PROSPERITAS VESTRA FINIS NOSTRA!

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# QUANTITATIVE MEASUREMENT OF EPICARDIAL ADIPOSE TISSUE AND CORRELATION WITH OTHER MARKERS FOR INCREASED CARDIOVASCULAR AND METABOLIC RISK IN PATIENTS WITH LONG-TERM DIABETES MELLITUS TYPE 1

**Thesis summary**

for the award of a doctoral degree

**SCIENTIFIC SPECIALTY:**

Medical radiology and radiology (including use of radioactive isotopes)

**Research supervisor: Prof. Boyan Balev, MD, PhD**

Varna 2022

# I. INTRODUCTION

Diabetes mellitus (DM) is one of the most common metabolic diseases and is characterized by impaired carbohydrates, protein and lipid metabolism. In recent years, diabetes incidence has been gradually increasing, becoming a serious threat to public health. Increased accumulation of visceral adipose tissue (VAT) is a risk factor for insulin resistance, which may reduce insulin sensitivity, increase the expression and secretion of anti-inflammatory cytokines in adipose tissue and trigger development of DM and cardiovascular disease (CVD).

In the present study we aim to examine EAT imaging methods, EAT role of as biomarker and its clinical significance as factor in increased cardiovascular risk in correlation with other known risk factors.

# II. TASKS

To achieve the aim of the dissertation, we set ourselves the following tasks:

1. To determine whether there is a statistically significant correlation between EAT measured by CT and MRI, patient lipid profile and BMI measured by DEXA.

2. To correlate EAT with inflammatory cytokines (IL1, IL6 and TNF-α) in order to assess cardiovascular risk in both groups of patients.

3. To determine whether there is a statistically significant correlation between EAT measured by CT and MRI and WC of patients in both groups.

4. To compare tomographic quantification accuracy of EAT by CT and MRI.

5. To determine whether there is a statistically strong correlation between EAT measured by CT and MRI and VAT of patients from both cohorts, measured with DEXA.

6. To determine whether there is a statistically significant correlation between EAT measured by CT and MRI and diabetes duration.

7. To develop an algorithm for estimating EAT volume by semi-automatic and manual segmentation.

# III. PARTICIPANS AND METHODS

The dissertation was prepared as part of the research project ”Cardiovascular and metabolitic risk associated with visceral adipose tissue in patients with type 1 diabetes mellitus”, supported by research Fund of the Ministry of Education and Sciensce (contract DN 13/3 от 14.12.2017) and is a part of sciensce project “ Changes the amount of visceral fat and its attitude to increased cardiovascular risk in patients with long-term type 1 diabetes”, supported by the research Fund at MU- Varna with contract 17022/2017.

The clinical trial was conducted after obtaining permission from the Research Ethics Commission at Medical University – Varna, protocol/decision №72, meeting on March 1st, 2018. All participants have signed an informed consent.

A total of 183 participants were studied, of which 124 with type 1 diabetes mellitus (T1DM) duration of at least 15 years, and 59 healthy controls without known cardiovascular and metabolic diseases. They were aligned by sex, age and BMI. It should be emphasized that, unlike most similar studies in international literature, the T1DM patients we studied were characterized by poor disease control. Study parameters were pre-defined and prospectively collected.

The study was conducted from June 2018 to December 2021 at the Clinic of Imaging, Interventional Radiology and Radiotherapy at the Saint Marina University Hospital, Varna.

Imaging, laboratory and anthropometric and demographic studies were conducted to evaluate EAT and search for clinically significant correlations. Information on diabetes duration and glycemic control was collected.

Medical history was taken from patient's data and accompanying documentation. The information was filled in a specially designed questionnaire, identical for people without and with diabetes. Appendix 1: Declaration of informed consent for measuring bone density and VAT with an X-ray machine in the Department of Imaging, Interventional Radiology and Radiotherapy at the Saint Marina University Hospital; Appendix 2: Declaration for informed consent for conducting MRI at the Saint Marina University Hospital; Appendix 3: Declaration of informed consent for conducting MRI at the Department of Imaging Diagnostics at the Saint Marina University Hospital.

Following anthropometric data were measured by standard methods for each participant in the study: body weight (with 0.5 kg accuracy), height (0.5 cm accuracy), WC at the iliac crest (up to 0.5 cm). BMI was calculated in kg/m2. The participants were divided into three BMI groups: normal (<25 kg/m2), overweight (25-29 kg/m2) and obese (>30 kg/m2). Information about demographic indicators such as: sex, age, education, social status, duration and method of treatment for T1DM patients, etc. was also collected.

## 1. CT Scan

To determine EAT volume and calcium score, we used native images from CT scans, performed on a dual-source multidetector Somatom Definition 64, Siemens Healthineers, Erlangen, Germany. The scan was native, without premedication, prospectively synchronized with ECG in diastole. A low-dose factory protocol was used to determine calcium score: 2 x 64 x 0.625 mm collimation, 120 kV tube voltage, 24-80 mA automatic amperage. Raw data were reconstructed in axial series with soft tissue kernel (B35f), with a slice thickness of 1 mm and 3 mm, respectively. CACS (Agatston score) was measured semi-automatically with specialized software on a Syngo workstation, Siemens Healthineers, Erlangen, Germany (fig.1).

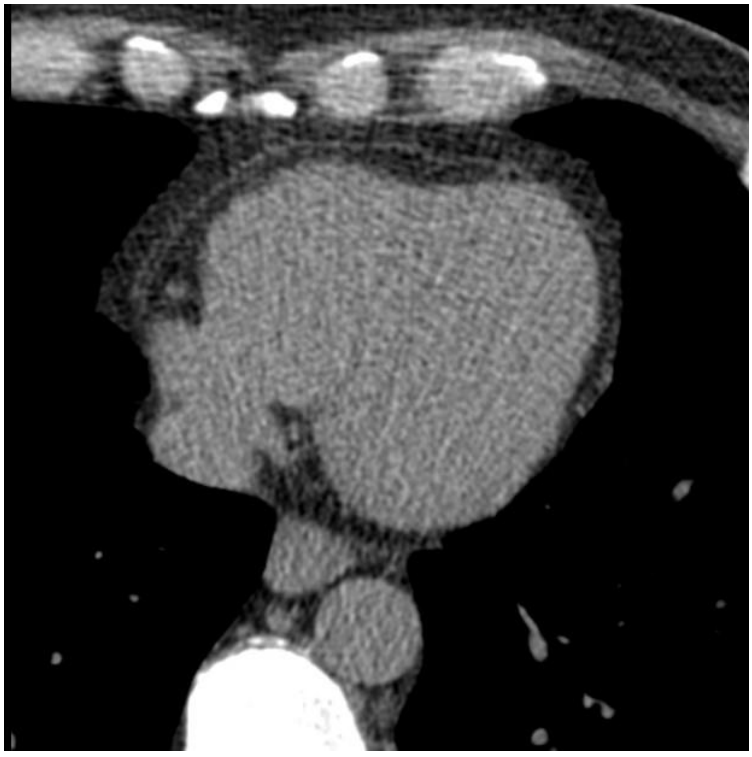


**Figure 1**: Non contrast cardiac CT imaging.

## 2. MRI

EAT was also evaluated in parallel by MRI, performed on a Magnetom Verio, Siemens Healthineers, Erlangen, Germany, field strength 3 T, without premedication. Using a specially modified turbo spin echo sequence we derived axial native series of images of the heart, loaded mainly at relaxation time T1 (TR 1200 ms, TE 30 ms). The sequence was performed with several breath-hold commands and prospective ECG synchronization. Obtained images were axial, with a slice thickness of 3 mm, without a distance between slices (fig,2 and fig.3).

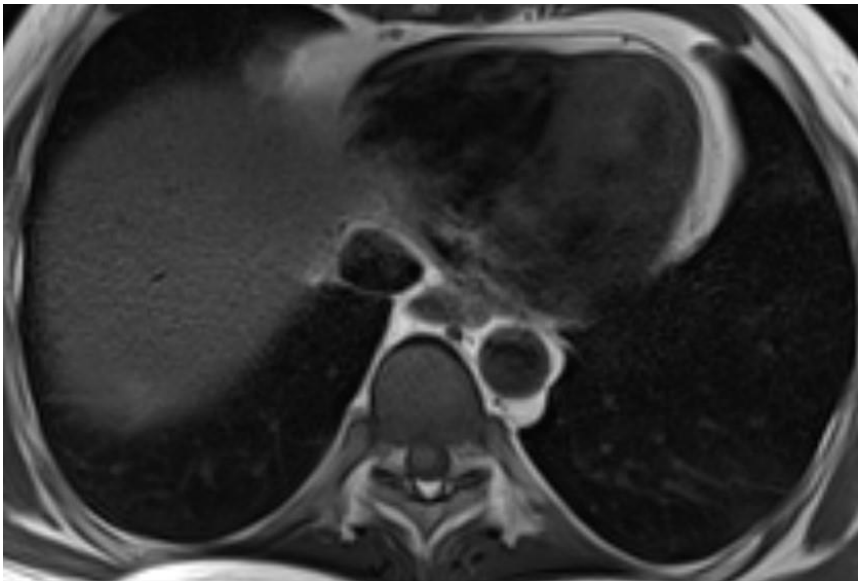


**Figure 2**: Cardiac MRT- axial series (T1- turbo spin echo sequence.

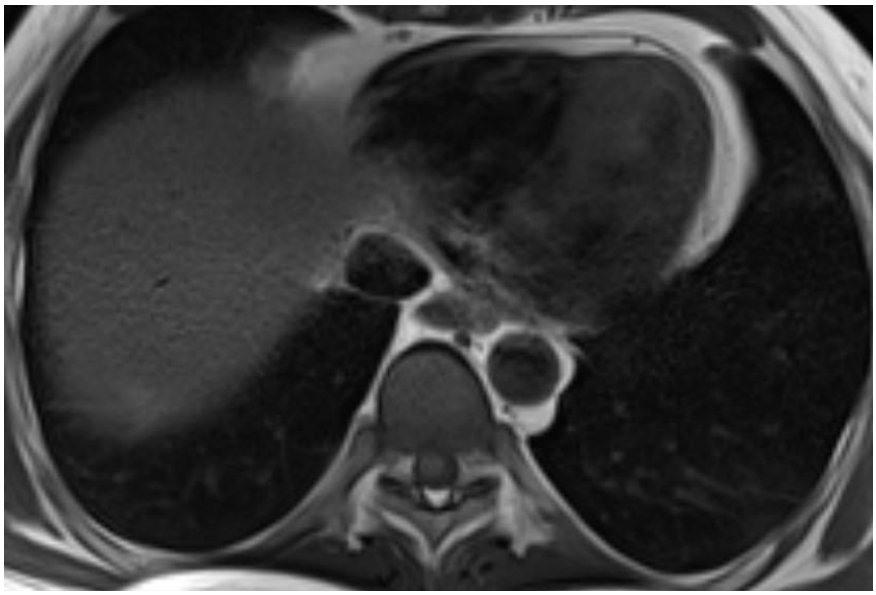


**Figure 3:** Cardiac MRT – T1 HASTE (Half- Fourier Acquisition Single-shot Turbo spin Echo imaging)

EAT volume was calculated on an offline workstation with 3D Slicer, version 4.1.0 (slicer.org). It is an open source software and allows semi-automatic and manual segmentation. We chose a threshold range for EAT between -200 and -30 HU for the semi-automatic segmentation of CT images. All voxels in this range were automatically selected by 3D Slicer. Selected voxels that do not belong to EAT were removed from the selected volume manually.

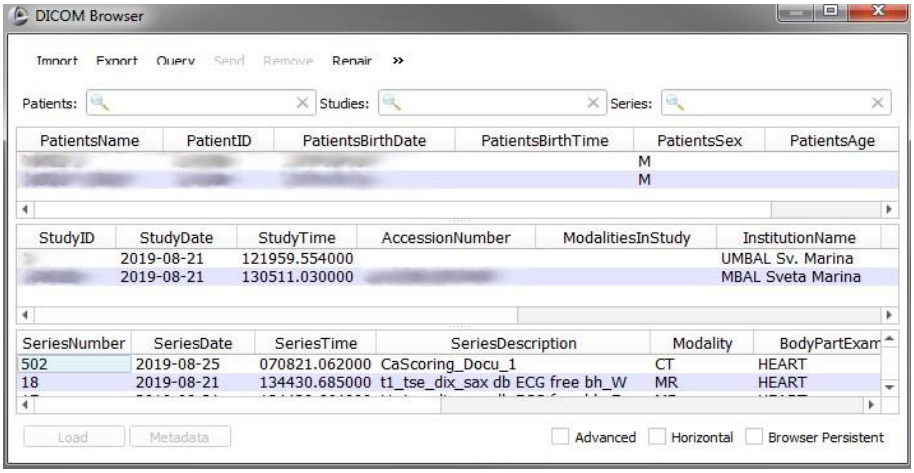



**Figure 4.** Loading DICOM data for processing. There is a list of available imaging examinations, loaded in memory and ready for processing.

