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RESEARCH ARTICLE

ULTRASOUND HISTOGRAM ANALYSES OF EPICARDIAL ADIPOSE TISSUE.

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Abstract

Objective: Adipose tissue is a major endocrine organ and plays a key role in energy homeostasis. Two types of adipose tissue such as white and brown adipose tissue exist having essentially different physiological function. The epicardial adipose tissue (EAT) is considered as reliable marker of visceral adiposity, however its composition is not entirely clear. The aim of our study was to examine the qualitative features of EAT by ultrasound histograms in obese and normal-weight adolescents.

Methods: 70 (mean age of 17.72 ± 1.20) randomly selected adolescents were involved in this study. Ultrasonographic histogram was used to assess the EAT structure by the contrast between the epicardial adipose tissue and left atrium (EAT/LA-value). Furthermore, anthropometric and biochemical parameters of cardiovascular risk were also obtained.

Results: The EAT/LA-value differed significantly between normal weight and obese youngsters (14.76 ± 0.83 vs. 26.22 ± 0.95 ; $p < 0.001$) and was associated with clinical parameters of obesity (body mass index (BMI): $r = 0.57$; $p < 0.0001$ and BMI percentile: $r = 0.72$; $p < 0.0001$), laboratory parameters of cardiovascular risk factors (ALT: $r = 0.38$; $p < 0.001$, adiponectin: $r = -0.33$; $p < 0.01$, hsCRP: $r = 0.24$; $p < 0.05$) and EAT thickness (EAT at end-systole: $r = 0.46$; $p < 0.0001$ and EAT at end-diastole: $r = 0.43$; $p < 0.0001$). Multiple regression analysis showed that the EAT/LA-value was associated with EAT at end-systole [B (95%CI) = 2.52 (0.94, 4.11); $p \leq 0.01$] and alanine aminotransferase (ALT) [9.23 (0.20, 18.26); $p \leq 0.05$ however, BMI proved to be the strongest independent predictor [0.16 (0.10, 0.21); $p \leq 0.001$].

Conclusion: The ultrasound histogram of epicardial adipose tissue seems to be non-invasive, low-cost and easy imaging approach that can

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qualitatively distinguish between different types and characteristics of adipose tissue.

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Introduction:-

Prevalence of obesity is increasing world-wide in association with sedentary lifestyle and consumption of calorie rich food. In addition, it is also linked with increasing risk of cardiovascular diseases (CVD) (coronary heart disease and stroke), hypertension, insulin resistance, type 2 diabetes, dyslipidaemia, and many types of cancer [1, 2].

Adipose tissue is an endocrine organ which regulates energy homeostasis. There are two types of adipose tissue (white adipose tissue and brown adipose tissue) having different functions regarding energy homeostasis regulation: white adipose tissue (WAT) stores the excess energy in the form of triglycerides (TGs) and brown adipose tissue (BAT) produces body heat (adaptive thermogenesis). BAT is abundant in small mammals and was thought to be limited to infants in humans providing support to survive cold temperature. In adults, BAT had been considered to be absent or at least with unremarkable physiological function [3]. However, recent investigations have shown that adults also have metabolically active BAT [4]. The studies on animals found that reduced amount or function of BAT leads to the obesity, dyslipidaemia, and insulin resistance. Whereas, increased amount or function of BAT protects against weight gain and its co-morbidities [5, 6, 7]. In the human studies the inverse relationship between the WAT and BAT was confirmed [6, 8, 9, 10]. Furthermore, BAT is considered to be a potential target for the treatment of obesity and its metabolic complications [3, 11].

Adipose tissue that directly surrounds the heart, is known as epicardial adipose tissue (EAT) [12] and its echocardiographic determination has been introduced for the first time by Iacobellis et al. [13, 14]. The EAT is considered as a special form of visceral fat reflecting intra-abdominal fat deposition [15, 16], however, recently it has been described as brown adipose tissue [17]. Investigators who confirmed that epicardial adipose tissue has some features of BAT used invasive methods primarily represented by gene expression in adipose tissue [17, 18, 19]. Despite the fact that non-invasive methods are quite expensive for clinical practice the methods such as magnetic resonance imaging (MRI) or computed tomography (CT) scanning for detection of the BAT [20, 21, 22] have been performed.

