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Difference in breast size between the first and third trimesters of pregnancy and changes in a woman's body size, subcutaneous fat tissue, and biochemical parameters

Janina Tutkuviene[1]✉, Simona Gervickaite[1], Jonas Drasutis[1], Indre Sakalauskaite[1], Renata Simkunaite-Rizgeliene[1], Valerija Jablonskiene[2], Arunas Barkus[1], Egle Marija Jakimaviciene[1], Dovile Karciauskaite[2], Arvydas Kaminskas[2], Vytautas Tutkus[1], Jonas Tutkus[1], Laura Nedzinskiene[1], Ramune Cepuliene[1], Jonas Algis Abaravicius[2] & Diana Ramasauskaite[3]

The greatest changes in the breasts occur during pregnancy, but during this time the entire woman's body also undergoes specific modification, however, only a few articles discuss breast changes during pregnancy. We aimed to analyse the variation of breast volume in relation to body size, subcutaneous fat tissue and biochemical parameters during pregnancy. The same female (N = 83) was studied twice – in the first and third trimester of pregnancy. Breast volume was calculated according to the formula of Kramer&Drexler (1981). Breast volumes varied greatly both at the ca. 10th week (544–3500 cm³) and at ca. 35th week (689–3730 cm³) of pregnancy. Only 35–67% (depending on the trimester) of women's breast volume categories (small, medium, large) matched their body fat categories (lean, average, obese). Breast size was mainly related to skinfolds located on the trunk and upper limbs. The largest changes in body parameters occurred in the group of small-breasted and thin women. In addition, the greatest increase in high-density cholesterol during pregnancy was seen in women with medium to large breasts (which may represent a positive effect of pregnancy), but higher levels of low-density lipoprotein cholesterol in early pregnancy were associated with a higher likelihood of larger breasts.

Keywords Breast volume, Pregnancy, Body size, Subcutaneous fat tissue, Biochemical parameters

Several hypotheses have been proposed to explain the evolutionary emergence of permanent breast morphology in women, including those related to sexual selection and its potential role as a cue of maturity, health, and reproductive capability[1]. It has also undergone shaping by natural selection as a nursing and thermoregulatory organ, as well as a byproduct of other evolutionary changes, such as an increase in subcutaneous fat tissue and hormonal changes[2]. In our days, breast size plays an important role while rating female's sexual attractiveness[1,3–7]. According to the popular press, female's breast size has increased during the last decades, however, this assertion is based mostly on data from bra sales[8,9].

Unfortunately, there is a lack of studies on normal variation of human breast morphology in relation to other body parameters and general health status[10–12]. Moreover, there is no unique methodology for the assessment of breast size, shape, position, volume or weight: different techniques, various parameters and formulas have been used, therefore it is difficult to compare few existing studies on breast size[13–29].

[1]Department of Anatomy, Histology and Anthropology, Faculty of Medicine, Vilnius University, M.K. Ciurlionio Str. 21/27, Vilnius LT-03101, Lithuania. [2]Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Faculty of Medicine, Vilnius University, M.K. Ciurlionio Str. 21/27, Vilnius LT-03101, Lithuania. [3]Clinics of Obstetrics and Gynaecology, Faculty of Medicine, Vilnius University, Santariskiu Str. 2, Vilnius LT-08406, Lithuania. ✉email: janina.tutkuviene@mf.vu.lt

The majority of studies on female's breast size have been published for plastic surgeons, and focus on defining an aesthetically pleasing template for breast shape and proportions[30–37]. However, beyond considerations of attractiveness, the correlation between breasts and other body composition parameters, also relationship between breast size, body fat, biochemical markers and overall health status – that is far more significant from the perspective of life expectancy and quality of life[38–47].

Few recent studies showed that women with different breast size have demonstrated diversity in BMI and other anthropometric indices[12,48–50]. Moreover, female's breast size and different parameters vary substantially between populations and within the same population[51], anatomic variety may cover breast volume and shape – width, length, protrusion and position on the chest wall[19,29]. However, it is still not clear how breast size is related to body size, shape and general health status of a female, especially during pregnancy[52–58].

It is well known, that large breast size is related to breast pain[59], postural disorders[60,61], thoracic, spine, shoulder, back and neck pain, headache, intertrigo, shoulder grooving[62–64], self-image dissatisfaction and social distress[63,65]. Moreover, recently, breast size, as well as higher body weight or BMI, was related to increased risk for breast cancer[40,42,66,67] and type 2 diabetes mellitus[39]. Additional evidence suggests that larger breast volume by itself may contribute to an increased risk of breast cancer, even after adjusting for BMI and other variables[40], but cancer risk is particularly increased when combined with a higher BMI[67]. However, data on breast size, especially, in relation to female's health are sparse and varies substantially within and between populations[55,68–71].

Moreover, no other organ undergoes such dramatic changes during its development (which includes also the pregnancy period) as does the breast. Majority of studies on breast development are performed on animal (mice, ewe and others) mammary gland[54,56,72–74]. It is well known that development of mammary gland starts at the embryonic period, undergoes spurt during puberty, but reaches maximal development during pregnancy[75]. During the first trimester of pregnancy, cells of the primitive buds in pregnant females' mammary gland begin to increase greatly in number due to the active cell division and enlargement of cytoplasm. Two main epithelial lineages (myoepithelial cells and luminal epithelial cells) of the mammary ducts originate from the mammary epithelial stem cells[76]– the mammary ducts and alveoli, the milk-producing units are generated. The number of well-formed lobules gradually increases and exceeds the amount of the intralobular and interlobular stroma.

The studies on the ratio and interaction between stroma and parenchyma show that the latter relationship is a dynamic process. During the pregnancy, these internal changes lead to variation in breast size, shape and volume, and breast enlargement is one of the first visible signs at the beginning of pregnancy. Several studies have examined breast size in women during pregnancy, focusing on different variables: changes in maternal breast size during pregnancy related to the health status and biological sex of the offspring, as well as lactation capacity[24,77–80]. However, we did not find any study that compared changes in breast volume during pregnancy with changes in BMI, subcutaneous fat tissue topography, and biochemical parameters. Most volumetric assessment techniques demonstrated limited practicability and were not used for pregnant women.