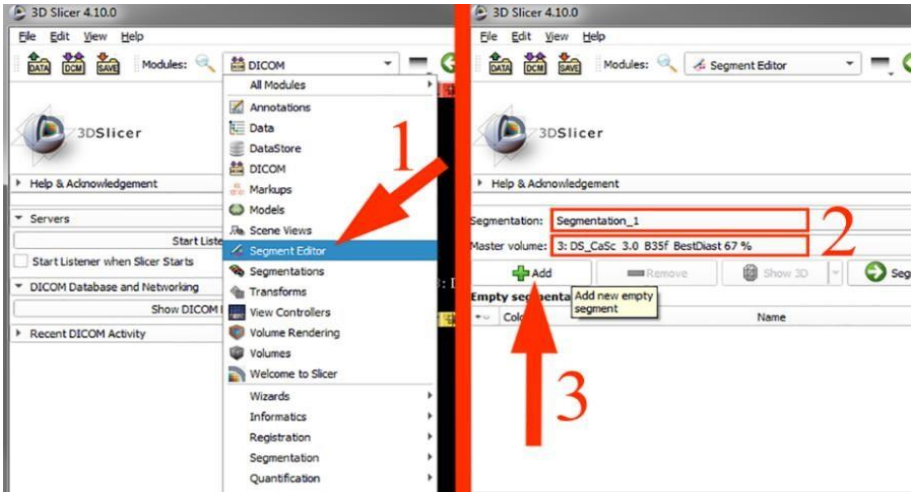



**Figure 5.** Preparation of loaded DICOM images for segmentation. Step 1: from the drop-down menu select the “Segment Editor” module. Step 2: input a name for the segmentation and select the series of images for processing. Step 3: create a new sample volume using the “+Add” button.

EAT thickness was measured over the free edge of the right ventricle, over the right coronary artery, and over the left coronary artery before it branches into left circumflex artery (LCX) and left anterior descending (LAD).

The settings of the selected range are shown in Figure 6. All voxels in this range are automatically selected by 3D Slicer (Figure 7). Selected voxels not belonging to EAT, as well as fat-equivalent voxels above the level of the aortic root, were deleted from selected volume manually with the “Erase” tool (Figure 8). This is a “brush”-like tool of different sizes, which applies its effect as a round stamp on a given slice, eliminating the selection in the stamped area. It is possible to configure the "brush" to apply its effect as a three-dimensional sphere (on several adjacent slices), instead of a two-dimensional circle.

If manual edits need to be adjusted, a similar “Paint” tool or a polygonal “Draw” tool for delineating the area can be used (Fiure 9). It is possible to go back a few steps using standard “Ctrl + Z/Y” (Undo/Redo) key combinations. The finalized selection is quantified automatically by 3D Slicer in cm3 (Figure 8). The software automatically multiplies the area by the slice thickness and sums the volumes of all processed slices to determine the final volume.

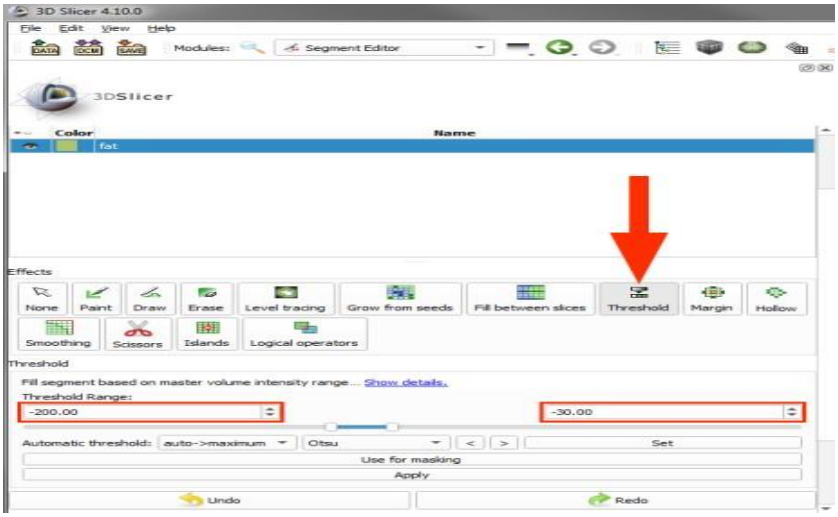



**Figure 6**. Setting the range for automatic selection: the new sample volume is created (marked in the blue bar). After its creation, the “Threshold” button is selected in the same window below (indicated by a red arrow) and the range limits are set in the boxes marked in red (in this case in HU).

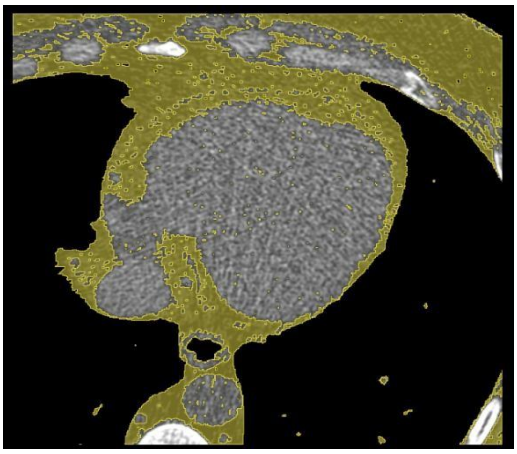


**Figure 7**. Automatic segmentation: after setting the range limits and selecting the "Apply" button, the entire volume of voxels, having density within the range, is selected. The selected voxels are marked in yellow (color and transparency can be manually adjusted, according to individual preferences).

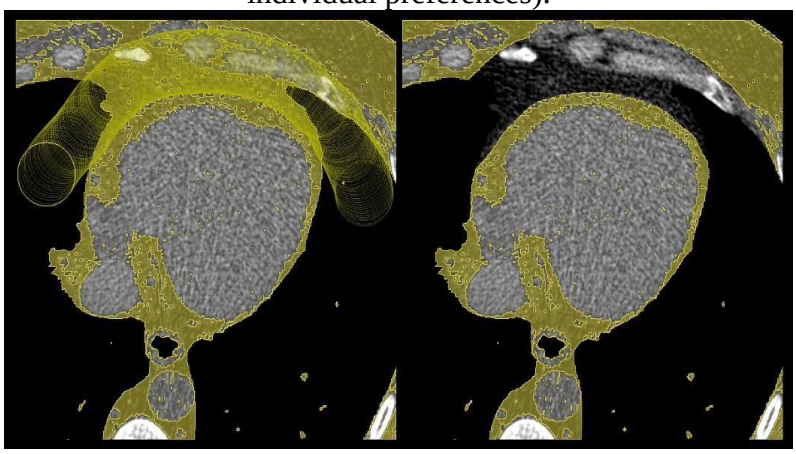


**Figure 8.** Deletion of unwanted parts of the selected volume with the “Erase” tool. The path of applying the "brush" is shown on the left. The result of its application is shown on the right.

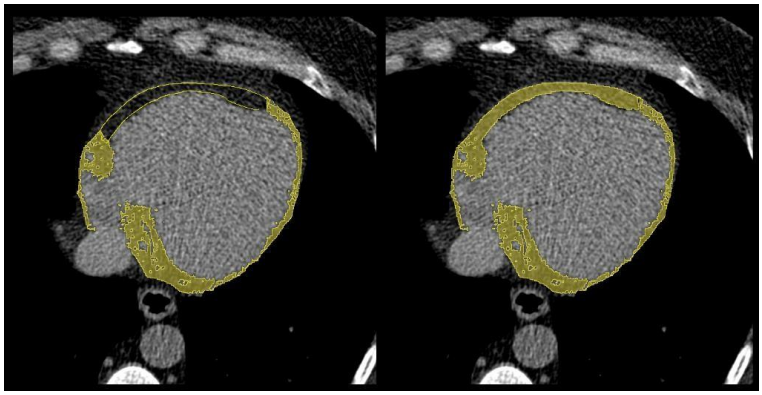


**Figure 9.** Recovering an incorrectly deleted part of the selection using the “Draw” tool. The desired area is outlined, either freely, holding the left mouse button or with a polygonal figure bounded by single-click anchor points, the outline is shown in the left image. Marking the area ends by pressing "Enter". On the right is the final result, a section in which all fat-equivalent voxels outside the area of interest are manually deleted.

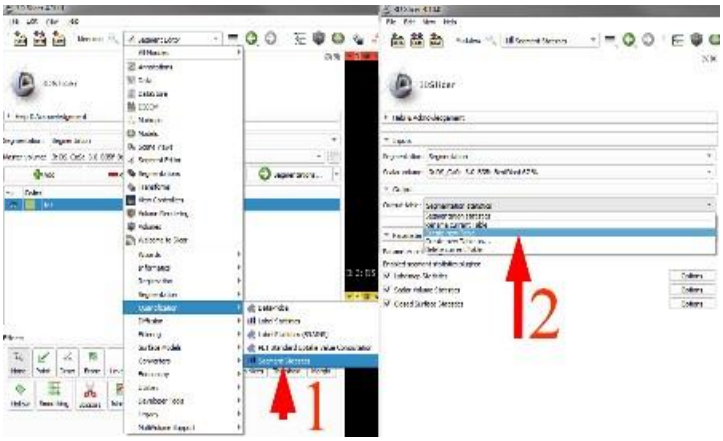



**Figure 10.** Sequence of commands for automatic calculation of selected volume. Step 1: from the drop-down menu select "Quantification", then "Segment Statistics". Step 2: in the new window create a new table and click "Apply", this generates a table with the necessary calculations.

Semi-automatic range selection cannot be used for segmentation of MRI images due to the subjective nature of the relaxation time data for T1-loaded images. Quantitative T1 maps, yet to come into widespread use, would be more suitable for this purpose. Segmentation of standard T1-loaded images is done manually, again in 3D Slicer, with the operator delineating EAT area of each slice using the Draw tool in a manner analogous to that described in Figure 7 for CT. The final selected volume is quantified by the program in cm3.

All measurements were performed by a team of three imaging specialists and each individual patient was measured at least by two. An excellent degree of agreement was established between interpreters. In rare cases of significant discrepancies (over 10% of the higher score), an additional team member was included in the arbitration.

Image processing (CT and MRI series) in 3D Slicer 4.10.1 takes approximately 40 minutes for radiologists with routine segmentation experience (treated at least 30 patients) and approximately one hour on the first attempt. Work is accelerated by using a graphics tablet as a workstation peripheral.

## 3. DEXA

All 183 patients underwent whole body DEXA examination to determine bone density, total and regional adipose tissue with a Lunar Prodigy machine (GE Healthcare, Madison, WI USA) by a certified X-ray technician. Prior to each examination, the instrument was calibrated according to manufacturer's instructions. For each examination, the software automatically locates the area between the upper edge of the second lumbar vertebra and lower edge of the

fourth lumbar vertebra, through which it measures the cross-sectional area. It measures directly total and subcutaneous adipose tissue and reports VAT content as the difference between these measurements.

## 4. Laboratory analyses

Venipuncture of a 5 ml blood sample for serum isolation was taken from the cubital vein in the morning on an empty stomach from all patients on the day of the imaging examination. All samples were processed in the Clinical Laboratory at Saint Marina Univesity Hospital, Varna, routine hematological and biochemical analysis was performed, including complete blood count (CBC), lipid profile (total cholesterol, LDL and HDL) and other biochemical indicators.

## 5. Statistical methods

The specialized statistical package IBM SPSS v.25 was used for data processing. MS Excel was used for presenting the obtained results in tabular and graphical form. In some cases we grouped patients for the purposes of statistical processing.

### 5.1.1. Pearson correlation coefficient test

It is a basic statistical method for evaluating hypotheses related to the statistical distribution itself. The test is used to evaluate results of studies in cases where it is not necessary to know the absolute magnitude of the trait itself and the size of the relationship, but is required to confirm whether the influence of the studied factor is significant or accidental.

### 5.1.2. Spearman's rank correlation coefficient analysis

Spearman's rank correlation coefficient analysis is used to assess the relationship between analyzed indicators. Estimation of the strength of the dependence between the variables is based on the results of the Spearman coefficient (rho $\leq 0.05$).

### 5.1.3. Comparative analysis

Comparative analysis was used for the assessment of the hypotheses of diabetic groups and controls. Both Independent t-tests were used, which are parametric and based on a comparison of arithmetic means between measurements between groups. Mann–Whitney U test compares mean values taking into account the nonparametric distribution of data.

### 5.1.4. Nonparametric tests

Fisher’s exact test is a non-parametric test and was used to determine the degree of association between compared groups by category.

### 5.1.5. Chi-squared test

Chi-squared test is a nonparametric test that was used to show the differences between groups based on category characteristics.

### 5.1.6. Descriptive statistics

It based on frequency analysis, including absolute and percentage data, averages (arithmetic mean, median, standard deviations, standard error). The obtained results were displayed in the form of a grapfic or table.

# IV. RESULTS

A total of 183 participants were studied, divided into two groups: 124 with T1DM and 59 healthy controls, corresponding in age and sex. Mean age of all participants was 43.47 ± 10.06 years, 95% CI: 42-44.94 years, rank: 19-67 years, median: 45 years IQR: 37-51 years. T1DM: 42.68 ± 10.4 years, median: 43 years; controls: 45.14 ± 9.17 years, mean difference between the two groups: 2.45 years; p=0.11 (t-test). Sex distribution: 54.1% men and 45.9% women. In patients with T1DM: 53.2% vs. 46.8%, in controls 55.9% vs. 44.1% (chi-square 0.73, Fisher's exact test 0.75). Mean duration of diabetes: 25.31 ± 8.22 years, 95% CI: 23.85-26.78, rank: 11-58 years, median: 24 years.

