contain the Scantibodies HBT blocking agent but do contain other components to minimize interference from heterophilic antibodies and anti-animal antibodies. The patient serum did not contain anti-TSH antibodies. A 10-fold dilution of the patient’s sample with the Elecsys diluent yielded a TSH result of 60 mIU/L, and 10-fold dilutions with two waste sera that had very low TSH concentrations (<0.01 mIU/L) yielded discrepant results: 33 mIU/L with the first low TSH serum and 66 mIU/L with the second (results corrected for the dilution factor).

We had no explanation for the different results obtained with the two sera without TSH. Treating the serum in HBT tubes had no effect on the Elecsys FT4 result (33.3 pmol/L after treatment), but decreased the Elecsys TSH value (30.3 mIU/L after treatment). The TSH result after this treatment remained high and disagreed with the results of the other methods. As already noted (13), HBT treatment does not guarantee a correct result. The addition of 100 mL/L mouse serum had a moderate effect on Elecsys TSH result (measured value, 84% of the expected value), and the further addition of 100 mL/L bovine serum did not decrease the TSH result. The reduction in measured TSH concentration after treatment in HBT tubes and after addition of mouse serum was consistent with interference from heterophilic antibodies. For this patient no complementary clinical data could be obtained. Having run out of serum, we were unable to undertake other investigations regarding either of these two patients.

In conclusion, interference from heterophilic antibodies in TSH assays has become exceptional but still exists (14–16). As expected (5), and clearly exemplified for the first time in TSH assays by these two patients, interference may occur even when immunoassays involving chimeric antibodies. The use of nonspecific blocking agents efficiently protects current immunoassays against interference from isotopic antibodies but probably protects less efficiently against idiotypic antibodies (5). Perfect protection against all interfering antibodies remains a goal difficult to reach, and possible interference should be considered, at the time of thyroid diagnosis or during the follow-up of primary hypothyroidism treatment, when the TSH concentration is not compatible with the clinical history or other thyroid function tests.

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References


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Nonglycosylated Ferritin Predominates in the Circulation of Women with Preeclampsia but Not Intrauterine Growth Restriction, Carl A. Hubel,1 Lisa M. Bodnar,1 Ariel Many,2 Gail Harger,2 Roberta B. Ness,2 and James M. Roberts1,2 (Magee-Womens Research Institute and Department of Obstetrics and Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, PA; 2 Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; and 3 Lis Maternity Hospital, Sournsky Tel Aviv Medical Center, Tel Aviv University, Tel Aviv, Israel; *address correspondence to this author at: Magee-Womens Research Institute, 204 Craft Ave., Pittsburgh, PA 15213; fax 412-641-1-503, e-mail rsica@mwri.magee.edu)

Three to five percent of pregnancies are complicated by preeclampsia, a multisystem disorder characterized by hypertension and proteinuria that occurs after 20 weeks of gestation. Although widespread inflammation and endothelial dysfunction appear to be central maternal abnor-
malities in preeclampsia, the pathogenesis of the syndrome remains poorly understood (1).

The intracellular iron storage protein ferritin can hold up to 4000 iron atoms. Low concentrations of ferritin are found in normal serum because of active secretion from reticuloendothelial or parenymal cells (2, 3). During normal pregnancy, serum ferritin concentrations decrease with advancing gestation to reach a nadir at the third trimester (4). The metabolism of the serum iron and iron-binding proteins, ferritin and transferrin, is abnormal in women with preeclampsia (5–8). Median serum ferritin concentrations are approximately fivefold higher during preeclampsia than in normal pregnancy (6, 8, 9). The lowest quartile of ferritin concentrations at 28–30 weeks of gestation is associated with decreased risk of preeclampsia, premature rupture of membranes, and infant admission to the neonatal unit (4). The reasons for the increased serum ferritin with preeclampsia remain unclear. Serum ferritin is a reliable indicator of total body iron status in nondiseased individuals, with low concentrations diagnostic of iron deficiency. However, a high ferritin does not always signify iron excess.

Unlike intracellular ferritin, which is nonglycosylated and high in iron, normal serum ferritin is 60–80% glycosylated and very low in iron, suggesting active secretion rather than nonspecific leakage from cells (2, 10). Blood concentrations of ferritin increase with inflammation or infection as a result of augmented intracellular synthesis (2, 11). In such cases, the proportion of glycosylated serum ferritin is basically unchanged, and the serum ferritin is iron-poor, reflecting the fact that the normal mechanisms of ferritin production remain operative (2, 12). Serum ferritin can also be greatly increased by damage to ferritin-rich tissues, in which case more of the ferritin is nonglycosylated and contains iron (3, 10, 13). Liver disease is the most frequent cause of increased nonglycosylated ferritin in serum, but damage to other tissues can also cause this change.

