Published in final edited form as:

Metabolism. 1994 November ; 43(11): 1438–1441.

Variations in High-Density Lipoprotein Subclasses During the Menstrual Cycle

Paul T. Williams, Melissa A. Austin, and Ronald M. Krauss

From the Life Sciences Division, Lawrence Berkeley Laboratory, Berkeley: CA; and the Department of Epidemiology, School of Public Health and Community Medicine. University of Washington, Seattle, WA

Abstract

In a study of 41 healthy premenopausal women, plasma high-density lipoprotein-2a (HDL_{2a}) levels (ie, HDL of diameter 8.8 to 9.7 nm) were significantly higher during the luteal phase than during the follicular phase of the cycle. There was no significant variation in HDL_{2b} or any of the HDL₃ subclasses.

Plasma high-density lipoprotein (HDL) cholesterol and apolipoprotein (apo) A-I are reported to be higher during the luteal phase than during the follicular phase of the menstrual cycle [1–3]. This may be due in part to decreased plasma triglycerides and reduced hepatic lipase activity during the cycle's luteal phase [1,4–6]. These variations in HDL, triglycerides, and hepatic lipase presumably arise in response to hormonal changes. As compared with the follicular phase, the luteal phase is characterized by elevated estrogen, progesterone, and sex-hormone-binding protein [6,7]. Estradiol valerate administered orally to postmenopausal women significantly increases HDL₂ cholesterol and significantly decreases hepatic lipase activity [8].

Nondenaturing polyacrylamide gradient gel electrophoresis has been used to identify five HDL subclasses: HDL_{3c} (7.2 to 7.8 nm in diameter), HDL_{3b} (7.8 to 8.2 nm), HDL_{3a} (8.2 to 8.8 nm), HDL_{2a} (8.8 to 9.7 nm), and HDL_{2b} (9.7 to 12 nm) [9]. Prior studies have demonstrated that these subclasses show specific relationships to shared environment or inheritance [10], aging [11], sexual maturation [11], adiposity [11], alcohol intake [11], exercise [12,13], and other lipoproteins [14]. Variations in these subclasses during the menstrual cycle have not been previously described.

SUBJECTS AND METHODS

Patients

The women were originally sampled as part of a family study of the inheritance of low-density lipoprotein (LDL) subclass phenotypes [15]. The selection of kindreds took place primarily among the Mormon community in the San Francisco Bay Area [15]. The kindreds were not selected for lipid disorders or family history of coronary heart disease, but sequential sampling of kindreds for an LDL-subclass phenotype expression was used [15]. Persons were excluded from this analysis if they reported drinking, smoking, using birth control pills, or taking drugs known to affect plasma HDL. We have previously reported that there were 89 premenopausal women who had regular menstrual periods in this sample [11]. The day of the cycle was

determined from the following questionnaire item: "Do you have regular cycles or periods? If yes, then indicate starting date of last period." Forty-five women were sampled before we introduced this question into the questionnaire. Three additional women did not answer this question. Thus, the current analyses are based on 41 women.

Methods

All participants provided a single blood sample after an overnight fast. Plasma total cholesterol and triglyceride concentrations were measured on fresh samples by enzymatic techniques using the Gilford 3500 autoanalyzer (Ciba Corning Diagnostics, Oberlin, OH) [16]. The HDL cholesterol level was measured after precipitation with heparin-MnCl₂ [17,18]. Fresh plasma was ultracentrifuged at $d < 1.006$ g/mL, cholesterol was assayed in the infranatant, and the LDL cholesterol level was calculated from the formula of Friedewald et al [19]. Plasma apo A-I and apo B concentrations were determined by radialimmunodiffusion [20], and enzyme-linked immunosorbent assay [21,22], respectively. The coefficients of variation for apo A-I and apo B did not exceed 15%.