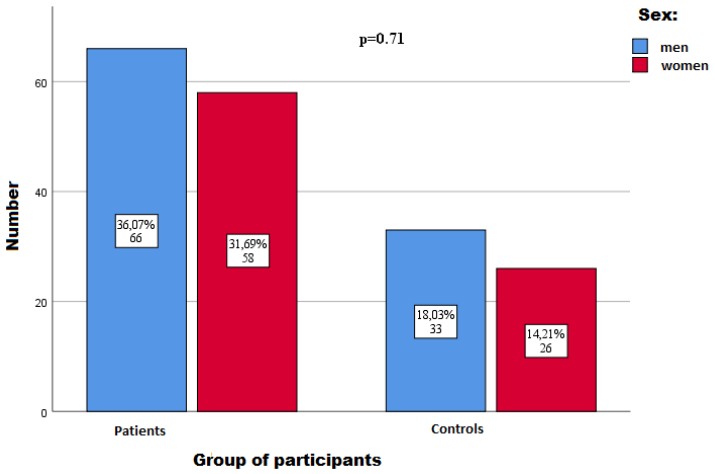



**Figure 11**. Distribution of participants by number and sex for both groups.

## 1. To determine whether there is a statistically significant correlation between EAT measured by CT and MRI and patient lipid profile and BMI measured by DEXA

We analyzed 183 patients and divided them into two groups: a group of diabetics with T1DM and healthy controls. Each of the two groups was divided by sex into men and women. BMI is used to determine normal weight in people of different heights, assessing obesity and malnutrition. BMI is measured in kilograms per square meter and is calculated using the following formula:

BMI = KG/M2

Where:

- BMI - Body Mass Index
- KG - Weight in kilograms

- M2 - Height in square meters

## 1.1. GROUP OF DIABETICS

A total of 123 patients were studied in the group of diabetics, 57 women and 66 men. All participants were divided into three groups according to BMI: BMI<25, BMI 25-30 and BMI> 30.

**Table 1**. Patients with T1DN. BMI - body mass index. MR/CT Vol – EAT volume measured by CT and MRI. r - Pearson correlation coefficient.

| Sex | BMI | Number | MR/CT Vol – r | MR/CT Vol – p |
|---|---|---|---|---|
| **Women** | <25 | 31 | 0.960 | <0.0001 |
| | 25-30 | 18 | 0.964 | <0.0001 |
| | >30 | 8 | 0.930 | 0.007 |
| **Men** | <25 | 32 | 0.988 | <0.0001 |
| | 25-30 | 25 | 0.991 | <0.0001 |
| | >30 | 9 | 0.878 | 0.004 |

### 1.1.1. Women with diabetes

In this group, we studied 31 women with T1DM and BMI <25. No statistically significant correlation was found between EFVCT / EFVMRI and BMI, but we established another statistically slight correlation, with a tendency toward statistical significance, between EFVCT Vol (cm3) and LDL-cholesterol levels (r = 0.324; p = 0.076) and HDL-cholesterol (r = 0.343; p = 0.059). There was a statistically significant strong correlation between MRI Vol (cm3) and total cholesterol levels (r = 0.538; p = 0.002) and a moderate correlation with LDL cholesterol levels (r = 0.420; p = 0.021). There was a statistically significant moderate correlation between total cholesterol and HDL cholesterol levels (r = 0.383; p = 0.031) and total cholesterol and LDL cholesterol (r = 0.930; p = 0.000).

**Table 2**. Pearson test to investigate the correlations between lipid profile and EAT volume measured by CT and MRI in diabetic women with BMI <25.

| indicators | EFVCT Vol cm3 | EFVMRI Vol cm3 | Total cholesterol |
|---|---|---|---|
| **HDL cholesterol** | r = 0.343; p = 0.059 | - | r = 0.383; p = 0.031 |
| **LDL cholesterol** | r = 0.324; p = 0.076 | r = 0.420; p = 0.021 | r = 0.930; p = 0.000 |
| **Total cholesterol** | - | r = 0.538; p = 0.002 | - |

In overweight women with T1DM(n = 18), analysis of the results showed a statistically significant strong correlation between EFVCT and BMI (r = 0.543; p = 0.024), as well as a statistically significant strong correlation between EFVMRI and BMI (r = 0.610 ; p = 0.007). In addition, we found a statistically significant correlation between EAT and lipid parameters: EFVCT and LDL-cholesterol (r = 0.655; p = 0.003), EFVCT and HDL-cholesterol (r = 0.559; p = 0.016), EFVMRI and LDL -cholesterol, (r = 0.614; p = 0.009) and EFVMRI and HDL-cholesterol (r = 0.526; p = 0.030).

**Table 3**. Pearson test to investigate the correlations between lipid profile and EAT volume measured by CT and MRI in women with T1DM and BMI 25-30.

| Показатели | EFVCT Volсм3 | EFVMRI Volсм3 |
|---|---|---|
| **BMI 25-30** | r = 0.543; p = 0.024 | r = 0.610; p = 0.007 |
| **LDL cholesterol** | r = 0.655; p = 0.003 | r = 0.614; p = 0.009 |
| **HDL cholesterol** | r = 0.559; p = 0.016 | r = 0.526; p = 0.030 |

Analysis of the results in women with T1DM and obesity did not show a strong correlation between EFVCT / EFVMR and BMI. Boundary correlations were found between BMI and LDL-cholesterol levels (r = 0.643; p = 0.086), as well as between EFVMRI and total cholesterol (r = 0.946; p = 0.117) and between EFVMRI and LDL-cholesterol (r = 0.652; p = 0.160).

**Table 4.** Pearson test to investigate the correlations between lipid profile and EAT volume measured by CT and MRI in women with T1DM and BMI >30.

| indicators | ***BMI >30*** | ***EFV MRI Vol cm3*** |
|---|---|---|
| **LDL cholesterol** | r = 0.643; p = 0.086 | r = 0.652; p = 0.160 |
| **Total cholesterol** | - | r = 0.946; p = 0.117 |

### 1.1.2. Men with diabetes

In the first subgroup we studied 32 men with T1DM and BMI <25. We found a strong correlation between BMI and EAT volume measured by CT (CT Vol cm3) (r = 0.586; p = 0.000), as well as statistically significant correlation between EFVMRI and BMI (r = 0.546; p = 0.002). In addition to total EAT volume, a strong correlation was found between BMI and adipose tissue volume in front of the right ventricle measured by CT (CT RVmm) (r = 0.399; p = 0.024) and MRI (MRI RVmm) = 0.430; p = 0.018). No statistically significant correlation with lipid parameters was found in this subgroup of patients.

**Table 5**. Pearson test to investigate the correlations between lipid profile and EAT volume measured by CT and MRI in diabetic men with BMI <25.

| indicators | CT Vol (cm3) | MRI Vol (cm3) | CT RV mm | MRI RV mm |
|---|---|---|---|---|
| **BMI<25** | r = 0.586; p = 0.000 | r = 0.546; p = 0.002 | r = 0.399; p = 0.024 | r = 0.430; p = 0.018 |

The second subgroup included 25 patients with T1DM and BMI 25-30. Analysis of the results showed a statistically significant strong correlation between EFVCT and BMI (r = 0.482; p = 0.015), between EFVMRI and BMI (r = 0.471; p = 0.023), and between EFVCT RV and BMI r = 0.261; p = 0.208). From the lipid profile, a statistically significant correlation was found between LDL cholesterol and total EAT volume measured with the two modalities, EFVCT (r = 0.469; p = 0.018) and EFVMRI (r = 0.444; p = 0.034).

**Table 6**. Pearson test to investigate the correlations between lipid profile and EAT volume measured by CT and MRI in diabetic men with BMI 25-30.

| indicators | EFVCT Vol cm3 | EFVMR Vol cm3 | EFVCT RVmm |
|---|---|---|---|
| **BMI 25-30** | r = 0.482; p = 0.015 | r = 0.471; p = 0.023 | r = 0.261; p = 0.208 |
| **LDL cholesterol** | r = 0.469; p = 0.018 | r = 0.444; p = 0.034 | - |

The third subgroup included only 9 male diabetics with T1DM and BMI over 30. Analysis of the results did not show a strong correlation between EFVCT / EFVMRI and BMI, as well as between EAT volume and lipid profile of the patient. The only statistically

significant correlation found was between LDL cholesterol and total cholesterol (r = 0.593; p = 0.092).

## 1.2. GROUP OF HEALTHY CONTROLS

In comparison with T1DM, the control group was twice smaller in number, but we selected patients with the same anthropometric data in terms of sex, age and body weight.

**Table 7**. Control group of healthy patients. BMI - body mass index. MR/CT Vol – EAT volume measured by CT and MRI. r - Pearson correlation coefficient.

| Sex | BMI group | Number | MR/CT Vol – r | MR/CT Vol – p |
|---|---|---|---|---|
| **Women** | <25 | 20 | 0.940 | <0.0001 |
| | 25-30 | 0 | - | - |
| | >30 | 5 | 0.928 | 0.023 |
| **Men** | <25 | 7 | 0.986 | <0.0001 |
| | 25-30 | 11 | 0.970 | <0.0001 |
| | >30 | 15 | 0.710 | 0.007 |

### 1.2.1. Healthy controls, men

Analysis of the results in men without DM and with BMI <25 did not establish a correlation between EFVCT / EFVMRI and BMI, but found a statistically significant strong correlation between EFVMRI and total cholesterol levels (r = 0.764; p = 0.046), between total cholesterol and those of LDL-cholesterol (r = 0.946; p = 0.001). There was a good correlation between EFVCT and total cholesterol levels with a tendency to statistical significance (r = 0.697; p = 0.082), as well as between EFVMRI and LDL cholesterol levels with a tendency to statistical significance (r = 0.728; p = 0.064).

**Table 8**: Pearson test to investigate the correlations between lipid profile and EAT volume measured by CT and MRI in healthy men with BMI <25.

| indicators | EFVCT Vol см3 | EFVMRI Vol см3 |
|---|---|---|
| **LDL cholesterol** | - | r = 0.728; p = 0.064 |
| **Total cholesterol** | r = 0.697; p = 0.082 | r = 0.764; p = 0.046 |

In men with BMI 25-30, there was only a statistically significant strong correlation between EFVMRI and BMI (r = 0.625; p = 0.040) and between EFVCT and BMI (r = 0.581;

p = 0.061) No statistically significant correlation with lipid parameters was found in this subgroup of patients.

**Table 9.** Pearson test to investigate the correlations between lipid profile and EAT volume measured by CT and MRI in healthy men with BMI 25-30.

| indicators | EFVCT Vol cm3 | EFVMR Vol cm3 |
|---|---|---|
| **BMI 25-30** | r = 0.581; p = 0.061 | (r = 0.625; p = 0.040 |

In the subgroup of men with BMI> 30, statistical significance was found between EFVCT and total cholesterol levels (r = 0.651; p = 0.019) and between EFVCT and BMI (r = 0.648; p = 0.012).

**Table 10**: Pearson test to investigate the correlations between lipid profile and EAT volume measured by CT and MRI in healthy men with BMI >30.

| indicators | BMI>30 | Total cholesterol |
|---|---|---|
| **EFVCT Vol cm3** | r = 0.648; p = 0.012 | r = 0.651; p = 0.019 |

### 1.2.2. Healthy controls, women

The first subgroup included 20 women. A statistically strong dependence in women with BMI <25 controls was found between EFVCT and total cholesterol levels (r = 0.467; p = 0.038) and between EFVCT and LDL-cholesterol levels (r = 0.541; p = 0.014). Statistically strong dependence was found between EFVMRI and total cholesterol levels (r = 0.502; p = 0.024) and between EFVMRI and LDL-cholesterol levels (r = 0.570; p = 0.009).

**Table 11**. Pearson test to investigate the correlations between lipid profile and EAT volume measured by CT and MRI in healthy men with BMI <25.

| indicators | EFVCT Vol cm3 | EFVMRI Vol cm3 |
|---|---|---|
| **LDL cholesterol** | r = 0.541; p = 0.014 | r = 0.570; p = 0.009 |
| **Total cholesterol** | r = 0.467; p = 0.038 | r = 0.502; p = 0.024 |

There were no women controls with BMI 25-30 in the second subgroup.

There were only 5 participants in the third subgroup, and no statistically significant correlation was found between EAT volume, lipid parameters and BMI.

## 2. Correlation of EAT with inflammatory cytokines (IL2, IL6 and TNF-α) to assess cardiovascular risk in both groups of patients

High levels of circulating cytokines such as IL-1, IL-6 and TNF-α have been shown over time to be responsible for the development of CAD and increased risk of cardiovascular disease. We set ourselves the task of correlating EAT volume measured by CT and MRI with the levels of established cytokines in patients with T1DM and healthy controls.