We asked whether, in women with preeclampsia, serum nonglycosylated ferritin was increased as a percentage of total serum ferritin, consistent with nonspecific release of ferritin from damaged cells, and whether glycosylated ferritin concentration was increased, consistent with increased synthesis. We examined the correlation of these variables with serum aspartate aminotransferase (AST), transferrin (negative acute-phase reactant), and serum iron. Because placental damage is a candidate source of ferritin and iron released into the circulation, we also measured these variables in normotensive women with intrauterine-growth-restricted (IUGR) fetuses, a condition with placental pathology similar to preeclampsia but without significant maternal vascular disease.

We studied 75 nulliparous women at Magee-Womens Hospital, either before or at the time of admission to the labor and delivery ward. The clinical data are summarized in Table 1 of the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue5/. Patients with chronic hypertension, renal or metabolic disease, or previous history of metabolic disorders were excluded. Small for gestational age (SGA) was defined as birth weight below the tenth centile, adjusted for race, gender, and gestational age, according to local standard growth curves. Ten of the 14 SGA pregnancies had clinical evidence of fetal growth restriction [abnormal umbilical Doppler (1) or asymmetric growth profile (9)]; insufficient information was available for the other four. Of the latter four, birth weight centiles were 4, 3, 3, and 1. Collectively, the SGA group will be referred to as having IUGR with the caveat that a few of these babies might have been constitutionally small without growth restriction. The criteria for preeclampsia, new-onset hypertension and proteinuria after 20 weeks of gestation, were as described previously (14, 15). The vast majority of women used prenatal vitamins containing iron. Information was unavailable for 5% of the women, but 89% of the remainder reported use of iron supplements during pregnancy.

Serum ferritin was measured with an immunoradiometric assay (Diagnostic Products Corp.). The detection limit of the assay is 1 μg/L. Nonglycosylated ferritin was measured after glycosylated ferritin was removed by binding to concanavalin A, according to the method of Worwood et al. (3) with minor modifications (13) (see also Fig. 1 in the online Data Supplement). Samples were assayed in duplicate. Ferritin and ferritin subtypes were also measured in a pool of serum during each assay. Interassay imprecision (CV) was 7.4% for total ferritin and 7.1% for the percentage of nonglycosylated ferritin. Serum total iron, total iron-binding capacity (TIBC), unsaturated iron binding capacity (UIBC), and the percentage saturation of iron-binding capacity were determined by colorimetric assay (ferrozine method; Sigma Diagnostics) as described previously (7).

Because the distributions for serum ferritin (total, glycosylated, and nonglycosylated), percentage saturation of TIBC, and iron were skewed, geometric means were calculated. Differences between groups were analyzed by ANOVA with the Bonferroni–Dunn post hoc test. Dichotomous variables were compared by use of the χ² test for homogeneity. Linear regression was used to calculate unadjusted correlations between clinical data and the serum analytes. Multivariable linear regression was used to determine the independent effect of pregnancy diagnosis (preeclampsia, IUGR, or normal) on each measure of ferritin, transferrin, and iron after adjusting for variables that might confound each relationship (gestational age at the time of blood sampling, maternal age, maternal prepregnancy body mass index, smoking status, presence or absence of labor at the time of blood sampling, and maternal race).

The clinical outcome characteristics of the patients are shown in Table 1 in the online Data Supplement. Gestational age at time of sampling was lower for women with preeclampsia than for controls (P < 0.002) and for women with preeclampsia compared with women carrying IUGR fetuses (P < 0.0001). Women with normal or IUGR pregnancies delivered at term (≥37 weeks), with the exception of one IUGR patient who delivered at 35 weeks. The
majority (23 of 28) women with preeclampsia delivered preterm. Ten of 28 women with preeclampsia had AST activities >64 U/L, consistent with the presence of liver damage. At least four of these women had HELLP (hemolysis, elevated liver enzymes, low platelets) or ELLP (elevated liver enzymes, low platelets) syndrome.

