

Plasma Lipids and Apolipoproteins A and B in Human Pregnancy

M. Pocoví, J. M.^a Ordovás and F. Grande-Covián

Departamento de Bioquímica
Facultad de Ciencias
Zaragoza-9 (Spain)

(Received on November 11, 1983)

M. POCOVI, J. M.^a ORDOVAS and F. GRANDE-COVIAN. *Plasma lipids and Apolipoproteins A and B in Human Pregnancy*. Rev. esp. Fisiol., 40, 183-190, 1984.

A cross sectional study was carried out in 200 normal pregnant women between 8-40th weeks of gestation, 25 women during delivery and 25 women 6 weeks after delivery. Plasma and lipoprotein lipids were measured using standard procedures. Apolipoprotein A (Apo A) and Apolipoprotein B (Apo B), were measured by electroimmunoassay. Plasma levels of Apo A were elevated in pregnant women but the elevations were not significant until 17-20 weeks of gestation. Apo A during pregnancy was significantly correlated ($p < 0.001$) with high density lipoprotein cholesterol (HDL-C). The level of Apo B increased progressively during pregnancy and it was significantly correlated ($p < 0.001$) with total cholesterol (TC), plasma triglycerides (TG) and phospholipids (PL). Apo A and Apo B levels returned to non pregnant values within the puerperium, whereas TC, TG and PL remained significantly elevated above controls ($p < 0.01$) 6 weeks post partum.

Key words: Apolipoprotein A, Apolipoprotein B, Lipoprotein, Human pregnancy.

It is well known that there is an increase in plasma cholesterol (TC), triglycerides (TG), phospholipids (PL) and lipoprotein lipids, during human pregnancy (7, 10, 16-18, 22-24, 30, 32, 33) but data on apolipoproteins are scarce in the literature (12, 29).

Recent research has given information about plasma lipoprotein composition and metabolism (26, 27, 31). In addition to their role in maintaining lipoprotein structure, some apolipopro-

teins play important regulatory roles by serving as cofactors for key enzymes of lipid metabolism (3, 9, 21) and in the binding of lipoproteins to cell receptors (11, 34). In this sense, the apolipoproteins not only participate in the transport of lipids but they play also an important role in the regulation of lipoprotein metabolism.

An analysis of apolipoproteins and their relationships with the plasma lipid fractions are, therefore, important in

characterizing the changes in lipid metabolism during human gestation. This is the purpose of the present study.

Materials and Methods

The cross sectional study was carried out in 200 normal pregnant women (primiparae and multiparae) visited in the Cazar Centre for Prevention of Subnormality (Saragossa, Spain). Demographic data and information on the state of health, medications and pregnancy itself were obtained by interview. Gestational age was determined by menstrual history. Apart from control of excess weight gain, no dietary instructions were given. The subjects had not received any medications known to affect plasma lipids and lipoproteins, at the time of blood sampling. Their ages ranged from 18 to 42 years with a mean of 27.8 years ($SD \pm 4.6$). They were distributed according to their gestational age in 8 groups of 25 subjects each. Another group of 25 women with age gestational ≥ 37 weeks, was studied at the time of delivery. The new born live babies had an average weight of 3,374 g ($SD \pm 450$). Twenty-five other women were studied 6 weeks after delivery. Because they were encouraged during pregnancy to breast-feed their babies, most of them (92 %) were lactating women. Finally, 25 non pregnant, healthy, premenopausal women of similar average age served as controls. None of them was taking oral contraceptives.

Venous blood samples were obtained after 12-16 hours fast and collected in chilled tubes containing 1 mg of Ethylenediaminetetraacetic acid disodium salt per ml of blood. Delivery blood samples were taken just after expulsion of the placenta. Plasma was separated immediately and stored at 0-4° C until further analysis.

Plasma TC and high density lipopro-

tein cholesterol (HDL-C) were determined using the HUANG *et al.* procedure (13). TG were measured by a semienzymatic method (4). Lipid phosphorous was measured after extraction with chloroform:metanol (2:1, v/v) using ZILVERSMIT's method (35). PL were calculated as lipid phosphorous times 25. Precipitation of low (LDL) and very low (VLDL) density lipoproteins was performed with magnesium chloride and sodium phosphotungstate (20). Apolipoproteins A and B were measured by electroimmunoassay (19) using antisera and standards purchased from Beringwerke AG (Marburg, W. Germany). The antiserum for apolipoprotein A included both anti A-I and A-II antibodies.

