Hypothesis/Commentary

Plasma Volume Expansion in Pregnancy: Implications for Biomarkers in Population Studies

Jessica M. Faupel-Badger,[1,2] Chung-Cheng Hsieh,[5] Rebecca Troisi,[3] Pagona Lagiou,[6,7] and Nancy Potischman[4]

[1]Division of Cancer Prevention, [2]Mammary Biology and Tumorigenesis Laboratory, Center for Cancer Research, [3]Division of Cancer Epidemiology and Genetics, and [4]Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, Maryland; [5]University of Massachusetts Medical School, Worcester, Massachusetts; [6]Department of Hygiene and Epidemiology, University of Athens Medical School, Athens, Greece; and [7]Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts

Abstract

There is a growing body of literature focused on endogenous hormone exposures during pregnancy and subsequent cancer risk for both mother and offspring. Examples of these studies include those focused on the biological mechanism for the association of preeclampsia with reduced risk of breast cancer for mother and female offspring or studies that have examined hormone concentrations during pregnancy between different ethnic groups who vary in their rates of breast cancer incidence. Although these studies seem relatively straightforward in conception and analysis, measurement of the concentration of hormones and other biomarkers in pregnant subjects is influenced by plasma volume expansion (PVE). During pregnancy, the maternal plasma volume expands 45% on average to provide for the greater circulatory needs of the maternal organs. Consequently, serum protein and hormone concentrations are greatly altered when comparing the pregnant with nonpregnant state. Assessing PVE also is complicated by the vast individual variation in PVE, ranging from minimal to a 2-fold increase. We propose that PVE needs to be evaluated when comparing biomarker concentrations during pregnancy in two populations that may differ with respect to PVE. Small body size is associated with lower PVE compared with higher body size. Therefore, we hypothesize that variation in PVE will influence the interpretation of differences in biomarker concentrations across population groups with respect to the etiologic significance of the biomarker to the disease under study (e.g., breast cancer). It is possible that some observations may be due only to differences in dilution between the two groups. We present PVE as a topic for consideration in population-based studies, examples of the types of studies where PVE may be relevant, and our own analysis of one such study in the text below. (Cancer Epidemiol Biomarkers Prev 2007;16(9):1720–3)

Introduction to Plasma Volume Expansion in Pregnancy

During pregnancy, maternal plasma volume increases to meet the greater circulatory needs of the placenta and maternal organs (e.g., uterus, breasts, skin, and kidneys), with an average increase of ~45% (1-5). There are vast differences among women, however, from a minimal change to a doubling in plasma volume (1, 6, 7). Several factors can influence plasma volume expansion (PVE) including maternal pre-pregnancy body mass index (BMI; ref. 8). In studies that have examined ethnic differences in PVE, populations that are on average shorter and weigh less exhibit less absolute change in plasma volume (9-11). This was well documented in a report comparing plasma volumes between Indians and Europeans (10). Furthermore, of studies examining PVE across different ethnicities, all show vast individual variation in PVE as well (9-11). Much of the variation is unexplained, although it is known that PVE is positively associated with parity (12), multiple births (13-15), higher birth weight (16, 17), and increased maternal pre-pregnancy BMI (8) and inversely associated with conditions of decreased fetal growth (e.g., intrauterine growth restriction) and compromised placental development (e.g., preeclampsia; refs. 1, 2, 18-20).

In clinical practice, PVE or 'hemodilution' is addressed by modifying the criteria for diagnostic biomarkers. For example, the cut-point for anemia or iron deficiency, which is defined by hemoglobin <12.0 g/dL in the

Received 4/3/07; revised 5/29/07; accepted 6/22/07.

Grant support: Division of Cancer Prevention and the Center for Cancer Research, National Cancer Institute (J.M. Faupel-Badger) and W81XWH-05-1-0314 Innovator Award and U.S. Department of Defense Breast Cancer Research Program, Office of the Congressionally Directed Medical Research Programs (P. Lagiou and C-C. Hsieh). J.M. Faupel-Badger is a fellow in the National Cancer Institute Cancer Prevention Fellowship Program.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Jessica M. Faupel-Badger, Mammary Biology and Tumorigenesis Laboratory, Center for Cancer Research, National Cancer Institute, MSC 4254, 37 Convent Drive, Bethesda, MD 20892. Phone: 301-443-3076; Fax: 301-402-0711. E-mail: badgerje@mail.nih.gov

doi:10.1158/1055-9965.EPI-07-0311

nonpregnant state, is changed to <11.0 g/dL in the first and third trimesters and <10.5 g/dL in the second trimester. This corresponds to the increase in plasma volume starting at 6 to 10 weeks of gestation that rises sharply through the second trimester, before beginning to plateau at 32 weeks (1).

