Increased Hemoglobin A1c in Obese Pregnant Women after Exclusion of Gestational Diabetes

To the Editor:

Preconception obesity is a strong predictor of glucose intolerance during pregnancy that has the potential to lead to adverse maternal and offspring effects. An increased prevalence of overweight offspring of obese mothers has been reported, despite normal values in an oral glucose tolerance test (OGTT) during pregnancy (1). Such findings might reflect effects of the intrauterine milieu, genetic background, or lifestyle factors related to maternal obesity or, alternatively, failure to diagnose glucose intolerance in obese pregnant women. We investigated whether increases in hemoglobin A1c (Hb A1c) at delivery as a surrogate marker of glycometabolic disturbances are prevalent in obese mothers, despite the exclusion of gestational diabetes (GDM) even after applying the stringent new criteria as recommended by the International Association of Diabetes and Pregnancy Study Groups (IADPSG).

We performed a retrospective analysis of 137 obese pregnant Caucasian women. Recruitment was through an ongoing cohort study on the long-term effects of preconception maternal obesity (PEACHES, Programming of Enhanced Adiposity Risk in CHildhood – Early Screening), which had been initiated in 15 recruiting maternity clinics in Bavaria, Germany. Inclusion criteria before delivery were an age ≥18 years, singleton pregnancy, full-term birth, and a pregestation body mass index (BMI) ≥30 kg/m². Women with a preexisting diagnosis of type 1 or type 2 diabetes mellitus or other chronic diseases were excluded. Ethics approval was obtained from the Ludwig-Maximilians-Universität München, and informed consent was provided by each participant.

The main exposure was the diagnosis or exclusion of GDM according to the results of a 75-g OGTT and the new IADPSG criteria or the former American Diabetes Association (ADA) criteria (Table 1). The primary outcome was an Hb A1c value ≥5.7% at delivery, a threshold that has been reported to be above the reference interval for late pregnancy (2, 3). Maternal EDTA-containing plasma samples were analyzed for Hb A1c via cation-exchange chromatography with a Tosoh G8 HPLC Analyzer (Tosoh Bioscience) in a central laboratory (interassay CV, ≤2.0%; analytical bias, ≤0.03% Hb A1c at a target of 5.44%). A complete blood count was performed at delivery. Within subgroups, we compared the prevalence of increases in Hb A1c by calculating the proportions with binomial 95% CIs (Table 1) and SAS software (version 9.2; SAS Institute).

According to the new IADPSG criteria, 38.9% (95% CI, 30.7%–47.5%) of the women had a GDM diagnosis, whereas the former ADA criteria yielded a percentage of only 8.0% (95% CI, 4.1%–13.9%). Subgroups did not differ significantly with respect to prepregnancy BMI, smoking during pregnancy, week of gestation of the OGTT, and parity. Iron deficiency anemia at delivery (4) was excluded by a complete blood count [mean corpuscular hemoglobin, 28.1 pg (median); mean corpuscular volume, 83.8 fl (median)].

The main finding was an increased prevalence of an increased Hb A1c of ≥5.7% at delivery in obese women—even considering the lower limit of the 95% CI—despite the exclusion of GDM even by the sensitive new IADPSG criteria (Table 1). Studies of Hb A1c levels during pregnancy for >500 pregnancies (2, 3) have yielded late-pregnancy estimates of the upper Hb A1c reference limit of 5.6%, compared with 6.2% in nonpregnant women. From these cross-sectional data, we used an Hb A1c value of ≥5.7% at delivery as a cut-off for potentially undiagnosed glycometabolic disturbances. An Hb A1c value above this threshold at delivery was associated with increased risks of newborns being large for their gestational age (odds ratio, 3.1; 95% CI, 1.3–7.6) and having hypoglycemia (odds ratio, 6.2; 95% CI, 1.3–29.0) (2), suggesting a potential role for Hb A1c as a surrogate risk marker. Indeed, recent research points toward a role for Hb A1c as a risk marker of cardiovascular disease and a predictor of mortality in nondiabetic adult populations, results that suggest that abnormal glycation, even at a subdiabetic level, may already reflect a considerable health risk (5). Besides indicating glycemic control, Hb A1c variation may be due to nonglycemic factors that lead to states of high or low glycation, such as ethnic disparities and disease conditions, including those that affect erythrocyte life span (4). In addition to biological variation, the potential influence of analytical variation in Hb A1c, measurements on decision thresholds needs cautious interpretation. In our study, both analytical and preanalytical variation was low, and we included only Caucasian mothers and excluded relevant microcytic hypochromic anemia. Our finding that the prevalence of increased Hb A1c values at delivery is still increased in seemingly nondiabetic obese preg-