Significance of differences was determined by the Student «t» test for non paired variates. Significance of correlations coefficients was estimated using the Geigy Scientific Tables (8). Probability values less than or equal to 0.02 were considered significant.

Results

Lipid levels in whole plasma, HDL-C, Apolipoprotein A (Apo A) and Apolipoprotein B (Apo B) from pregnant and non pregnant women are showed in table I. The plasma cholesterol level showed an overall 60 per cent increase during pregnancy, reaching a maximum value at 33-36 weeks and remaining still elevated at 6 weeks post partum. Little change occurred in HDL-C fraction. The total plasma TG concentration increased threefold during pregnancy with a maximum value in the last trimester, and being still significantly elevated ($p < 0.01$) at 6 weeks post partum. The plasma PL also increased during pregnancy, most of this increase took place in the first half of gestation. There was not significant change in Apo A until 17-20 weeks of gestation. At six weeks

Table I. Plasma lipids and apolipoprotein changes during pregnancy and post partum.
 Values shown are: mg/100 ml (mean $\pm$ SD). Total plasma cholesterol (TC). High density lipoprotein cholesterol (HDL-C). Total plasma triglycerides (TG). Total plasma phospholipids (PL). Apolipoprotein A (Apo A). Apolipoprotein B (Apo B). Number per group, 25.

Subjects	TC	HDL-C	TG	PL	Apo-A	Apo-B
Non pregnant women	165 $\pm$ 31.2	64 $\pm$ 17.0	56 $\pm$ 16.4	161 $\pm$ 28.5	235 $\pm$ 30.8	67 $\pm$ 26.3
8-12 wk gestation	180 $\pm$ 21.7	71 $\pm$ 10.8	71 $\pm$ 18.2	186 $\pm$ 26.9	234 $\pm$ 36.6	68 $\pm$ 9.5
13-16 wk gestation	194 $\pm$ 29.8	71 $\pm$ 9.5	93 $\pm$ 27.7	226 $\pm$ 41.0	254 $\pm$ 23.7	72 $\pm$ 23.6
17-20 wk gestation	205 $\pm$ 26.1	74 $\pm$ 12.1	106 $\pm$ 30.4	261 $\pm$ 45.7	275 $\pm$ 42.2	78 $\pm$ 16.3
21-24 wk gestation	229 $\pm$ 37.7	76 $\pm$ 10.9	131 $\pm$ 36.5	271 $\pm$ 49.0	295 $\pm$ 36.6	86 $\pm$ 20.4
25-28 wk gestation	229 $\pm$ 34.7	72 $\pm$ 13.3	140 $\pm$ 38.3	275 $\pm$ 40.1	274 $\pm$ 45.4	94 $\pm$ 15.8
29-32 wk gestation	239 $\pm$ 42.0	61 $\pm$ 12.4	197 $\pm$ 58.9	251 $\pm$ 53.8	261 $\pm$ 49.8	101 $\pm$ 23.7
33-36 wk gestation	269 $\pm$ 65.9	65 $\pm$ 13.0	200 $\pm$ 56.3	294 $\pm$ 53.5	275 $\pm$ 46.9	121 $\pm$ 27.3
37-40 wk gestation	254 $\pm$ 46.3	71 $\pm$ 11.6	186 $\pm$ 52.3	301 $\pm$ 44.2	254 $\pm$ 21.2	113 $\pm$ 26.6
Delivery	246 $\pm$ 36.1	72 $\pm$ 16.0	178 $\pm$ 67.4	259 $\pm$ 32.0	276 $\pm$ 55.1	98 $\pm$ 28.3
6 wk post partum	213 $\pm$ 43.0	69 $\pm$ 15.7	87 $\pm$ 29.0	224 $\pm$ 52.7	245 $\pm$ 42.1	73 $\pm$ 16.2