PVE and Population Studies

Highlighting diagnosis of anemia in clinical practice shows that PVE can have significant effects on biomarker concentrations. The implications of interindividual and between-group variability in PVE, however, have generally not been addressed in large population-based research studies involving pregnant subjects. In etiologic studies, this variability could introduce bias in the form of confounding if it were related to both the factor under study (e.g., preeclampsia) and the biomarker. Thus, accounting for PVE would be essential for a study focused on elucidating biomarkers involved in the causal pathway of a disease. For example, in preeclampsia, there is less PVE than in uncomplicated pregnancies (1, 2, 19). High concentrations of a biomarker such as sex hormone binding globulin (21) or soluble fms-like tyrosine kinase (1, 22, 23) among preeclamptic women compared with women who have uncomplicated pregnancies may reflect an etiologically relevant difference and/or the lower hemodilution in preeclampsia. We hypothesize that variation in PVE will affect the interpretation of differences in biomarker concentrations between individuals or population groups, especially with respect to etiologic significance, and that evaluation of PVE should be considered in population studies involving pregnant women.

Other examples in which individual variation in PVE may affect interpretation include when biomarkers are used to measure the success of nutritional intervention during pregnancy (24). In some areas of Asia, it is not uncommon for pregnant women to be deficient in multiple micronutrients (24, 25). To address this issue, one study among rural pregnant women in Nepal evaluated the effects of micronutrient supplementation on serum retinol, folate, riboflavin, and 25-hydroxyvitamin D concentrations (26). Assuming this population has wide variation in individual PVE, as has been shown in other populations, PVE may affect the perceived success of this intervention in individual women. Furthermore, variability in nutritional status and hydration across individuals could influence the apparent concentrations of nutrients of interest in this study via effects on PVE.

Measurement of PVE, Biomarker Concentrations, and Relation to Outcomes

PVE also may be an important factor in studies focused on understanding what biological features mediate associations of pregnancy characteristics with subsequent development of chronic disease in either the mother or offspring. For example, preeclampsia is associated with decreased breast cancer risk for both the mother and female offspring (27). Population-based studies of circulating biomarkers in preeclamptic and normotensive pregnancies have been conducted to elucidate possible biological mechanisms. Serum androgen concentrations at delivery are observed to be higher in preeclamptic women than in those with normal pregnancies (28). Progesterone concentrations also seem higher in maternal serum during the 27th week of pregnancy when comparing preeclamptic with normal pregnancies (29). Could the lower PVE that preeclamptic women experience relative to normotensive women explain their apparently higher concentration of serum hormones? And if so, are differences due to PVE of etiologic importance if the target tissues are exposed to the same total amount of the hormones?

Direct methods of measuring plasma volume are labor intensive and not easily adapted to large studies. Methods to approximate plasma volume involve labeling of albumin, with Evan's blue dye being the most common technique (11, 30). This method requires sampling plasma after injecting the patient with Evan's blue dye and allowing time (a minimum of 10 min) for sufficient mixing of the dye with plasma. The decay of Evan's blue dye is measured by the absorbance at 610 nm and plasma volume in milliliters per kilogram can be calculated from the absorbance measurement (30). In addition to patient monitoring during the dye injection and sample collection, it also is recommended that the patient observe a 30-min rest period before injection and that women in late pregnancy lay on their sides during injection to promote mixing of the dye (11, 30). Measuring Evan's blue dye at one point in time can approximate a measurement of plasma volume; however, to measure the increase in plasma volume, an individual would need to have the Evan's blue dye measurement completed more than once during pregnancy. These measurements across time points would then be used to determine the amount of PVE for the individual. This technique involves significant patient burden and is not practical for large studies.