The majority of studies on body size and shape during the pregnancy are limited to body weight and BMI changes in relation to pregnancy outcomes, risk for obesity, diabetes and other health disorders[81,82]. To our knowledge, changes of breast volume in relation to other body parameters during the pregnancy, multiple correlations between the breast volume, body size indices (body height, BMI, skinfolds, body composition) and biochemical parameters were not studied. Besides, it is not known, if changes in breast size during the pregnancy might be related to health status and may serve as a marker for health risk.

The aim of this study was to analyse the variation of breast size according to body size, subcutaneous adipose tissue and basic biochemical parameters in women during pregnancy. To achieve this goal, the following objectives were set: (1) To evaluate changes in breast volume, weight, BMI, subcutaneous fat tissue and blood serum biochemical parameters during pregnancy in females with different breast size and subcutaneous adipose tissue; (2) To determine the coincidence of women's breast size group and subcutaneous body fat tissue group during pregnancy; (3) To identify multiple relationships between breast volume, anthropometric and blood serum biochemical parameters during pregnancy in women with different breast sizes.

Materials and methods

The study was carried out in the maternity hospital of the city of Vilnius. A total of 105 healthy pregnant women were studied, but only 83 women were finally included in this prospective study after adjusting the inclusion criteria: only women without chronic diseases and pregnancy-related pathologies, aged 18–40 y., without implants, and with uniparous pregnancies were included in the study. Of these, 41 were primiparous and 42 were multiparous. Each woman was examined twice – at the first (M ± SD = 10.1 ± 2.1 week; min-max = 4–13 weeks) and at the third trimester (M ± SD = 35.0 ± 1.7 week; min-max = 29–39 weeks) of pregnancy. The average difference between measurements was 24.9 ± 2.7 weeks (min-max = 18–32 weeks). All examinations of the females were performed in the morning (8:00 a.m. to 12:00 p.m.)

For each pregnant woman only safe and non-invasive anthropometric methods were used. All measurements were performed by the same investigator (trained and checked for inter- and intra-observer error) and according to standard anthropometric methods[83,84]. Standardized anthropometric devices (Siber Hebner, Switzerland) were used: anthropometer, sliding calliper, spreading calliper, Holtain skinfold calliper, Gamma electronic weighing scale (SOEHNLE, Germany; step – 0.05 kg).

Height, weight, skinfolds (submental, pectoral I and pectoral II, axillary, subscapular, abdominal, suprailiac, tricipital and bicipital, forearm, femoral, calf) were measured. In addition, **body fat percentage (BF%)** was calculated according to W.E. Siri's (1961) formula[85]: BF% = [(4,95/BD) – 4,50] x 100. Body density (BD) was calculated applying J.H. Wilmore & A.R. Behnke (1970) formula[86]: BD = 1,06234–0,00068(X1) – 0,00039(X2) – 0,00025(X3), X1 – subscapular skinfold, X2 – triceps, X3 – femoral skinfold.

With respect to subcutaneous fat tissue (BF%), all pregnant females were divided into three groups: lean (subcutaneous fat tissue percentage was less than 25th percentile), average body build (percentage ranged from 25th to 75th percentile) and obese females (percentage was higher than 75th percentile).

Moreover, breast height (protrusion), vertical and horizontal diameters of both breasts were taken (Fig. 1). Breast volume was calculated using the following formula by R. Kramer & G. Drexler, 1981[87]:

Breast volume = 1/2 × 4/3 x Π x 1/4 x TD X VD X H

Π – pi number (3.14).

TD – transverse diameter of the breast base.

VD – vertical diameter of the breast base.

H – height (protrusion) of the breast contour.

With respect to the breast volume (cm³), all women were stratified into three groups taking the mean volume of both breasts: (1) Small breasts (breast volume – less than 25th percentile); (2) Average breasts (breast volume ranged from 25th to 75th percentile); (3) Large breasts (breast volume was higher than 75th percentile). There were no significant differences in time of measurements between the breast size groups ($p > 0.05$): the first measurement was performed on average at 10.3 (small breasts), 9.9 (medium breasts), and 10.1 (large breasts) weeks of pregnancy; the second – at 34.8 (small breasts), 35 (medium breasts), 35 (large breasts) weeks. The average interval between measurements was 25.5 (small breasts), 24.9 (medium breasts), and 25.5 (large breasts) weeks ($p > 0.05$).

Blood samples were taken under fasting conditions in the morning (from 8:00 a.m. to 12:00 p.m.) and were analyzed in the Centre of Laboratory Medicine of Vilnius University Hospital Santaros Klinikos. Specifically, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and prolactin levels in blood serum were determined using routine methods (Architect, Abbott, USA).

For each parameter mean, standard deviation, minimal and maximal values during the pregnancy were calculated. Symmetry, skewness and kurtosis were checked for the estimation of data distribution. Significant deviations from normal distribution were not found. Student's t-test or Chi-square tests were used to detect statistical significance of differences between the data of analysed sub-groups. Pearson's correlations were calculated and univariate logistic regression analysis (OR) as well as factor analysis (using principal components) were performed in order to estimate complex relationships between breast volume, subcutaneous fat and biochemical parameters. When analysing the odds ratio of breast volume with regard to body composition and biochemical parameters, all variables were divided into two groups according to the mean (mean and below being the reference group). Statistical analysis was performed using MS Excel and IBM SPSS Statistics 28.0.

Present study was approved by Lithuanian Bioethical Committee (2014-11-05; No. 6B-14–337), and was performed in accordance with the Declaration of Helsinki. All pregnant women gave informed consent to be included in the study.

Results

Changes in breast volume, body size, subcutaneous fat tissue and blood serum biochemical parameters during pregnancy

Body height was measured only once – at the beginning of pregnancy, because it was believed that the height of female should not change significantly during pregnancy. Meanwhile, women's body weight and BMI increased by 20.8%, and breast volume enlarged by 27.2% during the pregnancy ($p < 0.001$) (Table 1**)**. There was a particularly wide variation in breast volume of pregnant females – both at the beginning (from 543.6 cm³ up To 3500.4 cm³; mean [± SD] = 1165.9 [± 402.7] cm³) and the end (from 688.8 cm³ To 3729.9 cm³; mean [± SD] = 1482.9 [± 440.5] cm³) of pregnancy.

