

# Amniotic fluid insulin levels and fetal abdominal circumference at time of amniocentesis in pregnancies with diabetes

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## Abstract

**Aims** Fetal hyperinsulinism is a strong predictor for excessive growth and fetopathy in pregnancies complicated by diabetes. We examined (i) the relationship between measurements of amniotic fluid insulin (AF insulin) and fetal abdominal circumference (AC) at the time of amniocentesis, and (ii) whether there is a threshold for fetal AC percentiles which can identify low vs. high-risk levels of AF insulin without performing an amniocentesis.

**Methods** In a retrospective study, AF insulin from 121 pregnant diabetic women (32 pregestational; 89 gestational) was measured during the 3rd trimester as part of a diabetes management protocol. AC measurements were transformed into a continuous variable of percentile growth for gestational age (Hadlock). Division of the cohort according to deciles or quartiles of AC percentiles was performed to identify a threshold AC with a significant increase in elevated AF insulin, previously defined as AF insulin  $\geq 7 \mu\text{U/ml}$ . A receiver operator characteristic (ROC) curve was created and the negative predictive value (NPV) of the determined threshold was calculated.

**Results** AF insulin levels were significantly correlated with the AC percentiles ( $r = 0.3$ ,  $P = 0.0005$ ) by linear regression. No AC threshold could reliably identify a moderate elevated AF insulin  $\geq 7 \mu\text{U/ml}$  (NPV 77.2%), but an AC threshold  $\geq 75$ th percentile could identify with fetal hyperinsulinism with an AF insulin  $\geq 16 \mu\text{U/ml}$ . All 10 cases of AF insulin  $\geq 16 \mu\text{U/ml}$  were identified with a NPV of 100% (74/74).

**Conclusions** Our data indicate that an AC  $\geq 75$ th percentile determined by a 3rd trimester ultrasound examination may discriminate between pregnancies at low vs. high risk for AF insulin  $\geq 16 \mu\text{U/ml}$ . This AF insulin concentration corresponds to a level of hyperinsulinism reported to be associated with considerable neonatal and long term morbidity.

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**Keywords** diabetes, pregnancy, amniotic fluid insulin, fetal abdominal circumference, macrosomia

**Abbreviations** AC, abdominal circumference; AF insulin, amniotic fluid insulin; GDM, gestational diabetes; LGA, large-for-gestational age; ROC, receiver operator characteristic; GA, gestational age; BMI, body mass index

## Introduction

The principle of medical management of diabetes during pregnancy is based on normalization of maternal glucose levels. This approach has been shown to reduce neonatal morbidity and mortality in infants of mothers with gestational and pregestational diabetes. In studies in women with gestational diabetes (GDM), intensive therapy to achieve strict euglycemia can reduce the rate of macrosomia to below 10% [1], but this approach requires insulin therapy in up to two-thirds of the pregnancies [2,3].

A modification of this strategy, advocated by some researchers, has been to target pregnancies at increased risk for neonatal morbidity for intensive medical therapy by assessing the *in utero* fetal risk for developing neonatal complications. One approach is based on the measurement of amniotic fluid insulin levels (AF insulin). Fetal hyperinsulinism has been shown to be a strong predictor of diabetic fetopathy [4,5], and can be indirectly determined by AF insulin secondary to the urinary excretion of fetal insulin [6,7]. When AF insulin levels are elevated, insulin therapy is either initiated in GDM pregnancies or intensified in pregestational diabetic pregnancies to achieve strict euglycaemia [8,9]. Another fetal-based strategy has utilized ultrasound to identify excessive fetal growth as a clinical marker for presumed hyperinsulinism [10–12]. In this approach insulin therapy is reserved for pregnancies with accelerated growth of the fetal abdominal circumference (AC), which is predictive of newborn macrosomia [13,14].