In population-based studies, accounting for PVE in the data analysis by adjustment for factors highly predictive of PVE is more feasible. We reanalyzed data from a published study comparing maternal hormone concentrations in women from Boston and Shanghai (31) to determine if adjusting for correlates of PVE would affect the observed differences in estradiol concentrations. The women for this study were recruited from urban clinics affiliated with Beth Israel Hospital in Boston and three urban and one rural clinic in China affiliated with Shanghai Medical University. The women were all under the age of 40 years and were either Caucasian in Boston or Chinese in Shanghai. The purpose of the published study was to explore the hypothesis that fetal exposures, such as high *in utero* hormone concentrations, may be associated with the development of breast cancer in the offspring, using two populations with different disease incidence (31). Two serum samples, collected at 16 and 27 weeks of gestation, were used to evaluate estradiol, estriol, prolactin, progesterone, growth hormone, albumin, and sex hormone binding globulin. Given that women in Shanghai have approximately one fifth the incidence of breast cancer of women in Boston (32), it was hypothesized that the women from Shanghai would have lower hormone concentrations. In contrast, for every compound measured, the women from Shanghai had significantly higher concentrations with the exception of progesterone at 27 weeks. The results from this

Table 1. Percentage difference in maternal estradiol at 16 wks of gestation comparing Chinese with U.S. concentrations by strata of pre-pregnancy BMI, uniparous subjects only

	Adjusted for:			With further adjustment for:				
Pre-pregnancy BMI (kg/m^2)	Boston (*n*)	Shanghai (*n*)	Maternal age, gestational age	Albumin	Albumin, pre-weight	Albumin, height	Albumin, pre-BMI	Albumin, birth weight
<19.1	21	84	37.3*	39.5*	38.2*	35.2*	40.5*	40.8*
19.1-20.5	30	68	24.5*	31.0*	19.8	20.5	30.8*	31.0*
20.6-22.2	34	46	12.6	16.4	15.9	16.6	16.7	14.7
22.3+	36	22	34.8*	35.6*	34.3*	37.7*	34.3*	35.8*
All subjects	121	200	26.7*	30.5*	27.7*	29.0*	29.4*	30.6*

NOTE: Adjusted for maternal age, pre-pregnancy weight, pre-pregnancy BMI, height, and albumin measured at week 16, and offspring birthweight and gestational age.
*$P < 0.05$.

study were unexpected as Chinese women are known to have lower circulating estradiol concentrations than Caucasian women in the nonpregnant state (33-35).

We hypothesized that differences in PVE between the Chinese and U.S. populations could explain the higher estradiol concentrations observed among the Chinese. Asian women have lower values for several correlates of PVE, such as height, weight, and infant birth weight, when compared with Caucasian women (31, 36, 37). To assess whether the higher hormone concentrations present in Asian women were a result of less PVE compared with Caucasian women, we analyzed the data set from Lipworth et al. (31) adjusting for or stratifying on correlates of PVE. The analysis was restricted to uniparous (i.e., first pregnancy) women because parity is known to affect PVE (11). Regression analysis with log-transformed estradiol as the dependent variable and with adjustment for maternal age, pre-pregnancy weight, height, pre-pregnancy BMI, birth weight, and albumin concentration allowed calculation of percentage difference in estradiol concentrations between the Shanghai and Boston populations for subjects with complete data on covariates (131 subjects for Boston and 220 in Shanghai). Similar to results in the original study, among all uniparous women in our analysis, the estradiol concentrations in the women from Shanghai were 26.7% higher than the estradiol concentrations in the women from Boston, at 16 weeks of gestation, after adjusting for maternal age and gestational week. Table 1 presents the percentage difference among women at 16 weeks of gestation within the same category of pre-pregnancy BMI to attempt to further account for PVE. The differences in estradiol concentrations between the Boston and Shanghai populations were strongest at 16 weeks and adjustment for correlates of PVE, including maternal pre-pregnancy BMI and birth weight, had no effect. Results for analyses of estradiol concentrations at week 16 were similar when all subjects from the Lipworth study, uniparous and multiparous, were included in the analyses (data not shown).

Conclusions and Comments for Future Analysis of PVE

Our reanalysis of the estradiol data shows that the difference in estradiol concentrations between the Boston and Shanghai populations cannot be explained by adjusting for currently accepted correlates of PVE. Therefore, we have two possible conclusions. The difference in estradiol concentrations between these two populations may be real and due to factors other than differences in PVE. Alternatively, it is possible that pre-pregnancy BMI may not be a sufficient marker of PVE. In nonpregnant women, it may be possible to adjust for differences in plasma volume by including BMI in the model, as BMI is highly correlated with plasma volume in healthy, nonpregnant individuals (8). Whereas pre-pregnancy BMI is currently an accepted correlate for PVE, other factors (e.g., physiologic processes related to adaptation to pregnancy and size of fetus) besides maternal size can affect PVE, thus suggesting that pre-pregnancy BMI may not be the most adequate correlate of PVE, although it may be the best one in use at this time.

