# Maternal subcutaneous and visceral adipose ultrasound thickness in women with gestational diabetes mellitus at 24-28 weeks' gestation

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**Short title:** Maternal adipose ultrasound thickness and gestational diabetes

**Abstract**

**Objective:** To compare the sonographic measurement of maternal subcutaneous and visceral adipose thickness between pregnant women with gestational diabetes mellitus (GDM) and patients with non-diabetic pregnancies.

**Methods:** Adipose thickness was measured by transabdominal ultrasound in pregnant women attending our antenatal clinics at 24-28 weeks' gestation. All patients underwent a 75g oral glucose challenge as a diagnostic test for GDM.

**Results**: The study population comprised 56 women with a positive glucose challenge test and 112 non-diabetic pregnancies. Measurements of subcutaneous and visceral adipose tissues were converted into multiples of the median (MoM), adjusted for gestational age. The mean subcutaneous thickness MoM in patients with GDM was significantly higher compared to non-diabetic pregnancies (1.31 vs 1.07; p=0.011). Similarly, the mean visceral thickness MoM was higher in women with a positive oral glucose tolerance test compared to controls (1.61 vs 1.06; p<0.001). Multivariate logistic regression analysis demonstrated that visceral adipose thickness, but not subcutaneous thickness, was significantly and independently associated with GDM (OR 34, 95%CI 9.5–122.2).

**Conclusions**: Sonographic thickness of maternal visceral adipose tissue at 24-28 weeks' gestation was higher in women with GDM compared to non-diabetic pregnancies, independently from other known risk factors associated with GDM.

**Keywords:** gestational diabetes; adipose tissue; ultrasound; glucose tolerance; pregnancy

## Introduction

In recent years, obesity has become a public health problem in the industrialized world due to its increasing prevalence [1] and the association with cardio-vascular and metabolic disorders [2]. Obesity in pregnancy is a well-known risk factor for several fetal and obstetric complications, such as miscarriage, stillbirth, preeclampsia, macrosomia and gestational diabetes mellitus (GDM) [3,4]. It has been demonstrated that a body mass index (BMI) > 35 kg/m$^2$ is associated with a five-fold increase in the risk of developing GDM [5].

The body fat mass is distributed into two compartments: subcutaneous adipose tissue, which accounts for 85% of the total, and visceral adipose tissue, that represents the remaining 15% [6]. It has been shown that an excessive accumulation of adipose tissue into the visceral compartment is associated with an increased risk for metabolic disorders [7-10].

Some studies have investigated the measurement of maternal adipose compartments by ultrasound in patients with GDM, showing that an increased thickness of visceral adipose tissue in the first trimester of pregnancy was associated with subsequent development of metabolic disorders [11-14].

The aim of this study was to examine the sonographic measurement of maternal subcutaneous and visceral adipose thickness in pregnant women with GDM and in non-diabetic patients.

## Methods

In this prospective case-control study we included women with a singleton pregnancy attending our Centre for routine antenatal visits at 24-28 weeks' gestation and patients attending the diabetic clinic following the diagnosis of GDM. Cases of known pre-pregnancy diabetes mellitus, GDM diagnosed in the first trimester (fasting plasma glucose

level ≥ 92 mg/dl) [15], chronic drug therapy, previous epigastric surgery, fetal chromosomal and/or major structural abnormalities were excluded. In all pregnancies, gestational age was calculated based on the ultrasound measurement of crown-rump length before 14 weeks' gestation [16].

In accordance with the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommendations [15], all women underwent a 75-g oral glucose tolerance test of for the diagnosis of GDM between 24 and 28 weeks' gestation. The diagnosis of GDM was made using the IADPSG criteria as follows: fasting glucose ≥ 92 mg/dl or glucose levels ≥ 180 mg/dl after 60 minutes and/or ≥ 153 mg/dl after 120 minutes from glucose administration. For each woman with a positive oral glucose tolerance test, two cases with a negative test were enrolled as controls.