The latter increases coincided with the proliferation of subcutaneous fat tissue: almost all skinfolds increased statistically significantly during the study period (except for both pectoral, axillary and tricipital) (Table 1**)**. The relatively highest increase was detected for subscapular, bicipital and suprailiac skinfolds (14.7–16.5%).

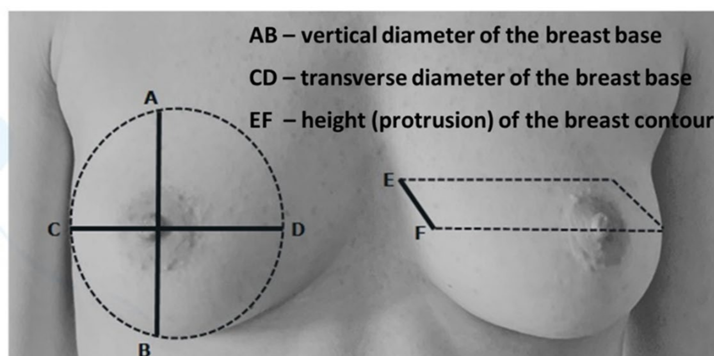

Fig. 1. Breast parameters that were used to calculate breast volume.

Parameter	First trimester (M = 10.1 weeks; SD = 2.1)			Third trimester (M = 35.0 weeks; SD = 1.7)			Relative change (%)	*p*
	N	M	SD	*N*	M	SD		
Breast volume (cm^3)	**83**	**1165.9**	**402.7**	**83**	**1482.9**	**440.5**	**27.2**	**< 0.001**
Height (cm)	83	167.5	5.7	-	-	-	-	-
Weight (kg)	83	64.9	9.9	83	78.4	10.2	20.8	**< 0.001**
BMI (kg/m^2)	83	23.1	3.5	83	27.9	3.6	20.8	**< 0.001**
Skinfolds (mm)								
Submental	83	11.8	4.2	83	13.2	4.6	11.7	**< 0.01**
Pectoral I	83	18.1	5.8	83	18.3	6.6	1.4	> 0.05
Pectoral II	83	16.2	6.0	83	17.2	6.2	6.5	> 0.05
Axillary	83	10.5	5.0	83	10.9	5.2	3.1	> 0.05
Abdominal	83	20.9	5.5	83	22.3	6.2	6.4	**< 0.05**
Suprailiac	83	23.6	6.8	83	27.1	6.5	14.7	**< 0.001**
Subscapular	83	19.4	7.0	83	22.6	7.3	16.5	**< 0.001**
Tricipital	83	21.9	6.5	83	22.8	6.8	4.1	> 0.05
Bicipital	83	12.6	5.4	83	14.5	5.8	15.0	**< 0.001**
Forearm	83	11.6	4.4	83	12.7	5.1	9.5	**< 0.01**
Femoral	83	25.3	5.8	83	28.2	5.4	11.6	**< 0.001**
Calf	83	24.0	5.8	83	25.7	6.5	6.9	**< 0.01**
Subcutaneus fat (%)	83	28.6	3.6	83	30.1	3.7	5.3	**< 0.001**
Biochemical parameters								
Prolactine (ng/ml)	83	41.0	22.6	83	389.6	219.3	849.4	**< 0.001**
Total cholesterol (mmol/l)	83	4.7	0.8	83	6.5	1.6	38.8	**< 0.001**
HDL cholesterol[A] (mmol/l)	83	1.8	0.3	83	2.1	0.5	15.6	**< 0.001**
LDL cholesterol[B] (mmol/l)	83	2.5	0.7	83	3.7	1.4	48.1	**< 0.001**
Triglycerides (mmol/l)	83	1.0	0.4	83	2.3	0.9	126.6	**< 0.001**

Table 1. Women's breast volume, body size, subcutaneous fat tissue and blood serum biochemical parameters during pregnancy. [A]HDL cholesterol – high-density lipoprotein cholesterol. [B]LDL cholesterol – low-density lipoprotein cholesterol.

Meanwhile, femoral, calf and tricipital skinfolds – traditionally attributed to gynoid body shape and related to the action of females' hormones – had increased relatively less (4.1–11.6%), however, among these folds, the femoral skin fold increased the most. In general, subcutaneous fat tissue had increased significantly ($p < 0.001$) during the pregnancy (Table 1).

Regarding biochemical parameters, a statistically significant ($p < 0.001$) increase was observed in blood serum levels of prolactin, total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride levels during the pregnancy (Table 1**)**. During pregnancy, the level of prolactin increased as much as 8.5 times, triglycerides - almost 1.3 times, LDL cholesterol - almost 50%, and HDL cholesterol increased less than 16%.

Finally, we examined whether breast size parameters differed based on other indicators (Table 2**)**. There were no significant differences in breast volume when stratifying women by age, education and biological sex of the newborn. However, multiparous women had slightly larger breast volume, confirming that the breast undergoes changes throughout the whole reproductive period. The amount of fat accumulated during pregnancy also differed between primiparous and multiparous women: primiparous women accumulated more fat and relative change of passive mass was greater (6.7% compared to 3.1% in multiparous women). However, the trend towards fat accumulation in the upper body was evident in both groups: primiparous women showed an increase in upper body skinfolds from 6.7 to 23% and in lower body – from 6.5 to 15%; whereas in multiparous women, these changes ranged from 5.7 to 12.1% in the upper body and from 7.4 to 8.2% – in the lower body.

Changes in breast volume, weight, BMI, subcutaneous fat tissue and blood serum biochemical parameters during pregnancy in females with different breast size and subcutaneous adipose tissue

The analysis of changes in different body parameters with respect to breast size showed that the largest relative increment in breast volume during pregnancy was detected in females with small breasts (more than 37%), while in women with large breasts – less than 21% (Table 3**)**. Changes in body weight during pregnancy were also most pronounced in females with medium breasts, while changes in BMI were similar for small and large breasts (Table 3**)**.