Electrophoresis of HDL in the ultracentrifuged $d \leq 1.20$ -g/mL fraction was performed on a 2-mL fresh plasma sample using the Pharmacia Electrophoresis Apparatus (GE 4-11) and slab gradient gels (PAA 4/30; Pharmacia, Piscataway, NJ) as described by Blanche et al [9]. Calculus (ie, transformation of variables) was used to convert the HDL distributions from the migration-distance scale to the particle-diameter scale [23]. The mean coefficients of variation for absorbency measured at individual diameter values were 23% within the HDL_{2b} interval, 17% within the HDL_{2a} interval, 23% within the HDL_{3a} interval, 33% within the HDL_{3b} interval, and 141% within the HDL_{3c} interval.

Statistics

A fourth-order polynomial equation was used to assess the relationship of menstrual cycle to HDL levels, using the number of days since the beginning of the last period as the independent variable (raised to the first, second, third, and fourth power) and HDL levels as the dependent variable (ie, $HDL = \alpha + \beta_1 \text{days} + \beta_2 \text{days}^2 + \beta_3 \text{days}^3 + \beta_4 \text{days}^4$). A variety of patterns can be approximated by this equation, including quadratic (convex or concave), cubic (eg. convex-concave or concave-convex), and fourth-order (convex-concave-convex or concave-convex-concave) approximations to the cycle. The fourth-order polynomial regression was said to be statistically significant ($P \leq 0.05$) if significance was achieved ($P \leq 0.05$) for every term of a fourth-order polynomial and for the overall F test of the regression model.

RESULTS

The women ranged in age from 14 to 49 years (mean $\pm$ SD: 35.9 ± 7.3 years), were generally nonoverweight (body mass index: 23.6 ± 4.6 kg/m²), and had generally normal lipoprotein concentrations (triglycerides: 95.3 ± 47.0 mg/dL; total cholesterol: 176.4 ± 33 mg/dL; HDL-cholesterol: 49.0 ± 11.2 mg/dL; apo A-I: 131.8 ± 13.1 mg/dL; and apo B: 76.1 ± 25.6 mg/dL). The associations between these lipoproteins and the number of days since the beginning of the last period did not achieve statistical significance in this sample.

A three-dimensional wire-frame graph (Fig 1) is used to display the relationship of HDL levels to the number of days since the beginning of the last menstrual period. This is performed over the entire HDL-diameter range. The height of the curve represents the changes in HDL from the overall sample mean. The base of the wire-frame graph represents the axes for the HDL particle diameter and number of days since the beginning of the last period. Thus, the surface of the graph shows variations in height that correspond to deviations from the mean value (see vertical axis) by diameter and day. Shading shows the correspondence between the vertical

axis and the elevation of the surface, ie, each shaded region represents a 2-unit increment in elevation of the curve. The specific example of protein-stained HDL of 9.1 nm is presented in Fig 2, which shows higher levels during the luteal phase than during the follicular phases of the cycle. Figure 1 shows that there were significant fourth-order fluctuations for most of the HDL_{2a} region (significant between 8.79 and 9.86 nm). Specifically, plasma HDL_{2a} levels were lower at 14 days and higher at 22 to 32 days after the beginning of the last period. Variations in levels of other subclasses were not significant.

DISCUSSION

Our results suggest that plasma HDL_{2a} levels were increased during the luteal phase, whereas HDL_{2b} remained constant. This is consistent with our previous observation that postmenopausal estrogen replacement primarily increases HDL_{2a} [11]. Estrogen and progesterone are elevated during the luteal phase. Both hormones are also elevated during pregnancy, however, during pregnancy the HDL_{2b} component is principally increased [24]. Many factors distinguish pregnancy from the luteal phase, including substantially higher estrogen and progesterone [24], higher triglyceride, cholesterol, and free fatty acid levels [25], greater insulin resistance [26], decreased very-low-density lipoprotein clearance [27], and decreased lipoprotein lipase activity [28]. In contrast, the luteal phase of the menstrual cycle is associated with lower plasma cholesterol [1,29], lower triglycerides [6,29], and lower plasma free fatty acid [30] as compared with the follicular phase. Postprandial lipemia [31], postheparin lipoprotein lipase activity [5], glucose tolerance [32], and insulin secretion [32, 33] all appear to be relatively constant during the normal menstrual cycle.