In all cases, after informed consent, a transabdominal ultrasound (RAB 4-8 MHz probe, Voluson E8 Expert, GE Medical Systems, Milwaukee, WI, USA) examination was carried out to measure fetal biometry, assess amniotic fluid and Doppler flow velocity in the umbilical artery. Additionally, a mid-sagittal section of the upper maternal abdomen was obtained, ensuring that the minimum possible pressure was applied with the ultrasound transducer on the abdomen (Figure 1a) as previously described [17]. On each image, the following landmarks were identified: skin, subcutaneous tissue, linea alba, liver and xiphoid process. The subcutaneous adipose thickness was measured as the maximum vertical distance from the skin line to the anterior edge of the linea alba. The visceral adipose thickness was measured on the same image from the posterior edge of the linea alba to the anterior surface of the left lobe of the liver (Figure 1b).

The measurements were undertaken off-line, on stored ultrasound images, by an operator (F.D.) who was not aware of any clinical information, including the results from the glucose challenge test. In 90 randomly selected cases, the measurements of subcutaneous and

visceral adipose thickness were carried out twice by the same operator (F.D.) and in 44 cases independently by two operators (F.D., F.C.).

Information on obstetric and neonatal outcomes were retrieved from the hospital records and entered into a computer database for analysis. Large for gestational age neonates were defined based on a birthweight at or above the 90$^{th}$ percentile.

The study was approved by the Institutional Review Board of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy (reference n.2955).

*Statistical analysis*

The distribution of subcutaneous and visceral thickness was made Gaussian after logarithmic transformation. Multiple regression analysis was used to determine which factors, among maternal age, ethnicity, BMI, parity and gestational age, were significantly associated with subcutaneous and visceral thicknesses in non-diabetic pregnancies. In all regression models, stepwise forward algorithms were performed to select variables at a p-value cut-off of 0.05.

The subcutaneous and visceral thicknesses, expressed as multiples of the median (MoM) of the normal group, were compared between patients with GDM and non-diabetic pregnancies.

The Student T-test and Pearson $\chi$2-squared test were performed for univariate comparisons of continuous and categorical variables, respectively. The Bland-Altman analysis was used to compare the measurement agreement and bias for a single examiner and between two examiners [18].

Logistic regression analysis was used to determine the variables that were significantly associated GDM. Univariate analysis was carried out to examine the individual variables contributing to GDM by assessing their odds ratios and 95% confidence intervals. Subsequently, multivariate logistic regression analysis with backward stepwise elimination

was performed to determine which of these variables provide a significant independent contribution in the logistic model.

The data were analyzed using the statistical package IBM SPSS 22.0 (New York, USA) and Excel for Windows 2010 (Microsoft Corp., Redmond, WA, USA).

**Results**

After exclusion of 8 patients with a first trimester plasma glucose level of $\geq$ 92 mg/dl and 4 women with known pre-pregnancy diabetes, measurements of subcutaneous and visceral adipose tissue were carried out in 173 cases. Of these, 5 patients were lost to follow-up, leaving a population of 56 women with a positive glucose challenge test and 112 non-diabetic pregnancies for data analysis.

The mean maternal age was 34.5 (SD 5.1) years and mean gestational age at ultrasound was 27.3 (SD 1.3) weeks. Mean subcutaneous and visceral adipose thicknesses in non-diabetic women were 9.3 mm (SD 3.6) and 9.7 mm (SD 2.2), respectively. The respective values in patients with GDM were 10.7 mm (SD 4.8) and 10.1 mm (SD 3.0). Regression analysis showed a significant association of subcutaneous and visceral thicknesses with gestational age at measurement. The regression equations are reported below:

Log (expected) subcutaneous adipose thickness = 1.774311 - (0.031241* gestational age);

Log (expected) visceral adipose thickness = 1.518001 - (0.0215341* gestational age).