**Table 12**. Independent Sample T-Test statistical analysis of the studied cytokines in patients with T1DM.

| | | Statistic | df | p |
|---|---|---|---|---|
| IL_6_R | Student's t | -0.420 | 62.0 | 0.676 |
| IL_6 | Student's t | -4.191 | 107.0 | < .001 |
| IL_1RL_1 | Student's t | 0.598 | 104.0 | 0.551 |
| TNFA | Student's t | 0.660 | 122.0 | 0.510 |

[a] Levene's test is significant (p <.05), suggesting a violation of the assumption of equal variances

**Table 13.** Independent Sample T-Test statistical analysis of the studied cytokines in healthy controls.

| | | Statistic | df | p |
|---|---|---|---|---|
| IL_6_R | Student's t | -0.0867 | 21.0 | 0.932 |
| IL_6 | Student's t | -2.5693 | 50.0 | 0.013 |
| IL_1RL_1 | Student's t | -0.4109 | 48.0 | 0.683 |
| TNFA | Student's t | 0.3614 | 57.0 | 0.719 |

[a] Levene's test is significant (p < 0.05), suggesting a violation of the assumption of equal variances

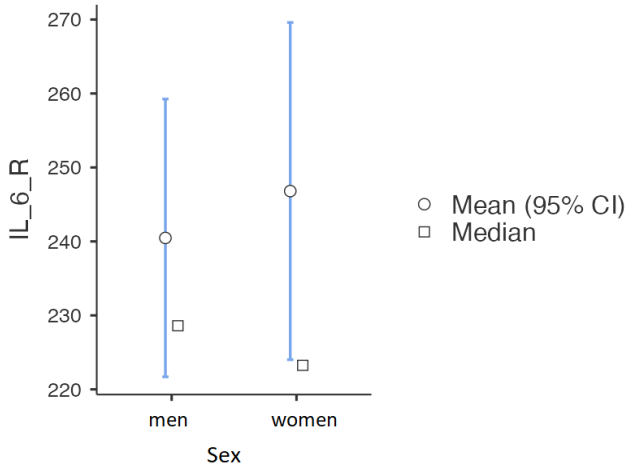
## 2.1. GROUP OF DIABETICS

We divided the group of diabetics (123 patients) by gender, and we found that increased values of inflammatory mediators are not present in all patients. IL-6-R ng / ml was detected in the least number of patients at least (32 women and 32 men,) IL-6 in 52 women and 57 men, IL-1-RL-1 in 50 women and 56 men, while the amount of TNF-α was found in the largest number of patients (58 women and 66 men).

**Table 14**: Group Descriptives

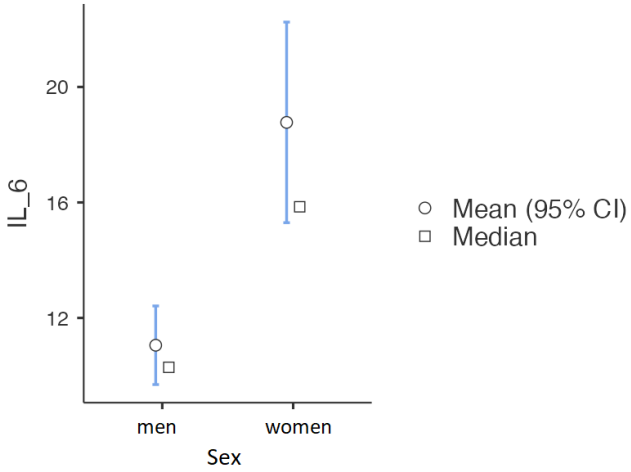
| | Group | N | Mean | Median | SD | SE |
|---|---|---|---|---|---|---|
| IL_6_R | мъж | 32 | 240.5 | 228.60 | 54.19 | 9.580 |
| | жена | 32 | 246.8 | 223.3 | 65.8 | 11.63 |
| IL_6 | мъж | 57 | 11.1 | 10.29 | 5.24 | 0.694 |
| | жена | 52 | 18.8 | 15.8 | 12.8 | 1.77 |
| IL_1RL_1 | мъж | 56 | 693.1 | 59.70 | 1059.96 | 141.643 |
| | жена | 50 | 581.5 | 51.8 | 832.2 | 117.69 |
| TNFA | мъж | 66 | 25766.1 | 8.27 | 44059.35 | 5423.331 |
| | жена | 58 | 20703.6 | 15.6 | 40854.2 | 5364.42 |

In patients with diabetes, there was a statistically strong relationship between IL-6-R ng / ml and EAT thickness around the left common carotid artery measured by CT (CT LM) ($r = 0.380$; $p = 0.002$) and a strong relationship between IL -6-R ng / ml and EAT thickness around the left common carotid artery measured by MRI (MRI LM) ($r = 0.384$; $p = 0.003$). A statistically strong correlation was found between the values of IL-6-R ng / ml receptor and TNF-α ($r = -0.290$; $p = 0.020$).



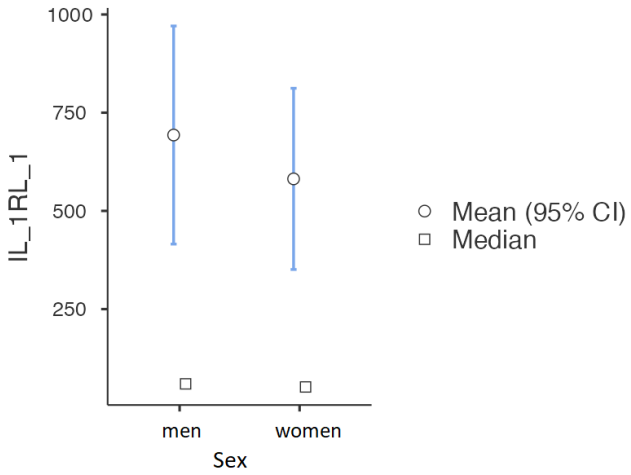

**Graph. 1**: Relationship between IL-6-R in man and woman.

There was also a statistically strong relationship between IL-6 pg / ml and total EAT volume measured by CT (CT Vol cm3) (r = -0.236; p = 0.014) and MRI (MRI Vol cm3) (r = -0.251 ; p = 0.012), as well as between IL-6 pg / ml and the amount of right ventricular EAT measured by CT RV (r = -0.206; p = 0.032) and MRI (MRI RV) (r = - 0.245; p = 0.014).



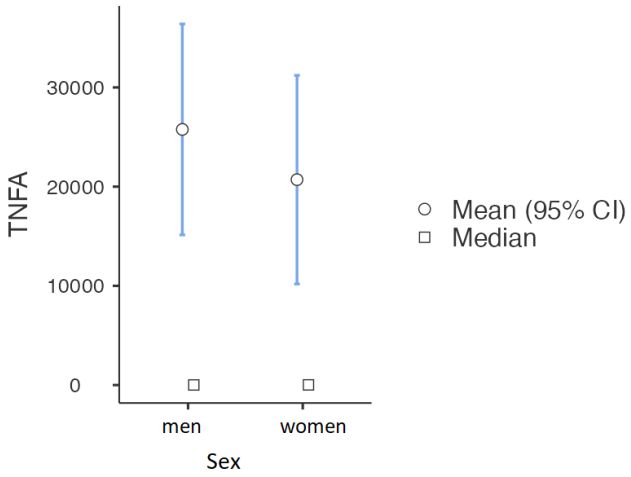

**Graph. 2:** Relationship between IL-6 in man and woman.

In the group of diabetics there was a statistically strong correlation between IL-1-RL-1 and the amount of EAT in front of the right ventricle measured by CT (CT RV) (r = 0.282; p = 0.004) and MRI (MRI RV) (r = 0.210; p = 0.006).




**Graph. 3**: Relationship between IL_1RL_1 in man and woman.

A statistically strong correlation was found between TNF-α and the amount of EAT around the common left coronary artery as measured by CT (LM) (r = 0.196; p = 0.030) and MRI (MRI LM) (r = 0.210; p = 0.025).




**Graph. 4**: Relationship between TNFA in man and woman.

### 2.2. GROUP OF HEALTHY CONTROLS

Logistic regression analysis in healthy patients did not show a significant relationship between the amount of cytokines and EAT volume.

**Table 15**: Group Descriptives

| | Group | N | Mean | Median | SD | SE |
|---|---|---|---|---|---|---|
| IL_6_R | men | 17 | 152.18 | 149.40 | 26.43 | 6.411 |
| | wom | 6 | 153.2 | 146.3 | 18.2 | 7.41 |
| IL_6 | men | 29 | 6.77 | 6.49 | 2.44 | 0.453 |
| | wom | 23 | 15.3 | 11.3 | 17.7 | 3.69 |
| IL_1RL_1 | men | 27 | 509.83 | 31.60 | 918.65 | 176.794 |
| | wom | 23 | 633.9 | 21.7 | 1214.1 | 253.16 |
| TNFA | men | 33 | 27280.98 | 7.72 | 45220.95 | 7871.957 |
| | wom | 26 | 23087.6 | 14.7 | 42960.4 | 8425.22 |

## 3. In this task, we aim to see whether there is a statistically significant correlation between EAT measured by CT and MRI and WC of patients in both groups

It is believed that WC depends on height, age and musculoskeletal system proportions. Increase in WC is associated with increased risk of obesity, high blood pressure, increased total blood cholesterol and development of cardiovascular disease (CVD). Normal WC in men is accepted to be less than 94 cm. In those over 94 cm, there is a moderately high risk of developing CVD, and over 103 cm – a high risk of CVD. Normal WC in women is less than 80 cm, for those over 80 cm, there is a moderate risk of CVD, and over 88 cm – a high risk of CVD.

In this task we used Spearman's rank correlation coefficient, which looks for associations between indicators. Correlations between 0 and 0.3 are weak, between 0.4-0.6 are moderate, and above 0.6 are strong. Positive correlation shows a parallel movement of associated indicators, ie. as one indicator increases, so does the other.

### 3.1. GROUP OF DIABETICS

The patients in this group were 124, (66 men and 58 women), into two subgroups. The first subgroup had normal WC, and in the second, WC was increased. The results of both subgroups were correlated with EAT volume measured by CT and MRI.

**Table 16**: Group Descriptives

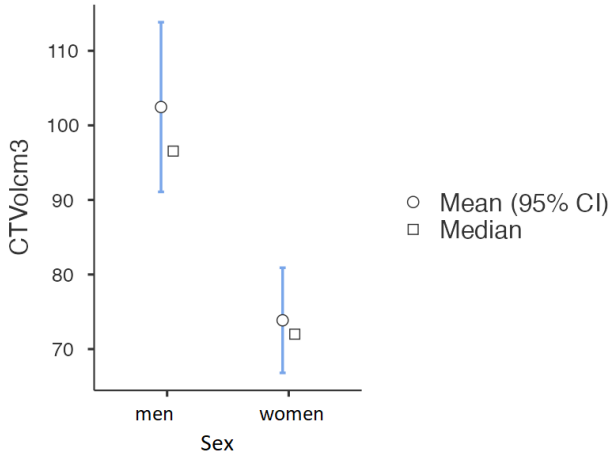
| | N | Mean | Median | SD | SE |
|---|---|---|---|---|---|
| men | 66 | 93.0 | 92.7 | 13.0 | 1.60 |
| wom | 58 | 85.6 | 84.5 | 12.1 | 1.58 |

### 3.1.1. Diabetics with normal WC

In patients with diabetes and normal WC (WC mean), there was a statistically strong correlation with EAT volume measured by CT (CT Vol cm3) (r = 0.335; p = 0.011) and MRI (MRI Vol cm3) (r = 0.359; p = 0.008).

**Table 17**. Pearson test to investigate correlations between normal WC and EAT volume measured by CT and MRI.

| indicators | CT Vol cm3 | MRI Vol cm3 |
|---|---|---|
| **WC mean** | r = 0.335; p = 0.011 | r = 0.359; p = 0.008 |




**Graph. 5**: EAT volume in men and woman.

### 3.1.2. Diabetics with increased WC

In patients with diabetes and increased WC, there a statistically strong correlation with EAT volume measured by CT (CT Vol cm3) (r = 0.568; p = 0.000) and MRI (MRI Vol cm3) p = 0.594; p = 0.000). There was also a strong correlation between WC and EAT volume measured in front of the free edge of the right ventricle by CT (RV) (r = 0.338; p = 0.006) and MRI (MRI RV) (r = 0.396; p = 0.002).

**Table 18**. Pearson test to investigate correlations between increased WC and EAT volume measured by CT and MRI.

| Indicators | CT Vol cm3 | MRI Vol cm3 | CT RVmm | MRI RVmm |
|---|---|---|---|---|
| WC mean | r = 0.568; p = 0.000 | r = 0.594; p = 0.000 | r = 0.338; p = 0.006 | r = 0.396; p = 0.002 |

## 3.2. GROUP OF HEALTHY CONTROLS

### 3.2.1. Controls with normal WC

This group included 29 patients and there was a statistically strong correlation between WC and EAT volume measured by CT (CT Vol cm3) (r = 0.508; p = 0.005) MRI (MRI Vol cm3) (r = 0.519; p = 0.005). A strong correlation was also found between WC and EAT volume measured in front of the free edge of the right ventricle of CT (RV) (r = 0.436; p = 0.018) and MRI (MRI RV) (r = 0.487; p = 0.009).

**Table 19**. Pearson test to investigate correlations between normal WC and EAT volume measured by CT and MRI in healthy controls.

| Indicators | CT Vol cm3 | MRI Vol cm3 | CT RVmm | MRI RVmm |
|---|---|---|---|---|
| WC mean | r = 0.508; p = 0.005 | r = 0.519; p = 0.005 | r = 0.436; p = 0.018 | r = 0.487; p = 0.009 |

### 3.2.2. Controls with increased WC

This group included 30 patients, with a statistically strong correlation between WC and EAT volume measured by CT (CT Vol cm3) (r = 0.588; p = 0.001) and MRI (MRI Vol cm3) (r = 0.421; p = 0.021). There was also a strong correlation between WC and EAT volume measured in front of the free edge of the right ventricle by CT (CT RV) (r = 0.525; p = 0.003).

**Table 20**. Pearson test to investigate correlations between increased WC and EAT volume measured by CT and MRI in healthy controls.

| Indicators | CT Vol cm3 | MRI Vol cm3 | CT RVmm |
|---|---|---|---|
| WC mean | r = 0.588; p = 0.001 | r = 0.421; p = 0.021 | r = 0.525; p = 0.003 |

## 4. To compare accuracy of EAT tomographic quantification by CT and MRI

EAT volume of 181 persons was quantified by CT (EFVKT), and of 172 persons by MRI (EFVMRT). Of the 181 patients examined by CT, 123 were patients with T1DM and 58 were healthy controls. MRI was performed on 114 patients with T1DM and 58 healthy controls. The discrepancy in the number of subjects was due to manifestations of claustrophobia during stay in the MRI machine or refusal of the participant of a certain examination.