The ultrasound histogram is a widely used non-invasive method for the identification of tissue characteristics and differences, and allows estimate to what extent a tissue is different from another. It delimits the contours of a tissue and measures its surface according to the obtained echo signals from tissue to partition the **B**-mode image into homogeneous zones of texture [23].

The aim of our study was to examine the characteristics of the epicardial adipose tissue using ultrasound histogram in obese and non-obese children and adolescents and to assess its relationship with the severity of obesity and the other cardiometabolic risk factors.

Materials and Methods

This observational study was performed at the Department of Paediatrics and Adolescent Medicine, Faculty of Medicine, P. J. Safarik University in Košice. Seventy randomly selected students (mean age: 17.72±1.2 years) attending 7th grade in Košice high schools district were included in the study. Out of them, 52 subjects were normal weight (BMI < 85th percentile) and 18 were overweight or obese (BMI ≥ 85th percentile). Normal weight and overweight/obese groups did not differ regarding age; other anthropometric and clinical parameters are shown in Table 1. Adolescents with secondary obesity were excluded, none of them were taking drugs or had a history of CVD. The study protocol was approved by the Ethics Committee of the University Hospital in Kosice, and the written informed consent was obtained from all research participants.

Epicardial adipose tissue thickness measurementThe thickness of epicardial adipose tissue (EAT) was measured in the parasternal long axis view at the end-diastole (EATd) and end-systole (EATs) in three cardiac cycles by transthoracic two-dimensional (2D) echocardiography (Aloka alfa 7 Prosound) according Iacobellis et al. [15, 16].

The ultrasonography epicardial adipose tissue histogram analysisUsing the Aloka alfa 7 Prosound system on a B-mode, the region of interest (ROI) was placed on both the EAT measurement area and the left atrium (LA) area in a square shape (2x2mm) where the contrast between the EAT and the LA should be displayed most clearly. The

echogenicity of each pixel included in the ROI was divided into 64 gradients (1 gradient corresponds approximately to 1 dB) by built-in computer on the basis of its intensity, and the frequency distributions of the gradients were shown as histogram. The histogram analyses provide information about the most frequent gradient (L-value), the number of the pixel that composes the L-value (M-value) and the mean of all pixels' gradient included in ROI (MN-value). As shown on the Figure 1., the total number of pixels included in each ROI was 64. In the LA, the L-value was 57, the M value was 17 and the MN value was 55.5. In the EAT, the L-value was 18, the M value was 13 and the MN value was 16.5. We used the EAT/LA-value, the difference between the L-value of EAT and the L-value of the LA, to assess the EAT/LA contrast (i.e. brightness of EAT as compared to LA). As shown on the Figure 1., the EAT/LA value is 39 (57-18). We applied this method similarly to the previous studies which used the difference between the L-value of the liver and the L-value of the kidney (L/K-value) for the assessment of the contrast between the liver and kidney [24, 25, 26].