After adjustment for gestational age, the mean subcutaneous thickness MoM in patients with GDM was significantly higher compared to non-diabetic pregnancies (1.31 vs 1.07; p=0.011). Similarly, the mean visceral thickness MoM was higher in women with a positive oral glucose tolerance test compared to controls (1.61 vs 1.06; p<0.001).

The mean difference and the 95% limits of agreement between paired measurements of subcutaneous and visceral thicknesses by the same observer were 0.092 mm (-2.166 to 1.982) and 0.016 mm (-1.994 to 1.961), respectively. The values in paired measurements by two different observers were 0.328 mm (-3.218 to 3.874) and 0.237 mm (-1.970 to 2.444), respectively.

Comparisons in maternal demographic and obstetric characteristics between the two study groups are shown in Table 1. Univariate logistic regression analysis demonstrated that maternal age, family history of diabetes mellitus, BMI, sonographic subcutaneous and visceral adipose thicknesses were significantly associated with GDM. However, the contribution of subcutaneous thickness did not remain independently significant in the multivariate model (Table 2).

**Discussion**

The findings of this study show that sonographic thickness of maternal visceral adipose tissue at 24-28 weeks' gestation was higher in women with GDM compared to non-diabetic pregnancies, independently from other known risk factors associated with GDM.

Previous studies have assessed body fat distribution in the non-pregnant population using computed tomography and/or magnetic resonance imaging. Neeland *et al.* [9] examined 732 patients with obesity and found an independent association between type 2 diabetes and the visceral fat mass, but not with subcutaneous adiposity or BMI. Similarly, Bray *et al.* [10] examined 1106 subjects and reported that the visceral adipose mass was independently associated with diabetes mellitus type 2, with no significant relationship between subcutaneous adipose tissue and glucose intolerance. Therefore, there is evidence that the visceral adipose tissue may be involved in the regulation of glucose metabolism and that excessive visceral adiposity is associated with increased insulin resistance.

Some studies investigated the relationship between glycemic status in pregnancy and sonographic thickness of maternal adipose tissue, mostly based on small groups of cases evaluated during the first trimester of pregnancy [11-14]. Bartha *et al.* [11] examined 30 women at 11-14 weeks' gestation and found a significant association between sonographic measurement of visceral adipose tissue and glycemia, insulinemia, and insulin sensitivity. Martin *et al.* [12] reported that a visceral adipose thickness above the upper quartile of the normal range in the first trimester of pregnancy was significantly more frequent in 6 cases that subsequently developed GDM compared to 56 controls. In another recent study carried out at 4-14 weeks' gestation in 94 pregnant women, Gur *et al.* [13] showed that visceral adipose tissue, more than BMI, was associated with the risk of developing GDM, dyslipidemia, hypertension and insulin resistance. However, the study population comprised only patients with obesity, who are known to be at increased risk for such disorders, and included 16 cases with a diagnosis of GDM. To the best of our knowledge, this is the first study examining the differences in the sonographic measurement of maternal adipose compartments at the time of diagnosis of GDM, which is usually made at 24-28 weeks' gestation, showing that visceral adipose thickness is higher in GDM patients compared to non-diabetic pregnancies. The main limitation of our study is the case-control design and therefore, larger screening studies should be carried out to confirm our findings.

The lack of an independent association between subcutaneous adipose thickness and GDM in our study is consistent with previous findings in non-pregnant individuals in relation to type 2 diabetes [8,9]. In adults, most of the weight gain is determined by progressive accumulation of adipose tissue into the subcutaneous compartment, with consequent increase in the BMI. It has been previously shown that pregnant women with obesity have a significantly higher risk of developing GDM [4] and therefore, measurement of the BMI is the most practical and effective method of quantifying the subcutaneous

adipose mass. Traditional risk factors for the development of GDM are increased maternal age, family history of diabetes mellitus and high BMI, and our data are consistent with these findings (Table 1). The present study also showed that the association between visceral adipose thickness and GDM remained significant when other variables were taken into account in the multiple regression model (Table 2), suggesting that increased visceral adiposity may be an independent factor associated with GDM. We could not show any significant difference between the study groups in the rate of Asian ethnicity, previous pregnancy with GDM and previous macrosomic baby, which are also considered as risk factors for GDM, and the likely explanation is that the prevalence of these conditions in our population was very low in both groups (Table 1).

