Daniele Luminoso

Avaliação ultrassonográfica de parâmetros do crescimento fetal-Relação com a hiperglicemia materna

Dissertação apresentada ao programa de Pós-Graduação em Ginecologia e Obstetrícia, área de concentração em Obstetrícia da Faculdade de Medicina de Botucatu-UNESP, para obtenção do título de mestre.

Orientador :Profa Titular Marilza V. Cunha Rudge
Co-Orientador: Prof. Dr. Marcos Consonni

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I also apologize for this work being in English, as it would have been extremely difficult for me to write this thesis in portuguese.

Sumário

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Resumo

Resumo

Introdução: A causa mais freqüente de mortalidade perinatal em gestantes diabéticas é a malformação congênita, enquanto a principal causa de morbidade é o crescimento fetal anômalo. Anormalidades na oferta de nutrientes ao feto provavelmente desencadeiam alterações na trajetória do crescimento fetal. A hiperglicemia materna é apontada como a primeira determinante do crescimento fetal excessivo, devido a oferta de grandes quantidades de glicose ao feto, o que desencadeia hiperinsulinemia fetal. O crescimento fetal excessivo pode ser identificado em fetos grandes para idade gestacional (GIG),definidos como aqueles com peso acima do percentil 90 ou pela macrossomia, definida por peso ao nascimento acima de 4000g. A típica fetopatia diabética é caracterizada pelo aumento de proteínas corporais totais, glicogênio e gordura, além do aumento (hiperplasia e hipertrofia) de órgãos internos como fígado, coração, tecido adiposo e tecido das ilhotas pancreáticas. Este aumento seletivo dos órgãos internos contribui para uma composição corporal desarmônica que pode não ser revelada pelo peso corporal ou altura. O controle glicêmico em gestantes diabéticas está relacionado com melhora da morbidade perinatal, mas as taxas de macrossomia nestas gestações continuam altas, quando comparadas com grupo controle. A avaliação ultrassonográfica é capaz de detectar fetos em risco para crescimento excessivo, usando a medida da circunferência abdominal (CA). Foi demonstrado e estabelecido que a CA fetal acima do percentil 75 para a idade gestacional está relacionada com fetos em alto risco para crescimento excessivo e aumenta a chance para que estes fetos sejam grandes para a idade gestacional (GIG). Este achado (CA > percentil 75) vem sendo utilizado como ferramenta adicional ao ajuste da dose de insulina naquelas pacientes que fazem uso desta medicação. Quando a circunferência abdominal fetal encontra-se acima do percentil 75, impõe-se controle glicêmico materno mais rigoroso e ajuste da dose de insulina. Quando ao contrário, a CA fetal encontra-se abaixo deste percentil, pode-se optar por controle glicêmico menos rigoroso. Muitos autores demonstraram que o abdome fetal é um bom

parâmetro ultrassonográfico preditor de crescimento fetal excessivo. No entanto, a predição de macrossomia fetal, pela estimativa de peso fetal ultrassonográfica sofre grande variação, e não é considerado parâmetro suficientemente sensível para este propósito. Sabe-se que o crescimento dos fetos de mães diabéticas é desproporcional, sendo a circunferência craniana o parâmetro menos afetado e o abdome fetal o mais afetado. Além destes parâmetros ultrassonográficos, o polidrâmnio, aumento do índice de líquido amniótico (ILA), também é achado frequente das gestações complicadas pelo diabetes mellitus, isto provavelmente devido a hiperglicemia fetal e conseqüente poliúria osmótica. Este achado está presente principalmente naquelas gestações com pobre controle glicêmico. A avaliação ultrassonográfica de parâmetros do crescimento fetal e do ILA é importante ferramenta adicional à avaliação dos níveis glicêmicos maternos nas gestações complicadas pelo diabetes mellitus. Considerando que a detecção de fetos com alto risco para crescimento excessivo e desproporcional é de grande importância para melhorar o resultado perinatal, e que a medida da circunferência abdominal fetal é o parâmetro mais sensível para avaliação do crescimento fetal excessivo e que o polidrâmnio está relacionado a gestações com pobre controle glicêmico, nossa hipótese é de que a hiperglicemia materna resultaria em alteração dos parâmetros ultrassonográficos fetais e do ILA e que a análise combinada destes parâmetros (ultrassonográficos e glicemia materna) identificaria aqueles fetos com alto risco para crescimento desproporcional. **Objetivo:** Avaliar a relação da glicemia materna sobre parâmetros ultrassonográficos do crescimento fetal e do índice de líquido amniótico (ILA) em gestações complicadas pelo diabetes mellitus. **Sujeitos e Métodos:**Trata-se de estudo observacional, analítico, retrospectivo com corte transversal no qual foram incluídas 305 gestações únicas complicadas por diabetes mellitus. Os seguintes parâmetros biométricos fetais, circunferência craniana (CC), circunferência abdominal (CA) e relação circunferência craniana e circunferência abdominal (CC/CA), assim como o índice de líquido amniótico (ILA), foram transformados em percentis e correlacionados com a média glicêmica materna diária (MG). As pacientes diabéticas foram divididas em 2(MG < 120mg/dL e MG ≥ 120mg/dL) ou 3 (MG < 100mg/dL entre 100 e

119mg/dL e ≥ 120mg/dL) grupos de acordo com a média glicêmica (MG) obtida do perfil glicêmico realizado no intervalo de 2 semanas do exame ultrassonográfico. Quando considerados os dois grupos de média glicêmica materna, foi denominado de grupo com controle glicêmico adequado aquele com MG < 120mg/dL e inadequado aquele com MG ≥ 120mg/dL. Quando considerados os três grupos de média glicêmica materna, foi denominado de grupo com bom controle glicêmico aquele com MG < 100mg/dL, grupo com MG aceitável aquele com MG entre 100 e 119mg/dL e controle glicêmico inadequado aquele com MG ≥ 120mg/dL. A relação entre MG e parâmetros ultrassonográficos foram primeiramente avaliados em todas as idades gestacionais. A seguir, para avaliar o impacto dos níveis glicêmicos maternos sobre os parâmetros ultrassonográficos em diferentes idades gestacionais, as pacientes também foram divididas de acordo com a idade gestacional (IG) em 2 (IG < 25 semanas e ≥ 25 semanas) e ou 3 (IG <25 semanas , entre 25 e 32 semanas e ≥ 33 semanas) grupos. **Resultados:** O percentil da circunferência abdominal fetal (CA) foi significativamente maior no grupo da média glicêmica materna acima de 120mg/dL (controle glicêmico inadequado) quando comparado com o grupo com MG<120mg/dL (percentil 60,5 vs 71,6; p=0,0227). Quando considerados 2 diferentes períodos gestacionais (<25 e ≥25 semanas), a circunferência abdominal fetal (AC) se demonstrou estatísticamente maior no grupo com controle glicêmico inadequado (≥120mg/dL) quando comparado com o grupo com controle glicêmico adequado (<120mg/dL) apenas no período gestacional ≥25 semanas de gestação (percentil 58,6 vs 71,6; p=0,0281). Quando considerados os 3 diferentes grupos de média glicêmica materna (MG), o percentil da circunferência abdominal (AC) manteve a diferença estatística acima de 33 semanas, quando comparados os grupos com bom controle glicêmico (MG<100mg/dL) e com controle inadequado (MG≥120mg/dL) (percentil 52,5 vs 89,3; p=0,0076) e quando comparados os grupos com MG aceitável (entre 100 e 119mg/dL) e com controle inadequado(MG≥120mg/dL) (percentil 57,5 vs 89,3; p=0,0090).Considerando a variável CC/CA (relação entre circunferência craniana e circunferência abdominal), esta apresentou diferença estatística nas idades gestacionais acima de 33 semanas entre os grupos com bom controle glicêmico e controle inadequado (percentil

35,5 vs 9,9; p=0,0313) e entre os grupos com controle glicêmico aceitável e inadequado (percentil 40,4 vs 9,9; p=0,0158). Quando considerada a idade gestacional, as variáveis CA (circunferência abdominal) e CC/CA (relação circunferência craniana e circunferência abdominal) não foram influenciadas pela média glicêmica materna abaixo de 33 semanas. Os outros parâmetros ultrassonográficos; CC (circunferência craniana), e ILA (índice de líquido amniótico) não demonstraram relação com as médias glicêmicas maternas. Houve correlação negativa (inversa) significativa entre média glicêmica materna e idade gestacional, ou seja quanto maior a idade gestacional, menor a média glicêmica. Houve correlação positiva significativa entre os parâmetros ultrassonográficos, CA e ILA e uma correlação negativa (inversa) significativa entre CC/CA e ILA. **Conclusão:** Em gestantes diabéticas, os parâmetros ultrassonográficos fetais CA e CC/CA apresentam correlação com os níveis glicêmicos maternos. Crescimento fetal excessivo e desproporcional é evidente em gestantes diabéticas com pobre controle glicêmico, e isto é evidente especialmente a partir de 33 semanas de gestação. O índice de líquido amniótico não apresenta correlação com controle glicêmico materno, mas tem correlação positiva com a CA fetal e correlação inversa com a relação CC/CA.

Dissertação

Sonographic Evaluation of Fetal Biometry and Amniotic Fluid Index- Relation with Maternal Mean Glycemic Levels in Pregnancies Complicated by Diabetes Mellitus

Padronizado de acordo com as normas para publicação da Revista Ultrasound in Obstetrics and Gynecology.

Sonographic Evaluation of Fetal Biometry and Amniotic Fluid Index- Relation with Maternal Mean Glycemic Levels in Pregnancies Complicated by Diabetes Mellitus

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KEYWORDS: Ultrasound, fetal biometry, diabetes, pregnancy, abdominal circumference, amniotic fluid index.

