Relation between abdominal subcutaneous fat tissue thickness and inflammatory markers during pregnancy

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Submitted: 4 January 2012
Accepted: 7 April 2012

Arch Med Sci 2014; 10, 4: 739–745
DOI: 10.5114/aoms.2014.44865
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Abstract

Introduction: Subcutaneous abdominal fat thickness (SCFT) is important for predisposition to metabolic and cardiovascular diseases. Our aim was to evaluate maternal SCFT and metabolic changes (such as insulin resistance and high inflammatory markers) during pregnancy.
Material and methods: A total of 92 pregnant women between 24–28 weeks of gestation were enrolled in the study. The SCFT was measured by ultrasonography and patients were divided into 2 groups according to thickness of maternal SCFT and body mass index (BMI). Groups were compared with each other for oral glucose loading test (OGL) results, and for haematological, biochemical and fetal biometric parameters.
Results: After analysis of frequency for SCFT, the most appropriate cut-off value for grouping patients was found to be 15 mm for SCFT. In 48 cases SCFT was over 15 mm. High C reactive protein (CRP) was found in 47.9% (23) of cases with SCFT over 15 mm. Serum haemoglobin A_{1c} (HbA_{1c}) level was significantly correlated with SCFT thickness. The most important factors for determination of OGL level were found to be serum HbA_{1c} level, BMI and SCFT. In obese subjects (BMI ≥ 25 kg/m^2), levels of inflammatory markers and SCFT thickness were higher. The CRP and γ-glutamyltransferase (GGT) levels were significantly correlated with BMI and SCFT.
Conclusions: High SCFT during pregnancy is associated with elevated inflammatory marker levels and HbA_{1c}. Pregnant women with thicker SCFT may be susceptible to the development of metabolic complications of pregnancy, such as gestational diabetes mellitus (GDM) and hypertension, as well as risk of future metabolic and cardiovascular disease.

Key words: pregnancy, adipose tissue, inflammation, subcutaneous fat tissue.

Introduction

Obesity plays a major role in the pathogenesis of several medical problems including metabolic and cardiovascular disease [1]. Although the body mass index (BMI) is a useful indicator of overall adiposity [2], it is still unclear whether the visceral or the subcutaneous part of abdominal fat is more deleterious for predisposition to metabolic and cardiovascular disease. The subcutaneous fat (SCFT) is different from the visceral adipose tissue (VAT) in the production of adipocytokines [3–5]. The SCFT releases 2–3 times more leptin than VAT [6]. There are some studies reporting that subcutaneous fat is associated with insulin resistance [7, 8]

although the role of SCFT in relation to insulin resistance is not well recognized [9].

There is no study in the literature about maternal subcutaneous fat thickness and metabolic changes (such as insulin resistance and high inflammatory markers) during the 2nd trimester of pregnancy. There is a marked increase in plasma levels of cholesterol and triacylglycerol during pregnancy, but it is unknown whether maternal subcutaneous fat is associated with these changes or whether some metabolic complications related to subcutaneous fat thickness are similar in pregnant and non-pregnant women. There might be an association between SCFT thickness and some pregnancy-related complications. Subcutaneous fat measurements over a cut-off point might help us to identify risk groups in which early and accurate detection of gestational metabolic disorders (gestational diabetes or hypertension) is essential.

Measurement of abdominal subcutaneous fat by ultrasound is an easy and non-invasive method. It also avoids the use of ionizing radiation. In this study we measured SCFT thickness in 24–28 week pregnant women and compared the results with oral glucose loading test results as well as haematological, biochemical and fetal biometric parameters to determine whether they might be early markers of gestational metabolic disorders.