The measurement of AF insulin has not been widely adopted in clinical practice because it requires an invasive diagnostic procedure. Therapeutic management of GDM using fetal AC is considered more and more to be useful [15–17]. But there is still concern about over- or underdevelopment when insulin therapy is administered in women with euglycemia or is still withheld in women with mild hyperglycemia respectively depending on the fetal AC. Our study investigated the relationship between AF insulin and fetal AC percentile to determine whether a threshold of fetal AC measurements at the time of amniocentesis could discriminate low and high risk levels of AF insulin.

## Patients and methods

Amniotic fluid insulin levels were analysed retrospectively in a cross-sectional study. The women attended the Diabetes Clinic of the Department of Obstetrics in the Vivantes Medical Centre between 1994 and 1998. The Clinic serves a predominately German population, although 30% of the women come from the Middle East. Study inclusion criteria were: (i) the presence of gestational or pregestational diabetes; (ii) amniocentesis with the measurement of AF insulin between 26 and 38 weeks of gestation; (iii) the absence of maternal vascular disease; (iv) a confirmed gestational age by an ultrasound examination performed before 20 weeks of gestation; (v) complete fetal biometry with measurement of the abdominal circumference performed at time of amniocentesis; (vi) singleton pregnancy; and (vii) absence of identified anomalies.

## Antepartum diabetic management

GDM was diagnosed by an oral glucose tolerance test with 75 g anhydrous glucose and determination of the glucose levels from capillary blood (Hexokinase) according to the guidelines of the German Diabetes Association [18]. GDM was defined as at least two abnormal values above the threshold of 5.0 mmol/l (90 mg/dl) for fasting glucose, 9.1 mmol/l (165) for the 1-h and 8.0 mmol/l (145) for the 2-h post-challenge glucose (adapted from O'Sullivan criteria) [18]. After diagnosis of GDM, or after the diagnosis of pregnancy in women with pregestational diabetes, prenatal care was transferred from the community physician clinics to the Diabetic Clinic. All women received diet education and instruction for self-monitoring of blood glucose (SMBG) using memory meters. In women with pregestational diabetes insulin therapy was adjusted using three to four daily doses of insulin to achieve preprandial glucose levels 5.0 mmol/l ( $\leq$  90 mg/dl) and post-prandial values  $\leq$  6.6 mmol/l (120) which results in mean glucose levels  $\leq$  5.5 mmol/l (100 mg/dl). Women with GDM on diet therapy performed glucose profiles (three preprandial and three post-prandial measurements) twice a week and insulin therapy was initiated when the mean daily glucose profile exceeded 5.5 mmol/l (100 mg/dl).

The determination of AF insulin was an integral part of the management protocol for pregnancies complicated by diabetes. This protocol had been approved by the local Ethics Committee when it had been established in 1989. AF insulin measurement in the early 3rd trimester was recommended in GDM pregnancies when: (i) borderline daily glucose profiles were present (mean daily glucose levels between 95 and 5.2–5.5 mmol/l (100 mg/dl) or (ii) clinical macrosomia was suggested with an estimated fetal weight by ultrasound which was above the 95th percentile for gestational age [19]. Insulin therapy was initiated when the AF insulin was elevated [8]. An AF insulin level  $\geq$  7  $\mu$ U/ml was previously determined to be the 90th percentile of our non-diabetic obstetrical population [20]. Between 24 and 36 weeks of gestation the AF insulin levels in non-diabetic pregnancies remained fairly constant showing a non-significant increase with increasing gestational age [20]. AF insulin measurement was recommended in pregestational diabetes in the early 3rd trimester to assess whether the maternal insulin dosage was sufficient to avoid fetal hyperinsulinism. When elevated AF insulin was found, insulin doses were increased to achieve fasting glucose  $\leq$  4.4 (80) and post-prandial values  $\leq$  6.1 mmol/l (110 mg/dl) corresponding approximately to mean daily glucose levels  $<$  5.0 mmol/l (90 mg/dl) [21]. Whenever insulin therapy was prescribed in women with either pregestational or gestational diabetes, daily fasting and three post-prandial glucose measurements and complete glucose profiles twice weekly were instituted.