Abstract

Objective: The aim of this study was to analyze the Sonographic Evaluation of Fetal Biometry and Amniotic Fluid Index (AFI) in Relation to Maternal Mean Glycemic Levels (MGL) in pregnancies complicated by diabetes mellitus. We hypothesized that maternal hyperglycemia would result in an increased of fetal growth parameters and AFI and that combined analysis of these would point out those fetuses at high risk for disproportional growth. **Methods:** This is an observational, retrospective, cross section, analytic study of 305 singleton pregnancies complicated by diabetes mellitus. Fetal biometric parameters (Head Circumference - HC, Abdominal Circumference - AC and Head Circumference to Abdominal Circumference ratio - HC/AC) and the Amniotic Fluid Index (AFI) were transformed in centiles and correlated to the daily maternal mean glycemic levels (MGL). The diabetic patients were divided according to the mean glycemic levels performed within two weeks of the ultrasound examination in two (< 120 and ≥ 120mg/dL) or three groups (< 100, 100-119 and ≥ 120mg/dL). The fetal sonographic parameters were evaluated overall gestational ages. In order to evaluate if mean glycemic level could have a specific influence on sonographic parameters in different gestation ages we have also subdivided the patients according to gestational age in two (< 25 and ≥ 25weeks) or three (< 25, 25-32 and ≥ 33weeks) groups. Kruskal-Wallis test was used as distribution comparison when three different groups were present, and when statistical significance was found, Mann-Whitney was used to test differences within groups. Mann-Whitney was also used as mean of comparison when two different groups were present. Correlation between the variables was assessed by linear correlation (Spearman). **Results:** We have performed five different analysis. The first one, was the evaluation of the difference between the ultrasound parameters in two different maternal MGL (<120mg e ≥ 120mg/dL) groups, independently of gestational age, i.e. considering all gestational ages. The fetal AC centile was significantly higher in the maternal mean glycemic level group (MGL) ≥ 120 mg/dL (inadequate controlled group) when compared with the adequate (< 120 mg/dL) glycemic level group (centile

60,5 vs 71,6; p=0,0227). The second analysis performed was the evaluation of the difference between the ultrasound parameters in two different maternal MGL (< 120mg/dL e ≥ 120mg/dL), first in the patients with gestational age < 25 weeks and second in the patients with gestational age ≥ 25weeks of gestation. Fetal AC was statistically larger in the inadequate MGL group (≥ 120mg/dL) compared with the adequate one (< 120mg/dL) only ≥ 25weeks gestation (centile 58,6 vs 71,6; p=0,0281), and not below it. The third analysis performed was the evaluation of the difference between the ultrasound parameters in two different maternal MGL (< 120mg/dL e ≥ 120mg/dL), first, in the patients with gestational age < 25 weeks, second, in the patients with gestational age between 25 – 32 weeks and finally in those patients with gestational age ≥ 33weeks. AC showed a statistically difference according to MGL only in the patients with gestational age ≥ 33weeks being larger in the MGL group ≥ 120mg/dL (centile 55,6 vs 89,3;p=0,0044. HC/AC showed a statistically difference according to MGL only in the patients with gestational age ≥ 33weeks being smaller in the MGL group ≥ 120mg/dL (centile 37,8 vs 9,9; p=0,0144). The forth analysis performed was the evaluation of the difference between the ultrasound parameters in three different maternal MGL (< 100, 100-119 and ≥ 120mg/dL), first, in the patients with gestational age < 25 weeks, second, in the patients with gestational age between 25 – 32 weeks and finally in those patients with gestational age ≥ 33weeks. The AC centile maintained a statistically significantly difference, ≥ 33weeks, when comparing the good (<100 mg/dL) and the inadequate glycemic level groups (centile 52,5 vs 89,3;p=0,0076) and the acceptable (100 – 119 mg/dL) and the inadequate glycemic level group (centile 57,5 vs 89,3; p=0,0090). Similarly the HC/AC centile had a statistically significant difference, ≥ 33weeks, between the good and the inadequate glycemic level group (centile 35,5 vs 9,9; p=0,0313) and between the acceptable and the inadequate glycemic level group (centile 40,4 vs 9,9; p=0,0158). AC and HC/AC centiles were not influenced by MGL below 33 weeks. The remaining ultrasound parameters (HC and AFI) did not show relation to MGL. The fifth and last analysis performed was the evaluation of linear correlation between all variables. There was a negative significantly correlation between MGL and gestational age. There was a positive significantly

correlation between fetal AC and AFI and there was an inverse significantly correlation between fetal HC/AC ratio and AFI. **Conclusion:** Fetal sonographic biometric parameters are related to maternal MGL in pregnancies complicated by diabetes mellitus. Accelerated fetal growth and disproportional fetal growth is evident in diabetic women with poor glycemic control starting from 33 weeks gestation. The AFI is not associated with glycemic control although has a positive correlation with AC and an inverse correlation with HC/AC.

EVIDENCE LEVEL II (Royal College of Obstetricians and Gynaecologists)

Introduction

The most frequent causes of perinatal mortality in diabetic women are congenital malformations, while the main cause of perinatal morbidity is abnormal fetal growth. Changes that influence the supply of nutrients to the fetus might lead to alterations of fetal growth trajectory. In gestational diabetes it always has been hypothesized that excessive fetal growth is deriving from the increased availability of maternal nutrients to the placenta. The increased intrauterine growth and fetal mass deposition in these cases may result from combined effects of nutrients and the permissive environment of fetal hyperinsulinemia [1].

Among all clinical complications caused by diabetes in pregnancy, excessive fetal growth remains an important perinatal concern. Excessive fetal growth can be identified on a fetus large for gestational age (LGA), defined as > 90th centile or by macrosomia, defined by a birth weight > 4000 gr. [2].

The typical diabetic fetopathy is characterized by increased amounts of total body protein, glycogen and fat; internal organs such as the liver, heart, adipose tissue, and pancreatic islet tissue are enlarged because of cellular hyperplasia and hypertrophy. This selective organomegaly contributes to a disharmonious body composition that may not be revealed by measurements of bodyweight and height [3].

According to Pedersen´s hypothesis, fetal overgrowth or macrosomia are consequences of increased maternal glucose [4].

Maternal glycemic control in diabetic pregnancies has showed to improve perinatal morbidity, but not eliminate the excess rate of macrosomia in these pregnancies when compared with controls [5]. The results of HAPO study demonstrate a linear association between increasing glycemia levels and birth weight above the 90th percentile and shoulder dystocia or birth injury [6].

Ultrasound evaluation can detect fetuses at risk of excessive growth by using the abdominal circumference (AC) measurement. It has been established that a fetus AC > 75th centile is at increased risk to be large for gestational age (LGA) and this has been used as an adding tool to adjust maternal insulin therapy. Contrary, a fetus AC < 75th centile is at low risk for LGA and allows the clinician for a relaxed maternal glycemic targets [7, 8]. It has been demonstrated that the fetus AC is a good predictor of birth weight, and can accurately estimate fetal size in 65% of gestations [9]. It also has been related to be a good predictor of fetal macrosomia and reflects the asymmetric growth in diabetic pregnancies [10, 11]. The fetal AC is a standard component of the basic fetal ultrasound examination for determining low and high risk fetal growth [7].

Wong at all [12], have showed that fetal growth rate assessed by ultrasound measurements of fetal biparietal diameter (BPD), femur length (FL) and AC are higher than those in low risk population, being the fetal BPD the least affected parameter among fetuses of women with diabetes mellitus. Nevertheless the accuracy of sonographic fetal weight estimation is considered insufficient, with a positive predictive value of 65% at the best for detecting a fetus of > 4000 gr., being the error between 250 to 500 gr., with more than 50% of these fetuses having their weight estimate within 10% of their actual birth weight. This is considering in the best hands of experts, which is not a reality for the majority of health services [4].

Regarding sonographic parameters in diabetic pregnancies, another common finding is polyhydramnios with a prevalence ranging from 1,5 to 66% of these pregnancies [13, 14]. This is probably due to fetal hyperglycemia and consequent fetal osmotic polyuria [13].

The presence of fetal polyuria until now has shown to be related to poor glycemic control, and iatrogenic preterm birth rates [13].

Studies have shown that the growth acceleration in large for gestational age (LGA) fetuses of diabetic mothers starts in the second trimester around 24weeks [15] and is evident primarily in diabetic women with poor glycemic control [16]. Sonographic evaluation of fetal abdominal circumference (AC), head circumference to abdominal circumference ratio (HC/AC) and the amniotic fluid

index (AFI) is important in addition to maternal glycemia in pregnancies affected by diabetes mellitus.

We hypothesized that maternal hyperglycemia would result in an increased of fetal growth parameters and AFI and that combined analysis of these, would point out those fetuses at high risk for disproportional growth.

Considering that the detection of fetuses at high risk for LGA is the main goal to improve perinatal outcome and that the fetal AC measurement is the most sensitive parameter to access the excessive fetal growth [8] and that polyhydramnios can be a marker of poor glycemic control [13, 14], the aim of this study was to analyze the sonographic evaluation of fetal biometry and amniotic fluid index in relation to maternal mean glycemic levels in pregnancies complicated by diabetes mellitus.

Patients and Methods

This study was approved by the Botucatu Medical School Ethical Committee for Human Research in Medical Sciences.

This is an observational retrospective cross section analytic study of 380 singleton pregnancies complicated by diabetes mellitus.

All patients with the diagnosis of diabetes in pregnancy i.e. overt diabetes or gestational diabetes mellitus (GDM) were included in this study. Gestational diabetes mellitus (GDM) was defined by the criteria of Carpenter and Coustan [17]. Data from the diabetic women who attended the Diabetes Prenatal Care Clinic of the Department of Obstetrics and Gynecology of Botucatu Medical School-Unesp which is a tertiary – level facility and at the Prenatal Care Clinic of Bauru Teaching Hospital, a secondary level facility, from January 2001 to August 2009 that have met the established criteria (see below) were included in this study.

The Prenatal Care Clinic and the Maternity Unit of the University Hospital -Botucatu Medical School -UNESP is an important national reference

center for diabetes in pregnancy and it's research group has established the Brazilian guidelines for the treatment and follow up of pregnancies complicated by diabetes mellitus. The Maternity Unit of Bauru Teaching Hospital has an important academic link to this university.

Data from the diabetic patients had been entered prospectively.