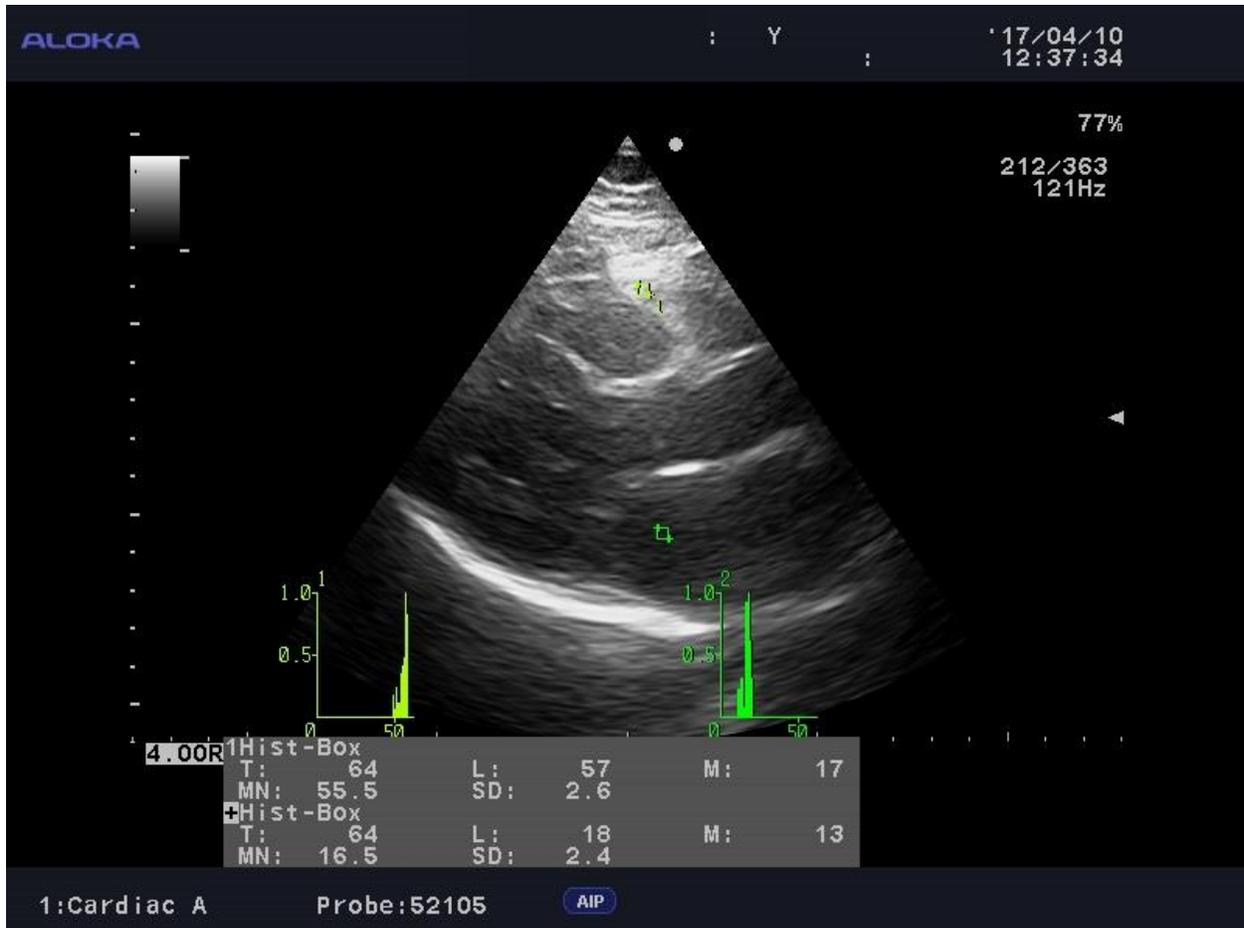


Fig 1: Ultrasonographic picture used for histogram analysis of the epicardial adipose tissue. See text for explanation.

The body mass index (BMI) percentile was calculated according to the World Health Organization's recommendations (22). Blood samples were drawn after 12-hours overnight fast. Total cholesterol, high-density lipoprotein (HDL) cholesterol, serum triacylglyceride (TAG), and plasma glucose levels were measured enzymatically on the Siemens ADVIA auto analyser, low-density lipoprotein (LDL) cholesterol was calculated using the Friedwald's formula (300). Insulin concentration was measured by sandwich ECLgA method on the Roche Modular Analytics E170 analyser. Uric acid (UA), alanine aminotransferases (ALT) and aspartate aminotransferases (AST) were measured by photometric kinect methods using a Siemens ADVIA biochemical auto analyser, high-sensitivity C-reactive protein (hsCRP) was measured by particle-enhanced immunoturbidimetric method. Immunoenzymatic assays were used for the quantitative measurement of serum

adiponectin. HOMA index (homeostasis model assessment of insulin resistance) was calculated according to the standard formula [27].

Results:-

In overweight and obese adolescents, the EAT/LA-value was significantly higher when compared to normal weight subjects. Overweight and obese adolescents had significantly higher EATs, EATd and MN(EAT) than normal weight subjects. BW, BMI, BMI percentile, glucose, insulin, HOMA-IR, UA, TAG and ALT were higher and HDL-cholesterol, adiponectin and hsCRP were lower in overweight/obese than normal weight adolescents (Table 1).

The EAT/LA-value correlated significantly positively with BW, body height, BMI, BMI percentile, ALT, hsCRP, EATs, EATd and MN(EAT) and negatively with adiponectin and MN(LA) (Table 2).