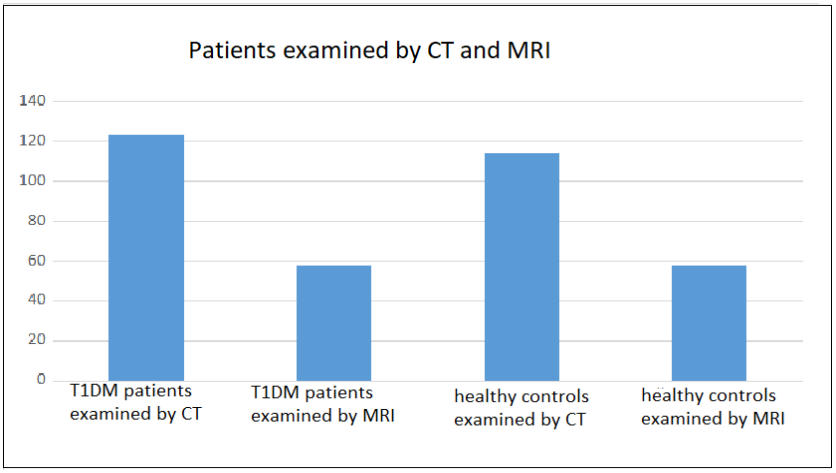
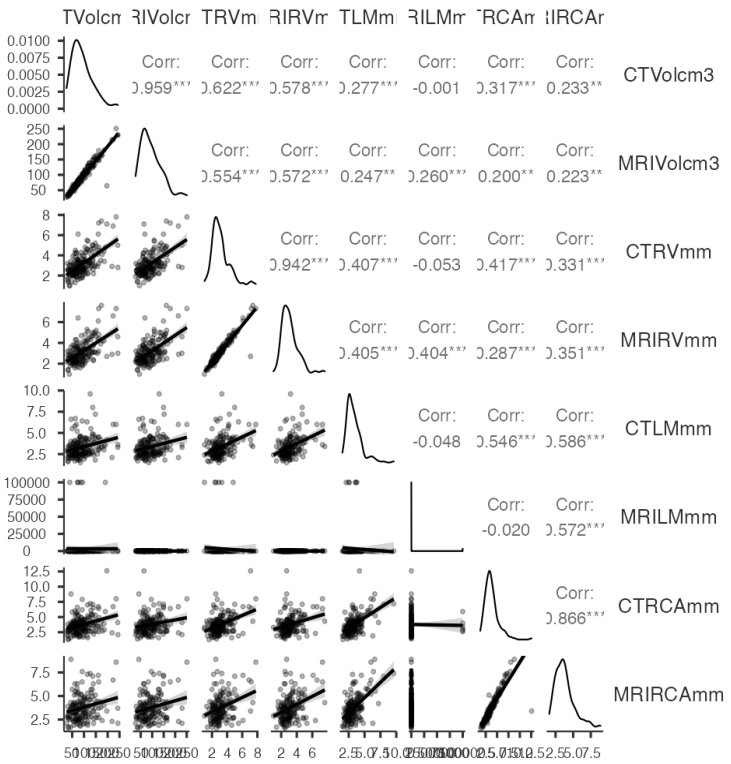


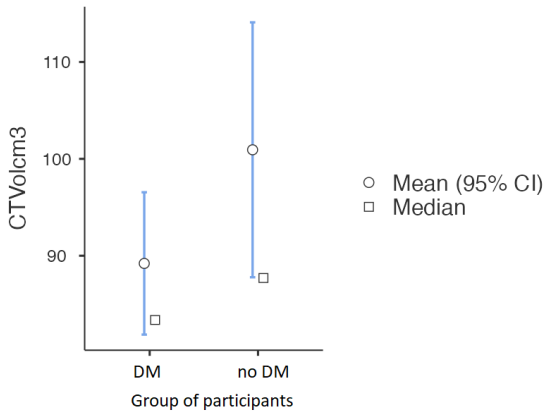

**Figure 12**. Number of CT and MRI examinations, performed in both groups.

When comparing EAT volume, measured in front of the free edge of the right ventricle, a statistically strong correlation was found between CTRV / MRIRV ($r = 0.942$; $p = 0.000$) in all patients. When comparing EAT volume around the left common coronary artery, we found again an excellent linear correlation between the two CTLM / MRILM methods ($r = 0.971$; $p = 0.000$) as well as for EAT volume around the right coronary artery CTRCA / MRRICA ($r = 0.866$; $p = 0.000$).

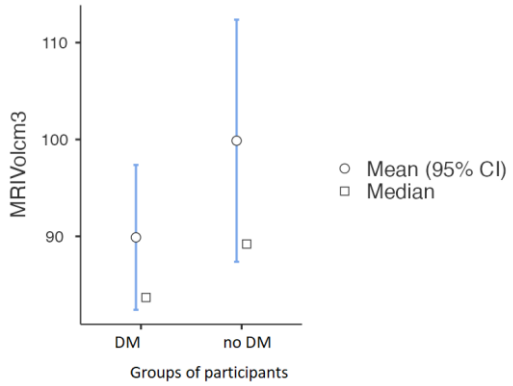
**Table 21.** Correlation of EAT indicators measured by CT and MRI




The mean value of EFVCT in patients with T1DM was 89.20 ± 41.57cm$^3$. The median was 83.37cm$^3$. EAT volume value, quantified by MRI, was extremely close, the mean value was 89.89 ± 40.73cm$^3$, median 83.69cm$^3$. In healthy patients, the mean value of EFVCT was 100.93 ± 51.13cm$^3$. The median was 87.70cm$^3$. In quantitatively determined EAT volume by MRI, the mean value was 99.87 ± 48.56cm$^3$, median 89.21cm$^3$. Non-parametric correlation analysis between individual measurements revealed an excellent linear correlation between EFVCT and EFVMP, Spearman's ρ = 0.95, p = 0.000.

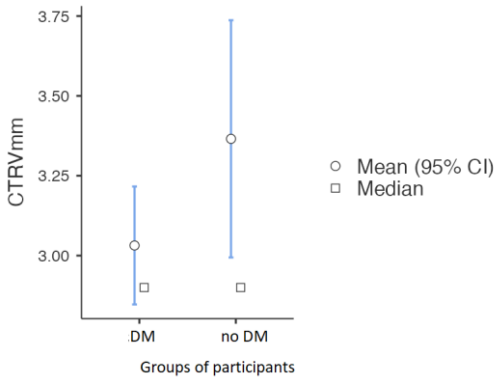



**Graph. 6**. Total EAT volume measured by EFVCT in both groups of patients.

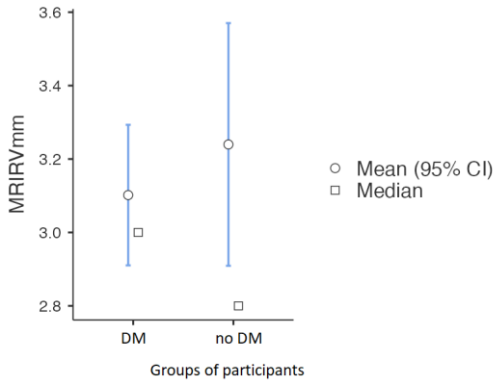



**Graph. 7**. Total EAT volume measured by EFVMRI in both groups of patients.

In addition to the total EAT volume in the two groups, we examined whether a strong correlation was found between the measurements in front of the free edge of the right ventricle, around the left common coronary and right coronary arteries by CT and MRI. The mean EAT volume in patients with T1DM in front of the free edge of the right ventricle CTRV was 3.03 ± 1.04 mm, median was 2.90 mm. When measured by MRI RV, mean volume was 3.10 ± 1.00 mm, median was 3.00mm. In healthy patients, mean CTRV was 3.37 ± 1.44 mm, median was 2.90 mm. For quantitatively determined MRIRV, the mean average value was 3.24 ± 1.28 mm, median was 2.80 mm. In non-parametric correlation analysis, an excellent linear correlation was found between the individual measurements of EAT volume by CTRV and MRIRV, Spearman's $\rho = 0.94$, $p = 0.000$.

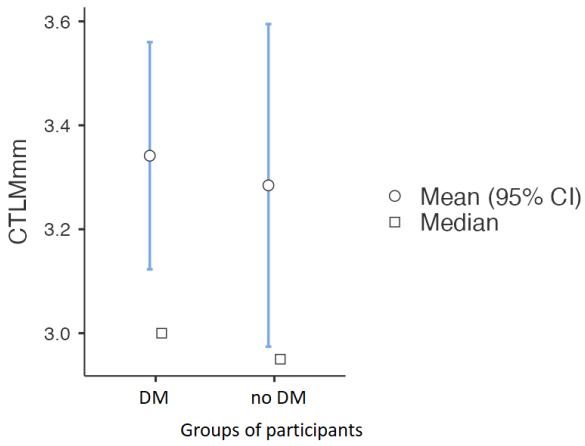



**Graph. 8***:* Measurements of EAT volume by CTRV in both groups of patients.

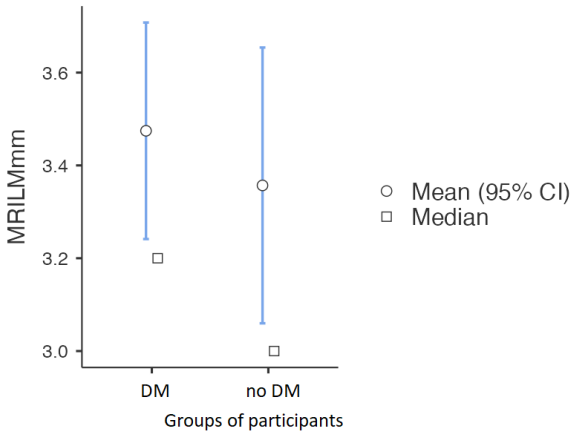



**Graph 9:**. Measurements of EAT volume by MRIRV in both groups of patients.

The mean EAT volume around the left common coronary artery in patients with T1DM measured by CTLM was 3.03 ± 1.04 mm, median was 2.90 mm. When measured by MRILM, the mean was 3.10 ± 1.00 mm and median was 3.00mm. In healthy patients, mean CTLM was 3.28 ± 1.21 mm and median was 2.90 mm. For quantitatively determined MRILM, mean value was 3.36 ± 1.15 mm, median was 3.00 mm. Non-parametric correlation analysis showed an excellent linear correlation between CTLM EAT volume and MRILM, Spearman's $\rho$ = 0.97, p = 0.000.

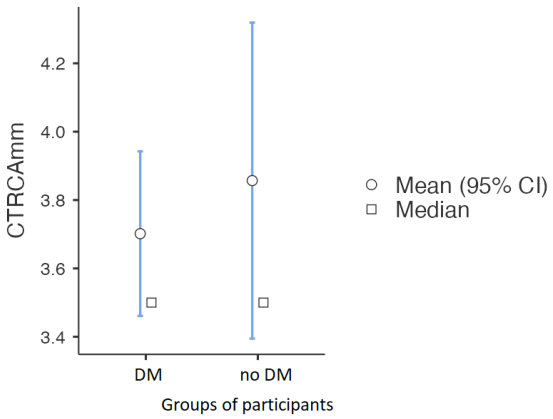



**Graph 10**. Measurements of EAT volume around the left common coronary artery by CTLM in both groups of patients.

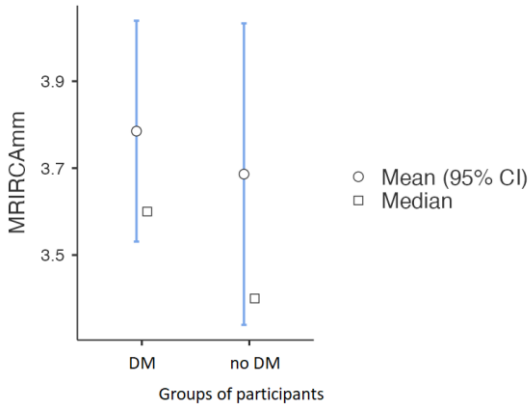



**Graph. 11**. Measurements of EAT volume around the left common coronary artery by MRILM in both groups of patients.

The mean EAT volume around the right coronary artery in patients with T1DM measured by CTRCA was 3.70 ± 1.36 mm, median was 3.50 mm. When measured by MRIRCA, the mean volume was 3.79 ± 1.38 mm, median was 3.60 mm. In healthy patients, the mean CTRCA was 3.86 ± 1.80 mm, median was 3.50 mm. For quantitatively determined MRRCA, the mean value was 3.69 ± 1.35 mm, median was 3.40 mm. Non-parametric correlation analysis showed an excellent linear correlation between CTRCA and MRIRCA EAT volume, Spearman's $\rho = 0.87$, $p = 0.000$.




**Graph. 12**. Measurements of EAT volume around the right coronary artery by CTRCA in both groups of patients.




**Graph. 13**. Measurements of EAT volume around the right coronary artery by MRIRCA in both groups of patients.

## 5. To determine whether there is a statistically significant correlation between EAT measured by CT and MRI and VAT of patients in the two study groups

To determine the correlation between EAT and VAT in both cohorts, we used Pearson correlation analysis and Mann-Whitney U test, because data did not follow normal distribution.