Study inclusion criteria were: *1*) documented overt diabetes or GDM, *2*) accurate gestational age determined by a trustable last menstrual period or by and early ultrasound scan performed before 14 weeks of gestation, *3*) singleton pregnancy, 4) record of a fetal biometry taken in the first ultrasound available after the 1st trimester (starting from 14 weeks gestation), 5) absence of fetal anomalies identified by ultrasound and absence of aneuploidy.

From the 380 patients of the database obtained from the 2 centers described above, 319 had complete mean glycemic levels, 301 patients had the HC measurements, 341 had the AC measurements, 301 had the HC/AC ratio measurements and 304 had AFI measurements.

All women were given individualized diets for the treatment of diabetes in pregnancy. Those requiring treatment with only diet were instructed to self-monitor blood glucose four times a day (fasting and 2-h postprandial) twice a week using memory-based reflectance meters. Women with sporadically elevated glucose values but not meeting the criteria for insulin therapy were tested more often. Insulin therapy was recommended when fasting glucose values repeatedly were ≥ 90mg/dl and/or 2-h postprandial values were ≥ 120 mg/dl [18, 19]. For the plasmatic glycemic measurements it was used the enzymatic method.

For the analysis it was considered the daily mean glycemic level of the glycemic profile performed within 2 weeks of the ultrasound examination that was selected for the study. The daily mean glycemic level (MGL) was calculated as average of fasting and 2-hour postprandial plasmatic glycemia from 6 am to 6 pm. The diabetic patients were divided according to the MGL performed within 2 weeks of the ultrasound examination in two (< 120mg/dL and ≥ 120mg/dL) or three groups (< 100mg/dL, between 100 and 119mg/dL and ≥ 120mg/dL).

When two MGL groups were taken into account, it was considered as adequate glycemic control a MGL < 120mg/dL and inadequate glycemic control a MGL ≥ 120mg/dL [20, 21, 22]. When three MGL groups were taken into account, it was considered good glycemic control a MGL < 100mg/dL, acceptable a MGL between 100 and 119 mg/dL and inadequate glycemic control a MGL ≥ 120mg/dL [20, 21, 22].

Sonographic examinations were performed by physicians experienced in fetal ultrasound. The ultrasound fetal parameters studied were the head circumference (HC), the abdominal circumference (AC), the head circumference to abdominal circumference ratio (HC/AC), and the amniotic fluid index (AFI). Fetal biometry and amniotic fluid index (AFI) were measured using a curvilinear array real time system with a 3,5 – 5,0 Mhz transducer (Toshiba Power Vision, Toshiba, Japan). Fetal HC was measured using the ellipse method in a transverse axial plane of the fetal head showing the central mid-line echo broken in the anterior third by cavum septii pellucidum, the thalami and 3rd ventricle in the absence of the cerebellum. Fetal AC was measured using the ellipse method in a transverse axial plane of the upper fetal abdomen showing stomach, left portal vein, a single rib on each side in the absence of the kidneys. The fetal HC/AC was calculated dividing the head circumference by the abdominal circumference. The AFI was measured by adding the vertical length of the deepest fluid pocket in each one of the four quadrant of the maternal abdomen avoiding umbilical cord and fetal parts.

In order to compare all the above mentioned ultrasound parameters independently of gestational age, the measurements in millimeters were transformed in centiles as described by Royston P et al [23]:

$$\text{Centile} = \text{inv. standard Gaussian distribution of } \frac{\text{Measurement} - \text{Mean}}{\text{Standard Deviation}}$$

“Measurement” is the ultrasound parameter measured in the study group. “Mean” and “Standard Deviation” are derived for each gestational age using regression equation of previously published references intervals 24, 25.

The fetal biometric parameters HC, AC, HC/AC and the AFI where analyzed and correlated to the maternal daily mean glycemic levels (MGL).

The relation between MGL and fetal sonographic parameters was evaluated considering the overall pregnancy, but in order to evaluate if mean glycemic level could have a specific influence in different gestation ages, we have also subdivided the patients according to gestational age in two (< 25 weeks and ≥ 25 weeks) or three (< 25 weeks, between 25 and 32 weeks, ≥ 33 weeks) groups. We have considered 3 moments of pregnancy to study fetal biometry and its possible relation to maternal mean glycemic levels based on, first, the non diabetogenic period of pregnancy, when maternal metabolism works in anabolism with favorable insulin sensitivity, and this period was established as that before 25 weeks of gestation [26, 27]. The second moment in pregnancy was established as that of maternal catabolism when the placental hormones induces physiological insulin resistance and when most gestational diabetes are identified, between 25 and 32 weeks of pregnancy [26], and the third period of study, that of the increased fetal growth in the third trimester of pregnancy, considered in this study as starting from 33 weeks of gestation [28]. When only two gestational ages groups were taken into account, we have considered 25 weeks of gestation as the marker of the two metabolic periods of pregnancy, dividing the maternal anabolic and catabolic periods of pregnancy.

In summary, we have performed 5 different analyses: the first one, was the evaluation of the difference between the ultrasound parameters in two different maternal MGL (<120mg e ≥ 120mg/dL) groups, independently of gestational age*, i.e*. considering all gestational ages; the second one was the evaluation of the difference between the ultrasound parameters in two different maternal MGL (< 120mg/dL e ≥ 120mg/dL), first in the patients with gestational age < 25 weeks and second in the patients with gestational age ≥ 25weeks of gestation; the third analysis performed was the evaluation of the difference between the ultrasound parameters in two different maternal MGL (< 120mg/dL e ≥ 120mg/dL), first, in the patients with gestational age < 25 weeks, second, in the patients with gestational age between 25 – 32 weeks and finally in those patients with gestational age ≥ 33weeks; the forth one was the evaluation of the difference between the ultrasound parameters in three different maternal MGL (< 100, 100-119 and ≥ 120mg/dL), first, in the patients with gestational age < 25 weeks, second, in the patients with gestational age between 25 – 32 weeks and

finally in those patients with gestational age ≥ 33weeks; the fifth and last analysis performed was the evaluation of linear correlation between all variables.

Statistical Analysis

Data were collected using Excel 2007 (Microsoft, Redmond, WA, USA) and statistical analyses were performed using the statistical package SAS 9,2 (SAS institute Inc., Cary, NC, USA).

We did not undertake power calculations for this retrospective analysis, and the sample size was determined by the size of the available database.

All variables were tested for normal distribution using Kolmogorov-Smirnov test. Even after different attempts of transformation none of the variables demonstrated a Gaussian fashion and were therefore expressed as median and ranges. Kruskal-Wallis test was used as distribution comparison when three different groups were present, and when statistical significance was found, Mann-Whitney was used to test differences within groups. Mann-Whitney was also used as mean of comparison when two different groups were present.

Correlation between the variables was assessed by linear correlation (Spearman).

Values of $p < 0,05$ on two tailed analyses were considered statistically significant.

Results

Three hundred and five diabetic pregnancies were included in this study, after excluding 75 patients from the original 380 patients, with incomplete data or that did not meet the inclusion criteria established.

The overall glycemic level median was 103mg/dL (ranges: 53-546mg/dL). Considering the 305 patients analyzed, 74% (n=237) had MGL < 120mg/dL and 26% (n= 82) had MGL ≥ 120mg/dL. When the patients were divided in three MGL groups, 137 (45%) had MGL < 100mg/dL, 92 (30%) had MGL between 100 and 119mg/dL and 76 (25%) had MGL ≥ 120mg/dL.

Regarding gestational age, the median of the 305 patients was 31 weeks and 4 days (ranges: 14 weeks and 5 days – 41 weeks and 2 days). Of those, 16% (n=50) were below 25 weeks of gestation, 45% (n=136) were between 25 and 32 weeks of gestation and 39% (n=119) were above 32 weeks of gestation.

Among the patients below 25 weeks of gestation, 28% (n=14) had MGL < 100 mg/dL, 26% (n=13) had MGL between 100 and 119mg/dL and 46% (n=23) had MGL ≥120 mg/dL (Figure 1). Among those patients between 25 and 32 weeks of gestation, 43% (n=58) had MGL< 100mg/dL, 29% (n=39) had MGL between 100 and 119 mg/dL and 29% (n=39) had MGL ≥ 120mg/dL (Figure 1) and among the patients above 32 weeks of gestation, 55% (n=65) had MGL < 100mg/dL, 34% (n=40) had MGL between 100 and 119mg/dL and 12% (n=14) had MGL ≥ 120mg/dL (Figure 1).

Regarding the fetal biometry parameters in the study population the median of the HC centile was 47 (ranges: 0-100), the median of the AC centile was 62 (ranges 0-100), the HC/AC centile median was 32 (ranges: 0-100) and the AFI centile median was 78 (ranges: 0-100).

As previously described, we have performed five different analyses.

The first one, was the evaluation of the difference between the ultrasound parameters in two different maternal MGL (<120mg e ≥ 120mg/dL) groups, independently of gestational age*, i.e*. considering all gestational ages.

In this case, the results were: the abdominal circumference (AC) centile showed statistically significant difference between the two groups of maternal MGL divided in MGL < 120mg/dL and ≥ 120mg/dL, being larger in the latter group (centile 60,5 vs 71,6; *p*=0,0227) (Figure 2 and table 1). All the others fetal ultrasound parameters (HC, HC/AC and AFI) didn't show difference when related to MGL in this analysis (table 1).

The second analysis performed was the evaluation of the difference between the ultrasound parameters in two different maternal MGL (< 120mg/dL e ≥ 120mg/dL), first in the patients with gestational age < 25 weeks and second in the patients with gestational age ≥ 25weeks of gestation.

In this case, the results were: the AC showed a statistically significant difference between the two MGL group only in the patients with a gestational age ≥ 25 weeks, being larger on the higher MGL (centile 58,6 vs 71,6; p=0,0281) (Figure 3 and table 3). The other ultrasound parameters (HC/AC, HC, and AFI) did not show differences in this analysis (Table 2 and 3).

The third analysis performed was the evaluation of the difference between the ultrasound parameters in two different maternal MGL (< 120mg/dL e ≥ 120mg/dL), first, in the patients with gestational age < 25 weeks, second, in the patients with gestational age between 25 – 32 weeks and finally in those patients with gestational age ≥ 33weeks.

