Amniotic Fluid Insulin at 14–20 Weeks' Gestation

Association with later maternal glucose intolerance and birth macrosomia

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OBJECTIVE — To examine the hypothesis that early second trimester amniotic fluid (AF) insulin concentration is elevated and later fetal growth is augmented in gravidas demonstrating later oral glucose intolerance.

RESEARCH DESIGN AND METHODS — In this prospective observational cohort study, AF was sampled at 14–20 weeks' gestation in 247 subjects, and 1-h 50-g oral glucose challenge tests (GCTs) were performed at ≥24 weeks. AF insulin was assayed by an automated immuno-chemiluminometric assay (8). Macrosomia was defined as birth weight above the 90th centile.

RESULTS — AF insulin concentration (range 1.4–44.5 pmol/l) correlated positively with gestational age and maternal weight. A logistic regression analysis, adjusted for maternal age and midpregnancy weight, showed increased AF insulin multiples of gestational age-specific medians to be associated with subsequently diagnosed gestational diabetes mellitus (GDM) (OR 1.9, CI 1.3–2.4, $P = 0.029$). Among 60 subjects with GCT values >7.2 mmol/l, each unit increase in AF insulin multiple of median (MOM) was associated with a threefold increase in fetal macrosomia incidence (3.1, 1.3–4.9, $P = 0.048$).

CONCLUSIONS — An elevated AF insulin concentration at 14–20 weeks' gestation is associated with subsequently documented maternal glucose intolerance. Among gravidas with GCT values >7.2 mmol/l, elevated early AF insulin concentration is associated with fetal macrosomia. Maternal glucose intolerance may affect fetal insulin production before 20 weeks' gestation.

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Insulin production by the human fetus can be identified as early as 11 weeks of gestational age (1). In the early midtrimester, an acute hyperglycemic stimulus in the human fetus does not appear to provoke insulin release in nondiabetic pregnancy (2) but may do so in the fetuses of diabetic women (1).

Trace amounts of insulin can be identified in amniotic fluid (AF) by radioimmunoassay as early as 12–16 weeks' gestation and has been measured at concentrations in the range of 6–24 pmol/l at 16 weeks' gestation (3). In the non–insulin-treated diabetic pregnancy, AF insulin appears to be of fetal origin, reaching the amniotic space from fetal urinary excretion (3). In the third trimester, increased AF insulin appears to be associated with maternal gestational diabetes mellitus (GDM), diabetic fetopathy (macrosomia and neonatal hypoglycemia) (4), and increased birth weight in the pregnancies of nondiabetic women (5).

Second-trimester AF insulin concentration has been associated, in case control and cohort studies, with subsequent maternal glucose intolerance (6,7). One study (7) examined relative birth weight (10) of infants born at 39.7 ± 1.9 weeks but did not demonstrate a statistically significant association of second-trimester AF insulin with birth macrosomia. However, this investigation (7) employed an insulin radioimmunoassay that did not detect insulin in 14% of the samples. Therefore, poor assay sensitivity and accuracy may have prevented detection of an association with birth macrosomia.

We have demonstrated the utility of a two-site dual monoclonal antibody chemiluminescent insulin assay in AF and the stability of insulin in this medium (8). We thought it might be possible that some gravidas with glucose intolerance, identified at 24–30 weeks' gestation, may have hyperglycemia early in the second trimester sufficient to augment fetal insulin production to the degree that it might be detected by this assay. Accordingly, we hypothesized that increased early second-trimester AF insulin concentration is associated with both subsequent diagnosis of maternal glucose intolerance (GDM) and fetal macrosomia at birth.

RESEARCH DESIGN AND METHODS

— We performed a prospective observational cohort study of 576 gravidas with singleton pregancies who underwent amniocentesis for fetal karyotype (based only on advanced maternal age) between 14–20 weeks' gestation from 13 June 1997 to 8 July 1998 (8). The AF specimens obtained for other indications were excluded, and all subjects had a sonographic estimate of gestational age at amniocentesis. Gestational age was assigned based on the first day of the last menstrual period, unless the estimate from the sonogram performed at amnio-

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Abbreviations: AF, amniotic fluid; GDM, gestational diabetes mellitus; MOM, multiple of median; GCT, glucose challenge test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

centesis was discrepant by >10 days, in which case the sonographic estimate was adopted.