**Table 22**: Group Descriptives

| | **Mean rater 1** | **Mean rater 2** | **Chronbach alpha** | **Interclass correlation** | **p-level** |
|---|---|---|---|---|---|
| CT EFV | 91.51 | 89.78 | .995 | .990 | 0.0001 |
| MRT EFV | 91.39 | 90.97 | .995 | .990 | 0.0001 |
| CT RV | 3.13 | 3.12 | 1.00 | .999 | 0.0001 |
| MRI RV | 3.14 | 3.14 | 1.00 | .999 | 0.0001 |
| CT LM | 3.31 | 3.31 | 1.00 | .999 | 0.0001 |
| MRI LM | 3.42 | 3.42 | 1.00 | .999 | 0.0001 |
| CTRCA | 3.73 | 3.74 | .999 | .998 | 0.0001 |
| MRIRCA | 3.74 | 3.74 | .999 | .999 | 0.0001 |

**Табл. 23.** X: Mann- Whitney тест за разпределение на данните.

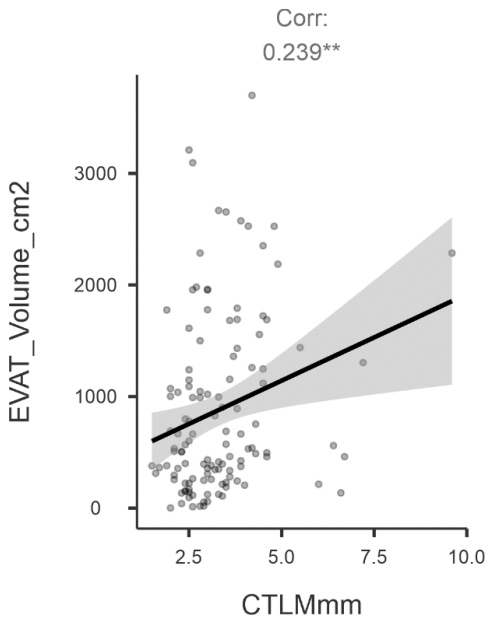
| | | | |
|---|---|---|---|
| CTVolcm$^3$ | Mann-Whitney U | 3169 | 0.227 |
| MRIVolcm$^3$ | Mann-Whitney U | 2955 | 0.256 |
| CTRVmm | Mann-Whitney U | 3333 | 0.477 |
| MRIRVmm | Mann-Whitney U | 3297 | 0.977 |
| CTLMmm | Mann-Whitney U | 3450 | 0.722 |
| MRILMmm | Mann-Whitney U | 3093 | 0.490 |
| CTRCAmm | Mann-Whitney U | 3558 | 0.978 |
| MRIRCAmm | Mann-Whitney U | 3091 | 0.486 |

## 5.1. GROUP OF DIABETICS

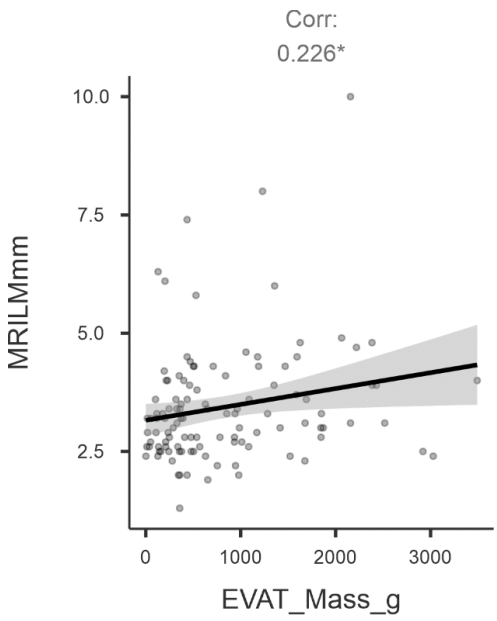
In T1DM patients, no statistically significant correlation was found for the total EAT volume measured by CT and MRI, but a statistically significant correlation was found between VAT and EAT around the coronary vessels. VAT was measured as volume per square cm and as total mass in grams.

- Statistical analysis of VAT in cm2 and EAT:

A strong correlation was found between EAT around the left common coronary artery measured by CT and MRI and VAT: EVAT Vol / CTLM (r = 0.239; p = 0.008), EVAT Vol / MRILM (r = 0.226; p = 0.016)

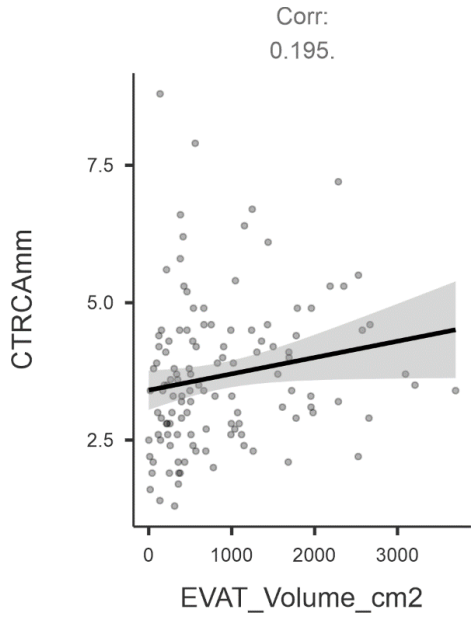



**Graph. 14**. Graphical representation of the statistically significant correlation between EVAT Vol / CTLM

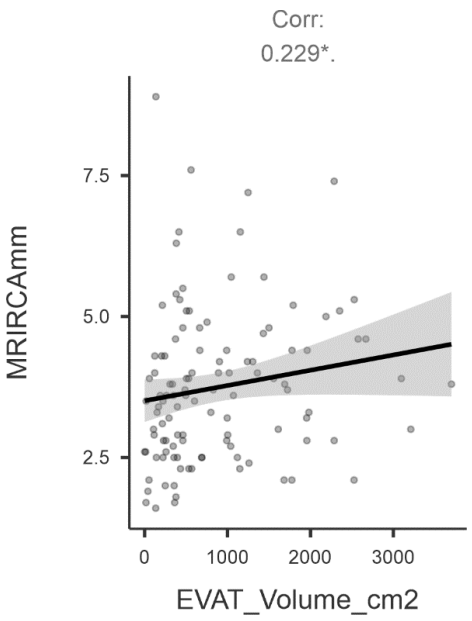



**Graph. 15**. Graphical representation of the statistically significant correlation between EVAT Vol /MRILM.

A statistically strong correlation was also found between EAT around the right coronary artery measured by CT and MRI, and VAT measured by DEXA, EVAT Vol / CT RCA (r = 0.195; p = 0.030), EVAT Vol / MRI RCA (r = 0.229; p = 0.014).



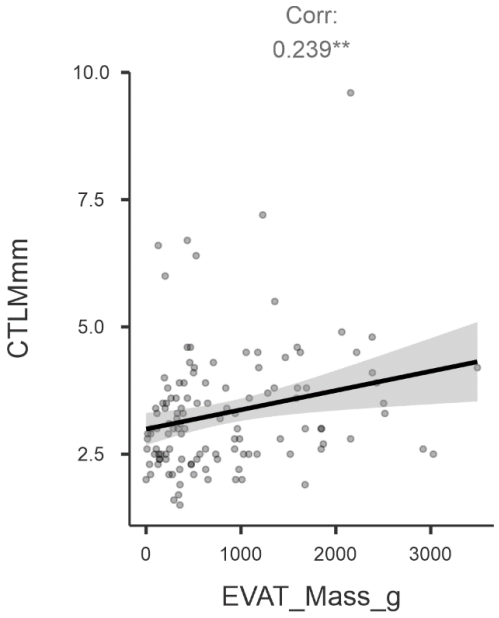

**Graph. 16**. Graphical representation of the statistically significant correlation between EVAT Vol / CT RCA.



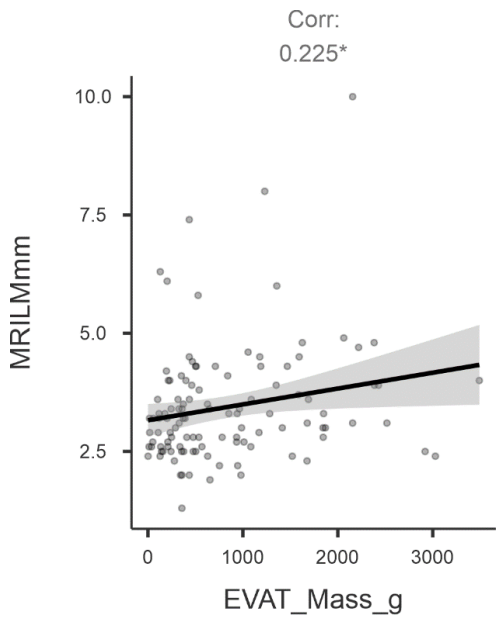

**Graph. 17**. Graphical representation of the statistically significant correlation between EVAT Vol / MRI RCA.

• Statistical analysis of VAT in grams and EAT.

A strong correlation was found between EAT near the left common coronary artery measured by CT and MRI and VAT EVAT Mass / CTLM (r = 0.239; p = 0.008), EVAT Mass / MRILM (r = 0.225; p = 0.016).

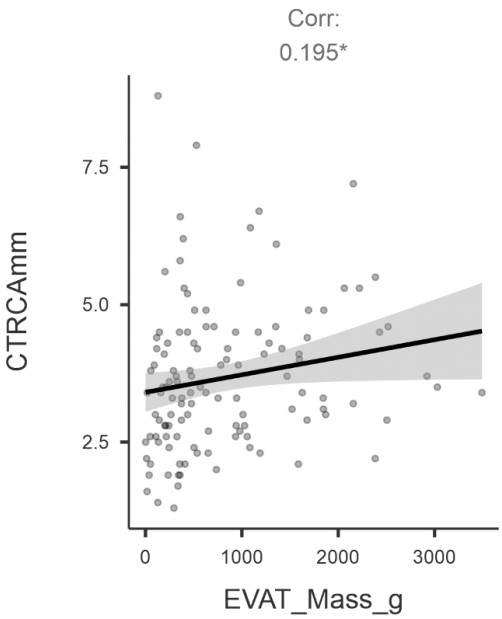



**Graph. 18**. Graphical representation of the statistical correlation between EVAT Mass / CTLM.

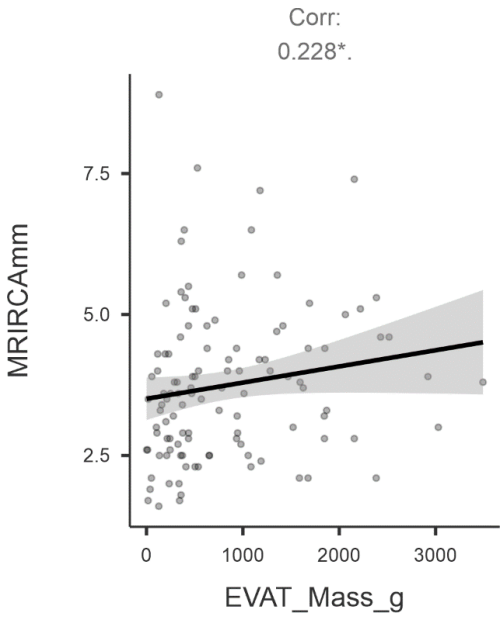



**Graph. 19**. Graphical representation of the statistical correlation between EVAT Mass / MRILM.

A statistically strong correlation was also found between EAT around the right coronary artery measured by CT and MRI and VAT measured by DEXA, EVAT Mass / CT RCA (r = 0.195; p = 0.031), EVAT Mass / MRI RCA (r = 0.228; p = 0.015).




**Graph. 20.** Graphical representation of the statistical correlation between EVAT Mass / CT RCA.




**Graph. 21**. Graphical representation of the statistical correlation between EVAT Mass / MRI RCA

### 5.2. GROUP OF HEALTHY PATIENTS

In the group of healthy controls, no statistically significant correlation was found between any of the EAT volume and VAT indicators.

## 6. To determine whether there is a significant correlation between EAT volume measured by CT and MRI and the diabetes duration

No statistically significant correlation was found between EAT volume and diabetes duration in both studied groups.

## 7. To determine whether there is a relationship between EAT volume and thickness, and age and sex of patients

### 7.1. CORRELATION OF EAT AND AGE OF PATIENTS

A statistically strong correlation was also found between EAT volume measured by CT and MRI and the age of patients in the diabetic group ($r = 0.406$; $<0.001$), while in the control group, in addition to a strong correlation between age and EAT volume ($r = 0.492$ $<0.001$) we also found a statistically strong correlation between the age of the patients and EAT thickness measured in front of the right ventricle ($r = 0.370$; $p = 0.004$).

**Table 23**. Correlation between the age of the patients and EAT volume in both studied groups.

| Indicators | CT Vol см3 | CTRV | MRI Vol см3 |
|---|---|---|---|
| **Age diabetics** | r = 0.406; < 0.001 | - | - |
| **Age controls** | - | r = 0.370; p = 0.004 | r = 0.492; < 0.001 |

## 7.2. CORRELATION OF EAT AND THE SEX OF PATIENTS

In order to compare the diabetics and healthy patients by sex, we used the Mann Whitney U test due to non-normal data distribution. We used biserial correlation due to the specifics of associated data (association between category data and numeric data).

