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Ultrasound assessment of maternal adipose tissue during first trimester screening for aneuploidies and risk of developing gestational diabetes

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Conflicts of interest

None

ABSTRACT

Introduction: The objective of the present study is to compare the sonographic measurement of subcutaneous adipose thickness and visceral adipose thickness during first trimester screening for aneuploidies between non-diabetic pregnant women and patients who develop first trimester or second trimester gestational diabetes mellitus (GDM). **Material and methods**: Adipose thickness was measured by the means of transabdominal ultrasound imaging in pregnant women attending our clinic for screening for fetal aneuploidies between 11 and 13 weeks. During the first trimester all patients were evaluated for fasting glycemia in accordance to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommendations. Patients with confirmed fasting glycemia (FPG) ≥ 92 mg/dl were diagnosed as 1st trimester GDM. Patients with FPG < 92 mg/dl underwent a 75g oral glucose tolerance test between 24 and 28 weeks. **Results:** The study population included 238 non-diabetic women, 29 women with 1st trimester GDM and 28 women with 2nd trimester GDM. Mean subcutaneous adipose thickness and visceral adipose thickness values in non-diabetic women were 9.8 mm (standard deviation: SD=4.9) and 7.2 mm (SD=3.5), respectively. Values in women with 1st trimester GDM were 12.8 mm (SD=6.5) and 9.9 mm (SD=4.4). In the 2nd trimester GDM group, the mean subcutaneous adipose thickness was of 11.1 (SD=4.6) and the mean visceral adipose thickness was of 10.5 mm (SD=5.3). Multiple logistic

regression analysis showed that visceral adipose thickness, but not subcutaneous adipose thickness, was significantly and independently associated both with 1st trimester GDM (OR 1.15, 95% CI 1.02 - 1.29) and with 2nd trimester GDM (OR 1.19 95% CI 1.05 - 1.34). **Conclusions:** Sonographic thickness of maternal visceral adipose tissue was greater in women with GDM compared to non-diabetic patients, independently from other known risk factors associated with GDM in the first and in the second trimester of pregnancy. Thus, this measurement may be considered of clinical use during first trimester screening.

Key words

Gestational diabetes; subcutaneous adipose thickness; Glycemia. Adipose tissue; First trimester screening; International Association of Diabetes and Pregnancy Study Groups;

Abbreviations

OGTT oral glucose tolerance test

VAT visceral adipose thickness

BMI body mass index

SAT subcutaneous adipose thickness

GDM gestational diabetes mellitus

DM pre-gestational diabetes

IADPSG International Association of Diabetes and Pregnancy Study Groups

ORs odds ratios

CI confidence interval

Key Message

We measured abdominal adiposity during first trimester screening for aneuploidies. We found that visceral adipose tissue is an independent risk factor for gestational diabetes already in the first trimester and not only in the late second trimester.

INTRODUCTION

Gestational diabetes (GDM) increases the risk of maternal and perinatal complications, both in the short term and in the long-term, the latter including a greater prevalence of type 2 diabetes in mothers and a higher likelihood of childhood obesity in the offspring.[1-3]

Although etiology of GDM is multifactorial[1,4], adipose tissue is thought to play a pivotal role in the development of this dysmetabolic condition.[5,6] However, it has not been established whether an increased preconceptional body mass index (BMI) or, alternatively, an excessive weight gain during pregnancy may be major determinants for the development of GDM.[7] Body fat mass is distributed into three compartments:[8] subcutaneous tissue, deep subcutaneous adipose tissue and visceral adipose tissue, but it is not clear which of the abovementioned is more effective in reducing insulin activity in pregnancy.[4-9] Our previous study showed how patients with GDM have an increased amount of visceral adipose tissue during second trimester of pregnancy.[10]

The aim of the present study is to measure maternal adipose tissue by the means of ultrasound imaging in patients undergoing first trimester screening for fetal aneuploidies[11] and to determine the role of such measurement in the evaluation of the risk of developing GDM in first (1st trimester GDM) and in second trimester (2nd trimester GDM).

