.[38] In the study by Zechner et al,[22] a relatively insensitive assay was used, which resulted in a limited population with Lp(a) levels not representative of those in the whole population. Only subjects with Lp(a) concentrations greater than 100 mg/L at any stage of pregnancy were included. In the study by Panteghini and Pagani,[23] women were studied in a cross-sectional design; given the large interindividual variation in Lp(a) concentrations, a longitudinal study design, as applied in the present study, is preferred.

Most of the secondary changes in lipoprotein metabolism seen during pregnancy and in diabetes mellitus are hormonally driven, and therefore differences in Lp(a) concentrations between pregnant IDDM women and normal pregnant women most likely reflect differences in hormonal status. However, at present, no consensus exists on the effect of normal pregnancy on Lp(a) concentrations. This makes definite conclusions as to whether Lp(a) fluctuations in pregnant IDDM women differ from those during normal pregnancy difficult.

In conclusion, in the present longitudinal study it was shown that in IDDM women Lp(a) concentrations increased during pregnancy. Although the prevalent opinion is that during normal pregnancy Lp(a) is increased as well, this remains to be confirmed. In this respect, choice of methodology is crucial. As compared with other studies of normal pregnant women, pregnant IDDM women showed similar changes in the other lipids, although in the present study no direct comparison was made. However, the results strongly suggest that no differences in secondary dyslipidemia, including Lp(a), exist during pregnancy in normal and IDDM women.

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