### 7.2.1. Group of diabetics

**Table 24.** Mann-Whitney U test data distribution.

| | | Statistic | p | | Effect Size |
|---|---|---|---|---|---|
| CTVolcm3 | Mann-Whitney U | 1179 | < .001 | Rank biserial correlation | 0.3732 |
| CTRVmm | Mann-Whitney U | 1685 | 0.252 | Rank biserial correlation | 0.1196 |
| CTLMmm | Mann-Whitney U | 1686 | 0.253 | Rank biserial correlation | 0.1194 |
| CTRCAmm | Mann-Whitney U | 1794 | 0.549 | Rank biserial correlation | 0.0627 |

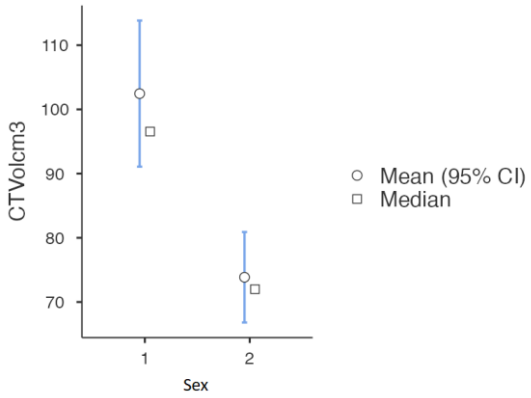
Interpretation is based on values from 0 to 1, with anything above 0.3 being moderate and strong as correlation. In this correlation we do not indicate the level of significance, because it explains the effect size, which indicates everything significant and turns it into percentages from 0 to 100.

**Table 25:** Descriptive statistics of the mean values measured between men and women

| | num | Mean/ср.аритм. | Median/медиана | SD/стнд.откл. | SE/станд.грешка |
|---|---|---|---|---|---|
| **men** | 66 | 102.46 | 96.55 | 47.16 | 5.805 |
| **wom** | 57 | 73.9 | 72.00 | 27.1 | 3.60 |

In the group of diabetics, 123 patients were studied, of which 66 men and 57 women. We found that EAT volume is significantly higher in men 102 ± 47.16 $cm^3$ with a median of

47.16 $cm^3$, compared to women, where EAT volume was 73.9 ± 27.1 $cm^3$, with a median of 72 $cm^3$.




**Graph. 22**. Graphic representation of EAT according to sex

### 7.2.2. Group of healthy patients

The interpretation here is based on values from 0 to 1, with anything above 0.3 being moderate and strong as a correlation. From the comparative analysis in healthy patients, a significant relationship was found between EAT volume and sex of the patients.

**Table 26**. Mann-Whitney U test data distribution

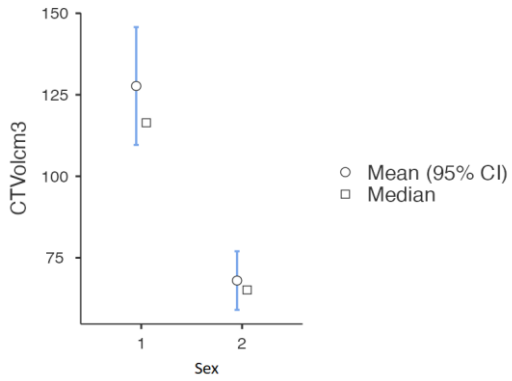
| | | Statistic | p | | Effect Size |
|---|---|---|---|---|---|
| CTVolcm3 | Mann-Whitney U | 128 | < .001 | Rank biserial correlation | 0.692 |
| CTRVmm | Mann-Whitney U | 281 | 0.034 | Rank biserial correlation | 0.326 |
| CTLMmm | Mann-Whitney U | 269 | 0.022 | Rank biserial correlation | 0.353 |
| CTRCAmm | Mann-Whitney U | 311 | 0.102 | Rank biserial correlation | 0.252 |

In this group we studied 58 patients, 32 men and 26 women. EAT volume was measured by CT, as well EAT thickness of EMT in front of the right ventricle, around the left common and right coronary artery. All EAT measurements were higher in men.

**Table 27**. Descriptive statistics of the mean values measured in men and women.

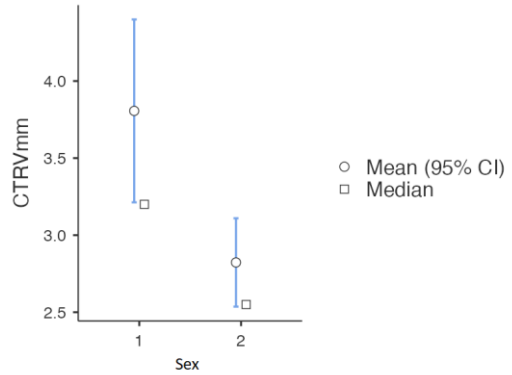
| | | num | Mean | Median | SD | SE |
|---|---|---|---|---|---|---|
| CTVolcm3 | men | 32 | 127.68 | 116.41 | 52.13 | 9.216 |
| | wom | 26 | 68.01 | 65.14 | 23.378 | 4.585 |
| CTRVmm | men | 32 | 3.81 | 3.20 | 1.71 | 0.303 |
| | wom | 26 | 2.82 | 2.55 | 0.746 | 0.146 |
| CTLMmm | men | 32 | 3.58 | 3.30 | 1.34 | 0.237 |
| | wom | 26 | 2.92 | 2.70 | 0.917 | 0.180 |
| CTRCAmm | men | 32 | 4.27 | 3.65 | 2.22 | 0.393 |
| | wom | 26 | 3.35 | 3.30 | 0.863 | 0.169 |

In men, EAT volume was approximately two times greater than in women. EFVCTVol $cm^3$ in men was 127.68 ± 52.13, $cm^3$ with a median of 116.41 $cm^3$, while in women EFVCTVol cm3 was 68.01 ± 23.37 $cm^3$, with a median of 65.14 $cm^3$.



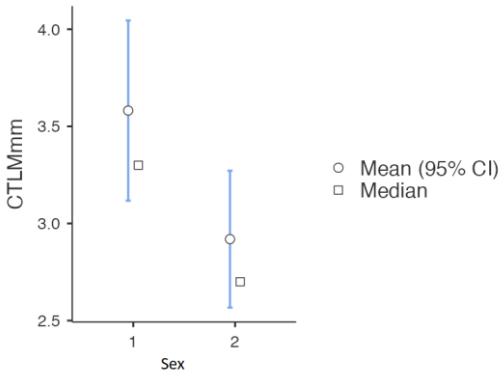

**Graph. 23**. Graphic representation of EAT volume according to sex.

In men, EAT thickness measured in front of the right ventricle by CT was 3.81 ± 1.34 mm, with a median of 3.20 mm, while in women CTRV was 2.82 ± 0.74 mm with a median of 2.55 mm.



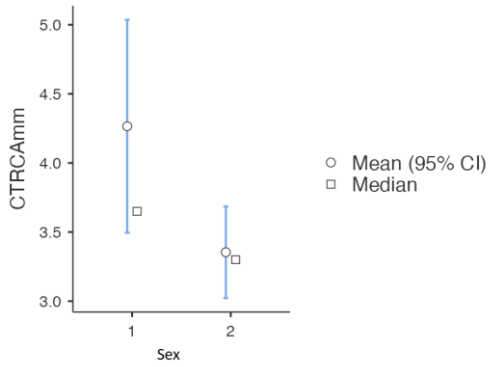

**Graph. 24**. Graphic representation of EAT thickness in front of the right ventricle according to sex

EAT thickness around the common left coronary artery measured by CT in men was 3.58 ± 1.34 mm, with a median of 3.30 mm., while in women it was 2.92 ± 0.91 mm, with a median of 2.70 mm.




**Graph. 25**. Graphical representation of EAT thickness around the left common coronary artery according to sex

In men, EAT thickness around the right coronary artery measured by CT was 4.27 ± 2.22 mm, with a median of 3.65 mm, and in women it was 3.35 ± 0.86 mm, with a median of 3.30 mm.




**Graph. 26**. Graphical representation of EAT thickness around the right coronary artery measured on CT according to sex.

From the studies and results obtained, we found that EAT volume was by 86.7% higher in men than in women in both studied groups. Another important finding is that EAT thickness was greatest around the right coronary artery in both groups of patients compared to that around the left common coronary artery and EAT thickness in front of the right ventricle.

# V. DISCUSSION

Our study is the first to evaluate the amount of EAT simultaneously measured by CT and MRI in correlation with other markers for cardiovascular risk assessment in patients with T1DM duration over 15 years. EAT is biologically active with a number of endocrine and paracrine functions (Talman et al., 2014). It is anatomically located between the myocardium and visceral pericardial layer, and is supplied by the coronary arteries (Sacks & Fain, 2007). Its location allows to be easily quantified by various imaging methods such as echocardiography, CT and MRI.

Over the years, several methods have been used to visualize EAT. Since 2003, the measurement of right ventricular EAT thickness has been used as an indicator of the severity of cardiac obesity (Iacobellis, Assael, et al., 2003; Iacobellis, Ribaudo, et al., 2003; Jeong et al., 2007). In several studies, CT was used to quantify EAT (Abbara et al., 2006; Taguchi, 2001; Wheeler et al., 2005). Abbara S et al. used 16-slice multidetector CT to examine 59 patients, measuring 32 different epicardial locations of the heart in each patient (Abbara et al., 2006)

Our study reliably confirmed excellent EAT quantification when measured by CT and MRI. After processing the data, we did not find a significant difference in median EAT volume values in diabetics and controls and this corresponds with the report by the team of Svanteson et al. 2019 in their study of asymptomatic patients with long-standing T1DM (Svanteson et al., 2019). The lack of significant difference can be interpreted as an expression of a different state of EAT, subject to partial reverse regulation by paracrine signals derived from the myocardium itself (Antonopoulos & Antoniades, 2017). According to EAT state, opposite effects on the coronary arteries and myocardium have been described (Antonopoulos & Antoniades, 2017). According to the results from the statistical analysis, EAT volume measurements by CT and MRI correlate well, which defines them as equivalent for accurate and reproducible quantification of epicardial volume. Our results did not reveal a statistically significant difference in EAT volume in patients with long-term T1DM and healthy controls.

According to previous studies such as Gorter et al., we confirmed that EAT quantification by CT is reproducible (Gorter, van Lindert, et al., 2008). It should be noted that quantitative EAT measurements do not require additional exposure to ionizing radiation, as segmentation and measurement of EAT thickness are derived from the obtained scanned

images acquired from clinical practice in other cardiac indications. Quantification is also possible in routine tomographic examinations of the thorax without ECG synchronization, but at the expense of loss of precision and a certain degree of subjectivity involved due to motor artifacts. Segmentation is a labor-intensive process, represented mainly in the field of scientific research. Different types of adipose tissue are often subject to segmental quantification due to their correlation with increased cardiovascular risk. Segmentation has potential to deliver accurate, detailed and reproducible data, and the use of flexible open source software allows for a wider application of this method. The segmentation algorithm presented by our team can be easily adapted for application in different types of tissues for new scientific research. Mahabadi et al. found in their study that increased EAT was associated with diabetes in 4 093 patients. In addition, they reported that EAT was directly related to the presence of cardiovascular risk factors in individuals without history of CAD and AMI (Mahabadi et al., 2009). Konishi et al. demonstrated a positive correlation between PAT volume and DM markers in 171 patients with suspected CAD (Konishi et al., 2010). Wang et al. reported that EAT was higher in 49 patients with diabetes compared with 78 non-diabetic controls (Wang et al., 2009b). In our study we found that there was no significant difference in the amount of EAT in the two groups of patients studied, the median EAT in patients with T1DM was 83.37 cm3 and in non-diabetic patients was 87. In a population of 402 patients, EAT was higher in men with arterial hypertension, hypercholesterolemia and smokers, but not in patients with diabetes (Rajani et al., 2013).

Iacobellis et al. (Iacobellis & Leonetti, 2005) found an association between EAT and impaired insulin resistance, as well as fasting glucose. Gorter et al. (Gorter, van Lindert, et al., 2008) reported a significant correlation between EAT and metabolic syndrome, emphasizing the close relationship between the systemic disease and VAT and its metabolic activity. Jorgun et al. reported that the strongest independent variables related to EAT thickness were metabolic syndrome, BMI, and age. In addition, they found that serum triglyceride levels were not associated with increased EAT thickness (Yorgun et al., 2013). Previous studies have shown a broad correlation between EAT and triglycerides (Wang et al., 2009b). Dong et al. found a very weak correlation between the two variables (Dong et al., 2013). In addition, Mookadam et al. (Goel et al., 2010) found no association between triglycerides and EAT thickness in echocardiography, as well as Hell et al. (Ginsberg, 1998; Hell, Ding, et al., 2016).

There is currently no consensus on normal EAT range (Sarin et al., 2008; Shmilovich et al., 2011). EAT has been reported to be positively associated with a number of cardiovascular risk factors and has a significant association with metabolic syndrome in patients with EAT >100 mL (Iacobellis, Ribaudo, et al., 2003; Jang et al., 2015; Lehman et al., 2010; Sarin et al., 2008). Gianluca Milanese et al. reported a median EAT of 82.62 cm3 in non-diabetic and 112.87 cm3 in diabetic patients examined by cardiac CT angiography. Patients with diabetes were significantly above the 100 cm3 EAT threshold, while patients without diabetes were mostly below (Milanese et al., 2019). In our study, healthy controls had a median EAT of 87.70 cm3 and patients with T1DM had a median EAT of 83.37 cm3. This may be due to weaker individual control over eating habits in controls.

Echocardiography has so far been accepted as the golden standard for examination and evaluation of the heart, EAT and valvular apparatus. The method is easy, inexpensive, non-ionizing and allows the examination to be performed at the patient's bedside. The disadvantage of this type of study is that it cannot measure the total amount of EAT, but only its thickness at a certain point (Jeong et al., 2007). In addition, the examination is dynamic and requires a high level of experience of the diagnostician.