Amniocentesis was performed under ultrasound guidance on an out-patient basis. All women gave written informed consent. AF insulin concentrations were measured by radioimmunoassay (Pharmacia RIA 100; Pharmacia, Uppsala, Sweden) with a lower limit of detection of 1  $\mu$ U/ml. Before amniocentesis was performed, a fetal biometry was obtained by an experienced ultrasonographer (W.H., M.B.). The fetal abdominal circumference was measured in the standard cross-section view of the abdomen at the level of the stomach and portal sinus of the liver. None of the women experienced a rupture of membranes

or preterm labour within 3 days after amniocentesis or before 37 weeks of gestation.

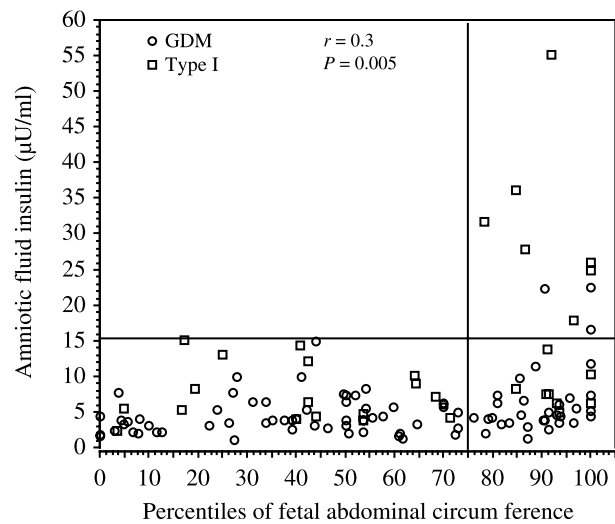
**Statistical analysis**

Normally distributed data are reported as mean and standard error, otherwise the median is given. AC measurements were transformed into a continuous variable of percentile growth for gestational age using the Hadlock formula [19]. Simple linear regression analysis was used to investigate the correlation between the level of AF insulin and the fetal AC percentile. For categorical examination of the best threshold for an increased risk for elevated AF insulin the AC percentiles were divided into deciles and quartiles.  $\chi^2$  tests and Bonferroni adjustment were applied to test for statistically significant ( $P < 0.05$ ) cut points of AC percentile for an increased risk for elevated fetal insulin. Receiver operating characteristic (ROC) curves were created to confirm the identified thresholds. The sensitivity, specificity, positive and negative predictive values of the threshold AC percentile for fetal hyperinsulinism were calculated.

**Results**

During the study period 340 women with GDM and 100 women with pregestational diabetes attended the Diabetes Clinic. AF insulin was determined in 171 (38.7%) women. Fifty women had to be excluded because they did not fulfill the study inclusion criteria, leaving 121 women for analysis. There was no statistically significant difference regarding maternal characteristics or median AF insulin levels between pregnancies studied and those excluded from the analysis (not shown). Our final study cohort consisted of 32 women with pregestational diabetes and 89 women with GDM.

Demographic and pregnancy characteristics of women with pregestational diabetes and gestational diabetes are displayed in Table 1. The mean gestational age at time of amniocentesis was  $33.7 \pm 0.5$  weeks for women with diabetes and  $32.8 \pm 0.3$  weeks for women with GDM. For the subsequent analysis we combined subjects with pregestational and gestational diabetes.



**Figure 1** Correlation of percentiles of the abdominal circumference (AC) according to gestational age and the level of amniotic fluid insulin (AF insulin) in pregnancies with preexisting (squares) or gestational diabetes (circles). All cases of severe hyperinsulinism (AF insulin  $\geq 16 \mu\text{U/ml}$ ) were associated with an AC  $\geq 75$ th percentile.