Others have reported that HDL_{2a} and HDL_{2b} differ in their apolipoprotein composition. The HDL_{2a} subclass contains particles that contain both apo A-I and apo A-II, whereas the HDL_{2b} subclass primarily consists of particles that contain apo A-I and no apo A-II [34,35]. Both apo A-I and apo A-II are increased by postmenopausal estrogen replacement [36]. Estrogen and progesterone are elevated during the luteal phase of the menstrual cycle [6]. Plasma concentrations of both apo A-I and apo A-II are highest in hysterectomized baboons treated with both estrogen and progesterone versus estrogen alone, progesterone alone, or no treatment [37]. Metabolic studies suggested that both estrogen and progesterone increased apo A-I and apo A-II production, and that progesterone increased the fractional catabolic rate of apo A-I but not apo A-II [37]. In contrast, estrogen does not appear to affect the fractional catabolic rate of either apo A-I or apo A-II [37]. Thus, elevated plasma HDL_{2a} during the luteal phase could arise from increased apo A-I and apo A-II production due to the combination of increased progesterone and estrogen.

In our study, the relationships between cycle day and plasma lipoproteins are likely to be attenuated (ie, diluted) by the imprecise timing of the cycle. The absence of data on the beginning of the next menses, hormone levels, and daily basal body temperatures introduces measurement error into the independent variable. This increases the probability of a type II statistical error (ie, the probability of missing an association), but will not inflate the probability of a type I statistical error (the probability of claiming an association when it does not exist) [38]. This may explain the lack of a significant variation in plasma triglycerides, HDL cholesterol, or LDL cholesterol, particularly if the effect of the menstrual cycle on these variables is less pronounced than its effect on HDL_{2a}.

Acknowledgments

We wish to thank Laura Hall, Charlotte Brown, and Bahareh Sahami for laboratory analysis of gradient gel electrophoresis.

Supported in part by Grants No. HL-02183, HL-24462, and HL-I8574 and First Independent Research and Transition Award No. HL-38760 from the National Heart, Lung, and Blood Institute, and conducted at the Lawrence Berkeley Laboratory (Department of Energy Grant No. DE-AC03-76SF00098 to the University of California).