An interesting trend in the shift of skinfolds during pregnancy was observed in relation to the breast size group: all changes were particularly pronounced, again, in females with small and medium breast sizes – the greatest increase was observed for bicipital, subscapular and suprailiac skinfolds of females with small breasts

Factors	First trimester BREAST SIZE Small	Medium	Large	Chi-square	df	*p*	Third trimester BREAST SIZE Small	Medium	Large	Chi-square	df	*p*
Age (mean)												
Education	30.3	30.3	30.8	166.0	164	> 0.05	30.0	30.5	30.5	166.0	164	> 0.05
Secondary	1	5	2	8.83	6	> 0.05	1	5	2	3.99	6	> 0.05
Vocational	0	0	2				0	1	1			
Non-university higher education	2	7	4				4	8	1			
University higher education	17	27	11				15	26	14			
Parity												
Primiparous	5	28	8	10.85	2	< 0.01	8	27	6	8.08	2	< 0.05
Multiparous	15	14	13				12	15	15			
Sex of the newborn												
Girl	10	20	11	0.13	2	> 0.05	10	24	7	3.18	2	> 0.05
Boy	10	22	10				10	18	14			

Table 2. *Cross-Tabulation* and *Chi-square* tests for social and infant related factors in women with different breast sizes.

Parameter	Relative change (%) Small breasts	*p*	Medium breasts	*p*	Large breasts	*p*
Breast volume (cm^3)	37.1	**< 0.001**	28.8	**< 0.001**	20.7	**< 0.01**
Weight (kg)	22.7	**< 0.001**	20.6	**< 0.001**	19.6	**< 0.001**
BMI (kg/m^2)	21.1	**< 0.001**	20.4	**< 0.001**	21.2	**< 0.001**
Skinfolds (mm)						
Submental	15.1	> 0.05	13.1	**< 0.05**	7.1	> 0.05
Pectoral I	2.3	> 0.05	5.9	> 0.05	−6.7	> 0.05
Pectoral II	14.3	> 0.05	13.0	> 0.05	−7.2	> 0.05
Axillary	4.0	> 0.05	6.0	> 0.05	−2.5	> 0.05
Abdominal	20.7	**< 0.01**	6.8	**< 0.05**	−4.1	> 0.05
Suprailiac	38.8	**< 0.001**	11.4	**< 0.001**	4.4	> 0.05
Subscapular	39.1	**< 0.001**	15.4	**< 0.001**	6.2	> 0.05
Tricipital	20.1	**< 0.01**	2.6	> 0.05	−3.3	> 0.05
Bicipital	44.7	**< 0.001**	11.6	> 0.05	4.5	> 0.05
Forearm	16.7	> 0.05	9.2	> 0.05	5.6	> 0.05
Femoral	15.5	**< 0.01**	14.1	**< 0.001**	4.0	> 0.05
Calf	3.7	> 0.05	9.3	**< 0.05**	5.2	> 0.05
Subcutaneus fat (%)	10.6	**< 0.001**	5.1	**< 0.01**	1.5	> 0.05
Biochemical parameters						
Prolactine (ng/ml)	847.2	**< 0.001**	874.0	**< 0.001**	803.1	**< 0.001**
Total cholesterol (mmol/l)	39.1	**< 0.001**	38.2	**< 0.001**	39.6	**< 0.001**
HDL cholesterol[A] (mmol/l)	9.7	**< 0.05**	17.7	**< 0.001**	17.5	**< 0.001**
LDL cholesterol[B] (mmol/l)	49.9	**< 0.001**	47.8	**< 0.001**	47.1	**< 0.001**
Triglycerides (mmol/l)	125.6	**< 0.001**	135.3	**< 0.001**	112.8	**< 0.001**

Table 3. Relative change (%) of breast volume, weight, BMI, subcutaneous fat tissue and blood serum biochemical parameters during pregnancy in females with different breast sizes. [A]HDL cholesterol – high-density lipoprotein cholesterol. [B]LDL cholesterol – low-density lipoprotein cholesterol.

(Table 3). In women with large breasts, changes in thickness of almost all skinfolds during the pregnancy were minimal or even negative.

Regarding biochemical parameters, the most pronounced increase in prolactin (8.5–8.7 times) and triglycerides (1.3–1.4 times) were found in women with small and medium breasts. However, total and low-density lipoprotein (LDL) cholesterol increased very similarly during pregnancy in all breast size groups, although the increase of HDL cholesterol was highest in medium- and large-sized breast groups of women (Table 3).

Very similar changes were observed when evaluating all changes in females with different amounts of subcutaneous fat tissue (Table 4): the largest changes in breast volume, weight, BMI, and subcutaneous adipose

Parameter	Relative change (%)					
	Lean	*p*	Average	*p*	Obese	*p*
Breast volume (cm³)	44.1	**< 0.001**	30.7	**< 0.001**	12.6	> 0.05
Weight (kg)	21.5	**< 0.001**	23.5	**< 0.001**	15.5	**< 0.01**
BMI (kg/m²)	20.6	**< 0.001**	22.2	**< 0.001**	18.5	**< 0.001**
Skinfolds (mm)						
Submental	18.3	**< 0.01**	11.7	**< 0.05**	8.3	> 0.05
Pectoral I	−7.9	> 0.05	0.4	> 0.05	9.2	> 0.05
Pectoral II	16.6	**< 0.05**	6.9	> 0.05	1.2	> 0.05
Axillary	12.3	> 0.05	−4.9	> 0.05	10.7	> 0.05
Abdominal	24.7	**< 0.001**	−2.7	> 0.05	11.7	**< 0.01**
Suprailiac	19.5	**< 0.05**	14.9	**< 0.001**	11.6	**< 0.05**
Subscapular	27.4	**< 0.001**	13.6	**< 0.001**	16.3	**< 0.01**
Tricipital	4.0	> 0.05	3.4	> 0.05	5.2	> 0.05
Bicipital	55.1	**< 0.001**	9.0	> 0.05	7.6	> 0.05
Forearm	26.6	**< 0.001**	4.6	> 0.05	8.9	> 0.05
Femoral	11.7	> 0.05	14.9	**< 0.001**	6.0	> 0.05
Calf	5.8	> 0.05	4.3	> 0.05	11.9	**< 0.01**
Subcutaneus fat (%)	5.5	**< 0.001**	4.8	**< 0.001**	5.9	**< 0.01**
Biochemical parameters						
Prolactine (ng/ml)	1069.2	**< 0.001**	804.7	**< 0.001**	764.1	**< 0.001**
Total cholesterol (mmol/l)	44.9	**< 0.001**	34.4	**< 0.001**	41.9	**< 0.001**
HDL cholesterol[A] (mmol/l)	9.7	**< 0.05**	14.9	**< 0.001**	23.6	**< 0.001**
LDL cholesterol[B] (mmol/l)	62.0	**< 0.001**	40.9	**< 0.001**	50.5	**< 0.001**
Triglycerides (mmol/l)	152.4	**< 0.001**	117.0	**< 0.001**	123.0	**< 0.001**