MATERIAL AND METHODS

Study design and setting

A single-center study was designed to recruit all consecutive women with singleton pregnancies undergoing first trimester combined screening for fetal aneuploidies at the Fetal Medicine and Surgery Centre in IRCCS Fondazione Ca' Granda, Ospedale Maggiore Policlinico, in Milan (Italy) from November 20th 2017 to December 5th 2018. The combined screening was offered to all women who accessed our centre during the first trimester of pregnancy and was performed when crown-rump length was between 45.0 mm (i.e., 11+2 gestation's week) and 84.0 mm (13+6 gestation's week).[11]

During the study period approximately 1000 women underwent first trimester screening for aneuploidies in our institute. Amongst this group, 157 pregnancies were multiple and 37 terminated in spontaneous abortions. For the performance of first trimester screening, patients

were randomly assigned to different out-patient departments. Our protocol was offered to all patients assigned to the "diagnosis and research" department, which is scheduled once a week for a total of 10 patients per session. These women received diagnostic, clinical and therapeutic treatment which did not differ in any from the outstanding protocols for pregnant women in our institute.

Patients who met any of the following criteria at the time of recruitment were not considered eligible for the study: presence of pre-gestational type 1 or type 2 diabetes, ongoing treatment with steroids, insulin or metformin, presence of scars in the epigastric region or previous bariatric surgery. Also, pregnancies complicated by fetal chromosomal abnormalities and/or major structural anomalies were excluded (Figure 1).

At the time of ultrasound examination, demographic variables (maternal age, weight, height) and obstetric variables (gestational age and parity) were collected.

Ultrasound measurements

In all cases, succeeding informed consent, transabdominal ultrasound examination was performed by a certified sonologists (RAB 4–8 MHz probe, Voluson E8 Expert, GE Medical Systems, Milwaukee, WI, USA). Gestational age was determined by fetal crown-rump measurement.[12] Fetal nuchal translucency and fetal heart rate were measured according to Fetal Medicine Foundation recomendations.[11] For the assessment of adipose tissue, subjects were placed in a supine position with their body in a straight line, their back being adjacent to the examination bed. A longitudinal scan of the upper maternal abdomen was obtained, applying the minimum pressure with the ultrasound transducer on the abdomen. The probe was placed perpendicular to the skin on the midline epigastrium. On each image the following landmarks were identified: skin, *linea alba*, liver, and xiphoid process. The subcutaneous adipose thickness (SAT) was measured as the maximum vertical distance from the skin line to the anterior edge of the *linea alba*, immediately caudal to the xiphoidal tip, capturing the image at the contact of the transducer with the skin to minimize the potential compression of the subcutaneous adipose fascia. The visceral adipose thickness (VAT) was measured on the same image from the posterior edge of the *linea alba* to the anterior surface of the liver, which is easily recognizable by ultrasound. The measurement was performed at the end of a normal expiratory act to avoid tension and distortion of the abdominal cavity (Figure 2). [13] This technique was introduced by Suzuki et al.[13] outside pregnancy and the sonographic index showed a high correlation with computed tomography measurements of VAT

and SAT. The sonographic index is characterized by excellent reproducibility and repeatability; in fact, in a review published by Valchos et al.[14] the coefficient of variation in several studies was reported to be less than 6%. We introduced the use of this technique during pregnancy[10], proving its high reproducibility by the means of a Bland-Altman analysis[15] which showed an agreement of measurements between different examiners.

Diagnosis of gestational diabetes

Universal screening for GDM was performed following the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommendations[16], as is routinely offered to all pregnant patients attending our institute. During the initial prenatal visit in the first trimester, fasting plasma glucose was dosed. Pre-gestational diabetes (DM) was diagnosed when fasting glucose levels were ≥126 mg/dL (7.0 mmol/L). Women with fasting plasma glucose ≥92mg/dL (5.1mmol/L) but <126mg/dL (7.0 mmol/L) were diagnosed as 1st trimester GDM. At 24–28 weeks of gestation, all women, except for those with diagnosis of DM or 1st trimester GDM, underwent a 75-g oral glucose tolerance test (OGTT). After a 72-h no carbohydrate restriction diet and a 10-h overnight fast, venous plasma samples were collected for the measurement of fasting, 1h and 2h plasma glucose levels. The diagnosis of 2nd trimester GDM was made using the IADPSG criteria as follows: fasting glucose ≥92 mg/dL (5.1 mmol/L) and/or glucose levels ≥180 mg/dL (10.0 mmol/L) after 60 min and/or glucose levels ≥153 mg/dL (8.5 mmol/L) after 120 min from glucose administration. All women resulting negative for OGTT screening were considered as non-GDM. Obstetric and neonatal outcomes were retrieved from the hospital records and entered into a computer database. The main outcome of this study was to evaluate the association between development of GDM and ultrasound estimation of adipose tissue thickness.