Of the 576 subjects, 329 did not meet additional inclusion criteria for the study because of antenatal care in a setting of universal gestational diabetes screening (18 subjects), performance of a 1-h 50-g oral glucose challenge test (GCT) between 24–30 weeks' gestation (26 subjects), freedom from later pregnancy complications other than gestational diabetes and idiopathic preterm labor (31 subjects), and maternal and birth-weight data that was not available at Women and Infants Hospital (254 subjects delivered in other hospitals or had records that were otherwise unavailable for extraction). The remaining 247 pregnancies were included in the investigation. Birth weight was measured in the delivery room by digital electronic scale after zeroing and extracted from the obstetrical record.

Glucose testing

Because of the large number of specimens required for the study, only residual AF samples were used for this study. Consequently, because we did not have access to these patients to perform immediate glucose tolerance testing, we sought data from a subsequent glucose screening test, performed in this cohort at 24–30 weeks' gestational age.

A 3-h 100-g oral GCT was performed on all of the subjects in whom the result of the 50-g GCT was >7.2 mmol/l. The diagnosis of GDM was made if at least two plasma-glucose values, using enzymatic methods, met or exceeded thresholds at the fasting, 1-h, 2-h, and 3-h samples of 5.3, 10.0, 8.6, and 7.8 mmol/l, respectively (9). Centile birth weight was calculated according to Thompson et al. (10), whose assessment allows for adjustment by gestational age, birth number, infant sex, and maternal stature.

AF specimens and assays

Amniocentesis was performed without regard to meals, between 8:30 A.M. and 3:00 P.M. Specimen handling, assay performance, and insulin stability under study conditions have been previously described (8). After centrifugation for cell culture, specimens were stored at −4°C within 6 h of amniocentesis and assayed for insulin within 9 ± 3 days. We employed an automated immuno-chemiluminometric assay (Access Immunoassay System; Beckman Coulter, Chaska, MN). Two monoclonal antibodies that are directed to epitopes specific to insulin form a solid-phase "sandwich." One antibody is bound to paramagnetic particles, and the other antibody is bound to alkaline phosphatase, which acts on a chemiluminescent substrate (11).

Because AF insulin concentrations increase between 14 and 20 weeks' gestation, we used weighted log-linear regression of median AF insulin concentration at each completed week of gestation during that time (8) to express amniotic-insulin values as multiples of the gestational age-specific median, as is done in expressing maternal α-fetoprotein values, which are also affected by gestational age. Both the laboratory personnel and the investigators were blinded to maternal and neonatal outcome at the time of the assay.

Data analysis

We used linear regression to estimate the effects of maternal weight and age on the log AF insulin multiple of the median (MOM). The logistic regression of the log AF insulin MOM was adjusted for maternal weight and age and was used to calculate ORs for the development of subsequent GDM and fetal macrosomia. Analysis of differences in the AF insulin concentrations between gravidas having screening glucose values above the threshold of 7.2 mmol/l and the remaining gravidas was by Student's *t* test applied to log-transformed values. Data distributions were expressed as the mean ± SD. Statistical significance was accepted at the $P = 0.05$ threshold. This investigation was approved by the Women and Infants Hospital's institutional review board on 22 July 1996.

RESULTS — Of the 247 subjects 91% were Caucasian, 3% were Black, 3% were Hispanic, and 2% were Asian. The 50-g oral GCT was performed at the gestational age of 26.7 ± 3.5 weeks, and the 100-g oral GCT was performed at 27.6 ± 4.3 weeks. A total of 60 (24%) subjects had a 50-g oral GCT plasma glucose value >7.2 mmol/l, and all 60 subjects had follow-up 3-h 100-g oral GCTs. Of these, nine subjects (15% of screen-positive subjects, 3.6% of the total subjects) had GDM, five of whom were Caucasian. All women with GDM were diagnosed following a routine glucose challenge screening. Median maternal age was 38 years (range 35–47). Median gestational age at amniocentesis for all 247 samples was 16 weeks (range 14–20), and median gestational age at birth was 40 weeks (range 27–43). At birth, 43 (17%) infants had birth weights ≥90th centile (10).

The AF insulin concentrations were normally distributed on a $\log_{10}$ scale (8). Because of a significant increase in AF insulin concentration with gestational age, we expressed insulin concentrations as MOMs for each week of gestational age (8). Among the 247 subjects, the AF insulin MOMs ranged from 0.13 to 7.25, with the median being defined as 1.0 (based on weighted regression of gestational age) (8).