Table 4. Relative change (%) of breast volume, weight, BMI, subcutaneous fat tissue and blood serum biochemical parameters during pregnancy in females with different subcutaneous fat percentage. [A]HDL cholesterol – high-density lipoprotein cholesterol. [B]LDL cholesterol – low-density lipoprotein cholesterol.

tissue (with a few exceptions) were again characteristic of lean and average-built females, but blood lipid parameters changes were very similar in females of different adiposity and breast size.

The coincidence (cross-tabulation analysis) of breast size group and subcutaneous body fat tissue group in females during pregnancy

During the first trimester of pregnancy, approximately half of the thin women had small breasts (55%), the remaining women had medium-sized breasts, and none had large breasts (Table 5**).** Almost 62% of normal body women had medium-sized breasts, more than 21% had small breasts and almost 17% had large breasts. None of the obese women had small breasts at the beginning of pregnancy, about one-third had medium-sized, and two-thirds had large breasts.

By the end of pregnancy, breast size of lean females shifted to the larger breast size groups, while breast size of obese females "overjumped" to smaller breast size group (Table 5).

It is clear that breast size did not correspond very often to a similar body type, and the different breast size groups may vary differently during pregnancy. Almost a third of females changed breast size group during pregnancy (Table 6): medium breasts changed size group relatively less, but 15% of women with small breasts changed to medium size at the end of pregnancy and 10% even to large breast group, and almost a third to large breasts of women (28.6%) switched to the medium-sized group at the end of pregnancy.

Multiple associations between breast volume, anthropometric and blood serum biochemical parameters during pregnancy in females with different breast size

Pearson's correlation coefficients were analysed in order to reveal linear relationship between breast size and other anthropometric and biochemical parameters (Table 7**).** There were differences (both at the beginning and at the end of pregnancy) in correlations between breast volume and all aforementioned parameters in females with different breast size.

The most statistically significant correlations were found in females with larger and medium breasts: breast volume in these women was most associated with skinfolds on the trunk and upper limbs at the beginning of pregnancy, as well as with femoral skinfold at the end of pregnancy (Table 7). Interestingly, in women with small breasts, breast volume was negatively related to height. However, during pregnancy, biochemical parameters had no specific and statistically significant linear correlations with breast volume within different breast size groups of females (Table 7).

Univariate logistic regression analysis was performed to identify complex relationships between breast size, subcutaneous fat tissue, and biochemical parameters in women during pregnancy (Table 8): in early pregnancy,

Subcutaneus fat tissue group, first trimester		Breast size group, first trimester			Total
		Small	Medium	Large	
Lean	n	11	9	0	20
	%	**55.0**	**45.0**	**0.0**	100.0
Average	n	9	26	7	42
	%	**21.4**	**61.9**	**16.7**	100.0
Obese	n	0	7	14	21
	%	**0.0**	**33.3**	**66.7**	100.0
Total	n	20	42	21	83
	%	24.1	50.6	25.3	100.0
p **< 0.001 (Chi-square > 20)**					
Subcutaneus fat tissue group, third trimester		**Breast size group, third trimester**			**Total**
		Small	**Medium**	**Large**	
Lean	n	7	11	2	20
	%	**35.0**	**55.0**	**10.0**	100.0
Average	n	11	22	9	42
	%	**26.2**	**52.4**	**21.4**	100.0
Obese	n	2	9	10	21
	%	**9.5**	**42.9**	**47.6**	100.0
Total	n	20	42	21	83
	%	24.1	50.6	25.3	100.0
p **< 0.05 (Chi-square = 9.5)**					

Table 5. The coincidence in breast size and subcutaneous fat tissue group of females during pregnancy (*Cross-Tabulation* analysis).

Breast size group, first trimester		Breast size group, third trimester			Total
		Small	Medium	Large	
Small	N	15	3	2	20
	Proc.	**75.0**	**15.0**	**10.0**	100.0
Medium	N	5	33	4	42
	Proc.	**11.9**	**78.6**	**9.5**	100.0
Large	N	0	6	15	21
	Proc.	**0.0**	**28.6**	**71.4**	100.0
Total	N	20	42	21	83
	Proc.	24.1	50.6	25.3	100.0
p **< 0.001 (Chi-square > 20)**.					

Table 6. The coincidence in breast size group at the beginning and the end of pregnancy (Cross-tabulation analysis).

the highest odds ratios for large breasts were again obtained in skinfolds located on the upper trunk and upper arms. As for biochemical parameters, having higher LDL ("bad") cholesterol increased the probability for larger breast size significantly (more than 4.6 times) in the beginning of pregnancy (but not at the end of pregnancy).