Statistical Analyses

The chi-squared test and ANOVA were used to compare the statistical significance of differences of categorical and continuous variables, respectively. Odds ratios (ORs) and corresponding 95% confidence interval (CI) for the association between GDM and maternal and obstetric characteristics were estimated by the means of multinomial logistic regression models.[15] Our final multivariate models included terms for adipose tissues (i.e., SAT and VAT) and other possible

confounders (i.e., maternal age, BMI at 12 weeks, gestational weight gain at 12 weeks, parity and family history of diabetes) which could modify the association between adipose tissue and GDM. All statistical tests were two-sided with a significance level set at <0.05. Statistical analyses were performed using SAS 9.4 statistical software (SAS Institute, Cary, NC, USA).

Ethical approval

The study was approved by the Institutional Review Board of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy (reference n.2955 - December 18th 2015) and all patients gave their written formal consent.

RESULTS

Overall, 401 women were identified as potential participants. Of these, 85 were excluded as they did not meet the inclusion criteria previously described. In particular, 9 presented with preconceptional DM type 1 or type 2 diabetes, 22 were unknown multiple pregnancies, 10 had a spontaneous abortion in the first trimester, 9 were undergoing treatment with metformin or steroids and 35 declined participation.

After ultrasound screening other 21 women were excluded: 2 were subsequently diagnosed as DM, 7 presented a fetal chromosomal anomaly and/or major structural abnormalities, 4 were undergoing treatment with steroids, 6 refused to undergo OGTT at 24-28 weeks and 2 were lost to follow up thus leaving 295 women for the analysis. Women with 1st trimester GDM were 29, women with 2nd trimester GDM were 28, and non-GDM women were 238 (Figure 1).

Maternal and obstetrical characteristics are reported in table 1. BMI at recruitment was significantly higher in women with GDM compared to non-GDM women (p<0.01). Women with GDM showed a significantly higher SAT (p<0.01). In particular, the mean SAT was 12.8 mm (standard deviation: SD=6.5) for 1st trimester GDM, 11.1 mm (SD=4.6) for 2nd trimester GDM, and 9.8 mm (SD=4.9) for non-GDM women. The pair-wise comparisons showed a significant difference in SAT mean values between 2nd trimester GDM and the non-GDM group (p-value with Bonferroni correction<0.01). No significant difference emerged from the comparison between 1st trimester GDM and the non-GDM group (p-value with Bonferroni correction=0.37) and from the

comparison between 1st trimester GDM and 2nd GDM (p-value with Bonferroni correction=0.35). Likewise, VAT was significantly higher ($p<0.01$) in women with 1st trimester GDM (mean=9.9 mm, SD=4.4) and in 2nd trimester GDM (mean=10.5 mm, SD=5.3 for) compared to non-GDM ones (mean=7.2, SD=3.5). The pair-wise comparisons showed a significant difference in VAT mean values between 2nd trimester GDM and the non-GDM group (p-value with Bonferroni correction<0.01). No significant difference emerged from the comparison between 1st trimester GDM and the non-GDM group (p-value with Bonferroni correction=0.19) and from the comparison between 1st trimester GDM and 2nd GDM (p-value with Bonferroni correction=0.99). According to the univariate analysis, higher values of BMI (OR=1.13, 95% CI: 1.04 -1.22), SAT (OR=1.10, 95% CI: 1.03-1.17), and VAT (OR=1.17, 95% CI: 1.08-1.28) significantly increased the risk of GDM in 1st trimester (Table 2). Higher values of BMI (OR=1.14, 95% CI: 1.05-1.23) and VAT (OR=1.20, 95% CI: 1.10-1.31) significantly increased the risk of GDM in 2nd trimester. In the multivariate analysis, only VAT was significantly associated with the risk of GDM (OR for 1st trimester GDM=1.15, 95% CI: 1.02-1.29 and OR for 2nd trimester GDM=1.19, 95% CI: 1.05-1.34). No further association was observed in the multivariate analysis.