Material and methods

This prospective cohort study was carried out at the Fatih University Faculty of Medicine, Obstetrics and Gynaecology Department, Ankara. Inclusion criteria were: maternal age between 20–35 years, singleton pregnancy with a live fetus, gestational age 24–28 weeks, and healthy women without any medical disorders. The exclusion criteria were: smokers or drug users in the index pregnancy, and women with a previous history of gestational diabetes mellitus (GDM) or any other systemic disease. Written informed consent was obtained from all subjects. Approval for this study was obtained from the Local Institutional Review Board of the Faculty of Medicine, Fatih University.

Body mass index was calculated as weight (kg)/height (m^2) in all cases. All measurements were obtained in the morning, after overnight fasting. Since our study population consisted of pregnant women, overnight fasting for 10 h was recommended before blood testing. Patients were advised not to eat anything after 10.00 pm and come for testing at or after 8.00 am. The study was performed using an Aloka Prosound SSD-3500SX (Aloka Holding Europe AG, Switzerland) 3.5 MHz transabdominal probe. All ultrasound examinations were performed by the same operator. The gestational age was calculated by the modified Naegele's rule. Last menstrual period-derived gestational age was compared with ultrasound-derived gestational age using fetal biometric parameters. Subcutaneous fat depth was measured from the subcutaneous fat layer to the outer border of the rectus abdominis muscle at the level of the linea alba [10]. After analysis of frequency for SCFT, the most appropriate cut-off value for grouping patients was found to be 15 mm for SCFT. This cut-off point was also very near to the median value of SCFT thickness. Patients were divided into 2 groups according to this value and compared with each other.

Venous blood samples were collected in the morning, after overnight fasting, from the brachial vein of the patients. Blood samples were immediately centrifuged, and plasma and serum samples were kept at –70°C until laboratory testing. Glucose was measured with a standard hexokinase reference method (Cobas Integra 800 analyser, Roche Diagnostics, Basel, Switzerland). An abnormal 50 g oral glucose loading test (OGL) was defined at a conventional cut-off point of ≥ 130 mg/dl and a 100 g, 3 h oral glucose tolerance test was applied to the patients with an OGL result over the cut-off point [11]. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), very-low-density lipoprotein cholesterol (VLDL), blood urea nitrogen (BUN), creatinine, γ-glutamyltransferase (GGT) and triglycerides (TG) were measured with enzymatic colour tests (Cobas Integra 800 analyser, Roche Diagnostics, Basel, Switzerland). Low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald formula [12]. Haemoglobin A_{1c} (HbA_{1c}) was determined by means of high performance liquid chromatography (Shimadzu, Kyoto, Japan) [13]. C-reactive protein (CRP) levels were measured by nephelometry (Beckman coulter Image, US) [14].

A power analysis was conducted before recruitment. Using an α level of 0.05, β level of 0.20, a power of 80% and effect size of 0.60, a total sample size of 92 cases was needed for the study.

Statistical analysis

Statistical analyses were conducted using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics are presented as mean values ± SD and median (interquartile range; IQR). The χ^2 test was used for comparison of categorical data. Independent samples *t* test was used for comparison of groups which were fit to a normal distribution. For groups that were not distributed normally, Mann-Whitney test was used for comparison. Spearman correlation coefficients were used to estimate the correlations between biochemical and obesity-related parameters and clinical characteristics. Backward multiple linear

regression analysis was performed to investigate the relationship between biochemical parameters as a dependent variable and obesity-related parameters and lipid profile as independent variables. Value of $p < 0.05$ was considered to be statistically significant.

Results

A total of 92 pregnant women were enrolled in the study. Patients were divided into 2 groups according to thickness of maternal SCFT. The SCFT was over the cut-off point (over 15 mm) in 52.2% (48) of cases. Women with SCFT > 15 mm had higher BMI than women with lower SCFT (27.34 kg/m^2 vs. 23.88 kg/m^2, $p < 0.001$). Comparison of SCFT subgroups revealed a significant difference in terms of OGL and GGT levels, which were higher in patients with high SCFT ($p = 0.003$, $p = 0.018$). Comparison for other parameters revealed no significant difference. The distribution of groups and demographic data of cases are shown in Table I.