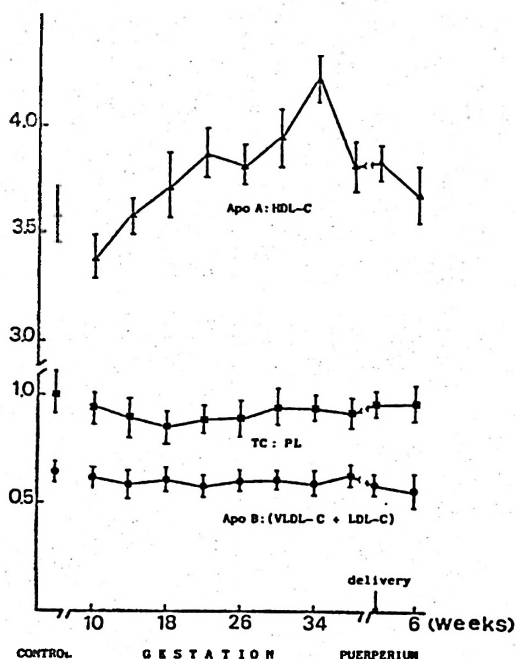


Fig. 1. Changes in the plasma apolipoprotein. A: High density lipoprotein ratio (Apo A: HDL-C) triangles; Total cholesterol: Phospholipid ratio (TC:PL) squares; and Apolipoprotein B: Low and Very low density cholesterol lipoprotein ratio (Apo-B: LDL-C + VLDL-C) circles; during pregnancy and puerperium. The results are means $\pm$ SEM.

post partum, the level of Apo A was similar to that of control subjects. Apo B increased progressively during pregnancy, it was significantly higher ($p < 0.01$) than control at 21-24 weeks. Apo B returned to non pregnant, control, value within the puerperium.

During pregnancy there were little changes in the TC:PL ratio (figure 1). Values for non pregnant, pregnant, at term and post partum women were practically identical. Little changes occurred in the Apo B: (VLDL + LDL) cholesterol ratio during pregnancy. By contrast, the Apo A: HDL-C ratio rose during pregnancy with a peak at 33-36 weeks of gestation, and subsequent decline towards non pregnant values at term.

Table II includes the correlation coefficients, and their significances, between apolipoproteins and plasma lipids during gestation. Apo A was significantly and positively correlated with HDL-C, TC and PL. Apo B showed high positive correlation ($p < 0.001$) with TC, TG and PL; but significant ($p < 0.01$) and negative correlation with HDL-C. High correlations ($p < 0.001$) were found between TC, TG and PL during pregnancy.

Table II. Correlation coefficients between apolipoprotein and lipid concentrations during pregnancy. Total cholesterol (TC). High density lipoprotein cholesterol (HDL-C). Triglycerides (TG). Phospholipids (PL). Apolipoprotein A (Apo A). Apolipoprotein B (Apo B).

Variable no.	Parameter	Correlation coefficient with variable no.					
		1	2	3	4	5	6
1	TC	—					
2	HDL-C	0.193*	—				
3	TG	0.617**	-0.228*	—			
4	PL	0.746**	0.261**	0.515**	—		
5	Apo-A	0.340**	0.593**	0.168	0.244*	—	
6	Apo-B	0.821**	-0.191*	0.622**	0.548**	0.077	—

* $p < 0.01$; ** $p < 0.001$. Others are non-significant.

Discussion

The increases during pregnancy in TC, TG and PL observed in this study, agree rather well with those found in other investigations (7, 10, 16, 18, 22, 24, 32). Some authors (17, 24, 33) demonstrated that in addition to the elevation in plasma lipid levels, there are also changes in the plasma lipoprotein composition. In the present work we have found little changes regarding HDL-C during pregnancy, except for the value obtained at 21-24 weeks, which was significantly higher than control ($p < 0.01$). Similar pattern of HDL-C during pregnancy has been also showed by others (17, 24). However, the protein moiety of HDL particles, mainly Apo A, rises during pregnancy and also the Apo A: HDL-C ratio. This rise in Apo A during pregnancy has been also described by SCHONFELD and PFLEGER (29) in regard to Apo A-I, the main component of the Apo A family. On the other hand, since the relation Apo A-I:Apo A-II does not change during pregnancy (12) this means that there is an elevation of whole Apo A, as shown by our data. These results indicate a change in the HDL particle composition throughout gestation. Our data show that, during pregnancy, Apo A was positively correlated with HDL-C, and this observation is consistent with the correlation reported by ALBERS *et al.* (1, 5) between Apo A-I and HDL-C in a group of women with and without hormone medication.