Although reanalysis of the data from the study by Lipworth et al. did not support our hypothesis that pre-pregnancy BMI would be a sufficient predictor to account for PVE, it may be worth considering this type of analysis in other studies with larger numbers or among different ethnicities. In addition, to fully account for any effects of PVE, it may be that new proxies or methods to evaluate PVE need to be developed for practical use in large studies. While new proxies or methods are being explored, other avenues to measure hormone exposure during pregnancy could be used to circumvent the issue of PVE. For example, studies focused on *in utero* hormone exposure and subsequent disease risk in the offspring could also examine cord blood samples for the measurement of hormones or other biomarkers (38). These samples would be insulated from variations in dilution present in the maternal circulation and may be more relevant to fetal exposure than conclusions based on maternal serum. In addition to cord blood, saliva or urine may also provide a source for hormone measurements that may be less influenced by PVE. Future studies with samples to measure the correlation of maternal serum hormone concentrations to concentrations in cord blood, urine and saliva would be helpful to determine if the magnitude increase in hormone concentrations seen in serum is reflected in the alternative method. It would also be necessary to evaluate whether the alternative method may also be influenced by PVE. Another approach would be to evaluate changes in a biomarker over the course of a pregnancy, perhaps elucidating different patterns in those that develop a pregnancy condition from those who remain normal.

In conclusion, given the vast individual and group differences in PVE, its implications for the interpretation of results from biomarkers studies should be considered. We hope this commentary begins a dialogue about the implications of PVE and its evaluation for the types of studies outlined above. Further evaluation of PVE is necessary to determine its potential effect on the interpretation of observed results with regard to etiology and the degree to which it needs to be addressed in study design and/or analysis.