The BMI was over 25 kg/m^2 in 66.3% (61) of all cases. The SCFT was significantly higher in women with high BMI (over 25 kg/m^2) ($p < 0.001$). The SCFT was found to be > 15 mm in 72.4% (44) of women with high BMI. The OGL was found to be significantly higher in women with BMI > 25 kg/m^2 ($p = 0.006$). Similarly, estimated fetal weight (EFW) was significantly higher in women with high BMI ($p = 0.012$) (Table II).

Table I. Comparison of patients according to SCFT thickness

Variables	All cases ($n = 92$)	< 15 mm ($n = 44$)	≥ 15 mm ($n = 48$)	Value of p
Age [years]	28 (7)	27 (5.5)	28.5 (7.75)	0.936
BMI [kg/m^2]	26.35 (3.67)	23.88 (3.12)	27.34 (4.15)	< 0.001
Gravidity, *n*	2 (1)	2 (1)	2 (1)	0.768
Parity, *n*	1 (1)	1 (1)	0 (1)	0.259
Gestational age [week]	25.25 (2.13)	25.35 (2.73)	25.15 (1.45)	0.564
BPD [mm]	64 (6.25)	64.15 (9.25)	63.5 (5.8)	0.841
FL [mm]	46 (3.25)	46 (5.5)	46 (2.25)	0.662
AC [mm]	208 (23.5)	209.5 (25.75)	205 (23.5)	0.760
EFW [g]	805 (209.5)	814 (224.75)	776 (232.5)	0.944
SCFT [mm]	15.25 (8.08)	11.2 (3)	18.95 (5.18)	< 0.001
Log CRP [mg/l]	0.71 ±0.28	0.61 ±0.17	0.80 ±0.33	0.002
Log HbA_{1c} [%]	0.66 ±0.04	0.64 ±0.04	0.67 ±0.04	0.001
FBS [mg/dl]	91 (18.50)	95 (14.75)	99 (14)	0.435
Urea [mg/dl]	15.6 (4.50)	15.35 (4.65)	15.71 (4.45)	0.799
Creatinine [mg/dl]	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)	0.883
TC [mg/dl]	238.66 ±46.65	240.50 ±51.08	238.23 ±43.28	0.572
TG [mg/dl]	191.11 ± 74.88	186.95 ±63.34	199.29 ±88.96	0.410
HDL-C [mg/dl]	70.74 ±17.00	68.35 ±14.90	73.29 ±19.74	0.647
LDL-C [mg/dl]	129.55 ±40.46	134.55 ±45.12	125 ±35.81	0.427
VLDL [mg/dl]	37.79 ±15.03	36.90 (12.76)	39.47 ±17.82	0.397
AST [U/l]	14 (4.25)	14.5 (4)	14 (4.5)	0.674
ALT [U/l]	12 (7.25)	13 (6.75)	12 (6.5)	0.513
GGT [U/l]	9.57 ±3.79	8.54 ±2.35	10.82 ±4.86	0.018
OGL [mg/dl]	109 (36.5)	99 (25.5)	116.5 (37.5)	0.003

BMI – body mass index, BPD – biparietal diameter, FL – femur length, AC – abdominal circumference, EFW – estimated fetal weight, SCFT – subcutaneous fat tissue, CRP – C-reactive protein, HbA_{1c} – haemoglobin A_{1c}, FBS – fasting blood sugar, TC – total cholesterol, TG – triglyceride, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, VLDL – very low-density lipoprotein cholesterol, OGL – oral glucose loading test, p < 0.05 significant

Table II. Relation between BMI and maternal SCFT, log CRP and log HbA_{1c}

Variables	BMI [kg/m^2]		Value of *p*
	< 25 (*n* = 31)	≥ 25 (*n* = 61)	
SCFT [cm]	11.55 (4.75)	16.60 (6.20)	< 0.001
Log CRP [mg/l]	0.63 ±0.2	0.75 ±0.3	0.059
Log HbA_{1c} [%]	0.65 ±0.03	0.66 ±0.05	0.252
OGL	100 (26.25)	116 (41.75)	0.006
EFW	765.5 (129)	855.5 (256.75)	0.012