Our results also show that the level of Apo A in non pregnant women were very similar, if not identical, to that reported by some authors (6, 14) but higher than that observed by others (5, 25). This can be due to the use of different methods of measurement.

HILLMAN *et al.* (12) reported an elevation of Apo B levels in a group of thirty-eight pregnant women sampled

between the 18-39th week of gestation. The new information provided by this study shows a progressive rise of Apo B throughout gestation. This progressive rise of Apo B is accompanied by a parallel elevation of cholesterol in the lipoprotein of density < 1.063 during pregnancy. This pattern of a balanced increase in the constituents of these lipoproteins is consistent with the idea of VLDL and intermediate density lipoproteins overproduction or underutilization in pregnancy (33). In this sense we have found a high positive correlation between Apo B levels during gestation and TC, TG and PL, and a negative correlation between Apo B and HDL-C, similar observations were reported in pregnancy and other clinical situations (2, 15, 28).

The cholesterol : phospholipid ratio did not change markedly during pregnancy. OLIVER and BOYD (22) found a pronounced increase of this ratio during pregnancy, but this has not been a general experience. The results of the present study thus agree with the majority of investigations (7, 23, 30, 32). No explanation can be given for the discrepant findings of OLIVER and BOYD.

Apolipoproteins A and B levels returned to non pregnant values in the post partum period. However, plasma TC, TG and PL remain significantly ($p < 0.01$) elevated 6 weeks post partum. The persistent elevation of these lipid levels six to seven weeks post partum has been previously observed (7, 22, 24).

Because most of the mothers (92 %) were lactating during the post partum, no comparison of the blood lipid and lipoprotein levels between lactating and non lactating mothers could be made.

Acknowledgements

Authors wish to thank to Dr. M. Jiménez for having supplied the samples of this study. This

study was supported by a grant from the «Comisión Asesora de Investigación Científica y Técnica n.º 3801» (Spain).

Resumen

Se lleva a cabo un estudio transversal con 200 mujeres embarazadas de 8 a 40 semanas de gestación, 25 mujeres en el momento del parto y 25 mujeres a las 6 semanas después del parto. Se determinan individualmente los lípidos y lipoproteínas del plasma utilizando métodos estándar y las apolipoproteínas A y B por electroinmunoanálisis. Los niveles de apolipoproteína A (Apo A) aumentan durante el embarazo, elevación significativa a partir de la 17-20 semana de gestación. Durante el embarazo la Apo A se encuentra correlacionada de forma significativa ($p < 0,001$) con el colesterol de las lipoproteínas de alta densidad. Los niveles de apolipoproteína B (Apo B) aumentan de forma progresiva durante el embarazo y están correlacionados ($p < 0,001$) con el colesterol total (CT) triglicéridos totales (TG) y fosfolípidos (FL). Los niveles de Apo A y Apo B recobran los valores normales durante el post-parto, mientras que a las 6 semanas del parto, el CT, TG y FL permanecen todavía elevados de forma significativa ($p < 0,01$) con respecto a los controles.