References

1. Blackburn ST, Loper DL. Maternal, fetal, and neonatal physiology. A clinical perspective. Philadelphia: W.B. Saunders Co.; 1992. p. 160–2, 171, 202–3, 222–8.
2. Brinkman CR. Maternal cardiovascular and renal disorders. Maternal-fetal medicine. Philadelphia: W.B. Saunders; 1984. p. 679–90.
3. Chesley LC. Plasma and red cell volumes during pregnancy. Am J Obstet Gynecol 1972;112:440.
4. Peck TM, Arais F. Hematologic changes associated with pregnancy. Clin Obstet Gynecol 1979;22:785.
5. Pritchard JA. Changes in blood volume during pregnancy and delivery. Anesthesiology 1965;26:393.
6. Pritchard JA, MacDonald PC, Gant N. Williams' obstetrics. New York: Appleton-Century-Crofts; 1985. p. 191–4.
7. Anderson HM. Maternal hematologic disorders. Maternal-fetal medicine. Philadelphia: W.B. Saunders; 1984. p. 795–832.
8. Dagher FJ, Lyons JH, Finlayson DC, Shamsai J, Moore FD. Blood volume measurement: a critical study prediction of normal values: controlled measurement of sequential changes: choice of a bedside method. Adv Surg 1965;1:69–109.
9. Harrison KA. Blood volume changes in normal pregnant Nigerian women. J Obstet Gynaecol Br Commonw 1966;73:717–23.
10. Hutchins CJ. Plasma volume changes in pregnancy in Indian and European primigravidae. Br J Obstet Gynaecol 1980;87:586–9.
11. Hytten F. Blood volume changes in normal pregnancy. Clin Haematol 1985;14:601–12.
12. Campbell DM, MacGillivray I. Comparison of maternal response in first and second pregnancies in relation to baby weight. J Obstet Gynaecol Br Commonw 1972;79:684–93.
13. Fullerton WT, Hytten FE, Klopper AI, McKay E. A case of quadruplet pregnancy. J Obstet Gynaecol Br Commonw 1965;72:791–6.
14. Letsky E. The haematological system. In: Hytten FE, Chamberlain GVP, editors. Clinical physiology in obstetrics. Oxford: Blackwell Scientific Publications; 1980. p. 43–78.
15. Rovinsky JJ, Jaffin H. Cardiovascular hemodynamics in pregnancy. I. Blood and plasma volumes in multiple pregnancy. Am J Obstet Gynecol 1965;93:1–15.
16. Hytten FE, Paintin DB. Increase in plasma volume during normal pregnancy. J Obstet Gynaecol Br Emp 1963;70:402–7.
17. Pirani BB, Campbell DM, MacGillivray I. Plasma volume in normal first pregnancy. J Obstet Gynaecol Br Commonw 1973;80: 884–7.
18. Bentley DP. Iron metabolism and anaemia in pregnancy. Clin Haematol 1985;14:613.
19. Salas SP, Marshall G, Gutierrez BL, Rosso P. Time course of maternal plasma volume and hormonal changes in women with preeclampsia or fetal growth restriction. Hypertension 2006;47:203–8.
20. Salas SP, Rosso P, Espinoza R, et al. Maternal plasma volume expansion and hormonal changes in women with idiopathic fetal growth retardation. Obstet Gynecol 1993;81:1029–33.
21. Wolf M, Sandler L, Munoz K, et al. First trimester insulin resistance and subsequent preeclampsia: a prospective study. J Clin Endocrinol Metab 2002;87:1563–8.
22. Levine RJ, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med 2006;355:992–1005.
23. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672–83.
24. Ahmed F, Mahmuda I, Sattar A, Akhtaruzzaman M. Anaemia and vitamin A deficiency in poor urban pregnant women of Bangladesh. Asia Pac J Clin Nutr 2003;12:460–6.
25. Jiang T, Christian P, Khatry SK, Wu L, West KP, Jr. Micronutrient deficiencies in early pregnancy are common, concurrent, and vary by season among rural Nepali pregnant women. J Nutr 2005;135:1106–12.
26. Christian P, Jiang T, Khatry SK, et al. Antenatal supplementation with micronutrients and biochemical indicators of status and subclinical infection in rural Nepal. Am J Clin Nutr 2006;83:788–94.
27. Innes KE, Byers TE. Preeclampsia and breast cancer risk. Epidemiology 1999;10:722–32.
28. Troisi R, Potischman N, Roberts JM, et al. Maternal serum oestrogen and androgen concentrations in preeclamptic and uncomplicated pregnancies. Int J Epidemiol 2003;32:455–60.
29. Tamimi R, Lagiou P, Vatten LJ, et al. Pregnancy hormones, preeclampsia, and implications for breast cancer risk in the offspring. Cancer Epidemiol Biomarkers Prev 2003;12:647–50.
30. el-Sayed H, Goodall SR, Hainsworth R. Re-evaluation of Evans blue dye dilution method of plasma volume measurement. Clin Lab Haematol 1995;17:189–94.
31. Lipworth L, Hsieh CC, Wide L, et al. Maternal pregnancy hormone levels in an area with a high incidence (Boston, USA) and in an area with a low incidence (Shanghai, China) of breast cancer. Br J Cancer 1999;79:7–12.
32. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002: Cancer incidence, mortality and prevalence Worldwide. In: IARC, editor. CancerBase no. 5. version 2.0 2006. Lyon: IARC Press; 2004.
33. Bernstein L, Yuan JM, Ross RK, et al. Serum hormone levels in premenopausal Chinese women in Shanghai and white women in Los Angeles: results from two breast cancer case-control studies. Cancer Causes Control 1990;1:51–8.
34. Key TJ, Chen J, Wang DY, Pike MC, Boreham J. Sex hormones in women in rural China and in Britain. Br J Cancer 1990;62:631–6.
35. Ursin G, Wilson M, Henderson BE, et al. Do urinary estrogen metabolites reflect the differences in breast cancer risk between Singapore Chinese and United States African-American and white women? Cancer Res 2001;61:3326–9.
36. Lagiou P, Hsieh CC, Trichopoulos D, et al. Birthweight differences between USA and China and their relevance to breast cancer aetiology. Int J Epidemiol 2003;32:193–8.
37. Wen SW, Kramer MS, Usher RH. Comparison of birth weight distributions between Chinese and Caucasian infants. Am J Epidemiol 1995;141:1177–87.
38. Troisi R, Potischman N, Roberts JM, et al. Correlation of serum hormone concentrations in maternal and umbilical cord samples. Cancer Epidemiol Biomarkers Prev 2003;12:452–6.