In this case, the results were: AC showed a statistically difference according to MGL only in the patients with gestational age ≥ 33weeks being larger in the MGL group ≥ 120mg/dL (centile 55,6 vs 89,3;p=0,0044) (Figure 4 and table 5); HC/AC showed a statistically difference according to MGL only in the patients with gestational age ≥ 33weeks being smaller in the MGL group ≥ 120mg/dL (centile 37,8 vs 9,9; p=0,0144) (Figure 5 and table 5). The other two ultrasound parameters (HC, AFI) did not show statistically difference, when considering these two MGL groups, in any of the three different gestational age groups (Tables 2, 4 and 5).

The forth analysis performed was the evaluation of the difference between the ultrasound parameters in three different maternal MGL (< 100,

100-119 and ≥ 120mg/dL), first, in the patients with gestational age < 25 weeks, second, in the patients with gestational age between 25 – 32 weeks and finally in those patients with gestational age ≥ 33weeks.

In this case, the results were: in the patients with gestational age < 25 weeks gestation sonographic parameters (AC, HC/AC, HC, and AFI) did not show differences according to MGL (table 6). In the patients between 25 – 32 weeks gestation Kruskal-Wallis showed a statistically significant difference in the HC/AC according to different MGL (p=0,0319) (Table 7).The analysis performed on HC/AC with the Mann-Withney test in order to identify differences within MGL groups did not show statistically difference. The others biometric sonographic parameters (AC, HC, AFI) did not change according to MGL in this gestational age interval (Table 7). In the group of patients ≥ 33weeks gestation there was a statistically significant difference in the AC between the good (MGL < 100mg/dL) and the inadequate (MGL ≥ 120mg/dL) mean glycemic level groups (centile 52,5 vs 89,3; p=0,0076) and between the acceptable (MGL 100 – 119mg/dL) and the inadequate (MGL ≥ 120mg/dL) mean glycemic level groups (centile 57,5 vs 89,3; p=0,0090), but there was no difference between the good and the acceptable MGL groups (centile 52,9 vs 57,5; p=0,8529) (Figure 6 and table 8). In the same gestational age period there was a statistically significant difference in the HC/AC between the good (MGL < 100mg/dL) and the inadequate (MGL ≥ 120mg/dL) mean glycemic level groups (centile 35,5 vs 9,9; p=0,0313) and between the acceptable (MGL 100 – 119mg/dL) and the inadequate (MGL ≥ 120mg/dL) mean glycemic level groups (centile 40,4 vs 9,9; p=0,0158), but there was no difference between the good and the acceptable MGL groups (centile 35,5 vs 40,4; p=0,5115) (Figure 7 and table 8).The other two ultrasound parameters (HC, AFI) did not show statistically difference, when considering three MGL groups, in any of the three different gestational age groups (Tables 6, 7 and 8).

The fifth and last analysis performed was the evaluation of linear correlation between all variables.

In this case, the results were: there was a statistically significant inverse correlation between MGL and gestational age (r=-0,23239; p<0,0001).

There was a statistically significant positive correlation between fetal AC centile and MGL (r=0,1942; *p*=0,0008) (Figure 8) and an inverse correlation between fetal HC/AC centile and MGL (r=-0,14914; *p*=0,0147) (Figure 9). No statistically significant correlation was found between HC or AFI centiles and MGL (*p*=0,0583 and *p*=0,1635 respectively) (Figure 10 and 11). Another statistically significant positive correlation was found between fetal AFI and fetal AC centiles (r=0,33881; *p*<0,0001) (Figure 12) and an inverse one between AFI and fetal HC/AC centiles (r=-0,24168; *p*<0,0001) (Figure 13).

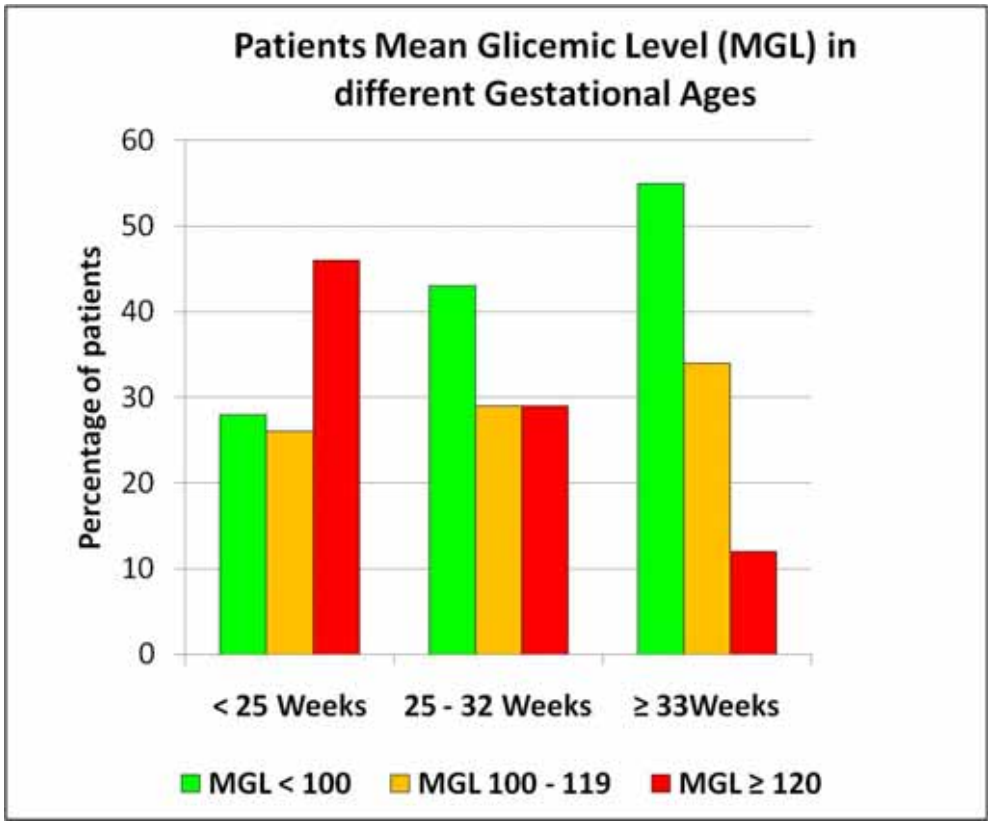

Figure 1: percentage of patients with 3 different mean glycemic levels (<100, 100-119 and ≥ 120 mg/dL) in 3 different gestational ages intervals (<25, 25–32 and ≥33 weeks).

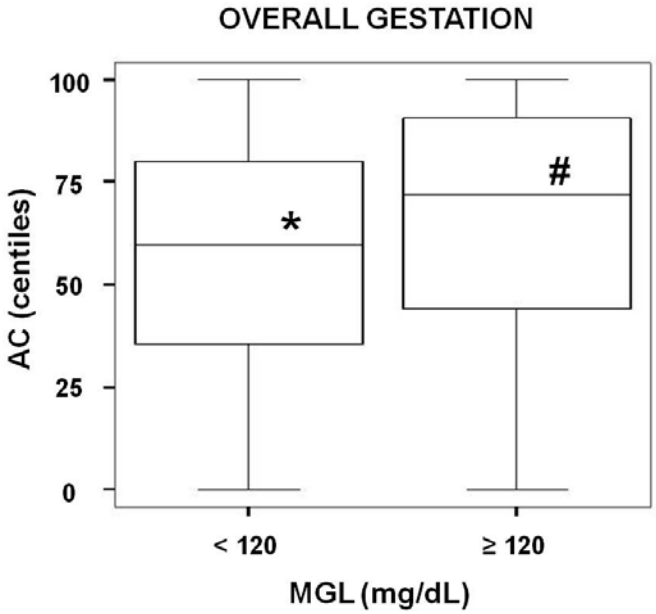

Figure 2: box-plot showing comparison of fetal abdominal circumference (AC) centiles, expressed as median, interquartiles and ranges, between two maternal mean glycemic level (MGL) groups during overall gestation.
*# Mann-Withney test significant difference p = 0,0227.

USG	MGL IN ALL GESTATIONAL AGES				
	MGL < 120 mg/dL		MGL ≥ 120 mg/dL		P value §
	n	Centiles: median (ranges)	n	Centiles: median (ranges)	
HC	200	46,6 (0,0 – 99,3)	67	46,5 (1,1-100)	0,4810
AC	225	60,5 (0,0-100)	73	71,6 (0,1-100)	**0,0227**
AFI	204	76,0 (0,5-100)	59	80,9 (0,0-100)	0,5150
HC/AC	199	34,1 (0,1-100)	68	27,9 (0,0-100)	0,1923

Table 1: comparison of fetal sonographic (USG) parameters, expressed in centiles, between two different maternal mean glycemic level (MGL) groups in all gestational ages. HC, head circumference; AC, abdominal circumference; AFI, amniotic fluid index; HC/AC, head circumference to abdominal circumference ratio.
§Mann-Whitney test.

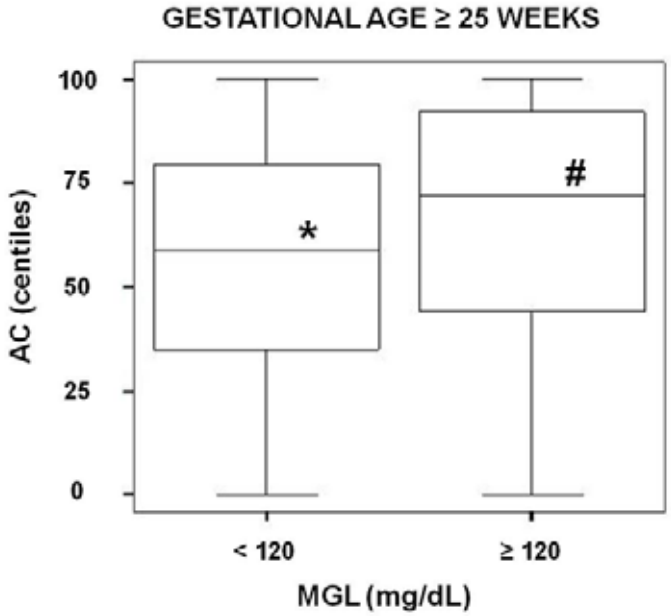

Figure 3: box-plot showing comparison of fetal abdominal circumference (AC) centiles, expressed as median, interquartiles and ranges, between two maternal mean glicemic level (MGL) groups in a gestational age ≥ 25 weeks.
*# Mann-Withney test significant difference p = 0,0281.