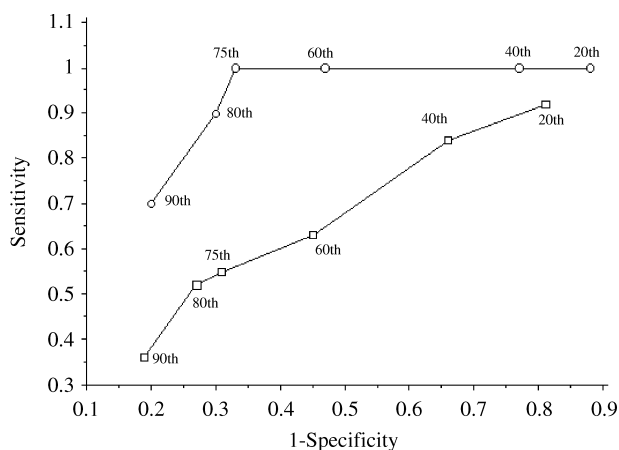
The mean AC percentile for gestational age at time of amniocentesis was  $58.5 \pm 2.8$  with 24% of the subjects having AC measurements  $> 90$ th percentile for gestational age. The median AF insulin was  $4.9 \mu\text{U/ml}$  (range  $1.1\text{--}55.2 \mu\text{U/ml}$ , data not normally distributed). Elevated AF insulin levels ( $\geq 7 \mu\text{U/ml}$ ) were seen in 38 of the 121 (31.4%) pregnancies. There was no significant correlation between gestational age and AF insulin ( $r^2 = 0.01$ ,  $P > 0.5$ ), thus adjustment for gestational age had not been performed.

Linear regression analysis showed a significant correlation between the AC percentile and AF insulin level ( $P = 0.005$ ,  $r = 0.37$ ) (Fig. 1), which was unchanged after removing an outlier within an AF insulin level of  $55.4 \mu\text{U/ml}$ . When analysed separately, the correlation of the AC percentile and AF insulin level was similar in pregnancies with pregestational diabetes ( $P = 0.03$ ,  $r = 0.37$ ) and those with GDM ( $P = 0.0036$ ,  $r = 0.3$ ).

**Table 1** Maternal characteristics (expressed as mean  $\pm$  SEM)

	Pregestational diabetes ( $n = 32$ )	Gestational diabetes ( $n = 89$ )
Age	$30.4 \pm 0.8$	$30.4 \pm 0.6$
Parity	$2.8 \pm 0.3$	$2.8 \pm 0.2$
Pre-pregnancy body mass index ( $\text{kg/m}^2$ )	$23.6 \pm 1.1$	$27.7 \pm 0.9$
Gestational age at diagnosis of GDM (weeks)		$28.6 \pm 0.8$
Oral glucose tolerance test:		
Fasting (mg/dl)		$99.1 \pm 3.4$
1 h		$195 \pm 5.8$
2 h		$159.9 \pm 6.0$
HbA <sub>1c</sub> (%)*	$6.4 \pm 0.3$	$6.1 \pm 0.6$
Gestational age at amniocentesis (weeks)	$33.7 \pm 0.5$	$32.8 \pm 0.3$

\*Normal range 4.1–6.0%.



**Figure 2** Receiver operator characteristic (ROC) curves for the percentiles of the abdominal circumference according to gestational age as a predictor of an amniotic fluid insulin level  $\geq 7 \mu\text{U/ml}$  (squares) and  $\geq 16 \mu\text{U/ml}$  (circles).

**Table 2** Rate of the threshold fetal abdominal circumference (AC) for severe hyperinsulinism (75th percentile) in pregnancies with and without highly elevated amniotic fluid (AF) insulin ( $\geq 16 \mu\text{U/ml}$ )

	AF insulin < $16 \mu\text{U/ml}$ ( $n = 111$ )	AF insulin $\geq 16 \mu\text{U/ml}$ ( $n = 10$ )
AC < 75th percentile ( $n = 74$ )	74	0
AC $\geq 75$ th percentile ( $n = 47$ )	37	10

The sensitivity of the 75th percentile AC to identify a pregnancy with severe hyperinsulinism was 100%, the specificity 66.6%, the positive predictive value 21.3% and the negative predictive value 100%.