Systematic studies on EAT as a new marker of cardiovascular risk have only emerged in the last decade. In 2001, Taguchi et al. have shown that in Japanese men, EAT measured by non-contrast CT shows a stronger relationship with the presence of CAD, compared to other fat deposits or can be considered as a cardiovascular factor in predicting CAD and its severity (Taguchi, 2001).

Several studies have been published using a semi-automated technique for measuring the amount of EAT by CT (Alexopoulos et al., 2010; Gorter, de Vos, et al., 2008). The mean EAT volume found in the study group ranged from 68 ± 34 up to 124 ± 50 ml (J. Ding et al., 2008; Mahabadi et al., 2009). In a study involving patients from the Framingham group, mean EAT volume was 110 ± 41 ml in women, and 137 ± 53 ml in men (Rosito et al., 2008). In 2011 Shmoilovich et al. published a study aimed at determining the upper limit of normal EAT volume by CT scan in a population of patients with low cardiovascular risk. In this group of 255 patients, the 95th percentile of EAT volume, indexed according to body surface area, was 68.1 ml/m2 (Shmilovich et al., 2011). In another study, Louise Hindso et al. examined the hearts of 132 patients after autopsy with postmortem 64-slice CT. The mean EAT volume was 73 ml in men and 64.8 ml in women, representing about 20.4 ± 10.2% and 21.9 ± 9.5%

of total heart volume (Hindsø et al., 2017). In our study, EAT volume of 123 patients with T1DM measured by CT was 89.20 ± 41.57 ml, and on MRI was 89.89 ± 40.73 ml, while in healthy patients EAT volume measured by CT was 100.93 ± 51.13 ml, and by MRI 99.87 ± 48 ml.

In our study we found a statistically significant correlation between EAT volume measured by CT and age and sex of patients in both studied groups. EAT volume in men was almost twice higher than in women. Also, EAT thickness was found to be greatest around the right coronary artery in both sexes, followed by EAT thickness around the left common coronary artery and thickness around the free wall of the right ventricle. Abbara et al. found a significant difference in EAT thickness and distribution between sex and age. They found that total EAT content in patients over 65 years of age was on average 22% higher than in patients under 65 years of age. These data show significant statistical significance, with the amount of EAT in front of the right ventricle of these patients being increased by 36% and in front of the lateral wall of the left ventricle by 38%. (Abbara et al., 2006).

Central obesity or increased VAT strongly correlates with the development of metabolic syndrome and CAD (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), 2002). There are various ways to estimate VAT volume and amount, including EAT, which is a type of VAT (Ahn et al., 2008). In recent years, there were several studies that measured EAT by ultrasound as an easy and reliable indicator (Iacobellis, Ribaudo, et al., 2003).

In our study, we measured the amount of EAT by CT and MRI, thus, in addition to thickness, we also measured total EAT volume, which gave us a more accurate estimate for determining cardiovascular risk. It is widely known that EAT thickness measured by echocardiography has a good correlation with VAT measured by CT and MRI (Pierdomenico et al., 2013). In our study, the volume and amount of VAT was measured with whole-body DEXA, with VAT measurement area located between the upper edge of the second lumbar vertebra and lower edge of the fourth lumbar vertebra. Park et al. conducted a study on the relationship between EAT and BMI, involving 643 patients (302 men, 341 women; age 59 ± 11 years), with patients divided into two groups: BMI <27 and BMI> 27. They found that EAT thickness measured in front of the right ventricle by echocardiography was greater in patients with BMI <27 (Park et al., 2010).

Our study showed that in men with T1DM there was a significant correlation between EAT volume and BMI in the BMI 25-30 group. The result was expected and coincided with the reported findings of the teams of Mohammadzadeh et al. and Kim et al. (10, 13). In obese diabetic patients with BMI> 30, there was also an association between EAT volume and BMI and total cholesterol, despite the relatively small number of patients in this subgroup. In women with T1DM, only in the BMI 25-30 group we found a statistical relationship between EAT volume and BMI, but in all BMI groups of diabetic women, there was a significant statistical relationship between EAT volume and HDL- and LDL-cholesterol levels. In controls we found a correlation between EAT volume and BMI only in the male group in the range of BMI 25-30 and BMI> 30, but all controls showed significant correlations between EAT volume and LDL- /total cholesterol. The association we found between EAT volume and cholesterol levels is analogous to the results of Manno et al., published in 2019, which confirms the link between increased EAT volume and higher LDL-cholesterol levels (12).

EAT is considered a visceral fat depot and thus its thickness is thought to correlate with BMI as a measure of obesity. However, the relationship between EAT and BMI has been inconsistent in previous studies, with some studies reporting moderate to strong associations, while others showing either a weak or insignificant association. In a meta-analysis discussing the relationship between EMT and obesity measures, a moderate relationship between EMT and BMI was found, which was confirmed by us in the groups with BMI 25-30 and BMI> 30 in men. In addition, unlike other visceral fat depots, the size of EAT adipocytes is not related to BMI. In a meta-analysis, discussing the relationship between EAT and obesity measures, a moderate relationship between EAT and BMI was found, which was confirmed by us in the subgroups with BMI 25-30 and BMI> 30 in men.

Increased BMI and adipose tissue distribution, especially VAT, is a risk factor for the development of CVD and CAD (Kazlauskaite et al., 2010). Increased WC is a sign of obesity and has been recognized as an important risk factor for the development of metabolic syndrome (Rexrode et al., 2001; Visscher et al., 2001). WC is a quick and inexpensive method for assessing VAT (Shetty et al., 2012). In addition to this, the link between WC, EAT and subclinical inflammation has been already proven by Akbas et al. In our study, we divided the two cohorts of patients into two subgroups, with normal and increased WC. The results obtained show a moderate correlation between EAT and normal circumference in patients with T1DM and a statistically strong correlation between EAT volume and increased WC in

both groups of patients, as well as a statistically strong correlation between increased WC and EAT thickness in front of the right camera. In this regard, risk factors such as obesity, lack of physical activity and smoking are associated with chronic low-grade inflammation and increased cardiovascular risk (Akbas et al., 2014).

The link between HF and obesity has long been recognized (Abel et al., 2008). Obesity is an independent risk factor for HF, especially in HFpEF. But some studies show that HF patients with higher BMI and WC have better prognosis than patients who are weak. This phenomenon is defined as the "obesity paradox" (Carbone et al., 2017; Lavie et al., 2016). Although the detailed mechanism behind this discrepancy is unclear, inflammation may be involved (Karason & Jamaly, 2020). In our study, patients in both study groups had no history of cardiovascular events or HF. It is widely accepted that obesity can stimulate systemic inflammation (Berg & Scherer, 2005; Ghigliotti et al., 2014). In obesity, VAT, along with EAT and PAT, increase the expression of various proinflammatory cytokines, including TNF-α, IL-6 and IL-1, while reducing the expression of anti-inflammatory cytokines such as IL-10 and adiponectin (Jahng et al., 2016). In our study, we found a correlation between IL-1, IL-6 and TNF-α with EAT around coronary vessels and in front of the right ventricle, which corresponds to those described in the literature.

Proinflammatory adipocytokines and lipids secreted by EAT can affect cardiomyocytes and extracellular matrix in a paracrine manner (Patel et al., 2017). Thus, EAT may mediate the impact of systemic inflammation on the adjacent myocardium (Packer, 2018). This may partly explain heart inflammation in obese people. On the other hand, in HF patients, damaged cardiomyocytes can secrete proinflammatory cytokines, such as IL-6 and TNF-α, which can cause EAT lipolysis, leading to cardiac cachexia and deterioration (Oikonomou & Antoniades, 2019).

According to the results of statistical analysis, EAT measurements by CT and MRI correlated well, therefore both imaging methods have the capacity to accurately and reproducibly quantify EAT volume. Segmentation is a labor-intensive process, represented mainly in the field of scientific research. Different types of adipose tissue are often subject to segmental quantification due to their correlation with increased cardiovascular risk. Segmentation has the potential to deliver accurate, detailed and reproducible data, and use of flexible open source software allows for wider application of this method. The segmentation

algorithm presented by our team can be easily adapted for application in different types of tissues in service of new scientific research.

From the results obtained in our study, we concluded that EAT measurement can serve as potential diagnostic tool for stratification of cardiovascular risk in both T1DM and healthy patients.

In the future, more prospective studies are needed on quantitative and qualitative EAT measurement and its assessment as new biomarker for development of CAD and cardiovascular disease, in order to be diagnostically and predictively applicable.

# VI. CONCLUSIONS

1. We found a significant correlation between the two imaging methods (CT and MRI) for measuring the amount of EAT. After processing the data, we did not find a significant difference in median EAT volume values in diabetics and controls. An algorithm for semi-automatic and manual segmentation was developed.
2. We found a statistically strong correlation between WC and the amount of EAT in both groups of patients studied.
3. We found a statistically strong correlation between the amount of EAT and studied cytokines (IL-6, IL-1, TNF) in the group of diabetics. No statistically significant correlation was found in healthy patients.
4. We found a statistically strong correlation was between increased VAT and PAT in patients with T1DM. No correlation was found in healthy controls.
5. No correlation was found between EAT volume and diabetes duration.
6. We found a statistically significant correlation between the amount of EAT, BMI and lipid profile in both study groups.
7. Increased amount of EAT can be considered a risk factor for the development of CVD, both in diabetics and in healthy patients.

# VII. CONTRIBUTIONS

1. This is the first study in Bulgaria, conducted to assess EAT as a risk factor for the development of cardiovascular and metabolic risk.
2. This is the first study in Bulgaria, conducted to assess EAT in patients with T1DM duration over 15 years old.
3. An algorithm for semi-automatic and manual segmentation of EAT examined by CT and MRI was developed for the first time in Bulgaria.
4. A study was performed simultaneously by CT and MRI to measure EAT in patients with T1DM duration over 25 years for the first time.
5. We affirm that a CT scan of the heart can be considered the golden standard for measuring simultaneously EAT and calcium score, and thus with one imaging examination we can identify two risk factors for CAD in patients with diabetes.

# VIII. LIST OF PUBLICATIONS IN CONNECTION WITH THE DISSERTATION

## 1. Full-text publications indexed in *Web of Science*/*Scopus*

1. Valchev G, Popova R, **Shemeri SE**, Bocheva Y, Usheva N, Galcheva S, Iotova V, Yotov Y. Applications of Routine Cardiac MRI Pulse Sequences – A Contemporary Review, J of IMAB. 2019 Oct-Dec;25(4):2718-2722; DOI: 10.5272/jimab.2019254.2718
2. Valchev G, Kaloyanova D, **El Shemeri S**, Bocheva Y, Boyadzhieva M, Usheva N, Galcheva S, Iotova V, Yotov Y. Semi-automatic and manual segmentation of epicardial adipose tissue from CT and MRI images with SD Slicer. Rentgenologiya i radiologya. 2021;60(3):177-182.

## 2. Other full-text publications

1. Valchev G, **El Shemeri S**, Kaloyanova D, Chalakova T, Tsochev K, Usheva N, Bocheva Y, Boyadzhieva M, Yotov Y, Iotova V. Correlation between coronary calcium score and epicardial fat in patients with long-term type 1 diabetes and healthy controls – preliminary results. Journal of the Union of Scientists - Varna Medicine and Ecology Series. 2020 Dec 5;25(2):5–11.
2. **El Shemeri S**, Kaloyanova D, Valchev G, Chalakova T, Bocheva Y, Usheva N. Epicardial adipose tissue as imaging biomarker for assessing cardiac and metabolic risk in patients with type 1 diabetes duration over 15 years. Journal of the Union of Scientists - Varna Medicine and Ecology Series. 2021. Article in press.
3. Tsochev K, Yotova V, Chalakova T, Yotov Y, Usheva N, Bocheva Y, **El Shemeri S**, Stefanova T, Pancheva R. Determination of the risk profile in patients with type 1 diabetes mellitus with a long history of alcohol use and metabolic control. Pediatrics Congress, Borovets, September, 2021.
4. Tsochev K, Iotova V, Shemeri S, Chalakova T, Yotov Y, Bocheva Y, Chausheva G, Boyadzhieva M, **El Shemeri S**, Usheva N. Role of education and its relationship to metabolic control in patients with long-term type 1 diabetes

mellitus. Journal of the Union of Scientists - Varna Medicine and Ecology Series. 2021. Article in press.

## 3. Abstracts indexed in *Web of Science*

1. Tsochev K, Usheva N, Iotova V, Yotov Y, Chalakova T, Hadzhieva E, Boyadzhieva M, Bocheva Y, **El Shemeri S**, Pancheva R. Evaluation of alcohol consumption in relation to metabolic control in patients with long standing type 1 diabetes mellitus. Abstracts for the 47th Annual Conference of the International Society for Pediatric and Adolescent Diabetes (ISPAD). October 13–15, 2021(Virtual). Pediatric Diabetes. 2021;22(Suppl. 30):eP227

## 4. Poster published

1. S**. El Shemeri**, D. Kaloyanova; "3D Slicer- An Accessible Option for Semi-Automatic Fat Segmentation and Quantification" ECR 2021; DOI: 10.26044/ecr2021/C-14682.


## Acknowledgment

Cardiovascular and metabolic risk associated with visceral fat mass in patients with type 1 diabetes mellitus. Supervisor: Prof. Violeta Iotova, MD, PhD, DSc