The log AF insulin MOMs were significantly associated with maternal mid-pregnancy weight (clinically recorded weight closest to 20 weeks' gestation) (log AF insulin MOM = 0.00123 lbs body wt − 0.196, $P = 0.015$) but not significantly with maternal age in this selected older age group. Subjects with GCT values >7.2 mmol/l did not have a statistically significant elevated log AF insulin MOM compared with those who had lower GCT values (0.02 ± 0.21 vs. 0.01 ± 0.22 log MOM [mean ± SD], respectively). Also, regression of log AF insulin MOM on the value of the subsequent 50-g oral GCT was not significant among all 247 subjects and among the 60 subjects with a GCT value >7.2 mmol/l.

The logistic regression analysis demonstrated a significant association of AF insulin concentration with subsequently diagnosed maternal GDM. We observed that a 1.0-unit increase in AF insulin MOM corresponds to a 77% increase in the odds of a subsequent diagnosis of GDM (OR 1.77, 95% CI 1.28–2.26), an effect that persists after adjusting for maternal age and weight (1.87, 1.33–2.41), estimating a near doubling in the probability of a later diagnosis of GDM with every MOM increase in AF insulin.

In this cohort, AF insulin values were not associated with birth weight. A total of 137 subjects had AF insulin MOM below the median, and 110 subjects had AF insulin MOM at or above the median. Mean ± SD birth weight in each group was not statistically significant (3,368 ± 577 vs. 3,475 ± 639 g, respectively, $P = 0.17$). Moreover, regression of birth

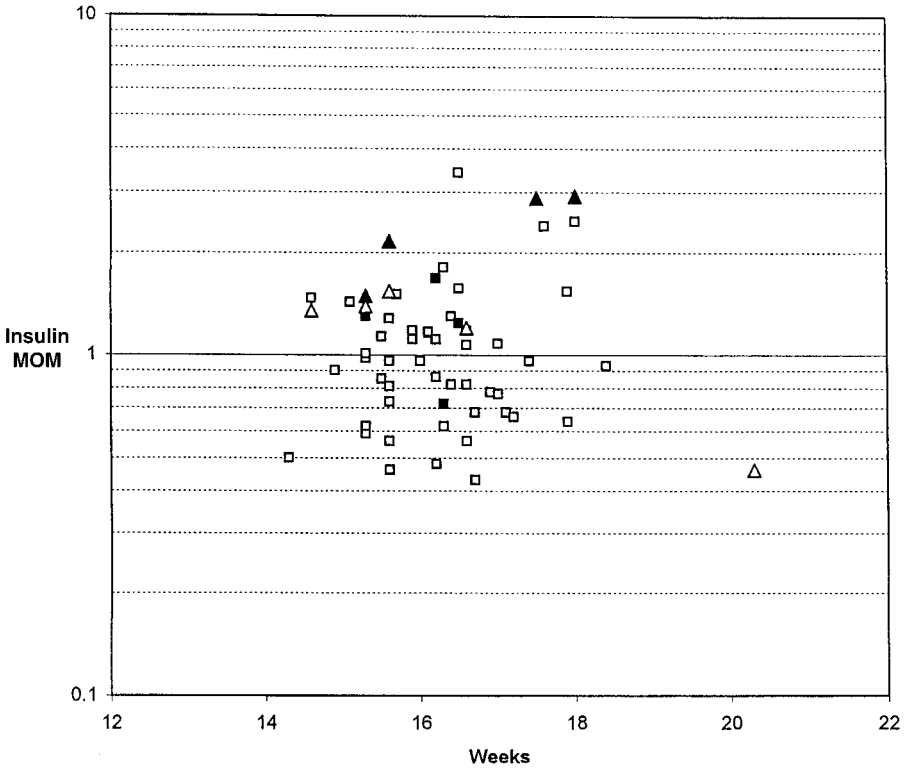

Figure 1—*AF insulin, expressed as multiples of the age-specific median (MOM) arrayed on logarithmic scale versus the gestational age at amniocentesis among 60 gravidas with 50-g GCT results >7.2 mmol/l. Squares denote patients without gestational diabetes; triangles denote patients with gestational diabetes; open icons denote pregnancies with offspring at or less than the 90th centile birth weight; and closed icons denote pregnancies with birth weight greater than the 90th centile.*

weight on AF insulin MOM was also not statistically significant ($F = 1.0$, $P = 0.31$).