We also investigated the relation between gestational age and SAT and VAT according to linear regression. We observed no association between SAT (β coefficient 0.03; 95% CI -1.23 to1.30) and VAT (β coefficient -0.09; 95% CI -1.07 to 0.89) and gestational age at the time of the measurement (data not shown).

DISCUSSION

Our paper clearly indicates that VAT is strongly related to early and late GDM. The visceral compartment, estimated by ultrasound measurement at 11-13 weeks, seems to play a relevant and stronger role than SAT, BMI and gestational weight gain during first trimester in patients with a diagnosis of GDM. The association between VAT and GDM remains significant in the multivariate regression model, adjusted for the other variables considered. In our case VAT is a predictive factor for GDM at 11-13 weeks as well as for GDM diagnosed at 24-28 weeks of gestation. It can be hypothesized that BMI does not reflect the individual adiposity compartments and in many cases may be an expression of a large SAT. Weight gain may be associated with an expansion of the subcutaneous compartment, whereas VAT is a pre-existing and predisposing factor already in the first weeks of pregnancy. In concordance with our results, Bartha et al.[18]

examined 30 women at 11–14 weeks of gestation and found a significant association between sonographic measurement of visceral adipose tissue and glycemia, insulinemia and insulin sensitivity. Martin et al.[19] reported that a VAT above the upper quartile of the normal range in the first trimester of pregnancy was significantly more frequent in cases than in controls. Only one study conducted by Yang et al.[17] on 333 singleton pregnancies in the first trimester reported that SAT was a statistically significant predictor of GDM. In a recent prospective study, De Souza et al.[20] measured SAT, VAT and total adipose tissue depth in a cohort of 485 women, using a different ultrasound technique at 11–14 weeks of gestation. They showed that elevated first-trimester measurements of VAT and total adipose tissue independently, but mostly VAT, predict the risk of developing hyperglycemia later in pregnancy. As reported by De Souza et al.[22], our study confirms the role of VAT in predicting GDM during the second trimester. Moreover, our data suggest that GDM diagnosed according to the IADPSG criteria in the first trimester is associated with increased VAT values. Furthermore, our recent publication showed that sonographic thickness of maternal VAT at 24-28 weeks of gestation is higher in women with GDM compared to non-GDM women, independently from other known risk factors associated with GDM.[10]

The strength of our study is the adoption of a standardized ultrasound protocol to measure abdominal adiposity at the same time of fetal nuchal translucency. Furthermore, this technique is not affected by intestinal contests or by gastrointestinal fullness. We have already proven the good inter and intra operator reliability of these measurements.[10, 13] This technique is highly reproducible; it is relatively simple to learn and can be quickly performed. Moreover, it can be applied at any gestational age since the measurement performed at the level of the xiphoid process is not affected by the increase of uterine volume during the different trimester of pregnancy. The limitations of this study may be represented by the predominant enrollment of Caucasian women and by the limited number of diabetic women studied. Further analysis on the evaluation of glucose homeostasis and insulin sensitivity in these patients may also represent an interesting future perspective of the study.

Measurement of VAT could be an instrument to evaluate the risk of developing GDM both at the beginning of gestation and during the second trimester. According to this hypothesis, the inclusion of VAT measurement in the first trimester of pregnancy may provide an additional contribution to

early identification of women at risk of developing GDM as is possible for other maternal and fetal complications of pregnancy such as preeclampsia or intrauterine growth restriction.[23] In addition, a recent study[24] has reported that, despite early testing, follow up and therapy, in women with first trimester GDM, adverse maternal and neonatal outcomes are more comparable to those of women with pre-gestational type 2 diabetes than to those with GDM diagnosed after 24 weeks of gestation. These women represent a high-risk cohort, which requires an early identification of risk factors. In our opinion, VAT may represent an additional factor in the identification of pregnancy complications and, as such, may provide more information about pathophysiology and prognosis.