Dividing the cohort according to AC percentiles revealed a significant stepwise increase in AF insulin  $\geq 7 \mu\text{U/ml}$  at the 80th percentile. AF insulin levels were elevated in 22.8% (18/79) when the AC was < 80th percentile, and in 47.6% (20/42;  $P = 0.04$ ) when the AC was  $\geq 80$ th percentile with a negative predictive value (NPV) of 77.2% (61/79). By only using ultrasound AC measurements at 80th percentile to identify pregnancies at low risk for AF insulin  $\geq 7 \mu\text{U/ml}$  18 of 38 (47.3%) pregnancies with elevated AF insulin would have been missed. The ROC curve confirmed that there was no clear threshold of the fetal AC to identify an AF insulin  $\geq 7 \mu\text{U/ml}$  (Fig. 2).

Since the NPV of the AC identifying a fetus with AF insulin  $\geq 7 \mu\text{U/ml}$  was low, we examined the correlation diagram of Fig. 1 for an AF insulin level which was associated with a higher NPV. An AF insulin level  $\geq 16 \mu\text{U/ml}$ , present in 10 of 121 (8.3%) pregnancies, could be identified by the 75th percentile of the AC with a negative predictive value of 100% (74/74,  $P < 0.0001$ ) (Table 2). There was no case of AF insulin level  $\geq 16 \mu\text{U/ml}$  when the AC was < 75th percentile. The ROC curve confirmed the threshold of an AC > 75th percentile to identify an AF insulin level  $\geq 16 \mu\text{U/ml}$  with an excellent rela-

tionship between sensitivity and specificity. When the AC exceeded the 75th percentile, hyperinsulinism was present in 21.3% of the pregnancies. For simplicity we refer in the further text to an AF insulin  $\geq 7 \mu\text{U/ml}$  as moderate and to an AF insulin  $\geq 16 \mu\text{U/ml}$  as severe hyperinsulinism.

The median AF insulin was significantly higher in pregnancies complicated by pregestational diabetes (8.35  $\mu\text{U/ml}$ ) compared with pregnancies complicated by GDM (4.1  $\mu\text{U/ml}$ ,  $P < 0.0001$ ). Similarly, moderately elevated AF insulin levels were significantly more common in pregnancies with maternal pregestational diabetes, occurring in 20 (62.5%) of 32 pregnancies with pregestational diabetes and in 18 (20%) of 89 pregnancies with gestational diabetes ( $P < 0.0001$ ). Severely elevated AF insulin levels occurred in seven (21%) and three (3.4%) pregnancies with pregestational and gestational diabetes, respectively ( $P = 0.003$ ).

## Discussion

Our study has two important findings relating to fetal-based management of diabetic pregnancy. First, we found a positive correlation between the ultrasound measurement of fetal AC percentile and an established biochemical marker for fetal hyperinsulinism, the AF insulin level. This supports the pathophysiology of accelerated growth in diabetic pregnancies. An association between macrosomia at birth and elevated AF insulin at third trimester as well as at time of birth has been well documented [22–24]. Second, our findings suggest that using AC measurements with a threshold  $\geq 75$ th percentile for gestational age may be useful in identifying severe hyperinsulinism. In our study the AC threshold of the 75th percentile did not miss AF insulin above  $16 \mu\text{U/ml}$ .