Multivariate analyses (Table 2 in the online Data Supplement) were generally concordant with bivariate analyses (Table 1). As shown in Table 1, serum total ferritin concentrations were approximately fivefold higher in women with preeclampsia than in women with normal pregnancies (P <0.0001) and approximately threefold higher than in women carrying IUGR fetuses (P <0.0005). Ferritin did not differ in women with IUGR fetuses compared with controls. Eleven women with normal pregnancies (33%) and 3 with IUGR fetuses (21%) had ferritin concentrations <12 μg/L; concentrations of the nonglycosylated ferritin fraction were too low to be accurately determined in these samples and thus were not included. The glycosylated and nonglycosylated ferritin concentrations reported in Table 1 are therefore an overestimate of the actual values for the healthy control and IUGR groups as a whole. Nevertheless, glycosylated ferritin was double (P <0.01) and nonglycosylated ferritin was increased fivefold (P <0.0001) in women with preeclampsia compared with those with a normal pregnancy. Nonglycosylated ferritin was also increased in preeclamptic women compared with women with IUGR fetuses (P <0.01), but glycosylated ferritin did not differ significantly (P = 0.11).

Nonglycosylated ferritin expressed as a percentage of total serum ferritin was significantly higher in preeclamptic (54.8%) than in normal (35.7%; P <0.0001) and IUGR (38.1%; P <0.01) patients (Table 1). There was no corre-
tion between total ferritin concentration and percentage of nonglycosylated ferritin among either preeclamptic ($r = 0.29; P = 0.14$) or nonpreeclamptic ($r = 0.1; P = 0.58$) women, suggesting that glycosylation percentages were independent of total ferritin concentration.

Only five women in the preeclampsia group delivered (and provided blood samples) at term ($\geq 37$ weeks). Nevertheless, when comparisons were restricted to samples collected at term, the pattern of significant differences presented in Table 1 remained essentially unchanged (data not shown). Therefore, the earlier mean gestational weeks at blood collection of the preeclampsia cohort does not explain the increased ferritin and increased percentage of glycosylated ferritin in this group. This is also supported by the multivariable analysis (Table 2 in the Data Supplement).

Within the preeclampsia group, TIBC showed a negative correlation with glycosylated ferritin ($r = -0.51; P < 0.01$) and total ferritin ($r = -0.42; P < 0.03$) but not with nonglycosylated ferritin ($r = -0.36; P = 0.07$). Likewise, TIBC was negatively correlated with glycosylated ferritin ($r = -0.79; P < 0.01$) and total ferritin ($r = -0.60; P < 0.03$), but not with nonglycosylated ferritin, in women with IUGR pregnancies. In contrast, TIBC did not correlate with any of the ferritin measures in normal pregnancy controls. In women with preeclampsia, AST concentrations, an indicator of hepatocellular damage, correlated with serum nonglycosylated ferritin ($r = 0.55; P < 0.003$) and total ferritin ($r = 0.53; P < 0.004$), and to a lesser degree with glycosylated ferritin ($r = 0.43; P < 0.03$). These data indicate that serum nonglycosylated ferritin concentrations are greatly increased in proportion to total serum ferritin, suggesting that cellular damage contributes to the four- to fivefold increase in mean ferritin values observed in women with preeclampsia. In addition to nonspecific leakage from cells, the synthesis of ferritin is also likely to be increased in women with preeclampsia because the mean serum glycosylated ferritin concentration was double that in the normal-pregnant women.

Hepatocellular damage is implicated as a source of serum ferritin in women with preeclampsia because serum AST concentrations correlated with serum nonglycosylated ferritin, correlated to a lesser degree with total and glycosylated ferritin, and did not correlate with iron, TIBC, UIBC, or percentage saturation of TIBC. Increased total ferritin and the percentage of nonglycosylated ferritin were observed even in the subset of preeclamptic women with serum AST concentrations below that diagnostic of hepatic injury. It is possible that ferritin (we suggest especially nonglycosylated ferritin) is an exquisitely sensitive measure of incipient, subclinical hepatocellular injury (6).

The mean percentages of nonglycosylated ferritin were virtually identical in women with normal pregnancies and women with growth-restricted fetuses (without hypertension or proteinuria). Because IUGR and preeclampsia ostensibly share reduced placental perfusion, placental infarction, and other aspects of placental pathology, these data suggest that placental damage is not the primary reason for the predominance of nonglycosylated ferritin in the serum of women with preeclampsia.

In both preeclampsia and IUGR, TIBC (transferrin) showed a significant negative correlation with glycosylated ferritin ($r = -0.51$ and $-0.79$, respectively) but not with nonglycosylated ferritin. Glycosylated ferritin is a positive acute-phase reactant, and transferrin is a negative acute-phase reactant (2). Our data are thus consistent with the notion that inflammation-induced synthesis is a partial cause of increased ferritin in preeclamptic and IUGR pregnancies.

In conclusion, hyperferritinemia in patients with preeclampsia appears to be attributable to the combined effects of increased ferritin synthesis and the release of intracellular ferritin from damaged cells. Hepatocellular, rather than placental, damage is the likely reason for the predominance of nonglycosylated ferritin in many women with preeclampsia.

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