USG	MGL < 25 WEEKS GESTATION				
	MGL < 120 mg/dL		MGL ≥ 120 mg/dL		P value §
	n	Centiles: median (ranges)	n	Centiles: median (ranges)	
HC	21	77,7 (7,1-99,3)	18	63,8 (8,6-100)	0,6446
AC	25	70,2 (13,7-100)	19	69 (8,2-98,8)	0,5181
AFI	19	93,5 (20,3-100)	10	91,5 (68,3-99,9)	0,8739
HC/AC	20	39,9 (4,1-98,5)	19	41,2 (8,6-100)	0,9444

Table 2: comparison of fetal sonographic (USG) parameters, expressed in centiles, between two different maternal mean glycemic level (MGL) groups in patients below 25 weeks gestational age. HC, head circumference; AC, abdominal circumference; AFI, amniotic fluid index; HC/AC, head circumference to abdominal circumference ratio.
§Mann-Whitney test.

USG	MGL ≥ 25 WEEKS GESTATION				
	MGL < 120 mg/dL		MGL ≥ 120 mg/dL		P value §
	n	Centiles: median (ranges)	n	Centiles: median (ranges)	
HC	178	45,4 (0,0-90,1)	48	43,7 (1,1-99,5)	0,6855
AC	199	58,6 (0,0-99,3)	53	71,6 (0,1-100)	**0,0281**
AFI	184	74,4 (0,5-100)	49	77,2 (0,0-100)	0,8816
HC/AC	177	34,1 (0,3-100)	48	27,0 (0,0-100)	0,0623

Table 3: comparison of fetal sonographic (USG) parameters, expressed in centiles, between two different maternal mean glycemic level (MGL) groups in patients ≥ 25 weeks gestational age. HC, head circumference; AC, abdominal circumference; AFI, amniotic fluid index; HC/AC, head circumference to abdominal circumference ratio.
§Mann-Whitney test.

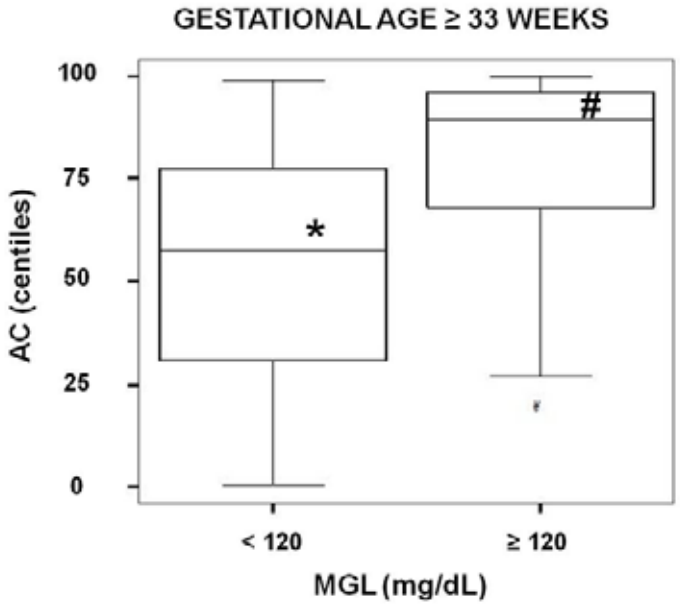

Figure 4: box-plot showing comparison of fetal abdominal circumference (AC) centiles, expressed as median, interquartiles and ranges, between two maternal mean glycemic level (MGL) groups in a gestational age ≥ 33 weeks.
*# Mann-Withney test significant difference p = 0,0044.

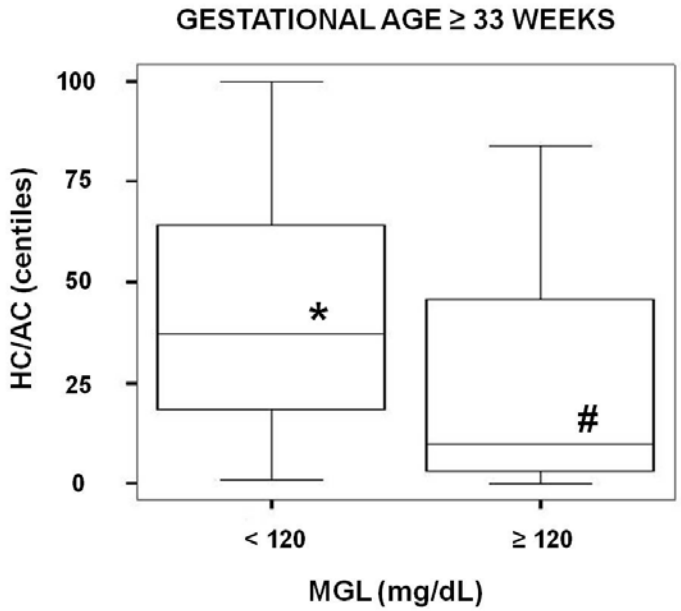

Figure 5: box-plot showing comparison of fetal head circumference to abdominal circumference ratio (HC/AC) centiles, expressed as median, interquartiles and ranges, between two maternal mean glycemic level (MGL) groups in a gestational age ≥ 33 weeks.
*# Mann-Withney test significant difference p = 0,0144.

USG	MGL 25 – 32 WEEKS GESTATION				
	MGL < 120 mg/dL		MGL ≥ 120 mg/dL		P value §
	n	Centiles: median (ranges)	n	Centiles: median (ranges)	
HC	90	42,8 (0,0-90,1)	35	43,4 (1,1-99,5)	0,9388
AC	97	60,5 (0,0-99,3)	39	65,9 (0,1-100)	0,6956
AFI	86	77,8 (9,0-100)	35	77,7 (0,0-100)	0,8040
HC/AC	90	27,8 (0,3-100)	35	28,5 (0,0-100)	0,7691

Table 4: comparison of fetal sonographic (USG) parameters, expressed in centiles, between two different maternal mean glycemic level (MGL) groups in patients between 25 – 32 weeks gestational age. HC, head circumference; AC, abdominal circumference; AFI, amniotic fluid index; HC/AC, head circumference to abdominal circumference ratio.
§Mann-Whitney test.

USG	MGL ≥ 33 WEEKS GESTATION				
	MGL < 120 mg/dL		MGL ≥ 120 mg/dL		P value §
	n	Centiles: median (ranges)	n	Centiles: median (ranges)	
HC	88	47,3 (0,1-89,9)	13	44,0 (20,8-86,2)	0,5679
AC	102	55,6 (0,5-98,7)	14	89,3 (18,1-100)	**0,0044**
AFI	98	77,7 (0,5-100)	14	76,7 (1,6-100)	0,6704
HC/AC	87	37,8 (0,8-99,8)	13	9,9 (0,0-83,9)	**0,0144**

Table 5: comparison of fetal sonographic (USG) parameters, expressed in centiles, between two different maternal mean glycemic level (MGL) groups in patients ≥ 33 weeks gestational age. HC, head circumference; AC, abdominal circumference; AFI, amniotic fluid index; HC/AC, head circumference to abdominal circumference ratio.
§Mann-Whitney test.

USG	MGL < 25 WEEKS GESTATION						
	MGL < 100 mg/dL		MGL 100 – 119 mg/dL		MGL ≥ 120 mg/dL		P value §
	n	Centiles: median (ranges)	n	Centiles: median (ranges)	n	Centiles: median (ranges)	
HC	12	78,4 (7,1-92,7)	9	77,7 (52,2-99,3)	18	63,8 (8,6-100)	0,8864
AC	13	66,6 (13,7-95,4)	12	75 (39,4-100)	19	69 (8,2-98,8)	0,3261
AFI	9	93,5 (20,3-96,5)	10	93,1 (49,6-100)	10	91,5 (68,3-99,9)	0,5779
HC/AC	11	59,7 (14,0-98,5)	9	28,1 (4,1-89,1)	19	41,2 (8,6-100)	0,2937

Table 6: comparison of fetal sonographic (USG) parameters, expressed in centiles, between three different maternal mean glycemic level (MGL) groups in patients below 25 weeks gestational age. HC, head circumference; AC, abdominal circumference; AFI, amniotic fluid index; HC/AC, head circumference to abdominal circumference ratio.
§ Kruskal-Wallis test.

	MGL 25 – 32 WEEKS GESTATION						
	MGL < 100 mg/dL		MGL 100 – 119 md/dL		MGL ≥ 120 mg/dL		P value §
	n	Centiles: median (ranges)	n	Centiles: median (ranges)	n	Centiles: median (ranges)	
HC	55	41,7 (1,0-81,1)	35	43,4 (0,0-90,1)	35	43,4 (1,1-99,5)	0,9586
AC	58	56,3 (0,0-99,3)	39	68,9 (8,0-97,9)	39	65,9 (0,1-100)	0,2782
AFI	51	79,4 (20,2-100)	35	74,4 (9,0-100)	35	77,7 (0,0-100,0)	0,9398
HC/AC	55	38,8 (0,3-100)*	35	20,4 (0,3-97,3)*	35	28,5 (0,0-100)*	**0,0319**

Table 7: comparison of fetal sonographic (USG) parameters, expressed in centiles, between three different maternal mean glycemic level (MGL) groups in patients between 25 – 32 weeks gestational age. HC, head circumference; AC, abdominal circumference; AFI, amniotic fluid index; HC/AC, head circumference to abdominal circumference ratio.
§ Kruskal-Wallis test.
* Mann-Withney test did not show statistically difference within MGL groups.