The measurement of VAT is simple, not invasive, and not expensive and it could be a further tool in combination with fasting plasma glucose, glycated hemoglobin and OGTT to identify high-risk pregnancies in each trimester.

CONCLUSION

Our study shows that an increased VAT augments the risk of developing GDM. Patients classified at an early stage as high-risk subjects for GDM may benefit of the introduction of preventive changes in lifestyle and medications, with the primary objective to reduce possible complications of diabetes in pregnancy.

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Figure legends

Figure 1. Clinical study design. DM1, pre-gestational type 1 diabetes; DM2, pre-gestational type 2 diabetes; GDM, gestational diabetes mellitus; OGTT, oral glucose tolerance test; FG, fasting glucose.

Figure 2. The measurement of subcutaneous and visceral adipose thickness.

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370 **Table 1.** Maternal and obstetric characteristics[a] of 57 women with gestational diabetes (GDM) and
371 238 non-GDM.

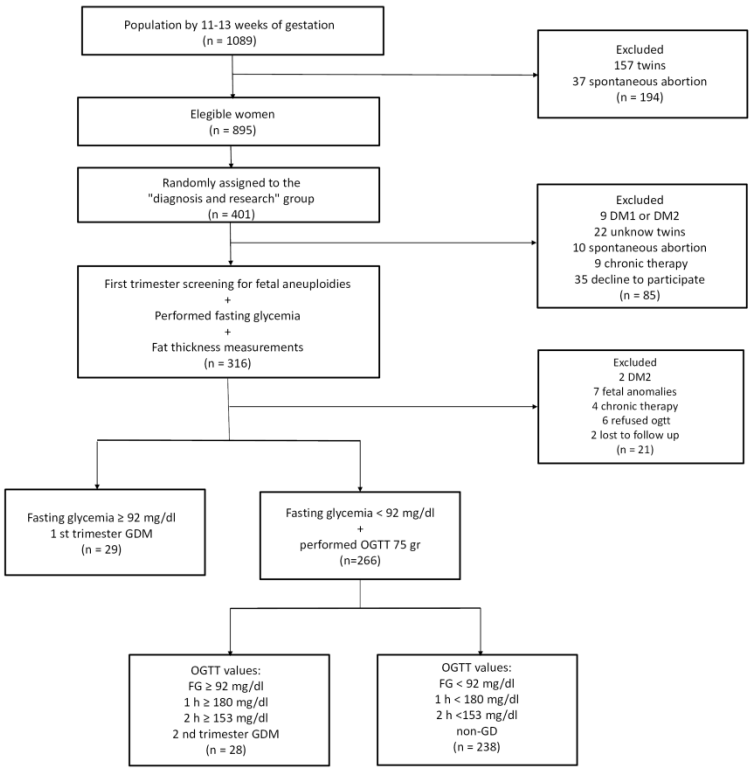
Variables	Gestational diabetes (GDM)		non-GDM (n=238)	p-value
	1st trimester (n=29)	2nd trimester (n=28)		
Maternal characteristics				
Age (years)	33.4 (4.3)	33.3 (4.1)	33.0 (4.3)	p=0.90
BMI at 12 weeks (Kg/m^2)	24.6 (4.8)	24.8 (4.8)	22.2 (3.9)	p<0.01
Gestational weight gain at 12 weeks (Kg)	2.0 (1.1)	1.6 (1.0)	1.7 (1.1)	p=0.25
Caucasian	29 (100.0)	26 (92.9)	234 (98.3)	p=0.56
Current Smoking	3 (10.3)	5 (17.9)	25 (10.5)	p=0.59
Multiparous	8 (27.6)	10 (35.7)	71 (29.8)	p=0.77
Family history of diabetes	1 (3.5)	2 (7.1)	10 (4.2)	p=0.75
In vitro fertilization	1 (3.5)	1 (3.6)	10 (4.2)	p=0.97
Previous gestational diabetes	0 (0.0)	1 (3.6)	1 (0.4)	p=0.14
Sonographic assessment (mm)				
Subcutaneous adipose thickness	12.8 (6.5)	11.1 (4.6)	9.8 (4.9)	p<0.01
Visceral adipose thickness	9.9 (4.4)	10.5 (5.3)	7.2 (3.5)	p<0.01
Crown rump length	61.3 (6.0)	61.1 (3.9)	61.4 (6.1)	p=0.96
Obstetric outcome				
Stillbirth	0 (0.0)	0 (0.0)	1 (0.4)	p=0.89
Cesarean	8 (27.6)	12 (42.9)	73 (30.7)	p=0.58
Vacuum Extraction (kiwi)	1 (3.5)	1 (3.6)	13 (5.5)	p=0.69
Birth weight (g)	3164.8 (419.0)	3344.1 (424.0)	3224.1 (453.3)	p=0.29
Birth weight percentile	51.8 (21.1)	60.6 (28.3)	52.6 (27.5)	p=0.32