We found AC measurements to be a relatively poor discriminator of moderate hyperinsulinism but excellent for severe hyperinsulinism. Kainer *et al.* examined the relationship between AF insulin and fetal growth measured at the time of amniocentesis in Type 1 diabetic pregnancies [25]. As in our study they found ultrasound AC measurements to be most useful in identifying pregnancies with highly pathological levels of AF insulin ( $> 20 \mu\text{U/ml}$ ) while 33% of cases with moderate elevated insulin levels would have been missed. Defining an AC exceeding the 50th percentile measurement by  $\geq 10 \text{ mm}$  as accelerated growth, this AC have had a sensitivity of 80% in identifying highly pathological hyperinsulinism [25]. Their definition of accelerated AC corresponds more or less to the 75th percentile of the AC in the 3rd trimester that we identified as a threshold for severe hyperinsulinism.

Weiss *et al.* have previously demonstrated that neonatal morbidity was largely limited to AF insulin levels which were increased two- to three-fold above normal [9]. They found that the risk of ‘biochemical fetopathy’ defined as hypoglycemia and biochemical dysregulation in the newborn occurred only when the AF insulin levels exceeded  $17 \mu\text{U/ml}$ . ‘Somatic fetopathy’, including the additional morbidity of excessive birth weight, was limited to even higher AF insulin levels ( $\geq 20 \mu\text{U/ml}$ )

[9]. Burkhart *et al.* have also reported that only markedly elevated AF insulin levels ( $> 18 \mu\text{U/ml}$ ) were associated with an increased risk of symptomatic diabetic fetopathy [26]. Long-term effects of fetal hyperinsulinism also appear to be restricted to the 'pathological' levels of AF insulin. Metzger *et al.* found in offspring of diabetic mothers an increased rate of childhood obesity when third-trimester AF insulin levels exceeded  $150 \text{ pmol/l}$  ( $\approx 20 \mu\text{U/ml}$ ). There was a significantly higher prevalence of impaired glucose intolerance at age 6 with AF insulin levels  $> 100 \text{ pmol/l}$  ( $\approx 14 \mu\text{U/ml}$ ) compared with pregnancies with lower AF insulin [4]. Thus the insulin level of  $\geq 16 \mu\text{U/ml}$ , which according to our data can be identified by an  $\text{AC} \geq 75\text{th}$  percentile, corresponds closely to levels reported by other researchers to be associated with morbidity.

Our study has several limitations. First, it is a retrospective analysis of pregnancies with pregestational diabetes, which were receiving insulin therapy, and pregnancies with gestational diabetes, which were mainly untreated with insulin at time of amniocentesis. In our analysis we combined pregnancies with maternal pregestational diabetes and gestational diabetes, but we excluded those women with vascular disease whose fetuses were at risk for restricted rather than excessive growth. While the incidence of hyperinsulinism and diabetic fetopathy is increased in pregnancies with pregestational diabetes the pathophysiology when present does not appear to be distinguishable between infants born to women with pregestational vs. gestational diabetes. Thus it seemed reasonable to combine the two groups. Second, the clinic protocol selected a biased spectrum of diabetic pregnancies (38.7%) for amniocentesis. In those with GDM, amniocentesis was limited mainly to pregnancies with borderline elevated maternal glycaemic values or with clinical evidence of macrosomia. In pregestational diabetic pregnancies there was clear selection bias as only 32% of the clinic population underwent AF insulin measurement. If all cases had been examined routinely, we might have seen a better correlation due to a wider variation of AF insulin values and AC measurements.

The AC threshold of the 75th percentile found by our study to reflect severe hyperinsulinism was identical to the AC threshold which has been recommended for initiating insulin therapy in GDM [10,12]. Considering fetal growth in guiding diabetic therapy in pregnancy had been included in the recent guidelines for clinical management of GDM [15]. Advocates of an ultrasound-based approach should be reassured by our findings: by using an AC threshold of the 75th percentile no cases of severe fetal hyperinsulinism were found below this level, and by extension, would have been missed and gone untreated had this level been used to guide insulin therapy. Further research is needed to confirm our findings.

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