USG	MGL ≥ 33 WEEKS GESTATION						
	MGL < 100 mg/dL		MGL 100 – 119 mg/dL		MGL ≥ 120 mg/dL		P value §
	n	Centiles: median (ranges)	n	Centiles: median (ranges)	n	Centiles: median (ranges)	
HC	52	42,8 (0,1-89,9)	36	51,7 (13,3-87,7)	13	44,0 (20,8-86,2)	0,0627
AC	63	52,5 (0,5-98,7)*	39	57,5 (3,0-96,6)*	14	89,3 (18,1-100)#	**0,0145**
AFI	61	67,3 (0,5-100)	37	77,5 (17,9-100)	14	76,7 (1,6-100)	0,8104
HC/AC	51	35,5 (0,8-99,8)*	36	40,4 (4,7-94,9)*	13	9,9 (0,0-83,9)#	**0,0358**

Table 8: comparison of fetal sonographic (USG) parameters, expressed in centiles, between three different maternal mean glycemic level (MGL) groups in patients with gestational age ≥ 33 weeks. HC, head circumference; AC, abdominal circumference; AFI, amniotic fluid index; HC/AC, head circumference to abdominal circumference ratio.
§ Kruskal-Wallis test.
*# Mann-Withney test significant difference $p < 0,05$.
** Mann-Withney test no significant difference $p > 0,05$.

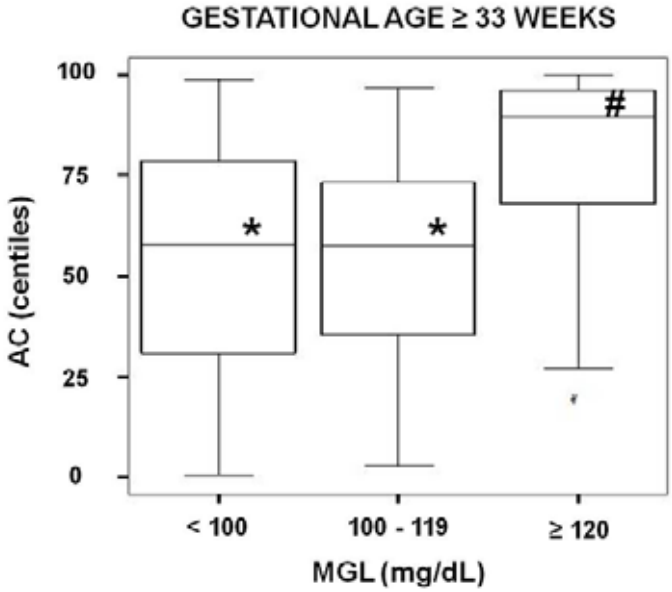

Figure 6: box-plot showing comparison of fetal abdominal circumference (AC) centiles, expressed as median, interquartiles and ranges, between three maternal mean glycemic level (MGL) groups in a gestational age ≥ 33 weeks.
*# Mann-Withney test significant difference p < 0,01.
** Mann-Withney test no significant difference p > 0,05.

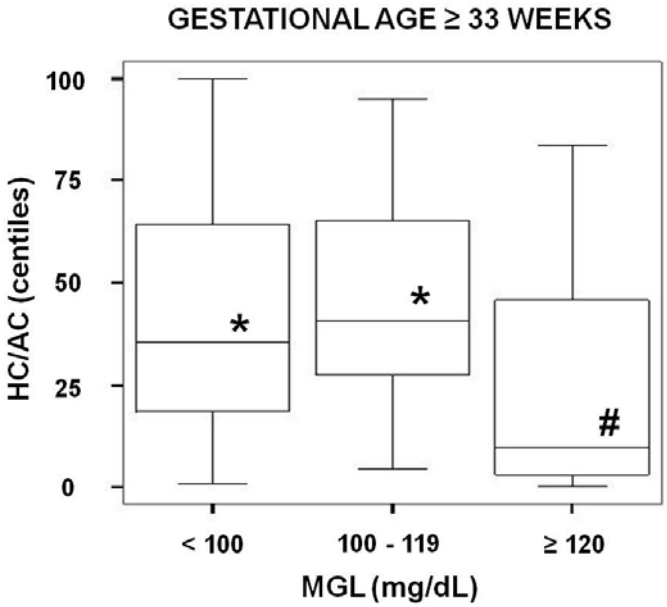

Figure 7: box-plot showing comparison of fetal head circumference to abdominal circumference ratio (HC/AC) centiles, expressed as median, interquartiles and ranges, between three maternal mean glycemic level (MGL) groups in a gestational age ≥ 33 weeks.
*# Mann-Withney test significant difference p < 0,05.
** Mann-Withney test no significant difference p > 0,05.

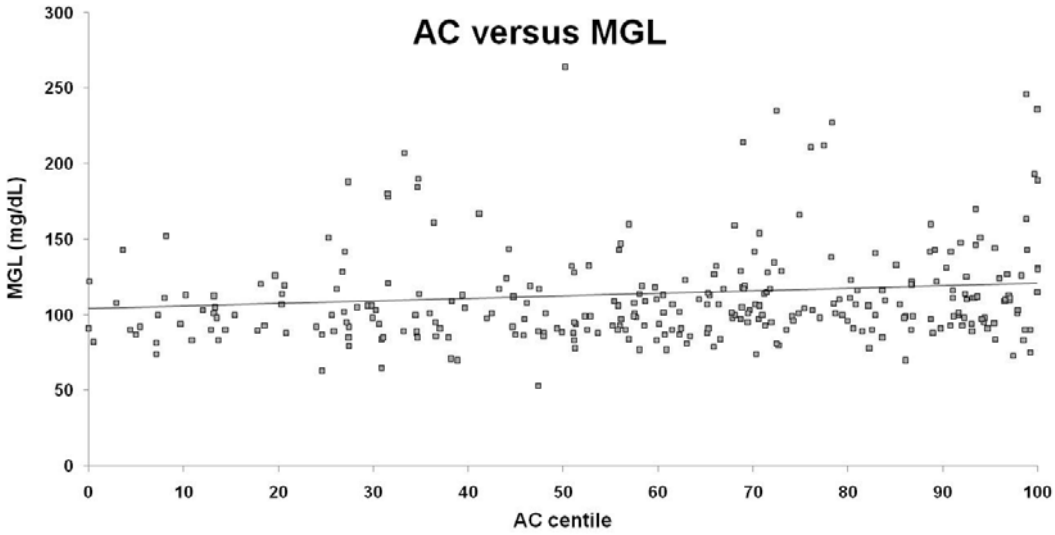

Figure 8: scatter-plot showing correlation between abdominal circumference (AC) centiles and maternal mean glycemic levels (MGL) expressed in mg/dL.
Pearson r=0,1942; p=0,0008.

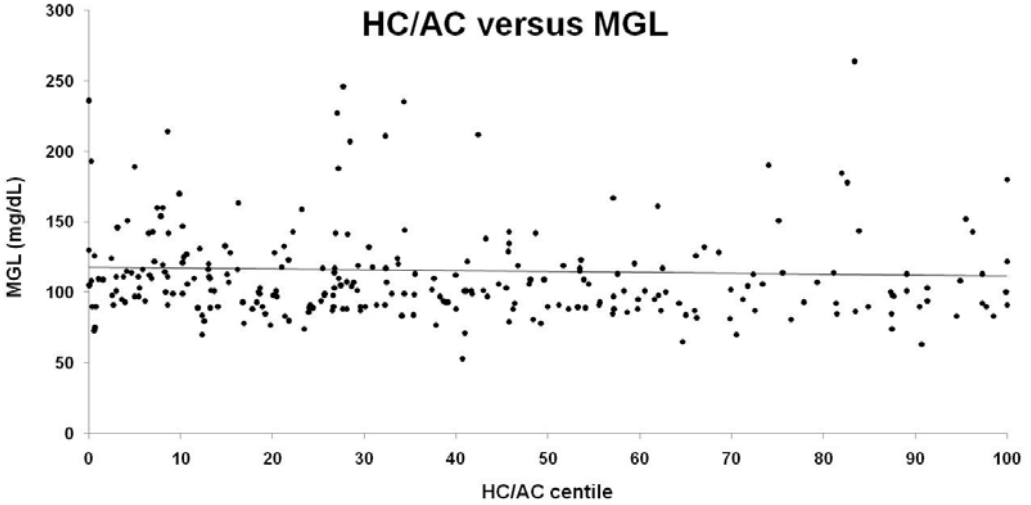

Figure 9: scatter-plot showing correlation between head circumference to abdominal circumference ratio (HC/AC) centiles and maternal mean glycemic levels (MGL) expressed in mg/dL.
Pearson r=-0,14914; p=0,0147.

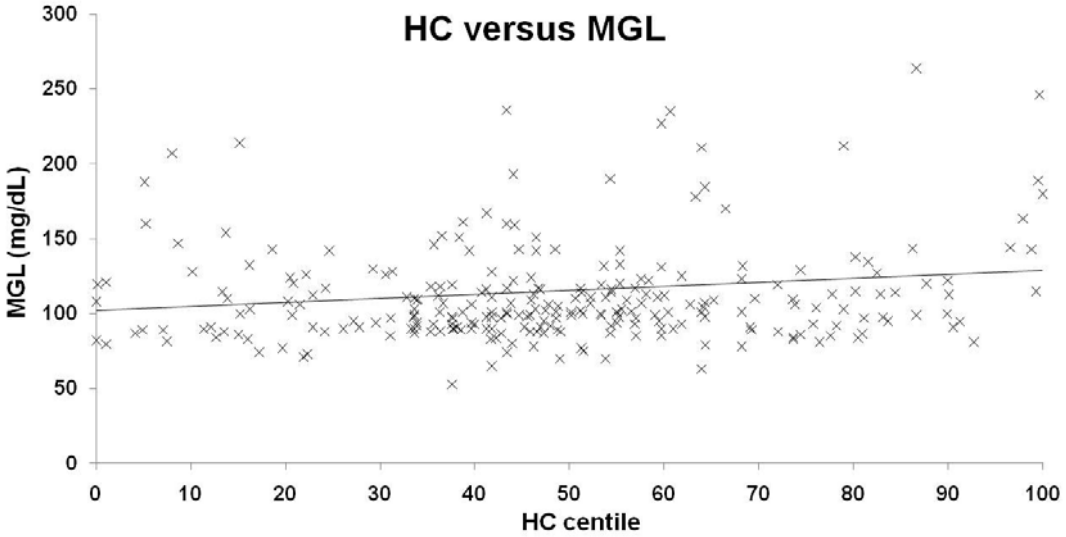

Figure 10: scatter-plot showing correlation between fetal head circumference (HC) centiles and maternal mean glycemic levels (MGL) expressed in mg/dL.
Pearson r=0,11602; p=0,0583.