Multiple linear regression analysis showed that the EAT/LA-value was associated with EATs and ALT (Crude effect, Model 1), however, after adjustment for BMI percentile, EAT/LA was no longer associated significantly with EATs and ALT (Model 2) pointed out on a strong association between the EAT/LA-value and BMI percentile (Table 3).

Discussion:-

To our knowledge this is the first study which assessed ultrasonographic features of the epicardial adipose tissue (EAT) on a simple histogram. In our study, we used the EAT/LA-value as a measure of the contrast between EAT and LA representing the relative brightness of the epicardial adipose tissue to the left atrium where fatty change hardly occurs. The EAT/LA-value differed significantly between normal weight and overweight/obese youngsters and it was associated with clinical parameters of obesity (e.g. BMI), laboratory parameters of cardiovascular risk factors (ALT, adiponectin, UA, hsCRP) and EAT thickness. However, BMI percentile proved to be the strongest significant independent predictor of EAT/LA-value.

The fact that the cardiovascular risk of obesity is related more with body fat distribution than with total body fat is known for more than 60 years [28]. Depending on the type of fat cells (adipocytes), there are clear differences between subcutaneous and visceral adipose tissue regarding their endocrine function, lipolytic activity, response to the insulin and other hormones [29, 30, 31]. Adipose tissue as an important endocrine organ in energy homeostasis consists of two types of adipose tissue: the white and the brown tissue. White adipose tissue stores excess energy while brown adipose tissue consumes the energy through the production of heat [32].

Epicardial adipose tissue exhibits features of intra-abdominal visceral fat and is considered as a reliable marker for visceral adiposity when assessed by echocardiography [13, 14, 17]. However, there are some data showing that EAT contains BAT as well. Published studies or studies focused on the identification of EAT as brown-like tissue used gene expression such as PGC1 α , UCP1 and PRDM16 mRNAs where adipose tissue was collected invasively usually during patient's operation [18, 19, 20]. Moreover, some of these invasive studies showed that, depending on the presence of different type of adipose tissue, different cardiovascular and metabolic risks are present in patients. Moreno-Santos et al. [18] showed that patients with type 2 diabetes and coronary artery disease exhibited a loss of brown-like fat features. This was also associated with higher prevalence of coronary lesions. Chechi et al. [33] confirmed that the presence of active brown adipocytes shares a functional association with the circulating plasma lipids in humans.

On the other hand, there are some non-invasive studies available for the identification of the BAT. Reddy et al. [34] provided first report for the reliable use of MRI to identify BAT in living adults. Since then more studies are available [34, 35, 36, 37]. Besides the MRI, some other non-invasive investigation methods like ¹⁸F-fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT) for the BAT identification were used [20, 21, 22].

In addition to functional differences, BAT and WAT show also different morphological characteristics. BAT is characterized by abundant of iron content and vascularization, and is multilocular with small lipid droplets presented. In contrast, in WAT the iron depot and vascularization is very low and unilocular large lipid droplets are depicted [32]. These characteristics could lead to different histogram values during the examination. Thus, the histogram can distinguish different adipose tissue features.

Since, it was for the first time used by Lowitz [38] the ultrasound histogram is the most useful method widely used in the clinical practice to evaluate various tissue pathology and tissue structures. The information derived from the local histograms is used to depict the tissues, to partition the B-mode image into homogeneous zones of texture, and estimate to what extent tissue is different from another [23]. Therefore, the calculation of „the sonographic hepato-renal index” is used as the method for ultrasound image optimization [39, 40].

In our study, by utilisation of the ultrasound histogram for the EAT assessment, similar findings were found. We confirmed difference in histogram pattern (EAT/LA-value) between normal weight and overweight/obese adolescents. Although, the EAT/LA-value correlated with EAT thickness, the strongest independent predictor was the BMI. Therefore, we suggest that the EAT thickness measured by ultrasound reflects quantitatively the visceral adipose tissue and the EAT/LA represents changes in tissue structure characteristic in association with obesity. Therefore, it seems that higher EAT/LA-value in obese subjects could be due to the less amount of BAT and higher amount of WAT characterized by large lipid droplets and very low vascularization within the EAT.