BMI – body mass index, SCFT – subcutaneous fat tissue, CRP – C-reactive protein, HbA_{1c} – haemoglobin A_{1c}, OGL – oral glucose loading test, EFW – estimated fetal weight, p < 0.05 significant

High CRP was found in 26.1% (24/92) of all cases, 32.8% (20/61) of women with high BMI (over 25 kg/m^2) and 47.9% (23/48) of women with high SCFT. The HbA_{1c} levels were within the normal range in all cases. To approximate a normal distribution, log transformed data of HbA_{1c} and CRP were used in the analysis for CRP and HbA_{1c} parameters. Log CRP and log HbA_{1c} were significantly higher in women with high SCFT (p = 0.002 and p = 0.001) (Table I). A borderline significant difference was observed between high BMI women versus low BMI in terms of log CRP (p = 0.059), although no significance was detected in terms of log HbA_{1c} levels (p = 0.252).

Correlation analysis demonstrated a positive correlation between SCFT versus log HbA_{1c}, BMI and GGT. A significant correlation was observed between HbA_{1c} vs. GGT and OGL. There were correlations of EFW with HDL, VLDL, TG, GGT and BMI (Table III).

Regression analysis showed that serum CRP levels were significantly associated with GGT and BMI (p = 0.002 and p = 0.037). There was a borderline association between CRP and SCFT (p = 0.063). Serum HbA_{1c} levels were significantly associated with SCFT thickness, TC, HDL, LDL and TG levels (p < 0.001, p = 0.033, p = 0.020, p = 0.037 and p = 0.019). The most important factors for determination of OGL level were HbA_{1c}, BMI, SCFT, HDL and VLDL (p = 0.001, p = 0.001, p = 0.020, p = 0.026 and p = 0.004). The CRP had borderline significance for prediction of serum OGL results (p = 0.060).

Discussion

Many researchers have demonstrated that obesity is a state of chronic low-grade inflammation [15–18]. Inflammation itself may be the cause of obesity-related disorders. Inflammatory cytokines such as CRP are associated with obesity and in turn with increased risk for insulin resistance, diabetes mellitus, hypertension and dyslipidaemia [19–24].

Both VAT and SCFT are more strongly associated with high CRP in women than in men [25]. For VAT, multiple prior studies have demonstrated a relation between pre-diabetic hyperglycaemia and diabetes [26–29]. However, for SCFT few studies have yielded significant relations with glycaemic disorders. It was demonstrated that both SCFT and VAT were important correlates of insulin resistance and cardiometabolic risk factors, and that SCFT was associated with peripheral insulin action at least as strongly as intra-abdominal fat [9, 30–32]. The SCFT was positively correlated with age and all risk factors for metabolic syndrome. In addition to the presence of insulin resistance, leptin levels have been shown to be correlated with SCFT [33]. Leptin is associated with vascular dysfunction [34–37], which may be a mechanism for development of metabolic diseases.

The most important point in our study was that the study population consisted of pregnant women. Similarly to the non-pregnant population, we detected significantly higher CRP and HbA_{1c} levels in pregnant women with high SCFT. The CRP was significantly higher in pregnant women with a BMI over 25 kg/m^2 than in women with a normal BMI. We believe that the presence of SCFT ≥ 15 mm is a very strong predictor of high CRP and HbA_{1c} levels in pregnant women. In particular, higher values of SCFT during 24–28 weeks of gestation may be associated with pregnancy-related complications that could be observed during later periods of gestation, and measurement of SCFT might help us to identify risk groups.