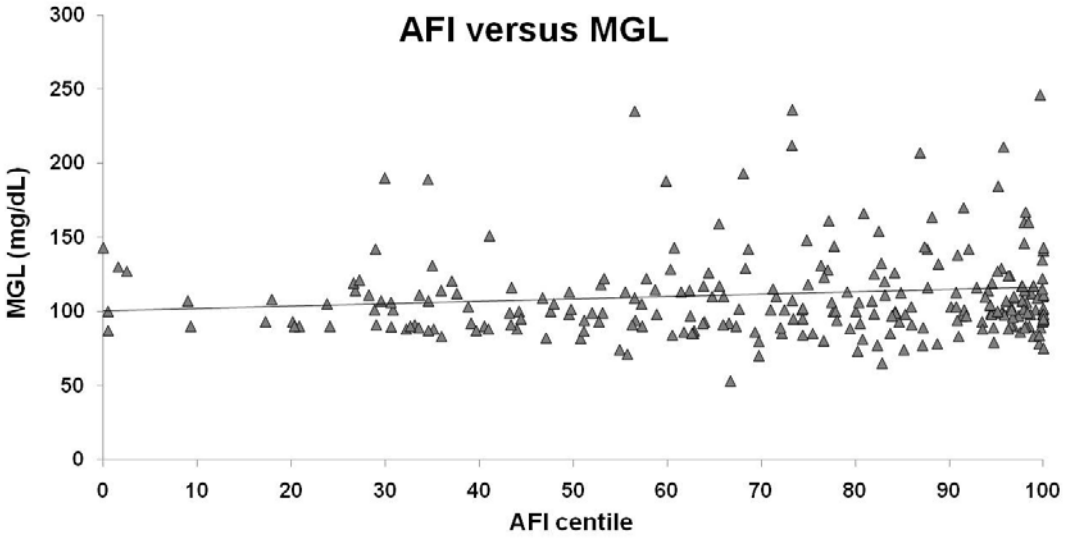

Figure 11: scatter-plot showing correlation between amniotic fluid index (AFI) centiles and maternal mean glycemic levels (MGL) expressed in mg/dL.
Pearson r=0,08617; p=0,1635.

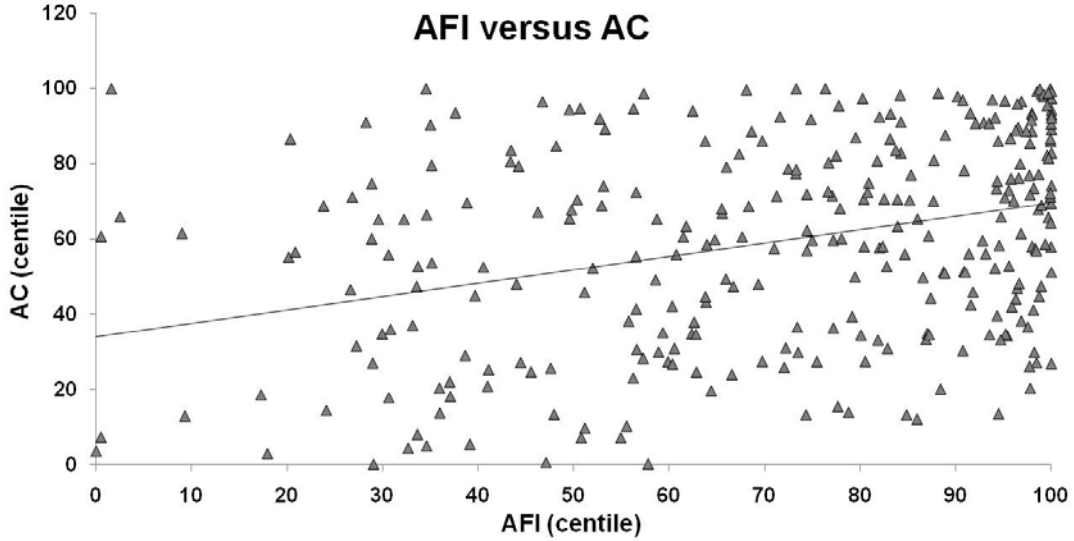

Figure 12: scatter-plot showing correlation between amniotic fluid index (AFI) centiles and fetal abdominal circumference (AC) centiles.
Pearson r=0,33881; p<0,0001.

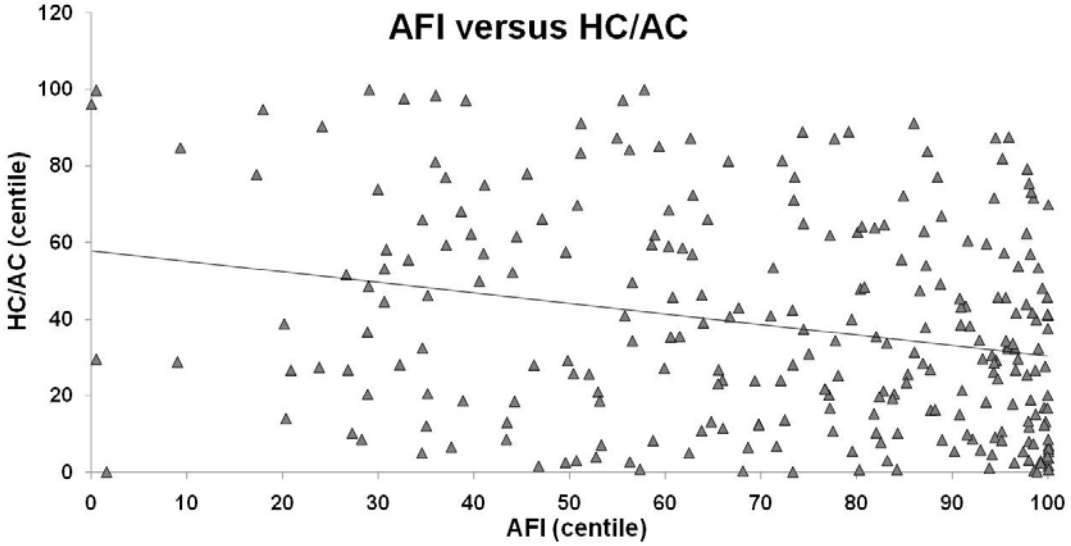

Figure 13: scatter-plot showing correlation between amniotic fluid index (AFI) centiles and fetal head circumference to abdominal circumference ratio (HC/AC) centiles.
Pearson r=-0,24168; p<0,0001.

Discussion

In this study we evaluated the sonographic parameters of fetal biometry and amniotic fluid index (AFI) in relation to maternal mean glycemic levels (MGL) in pregnancies complicated by diabetes mellitus. The key findings of our study were: 1) the fetal AC and fetal HC/AC, are related to maternal MGL. Almost 32% of our patients had fetal AC above the 75th centile and among those with inadequate glycemic control (MGL ≥ 120mg/dL) 46% showed a fetal AC above 75th centile in contrast to 28% in the adequate glycemic group (< 120mg/dL). 2) The impact of two maternal MGL (< 120mg e ≥ 120mg/dL) on sonographic parameters in 2 different phases of gestation (anabolic < 25 and catabolic ≥ 25weeks) showed larger fetal AC in the less controlled glycemic group only ≥ 25 weeks gestation. 3) The impact of three different MGL (< 100, 100-119 and ≥ 120mg/dL) in three different gestational ages showed larger AC and smaller HC/AC in the inadequate glycemic group (MGL ≥ 120 mg/dL) in later gestation (≥ 33 weeks gestation). The Kruskall-Wallis test showed a statistically significant difference in HC/AC according to MGL also between 25-32 weeks gestation, but Mann-Whitney test was not able to detect differences between the three different MGL groups. 4) Despite the absence of influence of the MGL on AFI, both the fetal AC and the HC/AC ratio presented correlation with the AFI (AC was positively correlated to the AFI (p<0,0001) and HC/AC ratio showed inverse correlation with AFI (p<0,0001)).

In diabetic pregnancies the sonographic evaluation of the fetus is important not only to detect fetal malformation but also to recognize deviation of fetal growth. Accurate prenatal estimation of birth weight may be useful in the management of pregnancy and in the delivery management of diabetic patients. The fetal AC is a standard component of the basic fetal ultrasound examination [7] and the combination with HC/AC could predict not only the LGA fetuses but also would be able to identify the disproportional LGA fetuses. These are high risk fetuses for obstetric complication like shoulder dystocia or birth injury. The results of anthropometric measurements in HAPO study confirms the link between maternal glycemia to neonatal adiposity, which may

be mediated by fetal insulin [6, 30]. Our results reinforce the flow chart of modified treatment of GDM-based serial ultrasound measurements of fetal AC proposed by Kjos and Shaefer-Graf [7]. In pregnant diabetic patients with fetal AC categorized as high risk (≥ 75th centile for gestational age), intensive medical therapy needs to be implemented. This is in accordance with other authors that have described that the fetal AC centile (above 70th or above 75th) could be a good predictor of excessive fetal growth .This parameter is related to maternal glycemic levels and has been used to adjust maternal insulin dose even on those patients with mildly abnormal glycemic levels [7, 8, 29].