Finally, **a factor analysis (based on principal components) was performed** in order to refine the multiple relationships between the breast volume and other studied indicators, to structure the independent factors and which parameters they affect. In the beginning of pregnancy (Table 9), breast size was mainly related to the indicators of the first factor, especially with trunk and upper arms' skinfolds, as well as BMI. Meanwhile, biochemical indicators stood alone under the influence of another factor, although high-density lipoprotein ("good") cholesterol was also negatively related to the first factor indicators and breast volume (via rotated factor loading – 0.41), which means a lower level of HDL ("good") cholesterol may be associated with large breast size and especially with upper trunk skinfolds (Table 9**).**

At the end of pregnancy (Table 10), in the overall structure of the factor analysis, breast size was mainly related to the indicators of the third factor, which means that larger breast size was associated with higher BMI but lower prolactin concentrations, although there was an indirect positive relationship between breast size and the first factor indicators (via a rotated factor loading of 0.39), i.e. with skinfolds (especially on the trunk and

Parameter	First trimester of pregnancy BREAST SIZE			Third trimester of pregnancy BREAST SIZE		
	Small	Medium	Large	Small	Medium	Large
N	20	42	21	20	42	21
Height (cm)	−0.34	0.21	−0.11	**−0.45***	0.19	−0.03
Weight (kg)	0.03	0.30	**0.51***	−0.23	0.11	**0.49***
BMI (kg/m^2)	0.22	0.22	**0.52***	0.13	0.02	**0.46***
Skinfolds (mm)						
Submental	0.21	0.17	0.11	0.33	**0.33***	−0.30
Pectoral I	0.29	0.28	0.20	0.39	**0.42****	−0.12
Pectoral II	0.26	**0.33***	0.13	0.37	**0.36***	0.09
Axillary	0.07	0.18	−0.24	0.36	0.13	−0.13
Abdominal	0.04	0.26	0.13	0.34	**0.31***	−0.03
Suprailiac	0.01	**0.31***	0.25	0.41	**0.42****	0.18
Subscapular	0.01	**0.49****	**0.53***	0.07	0.30	0.26
Tricipital	0.03	0.19	0.20	0.09	0.24	0.19
Bicipital	0.07	0.26	0.36	0.57	0.07	0.14
Forearm	0.18	0.19	0.36	0.29	0.29	0.07
Femoral	−0.14	0.28	0.18	0.40	**0.41****	0.22
Calf	0.19	−0.01	0.17	0.36	0.29	0.13
Subcutaneus fat (%)	−0.02	**0.43****	0.43	0.15	**0.34***	0.26
Biochemical parameters						
Prolactin (ng/ml)	0.39	0.28	−0.15	0.12	−0.11	−0.29
Total cholesterol (mmol/l)	−0.28	0.17	0.18	0.03	0.07	0.25
HDL cholesterol[A] (mmol/l)	−0.15	0.03	−0.13	−0.15	−0.17	0.08
LDL cholesterol[B] (mmol/l)	−0.28	0.21	0.13	0.03	0.25	0.08
Triglycerides (mmol/l)	0.09	0.19	−0.01	0.11	0.07	0.10

Table 7. Pearson's correlation coefficients between the breast volume, body size, subcutaneous fat and biochemical parameters during pregnancy in females with different breast sizes (statistically significant correlations are marked in bold). * Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level; [A]HDL cholesterol – high-density lipoprotein cholesterol; [B]LDL cholesterol – low-density lipoprotein cholesterol.

upper limbs). However, the level of triglycerides and cholesterol did not have a specific direct relationship with breast volume or skinfolds.

Discussion

Peculiarities of complex changes in breast volume, body size and subcutaneous adipose tissue during pregnancy

Although the breast reaches its final development only during pregnancy, these changes are less well studied, and only a few studies have been conducted recently[24,52–58,77–80]. Prolactin, progesterone, growth hormone, insulin like growth factor 1 (IGF-1), thyroid and the other hormones stimulate breast development – stroma, parenchyma, also fat tissue are under the influence[88–90]. However, it is not sufficiently clear how breast size changes during pregnancy in relation to subcutaneous fat topography and biochemical parameters, therefore our study focused mainly on these parameters.

Our study showed an especially wide variation in breast volume of pregnant females – both at the beginning and the end of pregnancy. Obviously, breast volume significantly increased (approximately by one quarter) in women during pregnancy. To our knowledge, there were no similar reference data (based on a similar methodology for determining breast size) from other geographic regions so as to compare changes in females' breast size during pregnancy. Nevertheless, several other studies have investigated changes in breast size during pregnancy using different methodologies, such as the three-dimensional surface assessment technique[24], as well as 3D scanning[80], but they also found a very similar relative change in breast volume (23–24%).

Our study confirmed that almost all general indicators of body size and shape (weight, BMI, subcutaneous fat tissue) increased significantly during pregnancy. That could be seen as obvious biological advantage – efficient fat storage for higher energy demands due to foetal development and preparation for lactation, however, further research into mechanisms of fat storage may provide better insights into the development and functions of body fat[91].

Our results showed that breast size was increasing during pregnancy in relation to woman's body size indices (weight, BMI, subcutaneous fat tissue), however, only about half of women retained the same category of body size, fatness and breast size, and even one third of female breasts "jumped" into another breast-size group during

Parameter	First trimester BREAST SIZE OR	95% CI	*p*	Third trimester BREAST SIZE OR	95% CI	*p*
Height (cm)	0.69	0.29–1.64	> 0.05	0.85	0.36–2.01	> 0.05
BMI (kg/m^2)	**3.26**	1.29–8.23	**< 0.05**	**2.74**	1.11–6.72	**< 0.05**
Skinfolds (mm)						
Submental	**3.35**	1.35–8.32	**< 0.01**	3.06	1.22–7.65	**< 0.05**
Pectoral I	**3.35**	1.35–8.32	**< 0.01**	2.47	1.01–6.02	**< 0.05**
Pectoral II	**5.16**	2.01–13.27	**< 0.01**	**3.43**	1.39–8.47	**< 0.01**
Axillary	2.26	0.93–5.47	> 0.05	1.67	0.70–3.99	> 0.05
Abdominal	**3.83**	1.51–9.74	**< 0.01**	2.24	0.93–5.42	> 0.05
Suprailiac	**2.95**	1.19–7.29	**< 0.05**	2.10	0.87–5.06	> 0.05
Subscapular	**6.13**	2.35–16.02	**< 0.001**	**3.40**	1.36–8.52	**< 0.01**
Tricipital	**5.04**	1.96–12.98	**< 0.01**	2.24	0.93–5.42	> 0.05
Bicipital	**4.71**	1.85–11.99	**< 0.01**	1.81	0.75–4.38	> 0.05
Forearm	**2.99**	1.21–7.37	**< 0.05**	**2.47**	1.01–6.02	**< 0.05**
Femoral	2.12	0.88–5.14	> 0.05	**3.43**	1.39–8.47	**< 0.01**
Calf skinfold	**2.61**	1.07–6.38	**< 0.05**	**3.85**	1.54–9.58	**< 0.01**
Biochemical parameters						
Prolactin (ng/ml)	1.44	0.60–3.48	> 0.05	0.77	0.33–1.84	> 0.05
HDL cholesterol[A] (mmol/l)	1.04	0.44–2.49	> 0.05	0.56	0.23–1.35	> 0.05
LDL cholesterol[B] (mmol/l)	**4.63**	1.82–11.77	**< 0.01**	1.33	0.55–3.21	> 0.05
Triglycerides (mmol/l)	1.94	0.80–4.72	> 0.05	1.39	0.58–3.30	> 0.05