Some investigators showed that lean patients exhibit greater BAT activity than obese subjects. Activation of BAT has been shown to be associated with changes in weight and adiposity in adults [8; 41; 42] and in children [35]. It is known that WAT can be changed to BAT during chronic cold exposure (browning or beiging process) [11], and recently it has been shown that BAT also can be transformed to WAT as a result of overnutrition [43]. These data could support the concept that BAT in epicardial adipose tissue of adolescents can be transformed to WAT in case of obesity or overweight.

Hu et al. [44], in MRI study, found that overweight and obese children have in the supraclavicular area significantly less total (functional and non-functional) BAT than lean, healthy children. There is a strong inverse relation between the MRI measures of BAT and body weight or BMI percentile in overweight and obese children [6].

In our present study, normal weight or lean adolescents had significantly lower MN (EAT) and EAT/LA-value. So, it seems that in the absence of obesity the different B-mode grey level obtained from histogram could be sign of other than WAT. Moreover, the EAT/LA-value showed on the negative correlation with adipocyte protective hormone adiponectin. It is presumed that lower EAT/LA-value of non-obese youngsters could be attributed to more BAT within the epicardial adipose tissue, however further studies are necessary to support this concept.

Data on correlation between the EAT and ALT, the biochemical marker of NAFLD, were published in previous study [45]. Our present study shows that the EAT/LA-value of the ultrasound histogram correlated well with laboratory parameters of cardiovascular risk factors including ALT as a marker of NAFLD. Although, obesity was the strongest predictor for EAT/LA value, further investigations are needed to reveal the relation between EAT characteristics and markers of NAFLD.

Limitation of study

In our study, we did not use invasive methods or reliable non-invasive methods such as MRI or CT to confirm what type of specific adipose tissue represented the different value in B-mode grey scale level. We also did not use other methods than biochemical markers for diagnosis of NAFLD.

Conclusion:-

Epicardial adipose tissue characteristics of normal weight adolescents differ significantly from features of overweight/obese youngsters. It is associated with obesity, parameters of cardiovascular risk factors and thickness of the epicardial adipose tissue, however, obesity is the strongest predictor. These findings suggest that the different histogram features of normal-weight and obese youngsters are attributed to different amount of brown and white adipose tissue of EAT in these groups and EAT characteristics are in relation with early markers of NAFLD. Further studies are needed to confirm these pathophysiological associations.

The ultrasound histogram of adipose tissue is non-invasive, low-cost and easy to perform imaging modality that can qualitatively make a distinction between different types of adipose tissue with intention to distinguish brown and white adipose tissue. These claims more studies to be performed for two purposes. First, is to identify and confirm what type of tissue of EAT or other visceral fat is equivalent to different B-mode grey level or EAT/LA-value in ultrasound histogram. Second is the standardization of this method for clinical practical use.

Conflict of interest

All the authors declare that there is no conflict of interest.

Ethical approval

“All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.”

Table 1:-Anthropometric and biochemical characteristics of the study groups

Parameters	Normal weight Mean \pm SD n=52	Overweight /obese Mean \pm SD n=18	p-value
Age (years)	17.74 \pm 1.16	17.67 \pm 1.34	ns
BW (kg)	61.84 \pm 9.59	85.16 \pm 16.33	<0.0001
Body height (cm)	172.64 \pm 9.99	173.55 \pm 10.33	ns
BMI (kg/m ²)	21.77 \pm 14.85	28.10 \pm 4.00	<0.0001
BMI percentile	40.18 \pm 24.00	94.83 \pm 4.00	<0.0001
Glucose (mmol L ⁻¹)	4.47 \pm 0.57	4.75 \pm 0.74	<0.05
Insulin (uIU/ml)	10.44 \pm 10.01	16.39 \pm 16.83	<0.001
HOMA-IR	2.13 \pm 2.35	3.77 \pm 4.79	<0.001
Total cholesterol (mmol L ⁻¹)	4.11 \pm 0.68	4.14 \pm 0.70	ns
TAG (mmol L ⁻¹)	0.88 \pm 0.46	1.07 \pm 0.67	<0.01
LDL cholesterol (mmol L ⁻¹)	2.35 \pm 0.50	2.49 \pm 0.58	ns
HDL cholesterol (mmol L ⁻¹)	1.57 \pm 0.04	1.28 \pm 0.07	<0.001
AST (ukat L ⁻¹)	0.38 \pm 0.01	0.44 \pm 0.034	ns
ALT (ukat L ⁻¹)	0.30 \pm 0.01	0.52 \pm 0.06	<0.01
Adiponectin (ng/ml)	8.99 \pm 0.63	6.12 \pm 0.57	<0.001
UA (umol L ⁻¹)	292.23 \pm 59.86	341.62 \pm 70.72	<0.0001
hsCRP (mg L ⁻¹)	0.79 \pm 0.80	1.63 \pm 1.59	<0.0001
EATs (mm)	2.21 \pm 0.06	3.89 \pm 0.32	<0.001
EATd (mm)	1.22 \pm 0.05	2.38 \pm 0.21	<0.001
EAT/LA-value	14.76 \pm 0.83	26.22 \pm 0.95	<0.001
MN(EAT)	31.93 \pm 7.4	42.00 \pm 5.00	<0.01
MN(LA)	16.00 \pm 5.57	15.24 \pm 8.1	ns