REFERENCES

1. Jones DY, Judd JT, Taylor PR, et al. Menstrual cycle effect on plasma lipids. *Metabolism* 1988;37:1–2. [PubMed: 3336283]
2. Mattsson LA, Silfverstole G, Samsioe G. Lipid composition of serum lipoproteins in relation to gonadal hormones during the menstrual cycle. *Eur J Obstet Gynecol Reprod Biol* 1984;17:327–335. [PubMed: 6541163]
3. Kim J-H, Kalkhoff RK. Changes in lipoprotein composition during the menstrual cycle. *Metabolism* 1979;28:663–668. [PubMed: 221787]
4. Woods NC, Graham TE. Effects of menstrual cycle phase and exercise training on serum lipids. *Can J Appl Sports Sci* 1986;11:88–93.
5. Tikkanen MJ, Kuusi T, Nikkila EA, et al. Variation in postheparin plasma hepatic lipase by menstrual cycle. *Metabolism* 1986;35:99–104. [PubMed: 3080655]
6. De Long RG, Austin KL, Richards L, et al. Lipid and hormonal profiles of Panamanian women during the menstrual cycle. *Int J Gynaecol Obstet* 1992;39:219–226. [PubMed: 1360917]
7. Takatani O, Okumoto T, Kosano H. Genesis of breast cancer in Japanese: A possible relationship between sex hormone binding globulin and serum lipid components. *Breast Cancer Res Treat* 1991;18:S27–S29. [PubMed: 1873554]
8. Tikkanen MJ, Nikkila EA, Kuusi T, et al. High density lipoprotein-2 and hepatic lipase: Reciprocal changes produced by estrogen and norgestrel. *J Clin Endocrinol Metab* 1982;54:1113–1117. [PubMed: 7076794]
9. Blanche PJ, Gong EL, Forte TM, et al. Characterization of human high-density lipoproteins by gradient gel electrophoresis. *Biochim Biophys Acta* 1981;665:408–419. [PubMed: 7295744]
10. Williams PT, Vranizan KM, Austin MA, et al. Familial correlations of high-density lipoprotein subclasses based on gradient gel electrophoresis. *Arterioscler Thromb* 1992;12:1467–1474. [PubMed: 1450178]
11. Williams PT, Vranizan KM, Austin MA, et al. Associations of age, adiposity, alcohol intake, menstrual status and estrogen therapy with high-density lipoprotein subclasses. *Arterioscler Thromb* 1993;13:1654–1661. [PubMed: 8218107]
12. Williams PT, Krauss RM, Vranizan KM, et al. Effects of weight loss by exercise and by diet on apolipoprotein A-I and A-II and the particle size distribution of high density lipoproteins in men. *Metabolism* 1992;41:441–449. [PubMed: 1556953]
13. Williams PT, Krauss RM, Stefanick ML, et al. Effects of low-fat diet, caloric restriction and running on lipoprotein subfraction concentrations in moderately overweight men. *Metabolism* 1994;43:655–663. [PubMed: 8177056]
14. Williams PT, Krauss RM, Vranizan KM, et al. Associations of lipoproteins and apolipoproteins with gradient gel electrophoresis estimates of high-density lipoprotein subfractions in men and women. *Arteriosclerosis* 1992;12:332–340.
15. Austin MA, King M-C, Vranizan KM, et al. Inheritance of low density lipoprotein subclass patterns: Results of complex segregation analyses. *Am J Hum Genet* 1988;43:838–846. [PubMed: 3195585]
16. Allain CC, Poon LS, Chan CSG, et al. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974;20:470–475. [PubMed: 4818200]
17. Warnick GR, Genderson J, Albers JJ. Dextran sulfate-Mg²⁺ precipitation procedure for the quantitation of high density lipoprotein cholesterol. *Clin Chem* 1982;28:1379–1388. [PubMed: 7074948]
18. Lipid Research Clinics Program: Manual of Laboratory Operations, vol I: Lipid and Lipoprotein Analysis. Bethesda, MD: National Institutes of Health; 1975. p. 51-59. NHLBI DHEW Publication No. (NIH)75-628