Landon et al demonstrated that LGA infants of diabetic mother had a significantly accelerated rate of fetal AC growth in the early third trimester, in contrast with head circumference [31]. Similarly in our study when using three different gestational ages, it was possible to demonstrate the increase in AC and decreased in HC/AC according to MGL ≥ 33weeks gestation, exactly at the period in which our diabetic population had the best glycemic control. In fact, only 12% had a mean glycemic level above 120mg/dL in the gestational period ≥ 33weeks, compared with 29% between 25 – 32 weeks and 46% below 25 weeks gestation (Figure 1), a trend also confirmed by a significant negative correlation between gestational age and MGL.These results are in accordance to Ben-Haroush et al that demonstrated that accelerated fetal growth is evident primarily in diabetic pregnant women with poor glycemic control and these fetuses cannot be identified by a single ultrasound examination at 29-34 weeks gestation [16]. It is therefore possible that hyperglycemia in the first half of pregnancy might not be sufficient to cause overgrowth or disproportional growth in this period of gestation, but might show its effects later in pregnancy even when a better glycemic control is achieved.

Our data suggests that the glycemic control levels above which we expect an increase in AC size and a disproportional fetal growth (HC/AC decrease) is 120 mg/dL, and it seems that this inadequate glycemic level acts in the second half of pregnancy and particularly in the period of physiologic increased fetal growth (starting from 33 weeks), at least in a strictly glycemic followed up population like ours. The suggestion that arise from our data, which

is in accordance with the recommendations of the 5th International Workshop-Conference on Gestational Diabetes Mellitus [32], is therefore to keep mean glycemic control levels below 120 mg/dL, even if the fetal biometric parameters seems to show a normal growth earlier in gestation. Another clinical recommendation arising from our data is that an increase in AC centile or a decrease in HC/AC centile between two sonographic evaluations in the second half of pregnancy should prompt a glycemic profile check up as this fetal biometric trend could be the result of an inadequate maternal glycemic level (≥ 120 mg/dL).

The analysis discussed above (three MGL in 3 different gestational periods) also showed that AC size and HC/AC did not change according to MGL in a gestational age < 25weeks. It is important to remember that the gestational period in which AC and HC/AC showed variation according to glycemic levels was the period in which our diabetic population had the best glycemic control (second half of pregnancy).This result is in accordance with Mulder EJH et al [9] that showed that poor glycemic control during periconception or during first trimester had a modest relation with midgestation AC growth, while maternal glycemia played a role in determining birth weight. Therefore, it seems that maternal hyperglycemia in early gestation has an impact on fetal growth later on in pregnancy. Another potential explanation for an increase in AC size despite good glycemic control is that maternal glycemia explains only a small fraction of neonatal weight [33, 34].

The relation of fetal AC and fetal HC/AC to maternal glycemic levels is an important and expected finding as the HC/AC is directly related to the excessive growth of the AC. Others, previously, have reported that the fetal HC is the least affected ultrasound parameter in diabetic pregnancies [12] as also demonstrated by our results. Considering that these are treated patients and clinically closely followed this is a very important finding meaning that, even on a mildly abnormal glycemic level (≥ 120 mg/dL) group, disproportional or increased fetal growth can be detected. Apparently HC/AC ratio could be, in a diabetic population, another sonographic parameter, beside AC, to identify fetuses that probably were exposed to hyperglycemia at a certain point in gestation.

Due to insulin treatment, many fetuses that would be LGA become adequate for gestational age (AGA), but they still are in high risk for future metabolic disorders due to an inadequate intrauterine environment, as many previously have postulated and demonstrated [35, 36, 37]. Fetal AC and HC/AC could be a useful tool to identify those fetuses at high risk for future metabolic disorders in order to give the opportunity of interfering positively on immediately and future outcome. The fetal programming could be interrupted with a modified therapy approach instituting intensive insulin treatment [7, 38].

An unexpected finding in our study was the absence of detected influence of MGL on AFI. We would expect AFI to be related with MGL, especially on the considered inadequate glycemic controlled group (MGL ≥ 120 mg/dL), as polyhydramnios has been reported to be related with poor glycemic control [13]. Our results are in accordance with those of Maganha et al [39] that studied the relation between AFI and glycemic control in 60 pregnancies complicated by diabetes mellitus type 1 and 2 followed in a specialized Brazilian multidisciplinary prenatal care service. No correlation was found between AFI and mean glycemic levels in this rigorous metabolic controlled and standardized treated population. Interestingly the mean glycemic level of their population (103mg/dL) was equal to the median MGL of our study population (103mg/dL). Even in the absence of a significant relation between amniotic fluid volume and MGL, the median centile of AFI in our study population was particular high (78th centile), and if we consider polyhydramnios as an AFI above the 95th centile, the prevalence of this sonographic finding, in our population, was also high (25%). We would have expected a median AFI around the 50th centile, like it happened to the HC (median centile of 47), another variable that was not influenced by glycemic level. By the contrary, as expected, when considering sonographic parameters that were influenced by MGL, the AC median centile was above the 50th centile (62nd centile) and the HC/AC was below it (32nd centile). This finding of a high AFI median centile and a high rate of polyhydramnios could be explained by the fact that these patients have been probably exposed to a certain degree of hyperglycemia during pregnancy. Probably, well controlled glycemic levels are not sufficient to keep a normal amniotic fluid volume. Another explanation could be the use of an AFI nomogram [25] that is not

customized for the Brazilian population. In fact, there could be differences in interval references between different world populations. Unfortunately it is not possible to rule out any of these two possibilities as we do not have a non diabetic control group of comparison. The third explanation could be the absence of a normal distribution in our AFI data. Borges et al have demonstrated that other factors such as maternal hydration with isotonic solution or water increases the AFI in women with previous normal hydramnios [40].

Although AFI was not correlated to MGL, the fetal AC and the HC/AC showed correlation with the AFI. On this study, AC was positively correlated to the AFI (p<0,0001) and HC/AC ratio showed a inverse correlation with AFI (p<0,0001).Considering that only AC size showed a positive correlation with MGL while HC and AFI did not, it seems that the correlation between fetal biometry and AFI is independent of MGL. In other words AFI seems, in our population, not to be correlated with MGL but only with fetal size. Larger fetuses have more amniotic fluid volume despite MGL. Those results agree in part with previous published data [41] that showed a positive correlation between AFI and fetal AC centile and between AFI and estimated fetal weight (EFW) centile in both 225 normal and 120 diabetic pregnancies. These authors concluded that there might be a relation between AFI and fetal size independent of diabetes. Perni SC et al [42], based on the association between body weight and urine output in neonates, conducted a study to assess the correlation between AFI and EFW centile in 426 normal pregnant women. They found a significant relationship only after 38 weeks of gestation.

The weaknesses of our study are: first, all variables evaluated in our population did not have a normal distribution and this did not allow us to compare our data using parametric statistics. Studies with larger sample and consequently normal distribution variables are needed to confirm our results. Second, our diabetic population belongs to a strictly clinically well followed up patients with mainly good or acceptable glycemic levels, confirmed by the fact that only 26% of the patients had daily mean glycemic level above 120mg/dL, and that only 19% of the patients had daily mean glycemic level above 130

mg/dL. A less well controlled glycemic population, that reflects not only the Brazilian reality but also the world reality where a good proportion of cases of gestational diabetes mellitus is either not recognized or treated only inadequately, might have shown different results [43]. Probably, poorer glycemic levels might have shown different results with earlier and more pronounced modification of fetal biometric parameters or might have shown modification of others USG parameters, such as AFI, that seemed not to change in our population. Third, for the best of our knowledge, there are not large sample Brazilian fetal biometric nomograms published in the literature. Therefore, in order to evaluate the size of our ultrasound parameters, the reference intervals used in this study were not build upon a Brazilian population, but upon an English and a north American one [24, 25]. This might have introduced a bias as these three different populations have different constitutional anthropometric characteristics.

In summary, our findings show that MGL is an important parameter in the treatment of diabetic patients and some sonographic parameters (AC and HC/AC) can be a marker to identify those fetuses at risk for over and disproportional growth. The fetal AC is a good predictor of excessive fetal growth and alone or in relation to HC (HC/AC) can be a marker of inadequate maternal glycemic control. Our results are according to previous reports that have demonstrated similar data. In this study group population we have also demonstrated that sonographic fetal biometric parameters are related to maternal mean glycemic levels (MGL) in pregnancies complicated by diabetes mellitus. Importantly, this study shows a correlation among these sonographic parameters as AC was positively correlated to the AFI and HC/AC showed a inverse correlation with AFI. These results adds information to the great amount of studies previously published about diabetic pregnancies and fetal growth and is in accordance to these previous results. From our point of view, sonographic evaluation of fetuses of diabetic pregnancies should include a thoroughly analysis of these parameters for best sensitive judgment in order to give more information to the clinician for best clinical management of these pregnancies with the aim of a better perinatal and long term outcome.

CONCLUSION

In conclusion, fetal sonographic biometric parameters are related to maternal MGL in pregnancies complicated by diabetes mellitus. Accelerated fetal growth and disproportional fetal growth is evident in diabetic women with poor glycemic control starting from 33 weeks gestation. The AFI is not associated with glycemic control although has a positive correlation with AC and an inverse correlation with HC/AC.

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Anexo

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Ética

Registrado no Ministério da Saúde
em 30 de abril de 1997

Botucatu, 29 de Novembro de 2010. Of. 554/10-CEP

Ilustríssima Senhora
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Prezada Drª Iracema,

De ordem do Senhor Coordenador deste CEP, informo que nesta data **(29/11/2010)**, foi autorizada a inclusão de um Sub-Projeto, abaixo transcrito, ao Projeto de Pesquisa (Protocolo CEP 504-2000) "Resultado das gestações complicadas pelo diabete e pela hiperglicemia diária materna – análise dos últimos 5 anos (1996 a 2000), de autoria de Maria Cristina Colella Oliveira, orientada por Vossa Senhoria, aprovado em 03/04/2000, na seguinte conformidade:

Sub-Projeto: (Protocolo CEP 504/A-2000) "Avaliação ultrassonográfica de parâmetro do crescimento fetal- Relação com a hiperglicemia materna", de autoria de **Daniele Luminoso** (aluno Mestrado do PPG-GOM), orientado pela **Profª Titular Marilza Vieira Cunha Rudge.**

Atenciosamente,

Alberto Santos Capelluppi
Secretário do CEP.