[a]Categorical variables are expressed as absolute frequencies (n) and percentages (%); continuous variables are expressed as mean and standard deviation (SD). Chi-squared test was used for testing differences in proportions for categorical variables (e.g., the proportion of current smokers) among the three groups (non-GDM, 1st trimester GDM and 2nd trimester GDM). Likewise, ANOVA was used for testing differences in means for continuous variables (e.g., the mean of maternal age) among the three groups (non-GDM, 1st trimester GDM and 2nd trimester GDM).

Table 2. Odds ratio (OR) and corresponding 95% confidence interval (CI)[a] for gestational diabetes (GDM) according to selected variables.

Variables	Univariate analysis				Multivariate analysis[b]			
	Gestational diabetes (GDM)				Gestational diabetes (GDM)			
	1st trimester		2nd trimester		1st trimester		2nd trimester	
	OR (95%CI)	p-value	OR (95%CI)	p-value	OR (95%CI)	p-value	OR (95%CI)	p-value
Age (years)	1.02 (0.93 - 1.12)	p=0.68	1.01 (0.92 - 1.11)	p=0.78	1.01 (0.91 - 1.11)	p=0.91	0.99 (0.90 - 1.10)	p=0.89
BMI at 12 weeks	1.13 (1.04 - 1.22)	p<0.01	1.14 (1.05 - 1.23)	p<0.01	1.02 (0.89 - 1.17)	p=0.80	1.06 (0.92 - 1.21)	p=0.45
Gestational weight gain at 12 weeks (Kg)	1.29 (0.94 - 1.78)	p=0.11	0.94 (0.63 - 1.38)	p=0.74	1.26 (0.89 - 1.84)	p=0.19	0.82 (0.54 - 1.24)	p=0.34
Subcutaneous adipose tissue (mm)	1.10 (1.03 - 1.17)	p<0.01	1.05 (0.98 - 1.13)	p=0.17	1.04 (0.94 - 1.14)	p=0.49	0.95 (0.86 - 1.06)	p=0.36
Visceral adipose tissue (mm)	1.17 (1.08 - 1.28)	p<0.01	1.20 (1.10 - 1.31)	p<0.01	1.14 (1.01 - 1.28)	p=0.04	1.19 (1.05 - 1.35)	p=0.01
Multiparous	0.90 (0.38 - 2.12)	p=0.80	1.31 (0.58 - 2.97)	p=0.52	0.69 (0.26 - 1.86)	p=0.47	1.20 (0.47 - 3.05)	p=0.70
Family history of diabetes	0.84 (0.10 - 6.85)	p=0.87	1.75 (0.36 - 8.44)	p=0.48	0.50 (0.04 - 5.71)	p=0.58	0.92 (0.12 - 6.84)	p=0.93

[a]ORs and corresponding 95% CI were estimated by multinomial logistic regression models; non-GDM as reference group. [b]Mutually adjusted.



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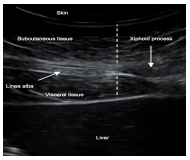

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