The CRP level is associated with BMI and waist circumference, which is a crude index of visceral obesity [15, 38]. However, correct measurement of waist circumference may be difficult during pregnancy. Also, correct calculation of BMI might be difficult due to weight gain and fluid retention. But SCFT can easily be measured in all pregnant women and this could correctly predict obesity and related complications during pregnancy.

Another important result of this study was the significantly higher GGT levels in women with SCFT over 15 mm. Serum GGT level is a possible marker of oxidative stress [39, 40]. It is associat-

ed with development of metabolic syndrome and glucose intolerance [41–43]. It was suggested in previous studies that GGT is associated with VAT, but not with SCFT [44, 45]. In this study we detected high serum GGT in patients with high SCFT thickness and a positive correlation was found between serum GGT and CRP levels.

Our findings underscore the positive association of SCFT with circulating markers of inflammation such as CRP and GGT. Thus, an increase in SCFT relative to overall body weight is associated with significantly elevated CRP concentrations, and this relation is also observed in pregnant women. Our results were similar to the results of the Framingham Heart Study [46]. They suggest that both abdominal SCFT and VAT have a role in inflammation and oxidative stress.

Another important point of this study was the detection of significantly higher OGL results in pregnant women with thicker SCFT. Women with high SCFT might be watched closely for the development of GDM during their pregnancy. We also observed significantly higher EFW in patients with high SCFT. In addition, there was a correlation between EFW versus lipid profile and BMI. These results may caution us about the prediction of macrosomia in patients with high SCFT and might help us in the prevention of macrosomia and related complications by regulation of lipid profile and dietary management.

A number of limitations of this study deserve comment. First, although the sample size is adequate, study groups were modest in size, so it is possible that the study was underpowered to detect differences between the groups. Secondly, we used only CRP and GGT as inflammatory and oxidative stress markers for prediction of metabolic disorders. However, other risk factors that could modify the condition must also be assessed separately. New studies with larger series including other inflammatory markers would increase the reliability and power of the study. And thirdly, perinatal results of cases were not evaluated. Long-term follow-up of cases and evaluation of perinatal results would be very important to correctly stratify the future risk. Addition of women with gestational diabetes to the study as another group would be more enlightening for the results of the study. However, we detected only one patient with GDM in our study. Therefore we only reported results concerning OGL.

In conclusion, CRP and GGT were significantly correlated with BMI and SCFT in pregnant women. These findings suggest that reducing obesity may prevent the elevation of cytokine levels, and as a result reduce the risk of metabolic complications during pregnancy, as well as the risk of future metabolic and cardiovascular disease.

Table III. Correlations between SCFT, OGL, BMI versus EFW and biochemical markers

		SCFT	OGL	BMI	TC	HDL	LDL	VLDL	TG	GGT	Log_CRP	Log_HbA_{1c}	CRP	HbA_{1c}	EFW
SCFT	**Rho**		0.198	**0.502***	–0.155	0.057	–0.155	0.056	0.051	**0.262***	0.213	**0.412***	0.213	**0.413***	–0.096
	p		0.067	**< 0.001**	0.177	0.618	0.182	0.626	0.658	**0.021**	0.058	**< 0.001**	0.058	**< 0.001**	0.376
OGL	**Rho**	0.198		**0.294***	0.022	0.105	–0.089	**0.266***	**0.262***	0.101	**0.338***	**0.229***	**0.338***	**0.230***	0.045
	p	0.067		**0.005**	0.850	0.362	0.445	**0.019**	**0.021**	0.379	**0.003**	**0.049**	**0.003**	**0.049**	0.684
BMI	**Rho**	**0.502***	**0.294***		–0.149	–0.165	–0.018	0.031	0.026	0.138	**0.235***	0.131	**0.235***	0.131	**0.228***
	p	**< 0.001**	**0.005**		0.187	0.143	0.876	0.788	0.818	0.223	**0.034**	0.255	**0.034**	0.254	**0.032**

**Correlation is significant at the 0.05 level*

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