1 Nonstandard abbreviations: OGTT, oral glucose tolerance test; Hb A1c, hemoglobin A1c; GDM, gestational diabetes; IADPSG, International Association of Diabetes and Pregnancy Study Groups; PEACHES, Programming of Enhanced Adiposity Risk in CHildhood – Early Screening (study); BMI, body mass index; ADA, American Diabetes Association.
nancies may point toward the need for further refinement of GDM screening and/or the presence of glyco- and metabolic disturbances beyond GDM in this high-risk group. Although requiring prospective assessment, Hb A1c analysis at delivery might be beneficial. An apparently normal OGTT value would indicate to physicians an “all clear” situation without a need for special postnatal care, whereas in conjunction with an increased Hb A1c value, it should raise awareness for the potentially hidden long-term risks of obese pregnancies.

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Accuracy of First and Second Generation Testosterone Assays and Improvement through Sample Extraction

To the Editor:

The monitoring of antiandrogen treatment in patients with prostate cancer, investigation of hyperandrogenism in women, and evaluation of infants with ambiguous genitalia require accurate measurement of low testosterone concentrations. However, testosterone immunoassays have been shown to be inaccurate and often to overestimate testosterone concentrations in the low range (1). A working group of the Endocrine Society recently reviewed this concern and presented several recommendations to ensure the accuracy of future testosterone testing for improvement of diagnosis and treatment of disease (2).

In the present study we evaluated the current situation with regard to the accuracy of 7 testosterone immunoassays, including 2 second generation assays, by comparison with isotope-dilution liquid chromatography–tandem mass spectrometry (ID-LC-MS/MS). In addition, we investigated the possible improvement of these immunoassays by diethyl ether sample extraction.

Serum from 50 men, 50 women, and 16 children (age 4–16 years) was collected, divided into aliquots, and stored (−20 °C) until analysis. All investigations conformed to the ethics standards of the Helsinki Declaration.

Total serum testosterone was measured singly (except in duplicate by ID-LC-MS/MS), within the same lot, and before and after extraction by ID-LC-MS/MS and 7 immunoassays. We used a slightly adjusted version of a previously published ID-LC-MS/MS method (Quattro Premier XE, Waters) (3). Validation showed that testosterone results were not affected by the modifications and were concordant with a reference method, GC-MS. All immunoassays are currently available, except for the second generation Architekt® (Abbott) assay, which will be available soon. None of the immunoassays investigated (Table 1) used an extraction step, as a prescribed procedure, before analysis. We extracted testosterone using diethyl ether as described previously (4). The dried extracts were reconstituted in a matrix recommended by the manufacturer.

The results of the extracted samples were corrected for baseline testosterone concentration of the reconstitution matrix and mean extraction recovery. By ID-LC-MS/MS, the 116 individual serum samples were categorized and subsequently analyzed in 2 groups, <4.0 nmol/L (n = 68) and >4.0 nmol/L (n = 48), because the immunoassays showed a nonlinear relationship over the entire measuring range. Samples with concentrations below the detection limit of the tested immunoassay or insufficient serum volume were excluded from data analysis (Table 1). For untreated and extracted samples in the higher testosterone range, all immunoassays showed a good correlation with ID-LC-MS/MS (r > 0.92; Table 1). In the lower range, the correlation coefficients of the immunoassays ranged from 0.59 to 0.92. The best correlation

References