References

- ALBERS, J. J., WAHL, P. W., CABANA, V. G., HAZZARD, W. R. and HOOVER, J.: *Metabolism*, 25, 633-644, 1976.
- AVOGARO, P., GAZZOLATO, G., BITTOLO BON, G., BELUSSI, F. and QUINCI, G. B.: *Clin. Chim. Acta*, 95, 311-315, 1979.
- BENGTTSSON, G. and OLIVECRONA, T.: *FEBS Lett.*, 128, 9-12, 1981.
- BUCOLO, G. and DAVID, H.: *Clin. Chem.*, 19, 476-482, 1973.
- CHEUNG, M. C. and ALBERS, J. J.: *J. Clin. Invest.*, 60, 43-50, 1977.
- CURRY, M. D., ALAUPOVIC, P. and SUENRAM, C. A.: *Clin. Chem.*, 22, 315-322, 1976.
- DE ÁLVAREZ, R. R., GAISER, D. F., SIMKINS, D. M., SMITH, E. K. and BRATVOLD, G. E.: *Am. J. Obstet. Gynecol.*, 77, 743-757, 1959.
- DOCUMENTA GEIGY: Tablas científicas, ed. Ciba-Geigy) Basel, 1966, p. 61.
- FIELDING, C. I., SHORE, V. G. and FIELDING, P. E.: *Biochem. Biophys. Res. Commun.*, 46, 1493-1498, 1972.
- GILLET, M. P. T., SILVA, T. B. and BESTERMAN, E. M. M.: *Atherosclerosis*, 33, 495-497, 1979.
- GOLDSTEIN, J. L. and BROWN, M. S.: *Ann. Rev. Biochem.*, 46, 897-930, 1977.
- HILLMAN, L., SCHONFELD, G., MILLER, J. P. and WULFF, G.: *Metabolism*, 24, 943-952, 1975.
- HUANG, T. C., CHEM, C. P., WEFLER, V. and RAFTERY, A.: *Anal. Chem.*, 33, 1405-1406, 1961.
- JACQUESON, A., DOUAY, O., LOREUX, C., RAPIN, J. and VOITOT, H.: *Ateliers Behring Deprintems*, 22, 40-45, 1980.
- KARLIN, J. B., JUHN, D. J., SCANU, A. M. and RUBESTEIN, A. H.: *Eur. J. Clin. Invest.*, 8, 19-26, 1978.
- KHOPP, R. H., WARTH, M. R. and CARROLL, C. J.: *J. Reprod. Med.*, 10, 95-101, 1973.
- KNOPP, R. H., MONTES, A., CHILDS, M., LI, J. R. and MABUCHI, H.: *Clin. Obstet. Gynecol.*, 24, 21-49, 1981.
- KONTTINEN, A., PYORALA, T. and CARPEN, E.: *Br. J. Obstet. Gynecol.*, 71, 453-458, 1964.
- LAURELL, C. B.: *Ann. Biochem.*, 15, 45-52, 1966.
- LOPES-VIRELLA, M. F., STONE, P., ELLIS, S. and COLWELL, J. A.: *Clin. Chem.*, 23, 882-884, 1977.
- NILSSON-EHLE, P.: In «Metabolic risk factors in ischemic cardiovascular disease» (Carlson L. A. and Pernow B., eds.). Raven Press, New York, 1982, pp. 49-57.
- OLIVER, M. F. and BOYD, G. S.: *Clin. Sci.*, 14, 15-23, 1955.
- PETERS, J. P., HEINEMANN, M. and MAN, E. B.: *J. Clin. Invest.*, 30, 388-394, 1951.
- POTTER, J. M. and NESTEL, P. J.: *Am. J. Obstet. Gynecol.*, 133, 165-170, 1979.
- REISEN, W. F., MORDASINI, R. C. and MIDDELHOFF, G. W.: *FEBS Lett.*, 91, 35-39, 1978.
- SCANU, A. M. and LANDSBERGER, F. R.: *Ann. N. Y. Acad. Sci.*, 348, 16-29, 1980.
- SCHAEFER, E. J., EISENBERG, S. and LEVY, R. I.: *J. Lipid. Res.*, 19, 667-686, 1978.
- SCHONFELD, G., LESS, R. S., GEORGE, P. K. and PFLEGER, B.: *J. Clin. Invest.*, 53, 1458-1467, 1974.
- SCHONFELD, G. and PFLEGER, B.: *J. Clin. Invest.*, 54, 236-246, 1974.
- SCHWARZ, O. H., SOULE, S. D. and DUNIE, B.: *Am. J. Obstet. Gynecol.*, 39, 203-213, 1940.

31. SMITH, L. C., POWNALL, H. J. and GOTTO, A. M.: *Ann. Rev. Biochem.*, 47, 751-777, 1978.
32. SVANBORG, A. and VIKROT, O.: *Acta Med. Scand.*, 178, 615-629, 1965.
33. WARTH, M. R., ARKY, R. A. and KNOPP, R. H.: *J. Clin. Endocrinol. Metab.*, 41, 649-655, 1975.
34. WINDLER, E., CHAO, Y. S. and HAVEL, R. J.: *J. Biol. Chem.*, 255, 8303-8307, 1980.
35. ZILVERSMIT, D. B., and DAVIS, A. K.: *J. Lab. Clin. Med.*, 35, 155-160, 1950.