BW - body weight, BMI - body mass index, HOMA-IR - homeostasis model assessment of IR, TAG - triacylglyceride, LDL - low-density lipoprotein, HDL - high-density lipoprotein, AST - aspartate aminotransferases, ALT - alanine aminotransferases, UA - uric acid, hsCRP - high-sensitivity C-reactive protein (hsCRP), EATs - epicardial adipose tissue end-systole, EATd - epicardial adipose tissue end-diastole, EAT/LA-value - difference between the L-value of EAT and the L-value of the LA, MN - mean of all pixels' gradient included in each ROI, LA- left atrium

Table 2:-Correlation between the EAT/LA-value and anthropometric, biochemical parameters, EAT thickness and histogram values

Parameters	EAT/LA-value
BW (kg)	r=0.60 p<0.0001
Body height (cm)	r=0.30 p<0.01
BMI (kg/m ²)	r=0.57 p<0.0001
BMI percentile	r=0.72 p<0.0001
Glucose (mmol L ⁻¹)	ns
Insuline (uIU/ml)	ns
Homa-IR	ns
Total cholesterol (mmol L ⁻¹)	ns
TAG (mmol L ⁻¹)	ns

LDL-cholesterol (mmol L ⁻¹)	ns
HDL-cholesterol (mmol L ⁻¹)	ns
AST (ukat L ⁻¹)	r=0.26 p=0.05
ALT (ukat L ⁻¹)	r=0.38 p<0.001
Adiponectin (ng/ml)	r= -0.33 p<0.01
UA (umol L ⁻¹)	r=0.20 p<0.08
hsCRP (mg L ⁻¹)	r=0.24 p<0.05
EATs (mm)	r=0.46 p<0.0001
EATd (mm)	r=0.43 p<0.0001
MN(EAT)	r=0.62 p<0.0001
MN(LA)	r= -0.28 p<0.05

BW - body weight, BMI - body mass index, HOMA-IR - homeostasis model assessment of IR, TAG - triacylglyceride, LDL - low-density lipoprotein, HDL - high-density lipoprotein, AST - aspartate aminotransferases, ALT - alanine aminotransferases, UA – uric acid, hsCRP - high-sensitivity C-reactive protein (hsCRP), EATs - epicardial adipose tissue end-systole, EATd - epicardial adipose tissue end-diastole, EAT/LA-value - difference between the L-value of EAT and the L-value of the LA, MN - mean of all pixels' gradient included in each ROI, LA- left atrium

Table 3:-Multiple linear regression analysis of the EAT/LA-value in Model 1 (Crude effect), and Model 2 (adjusted for BMI percentile)

	Model 1 (Crude effect)	Model 2 (adjusted for BMI percentile)
	B (95% CI)	B (95% CI)
ALT (ukat L⁻¹)	9.23 (0.20, 18.26)*	2.71 (-4.86, 10.28)
EATs (mm)	2.52 (0.94, 4.11)**	-0.09 (-1.62, 1.44)
BMI percentile		0.16 (0.10, 0.21)***
R² / Adjusted R²	0.26/0.24	0.53/0.51

* p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001

ALT - alanine aminotransferases, EATs - epicardial adipose tissue end-systole, BMI - body mass index, EAT/LA-value - difference between the L-value of EAT and the L-value of the LA

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