Table 8. Odds ratios for breast volume in relation to body size and biochemical parameters (univariate logistic regression analysis; statistically significant odds ratios OR are pointed in bold). [A]HDL cholesterol – high-density lipoprotein cholesterol. [B]LDL cholesterol – low-density lipoprotein cholesterol.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Subscapular skinfold	**0.88**	0.18	0.11		
Bicipital skinfold	**0.86**	0.28			
Tricipital skinfold	**0.83**	0.13			
Forearm skinfold	**0.80**	0.34		0.18	
BMI	**0.78**	−0.38			−0.17
Submental skinfold	**0.75**	0.48			
Suprailiac skinfold	**0.74**	0.14		0.28	0.21
Pectoral II skinfold	**0.73**	0.15		0.40	
Abdominal skinfold	**0.73**	0.18		0.19	
Breast volume	**0.71**	−0.17			−0.11
Calf skinfold	**0.67**			0.18	0.37
Femoral skinfold	**0.58**	0.15	−0.11	0.24	0.39
Pectoral I skinfold	**0.49**	0.22		0.47	
Axillary skinfold	0.28	**0.82**	−0.13		
LDL cholesterol[B]			**0.78**	−0.10	
Triglycerides	0.17	−0.20	**0.72**	0.20	
HDL cholesterol[A]	−0.41	0.18	**0.54**		−0.27
Prolactin				**0.88**	
Height	−0.13				**0.88**
% of Variance	39.2	8.2	7.8	7.7	6.6
Cumulative %	39.2	47.4	55.2	62.9	69.5

Table 9. Multiple relationships (factor analysis) between breast volume, body size and biochemical parameters in the *first trimester of pregnancy* (rotated factor loadings based on principal components analysis). [A]HDL cholesterol – high-density lipoprotein cholesterol. [B]LDL cholesterol – low-density lipoprotein cholesterol.

Variable	Factor 1	Factor 2	Factor 3	Factor 4
Tricipital skinfold	**0.82**		0.11	
Forearm skinfold	**0.81**	−0.11		
Subscapular skinfold	**0.81**		0.29	
Submental skinfold	**0.78**	−0.25		
Pectoral II skinfold	**0.78**	−0.31	0.10	0.12
Bicipital skinfold	**0.77**			0.11
Suprailiac skinfold	**0.76**	0.18		
Pectoral I skinfold	**0.72**	−0.31		
Abdominal skinfold	**0.72**	−0.21		0.13
Femoral skinfold	**0.69**	0.24	−0.12	0.16
Calf skinfold	**0.67**	0.24	0.16	0.13
Axillary skinfold	**0.55**	−0.25	−0.36	−0.10
Triglycerides		**0.76**		
LDL cholesterol[B]		**0.75**	0.13	−0.38
Height		**0.51**	−0.35	0.39
BMI	0.44		**0.67**	0.15
Prolactin	0.26		**−0.60**	0.21
Breast volume	0.39	0.18	**0.51**	0.26
HDL cholesterol[A]		0.21		**−0.85**
% of Variance	37.2	10.6	8.0	6.6
Cumulative %	37.2	47.8	55.8	62.5

Table 10. Multiple relationships (factor analysis) between breast volume, body size and biochemical parameters in the *third trimester of pregnancy* (rotated factor loadings based on principal components analysis). [A]HDL cholesterol – high-density lipoprotein cholesterol. [B]LDL cholesterol – low-density lipoprotein cholesterol.

the pregnancy. Besides, breast volume of pregnant females was mostly related to the localization of subcutaneous fat tissue at the upper part of the trunk and upper arms.

However, topography of the latter subcutaneous body fat, in general, is assumed as a health risk marker related to metabolic syndrome, cardiovascular and other associated diseases, whereas "pear shape" topography of subcutaneous fat may simply provide a safe lipid reservoir for excess energy and plays a protective role for type 2 diabetes and cardiovascular risk[44–46,92,93]. Nevertheless, further investigation is needed to fully understand the biological and clinical significance of topography and redistribution of fat accumulation during pregnancy.

Our study revealed, that changes in breast volume, body weight, BMI, and skinfolds were mostly prominent in females with small breasts, while in females with large breasts changes in all these measurements were less expressed. This phenomenon might be partly explained by individual variability in total amount and topography of subcutaneous fat tissue and sex-related fat in females[2,92–95]. During pregnancy, these indices differentiate and diverge much more significantly and present study did show that breasts of pregnant females were enlarging parallelly to body fat accumulation, yet, up to a certain (limitary) level. The relationship between breast volume and subcutaneous fat (in particular, located on the upper part of the body) was stronger at the beginning of pregnancy, especially, in females with medium and larger breasts. However, individual variability of sex-specific body fat, which is mainly located at the gluteofemoral region and female breasts, is poorly studied among pregnant women from different populations[91,93,95].

Generally, female enzyme activity is higher in subcutaneous than in visceral adipose tissue, and pregnancy may accentuate the sexually dimorphic distribution of fat, also enhance metabolic activity of fat tissue by regulating its "browning"[95]. On the other hand, it is not known how metabolic activity of subcutaneous fat tissue, located at various parts of the body, might change in different females during pregnancy or postpartum[92].