19. Friedewald WT, Levy RI, Frederickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without the use of the preparative centrifuge. *Clin Chem* 1972;18:499–502. [PubMed: 4337382]
20. Cheung MC, Albers JJ. The measurement of apolipoprotein A-I and A-II levels in men and women by immunoassay. *J Clin Invest* 1977;60:43–50. [PubMed: 194921]
21. Voller, A.; Bidwell, DE.; Bartlett, AA. *The Enzyme Linked Immunosorbent Assay (ELISA)*. London, UK: Nuffield Laboratories of Comparative Medicine, Zoological Society of London; 1979.
22. Weiler RJ, Hofstra A, Szentivanyi R, et al. The inhibition of labeled antigen precipitation as a measure of serum g-globulin. *J Immunol* 1960;85:130–137. [PubMed: 13843522]
23. Williams PT, Krauss RM, Nichols A, et al. Identifying the predominant peak diameter of high-density (HDL) and low density (LDL) lipoproteins by electrophoresis. *J Lipid Res* 1990;31:1131–1139. [PubMed: 2373962]
24. Silliman K, Tall AR, Kretchmer N, et al. Unusual HDL subclass distribution during late pregnancy. *Metabolism* 1993;42:1592–1599. [PubMed: 8246775]
25. Desoye G, Schweditsch MO, Pieffer KP, et al. Correlations of hormones with lipid and lipoprotein levels during pregnancy and postpartum. *J Clin Endocrinol Metab* 1987;64:704–713. [PubMed: 3546352]
26. Hornnes PJ. On the decrease of glucose tolerance in pregnancy. A review. *Diabete Metab* 1985;11:310–315.
27. Mancini M, Steiner G, Betteridge DJ, et al. Acquired (secondary) forms of hypertriglyceridemia. *Am J Cardiol* 1991;68:17A–21A.
28. Kinnunen PK, Unnerus HA, Ranta T, et al. Activities of lipoprotein lipase and hepatic lipase during pregnancy and lactation. *Eur J Clin Invest* 1980;10:469–474. [PubMed: 6788565]
29. Ahumada Hemer H, Valles de Bourges V, Juarez Ayala J, et al. Variation in serum lipids and lipoproteins throughout the menstrual cycle. *Fertil Steril* 1985;44:80–84. [PubMed: 4007194]
30. Pansini F, Bonaccorsi G, Genovesi F, et al. Influence of estrogens on serum free fatty acid levels in women. *J Clin Endocrinol Metab* 1990;71:1387–1389. [PubMed: 2229296]
31. Wendler D, Michel E, Kastner P, et al. Menstrual cycle exhibits no effect on postprandial lipemia. *Horm Metab Res* 1992;12:580–581. [PubMed: 1478617]
32. Toth EL, Suthijumroon A, Crockford PM, et al. Insulin action does not change during the menstrual cycle in normal women. *J Clin Endocrinol Metab* 1987;64:74–80. [PubMed: 3536985]
33. Bonora E, Zavaroni I, Alpi O, et al. Influence of menstrual cycle on glucose tolerance and insulin secretion. *Am J Obstet Gynecol* 1987;157:140–141. [PubMed: 3300346]
34. Cheung MC, Albers JJ. Characterization of lipoprotein particles isolated by immunoaffinity chromatography: Particles containing A-I and A-II and particles containing A-I but no A-II. *J Biol Chem* 1984;259:12201–12209. [PubMed: 6434538]
35. Nichols AV, Blanche PJ, Shore VG, et al. Conversion of apolipoprotein-specific high-density lipoprotein populations during incubation of human plasma. *Biochim Biophys Acta* 1989;1001:325–337. [PubMed: 2492827]
36. Applebaum-Bowden D, McLean P, Steinmetz A, et al. Lipoprotein, apolipoprotein, and lipolytic enzyme changes following estrogen administration in postmenopausal women. *J Lipid Res* 1989;30:1895–1906. [PubMed: 2621417]
37. Kushwaha RS, Foster DM, Murthy VN, et al. Metabolic regulation of apoproteins of high-density lipoproteins by estrogen and progesterone in the baboon (*Papio sp.*). *Metabolism* 1990;39:544–552. [PubMed: 2110609]
38. Fuller, WA. *Measurement Error Models*. New York, NY.: Wiley; 1987. p. 1-5.

Fig. 1.

Three-dimensional wire-frame diagram between HDL levels and number of days since the beginning of the last period in 41 menstruating women. A fourth-order polynomial equation was fitted at each diameter value. The height of the curve represents changes in HDL levels from the overall sample mean of the 41 women. The solid portions of the bar at the bottom of the figure designate the diameter values that achieve statistical significance ($P \leq 0.05$) for every term of a fourth-order polynomial and for the overall F test of the regression model.

Fig. 2.

Fourth-order polynomial regression between 9.1-nm diameter HDL levels and days since the beginning of the last period in 41 menstruating women. The mean absorbency for protein-stained HDL at 9.1 nm was 10.78 in these 41 women, and the Y-axis is the amount above or below the mean of the sample of women. Specifically, the formula was $HDL = -7.4445 + 3.5676 \cdot \text{day} - 0.5140 \cdot \text{day}^2 + 0.2630 \cdot \text{days}^3 - 0.0004 \cdot \text{day}^4$. Significance levels for each term were $P = 0.01$ for day, $P = 0.005$ for day^2 , $P = 0.026$ for day^3 , and $P = 0.002$ for day^4 .