Recent studies have shown that more than 70% of pregnant women that will develop GDM can be identified at 11-13 weeks' gestation, by combining information from maternal history, biophysical and biochemical markers into specific screening algorithms [19-21]. Large prospective studies can evaluate whether the inclusion of the visceral adipose thickness, measured in the first trimester of pregnancy, may provide an additional contribution in early identification of women at risk of developing GDM. First trimester screening for GDM has the main advantage of selecting a high risk group of women who may benefit from early dietary intervention or preventive medications, once their efficacy has been demonstrated by randomized controlled trials, with the primary objective to reduce the prevalence of GDM and its related feto-maternal complications.

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**Table 1.** Maternal demographic and maternal characteristics of the study groups

| Characteristics | GDM (n=56) | Controls (n=112) | P-value |
|---|---|---|---|
| **Maternal** | | | |
| Age (years) | 36.8 (4.8) | 33.4 (4.8) | <0.001 |
| Body Mass Index (Kg/m$^2$) | 26.8 (6) | 24.7 (3.3) | 0.018 |
| Ethnicity | | | 0.366 |
| White | 53 (94.6) | 108 (96.4) | |
| Black | 1 (1.8) | 0 (0) | |
| Asian | 2 (3.6) | 4 (3.6) | |
| Family history of diabetes | 20 (35.7) | 13 (11.6) | <0.001 |
| Previous gestational diabetes | 4 (7.1) | 2 (1.8) | 0.096 |
| Previous newborn >4000 g | 2 (3.6) | 2 (1.8) | 0.407 |
| | | | |
| **Obstetric** | | | |
| Gestational age at delivery (weeks) | 39 (1.2) | 39.1 (1.4) | 0.131 |
| Birth weight (g) | 3307 (456) | 3264 (508) | 0.592 |
| Birth weight percentile | 50.4 (26.6) | 45.7 (27.3) | 0.288 |
| Large-for-gestational-age neonates | 8 (14.3) | 9 (8) | 0.160 |
| Stillbirth | 1 (1.8) | 0 (0) | 0.333 |

Data are expressed as n (%) or mean (SD).

**Table 2.** Logistic regression analysis to examine the relationship between gestational diabetes and maternal demographic and biophysical variables.

| Variable | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | **OR** | **95% CI** | **P-value** | **OR** | **95% CI** | **P-value** |
| Maternal age (years) | 1.163 | 1.077 – 1.256 | <0.001 | 1.164 | 1.053 – 1.286 | 0.003 |
| Family history of diabetes | 4.231 | 1.909 – 9.375 | <0.001 | 4.470 | 1.690 – 11.825 | 0.003 |
| Body Mass Index ($Kg/m^2$) | 1.115 | 1.028 – 1.210 | 0.009 | 1.172 | 1.062 – 1.295 | 0.002 |
| Subcutaneous adipose tissue (MoM) | 1.537 | 0.623– 3.789 | 0.0351 | - | - | - |
| Visceral adipose tissue (MoM) | 35.92 | 10.716– 120.41 | <0.001 | 34.047 | 9.489 – 122.166 | <0.001 |

OR = Odds Ratio; CI = Confidence Intervals

**Figure legend**

**Figure 1.** Images showing the correct placement of the ultrasound transducer on the maternal abdomen (a) and the measurement of subcutaneous and visceral adipose thickness (b).