Summing up, our study revealed that during pregnancy, subcutaneous adipose tissue tended to accumulate relatively more in the upper body (which is more typical of android fat distribution). Approximately 20–30 years ago, it was found that during pregnancy, the topography of subcutaneous fat tissue in Lithuanian women was more gynecoid[96,97]. In addition, Lithuanian women who accumulated more subcutaneous fat in the upper body during pregnancy were more likely to suffer from metabolic syndrome later in life – at the age of 40–50[97]. In general, the so-called "V" shaped subcutaneous adipose tissue topography, based on numerous other studies[38,39,41,43–46,66], is associated with central obesity, metabolic syndrome, cardiovascular disease, type 2 diabetes, and breast cancer risk. On the other hand, research should be continued to investigate how subcutaneous adipose tissue changes after childbirth and whether this may be related to a woman's future health[98].

Peculiarities of complex changes in breast volume and serum biochemical parameters during pregnancy

Regarding biochemical parameters, our study showed that the most pronounced increase in prolactin and triglycerides was found, again, in women with small and medium-sized breasts, while the increase in high-

density lipoprotein ("good") cholesterol during pregnancy was greatest in women with medium and large-sized breasts. In contrast, low-density lipoprotein ("bad") cholesterol levels in early pregnancy were associated with the likelihood of larger breast size. In addition, in our study factor analysis revealed that lower levels of HDL ("good") cholesterol in early pregnancy may also be associated with large breasts, as well as skinfolds on the upper trunk and upper limbs. However, by the end of pregnancy, triglycerides and cholesterol had no specific direct relationship with breast volume or skinfolds, which may mean the positive effect of the pregnancy mechanism (itself) and the specific reorganization of body size and shape during its course in order to ensure an optimal biochemical condition.

The changing levels of biochemical parameters, probably, act as a buffer that responds to the needs of the woman's body, but it is difficult to predict changes in breast volume during pregnancy based only on changes in biochemical indicators.

These findings are consistent with studies suggesting that various adaptive mechanisms are activated during pregnancy, including changes in lipid metabolism[99–103]. During early pregnancy, lipogenesis is activated and lipolysis is inhibited – that results in increasing body fat accumulation, which is necessary for maternal needs and foetal development during pregnancy, and the mother switches to a catabolic condition[104–106]. As a result, by the end of pregnancy enhanced lipolytic activity leads to physiological dyslipidemia[104,107]. The activation of lipolysis also allows pregnant woman to use lipids as a maternal energy source and preserve glucose and amino acids for the foetus[108,109].

Prolactin activates lipid synthesis in liver, reduces the ability of fatty tissue to store fat, and, consequently, increases the level of lipids in serum and certain tissues. Circulating lipids are important for different processes – both for mother and foetus. Cholesterol is used by placenta for the steroid synthesis, fatty acids – for oxidation, etc. Maternal cholesterol is more important for the foetus during early gestation, while at late pregnancy foetal tissues have higher capacity to synthesize cholesterol. In addition, cholesterol is used for the synthesis of sex hormones (progesterone, estrogens), and progesterone is especially important at the beginning of pregnancy because it suppresses the effect of oxytocin (which stimulates contractions of the smooth muscles of the uterus)[103,110–112]. Triglycerides are insoluble, therefore, they do not directly cross the placental barrier, thus, essential fatty acids are transported as fatty acid-albumin complex and triglycerides of very low-density lipoproteins (VLDL), low- (LDL) and high-density (HDL) lipoproteins[105].

During late pregnancy, when catabolic processes were activated, we observed physiological dyslipidemia – all lipid fractions rose during the third trimester of pregnancy, however, triglycerides showed the largest increase during pregnancy. These findings showed that increasing prolactin concentration during pregnancy had activated triglyceride synthesis in liver and, consequently, serum triglyceride levels had augmented. After pregnancy, levels of cholesterol and triglyceride, usually, decrease during the 6–7 weeks postpartum[99–101].

In the future, it would be worthwhile to investigate additional body size and shape parameters, as well as biochemical markers that may be associated with changes in the breasts (including their glandular and adipose compartments) during pregnancy. It would be also important to examine how all these changes relate to lactation potential, fetal development, and women's health in later life, with the aim of identifying early predictors of health risks for both the mother and child.

Conclusions

1. Breast volume in pregnant women (regardless of their body size and subcutaneous fat category) was relatively more associated with the accumulation of subcutaneous fat tissue on the trunk and upper body, which is traditionally more commonly associated with metabolic syndrome, cardiovascular disease risk, type 2 diabetes, and breast cancer.
2. The biggest changes in body parameters occurred in the group of small-breasted and thin women. In addition, the largest increment of high-density lipoprotein (HDL; "good") cholesterol during the pregnancy was observed in medium and large-sized breast women, however, low-density lipoprotein (LDL; "bad") cholesterol in early pregnancy showed the higher likelihood of larger breasts. We hypothesize that pregnancy differentially affected fat accumulation in women with different breast sizes and body types, "optimizing" the fat reservoir, which may indicate a positive effect of pregnancy itself and specific reorganization of body size and shape during it.
3. Only half of women retained the same category of body build and breast size, and even one third of female breasts "jumped" into another breast-size group during the pregnancy. Special attention should be directed towards females with large breasts, even in the case of normal BMI, also to females whose breast size rapidly changes during pregnancy, as well as to females, who during pregnancy accumulate more fat on the trunk and upper body.

Data availability

The datasets used and/or analysed during the present study are available from the corresponding author upon reasonable request. All data generated or analysed during this study are included in this published article.

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Author contributions

J.T. raised the main conceptual idea, designed and supervised the study, took the lead in data interpretation, search for literature sources and writing the manuscript with the input from other authors; S.G. prepared raw data for calculations, helped with statistical analyses, provided suggestions for data interpretation, participated in the search for literature sources, contributed to the text writing; J.D. and I.S. conducted a study of pregnant women and collected data, contributed in revising the manuscript; R.S.R., V.J., E.M.J., D.K., A.K., contributed to data interpretation, search for literature sources and added ideas while writing text; A.B. helped with statistical analyses, data interpretation and contributed in revising the manuscript; V.T. and J.T. provided suggestions for data interpretation and ideas for introduction and discussion, contributed in revising the manuscript; L.N., R.C., J.A.B. took part in data interpretation and revising the manuscript; D.R. added ideas for study design, took part in data interpretation, writing and revising the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to J.T.

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