However, AF insulin values were associated with fetal macrosomia at birth. Among all 247 subjects, logistic regression of AF insulin MOM on fetal macrosomia (≥90th centile birth weight) demonstrated a crude OR of 1.39 (95% CI 1.10–2.68) and a maternal weight- and age-adjusted OR of 1.13 (0.87–2.39). However, among the 60 gravidas with GCT values of >7.2 mmol/l, the crude OR was 4.20 (1.87–6.53, $P = 0.010$), and the adjusted OR for macrosomia at birth was 3.13 (1.32–4.94, $P = 0.048$). This estimates a threefold increase in the incidence of fetal macrosomia with every 1.0-unit increase in AF insulin MOM. For example, among subjects who had a positive 1-h glucose screen (>7.2 mmol/l), those with a 2.0 AF insulin MOM value (found in 6 of the 60 subjects) would be predicted to have a threefold incidence of fetal macrosomia compared with those with a 1.0 MOM value, whereas those with a 2.6 MOM value (found in 3 of the 60 subjects) would have a fivefold risk of having a baby with a birth weight ≥90th centile.

The distribution of insulin MOM values among all 60 gravidas with GCT results >7.2 mmol/l is depicted in Fig. 1. Those patients who subsequently developed either GDM or delivered macrosomic offspring generally had AF insulin values above the median (1.0 MOM).

Figure 2 illustrates the number of normal weight and macrosomic offspring among women with and without GCT values >7.2 mmol/l, distributed as a function of their AF insulin MOM values. The proportion of infants with macrosomia increases with the AF insulin MOM value only in subjects with elevated GCT values. For example, among gravidas with GCT values ≤7.2 mmol/l, subjects with AF insulin MOM values <2.0 had a 17% incidence of macrosomia compared with a 30% incidence among those with AF insulin MOM values ≥2.0, a 1.8-fold increment in incidence. In contrast, among the 60 pregnancies with GCT values >7.2 mmol/l, the macrosomia incidence in each group was 9 and 43%, respectively, a 4.6-fold increment in incidence.

CONCLUSIONS — Third-trimester AF insulin concentration has been associated with birth weight in nondiabetic pregnancy (12), diabetic fetopathy in diabetic pregnancy (4), as well as childhood obesity and impaired glucose tolerance (13,14). This association has suggested that AF insulin concentrations may provide insight into fetal response to the diabetic environment.

Insulin can be identified in pancreatic islets in the early midtrimester (2). AF insulin concentration increases significantly during the 14–20-week period (3,8) and has been associated with birth weight in female offspring (16). Though acute fetal hyperglycemia does not appear to elicit insulin release in the fetuses of nondiabetic women during this period (2), increased pancreatic insulin content and glucose-mediated insulin release can be demonstrated in the fetuses of diabetic women during the 16–20-week period (1). The presence of AF insulin and C-peptide before 20 weeks has been associated with maternal diabetes (6,7,15).

Until recently, the lower limit of assay detection of insulin in AF was in the 6–12 pmol/l range (12,17,18), limiting its utility in early pregnancy. Previous studies by Paulin et al. (19) (16–18 weeks, 23 ± 18.0 pmol/l) and Cormbach et al. (20) (16–20 weeks, median 12 mmol/l, 97th percentile 26 pmol/l) reported insulin levels in this range. We recently reported improved insulin assay sensitivity in AF, detecting values of 0.24 to 7.41 μIU/ml (1.7 to 53.2 pmol/l) at 14–20 weeks gestation (8). Because of the association with gestational age, we expressed AF insulin values as MOMs, based on a regression of weighted insulin medians on gestational age (8).

This study confirms our earlier observations (6,7) that an early AF insulin concentration is a marker for subsequently diagnosed maternal GDM. We found an OR of 1.87 for GDM with each unit increase in the MOM insulin value, adjusting for maternal weight and age. This association suggests that, among older gravidas, some who were diagnosed with GDM may have had an earlier elevation of glucose intolerance sufficient to amplify

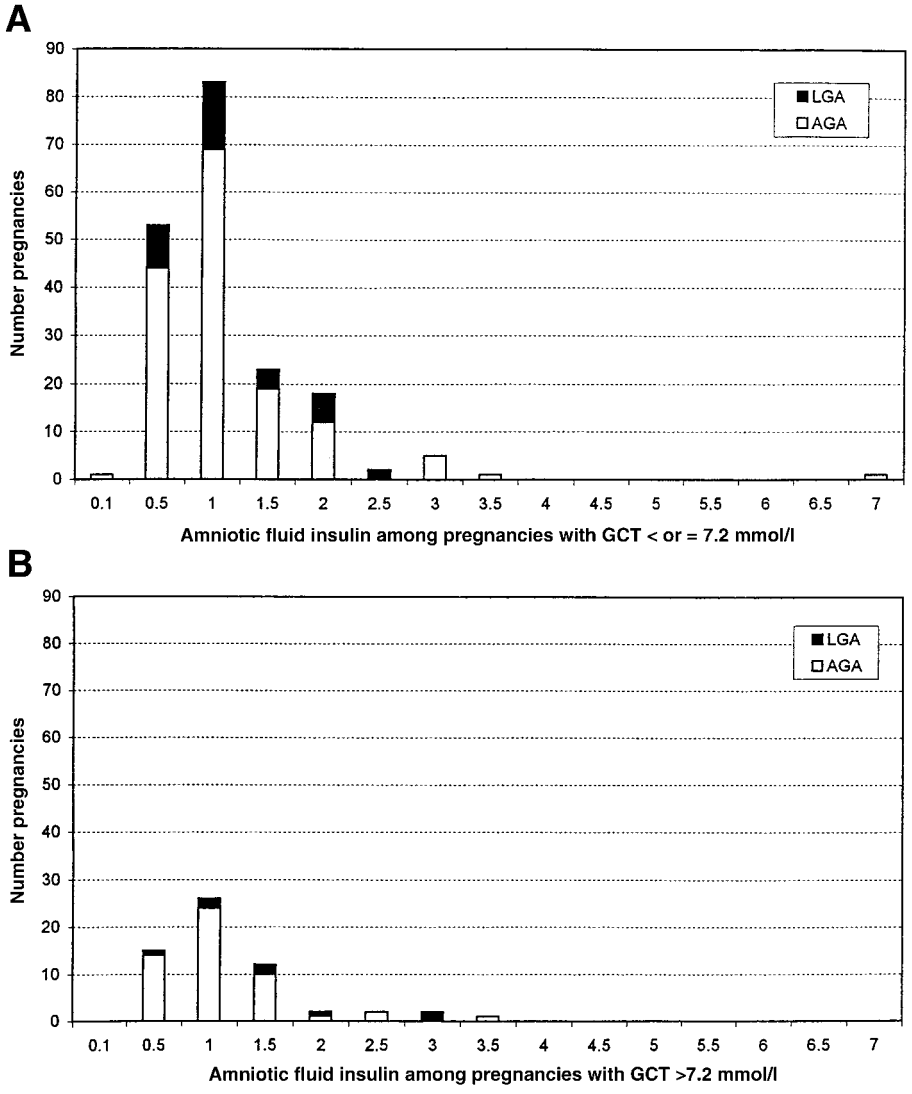

Figure 2—*The number of normal weight (□) and offspring macrosomic (■) offspring among women without (A) and with (B) GCT values >7.2 mmol/l distributed as a function of their AF insulin MOM values.*

fetal production and/or increased fetal excretion of insulin.

In addition, the present study demonstrates that (among the 60 gravidas with GCT values >7.2 mmol/l) the increasing MOM insulin values at 14–20 weeks' gestation have a significant association with subsequent fetal macrosomia (OR 3.13). However, the association between AF insulin concentration and birth macrosomia is not found in the overall cohort. This is because subjects with GCT values <7.2 mmol/l have a much narrower range of glucose values, thereby reducing the power of the association between GCT values and birth macrosomia in the overall group.

The association between AF insulin concentration and fetal macrosomia was not identified in our earlier study (7), which employed a less sensitive insulin assay with a lower limit of detection of 2.1 pmol/l, causing null values in 14% of the samples.

The association between AF insulin concentration and maternal glucose intolerance may be confounded by the transport of maternal insulin into the AF of women who may already have severe hyperinsulinemia. However, only antibody-bound insulin appears to be transported across the placenta (21). Neither maternal insulin nor insulin antibodies were measured in the present study. However, only 9 of 60 patients with an elevated glucose screen developed GDM, and none of the 247 subjects had a history of diabetes or insulin treatment.

The association between AF insulin concentration and fetal macrosomia may not be mediated by maternal effects, but may instead reflect genetic differences of β-cell regulation among fetuses. Though this investigation cannot exclude such effects, genetic differences among fetuses do not explain the association found between early AF insulin concentration and maternal GDM detected at 24–30 weeks' gestation.

The observations of this investigation suggest that 14–20-week AF insulin concentration may reflect fetal insulin response to maternal hyperglycemia, diagnosed later in pregnancy, sufficient to affect subsequent fetal growth. Confirmation of this association will require simultaneous AF sampling and maternal glucose tolerance testing in this early fetal period. Such data would suggest that early maternal metabolic imprinting may affect fetal growth.

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