DOI:10.14753/SE.2020.2396

SEMMELWEIS EGYETEM

DOKTORI ISKOLA

Ph.D. értekezések

2396.

JERMENDY ÁDÁM LEVENTE

Szív- és érrendszeri betegségek élettana és klinikuma

című program

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DOI:10.14753/SE.2020.2396

Clinical implications of measuring epicardial adipose tissue quantity

PhD Thesis

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Budapest

2019

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List of abbreviations

ALT: alanine aminotransferase

AST: aspartate aminotransferase

BMI: body mass index

BUDAPEST-GLOBAL: Burden of atherosclerotic plaques study in twins - Genetic Loci and the Burden of Atherosclerotic Lesions

CAD: coronary artery disease

CRP: C-reactive protein

CT: computed tomography

CTA: CT angiography

DCCT/EDIC: Diabetes Control and Complications Trial / Epidemiology of Diabetes Intervention and Complications

DZ: dizygotic

EAT: epicardial adipose tissue

ECG: electrocardiogram

FFA: free fatty acid

GLOBAL: Genetic Loci and the Burden of Atherosclerotic Lesions

HDL-cholesterol: high-density lipoprotein cholesterol

HU: Hounsfield unit

ICC: intra-class correlation coefficient

IL-1β: interleukin-1β

IL-6: interleukin-6

JNK: Jun N-terminal kinase

LDL-cholesterol: low-density lipoprotein cholesterol

MCP-1: monocyte chemoattractant protein-1

MDCT: multidetector-row CT

MESA: Multiethnic Study of Atherosclerosis

MRI: magnetic resonance imaging

mRNA: messenger ribonucleotide acid

MZ: monozygotic

NAFDP: non-alcoholic fatty pancreas disease

NAFLD: non-alcoholic fatty liver disease

NASH: non-alcoholic steatohepatitis

NFkappaB: nuclear factor-kappa-B

NSTEMI: non-ST-elevation myocardial infarction

OR: odds ratio

PAI-1: plasminogen activator inhibitor-1

ROI: region of interest

SAT: subcutaneous adipose tissue

SD: standard deviation

SPECT: single photon emission computed tomography

TIMI: thrombolysis in myocardial infarction

TNF-α: tumor necrosis factor-α

UCP-1: uncoupled protein-1

VAT: visceral adipose tissue

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1. Introduction

1.1. Epicardial adipose tissue

Type 2 diabetes mellitus and its cardiovascular complications carry a great burden for the health care system worldwide due to their high prevalence rate (1). Insulin resistance syndrome with the dysfunction of abdominal fat compartment plays an important role in the development of the disease (2, 3). In the last couple of years it was demonstrated that other fat compartments may also be involved in the insulin resistance syndrome and may contribute to the pathogenesis of atherosclerosis (4). Recently, the epicardial fat compartment has gained special attention in this regard (5-7).

In the 19th century it was believed that fatty degeneration of the heart is the main cause of every heart disease (8). *Richard Quain* was the most well-known scientist supporting this theory recognizing the relationship of increased fat volume on the epicardial surface with coronary artery obstruction. The diagnosis of fatty heart was very popular in the Victorian era but was later changed to the concept of fibrosus heart disease and chronic myocarditis. All these diagnoses were replaced by the ischemia-theory in the middle of the 20th century. Interestingly, it was recognized at that time that in 70% of the fatty heart diagnosis in Quain's pathological records corresponded with the ischemic heart disease. Although the relationship between increased epicardial fat quantity and different cardiac pathologies was described almost 150 years ago, medicine did not dedicate to much attention to this field. Cardiovascular research has begun to explore the role of different fat compartments with the pandemic spread of obesity and the dynamic development of radiological imaging technics (9). Lately, special attention was paid to the epicardial fat due to its anatomical proximity with the coronary arteries (10-12). While anatomical and biochemical characteristics of the epicardial fat compartment were described in early studies, its potential role in the pathomechanism of coronary artery disease (CAD) and other cardiac dysfunction has only been investigated recently.

1.1.1. Terminology

The exact terminology of fat compartments covering and surrounding the heart is not standardized; there are still many imprecise uses of these definitions in the literature. Nevertheless, the most widely used and accepted terms are summarized in **Table 1.**

Table 1. Terminology of fat compartments around the heart

Visceral fat	Adipose tissue around the visceral organs
Epicardial fat	Visceral fat between the myocardial surface and the visceral layer of the pericardium
Pericardial fat	Adipose tissue between the two pericardial layers (visceral and parietal pericardium) and fat depot on the external surface of the parietal pericardium
Paracardial fat	Fat deposits outside the parietal pericardium (extra-pericardial thoracic fat)
Perivascular (pericoronary) fat	Adipose tissue around the vessels (coronary arteries) irrespective of location
Ectopic fat	Lipid (triglycerides) deposits in non-adipose tissue (i.e. myocardium, liver, pancreas, etc.)

The epicardial fat as a part of the visceral fat is localized between the myocardial surface and the visceral layer of the pericardium. Pericardial fat involves adipose tissues between the two (visceral and parietal) pericardial layers and the fat depot on the external surface of the parietal pericardium. Paracardial fat contains fat deposits outside the parietal pericardium and therefore, sometimes is called as extra-pericardial intrathoracic fat. The coronary arteries are surrounded by the perivascular/pericoronary fat, irrespective of

location. The term of ectopic fat implies triglycerides deposits in non-adipose tissue of different organs such as myocardium, muscle, liver, or pancreas (13).

The clear distinction of epicardial fat from pericardial fat is of great clinical importance (14, 15). From embryological aspect they have different origins. While the epicardial fat - similarly to the visceral fat - originates from mesodermal cells, the pericardial fat has an ectodermal origin, similarly to subcutaneous fat. Moreover, there is also a difference in the blood supply between these two fat compartments. Epicardial fat is supplied by the small myocardial coronary arteries, on the other hand, the blood supply of pericardial fat is provided from the thoracic vessels. The amount of epicardial and pericardial fat compartments as percentage of total cardiac mass also differs (**Table 2**).

Table 2. Differences between epicardial and pericardial fat compartments

	Epicardial fat	**Pericardial fat**
Location	between the myocardial surface and the visceral pericardium	outside the visceral pericardium, between the visceral and parietal pericardium and on the external surface of the parietal pericardium
Embryologic origin	splanchnopleuric mesoderm	primitive thoracic mesenchyme
Blood supply	branches form the coronary arteries	non-coronary sources (branches from the internal mammary artery)
Amount	20% of total heart weight	20-40% of cardiac mass

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1.1.2. Imaging of epicardial adipose tissue

The most commonly used non-invasive modalities for the visualization and quantification of epicardial fat tissue are echocardiography, magnetic resonance imaging (MRI) and cardiac computed tomography (CT).

Echocardiography provides a simple, cheap and readily available assessment which pictures directly the epicardial adipose tissue (EAT) thickness on the free wall of the right ventricle. Importantly, this technique does not subject the patient to ionizing radiation. Echocardiographic assessment of EAT thickness requires parasternal short- and long-axis views in three following end-systolic phases **(Figure 1)**. Several studies have established the general EAT thickness under 7 mm in asymptomatic population (16). Nevertheless, this method has several disadvantages including the poor reproducibility and the high dependence on the observer's experience. In addition, it may not reflect accurately the whole quantity of the epicardial fat due to the two-dimensional nature of the measurement. Moreover, the method has poor intra- and interobserver variability, and its results may differ significantly from the measurements with CT (17, 18).

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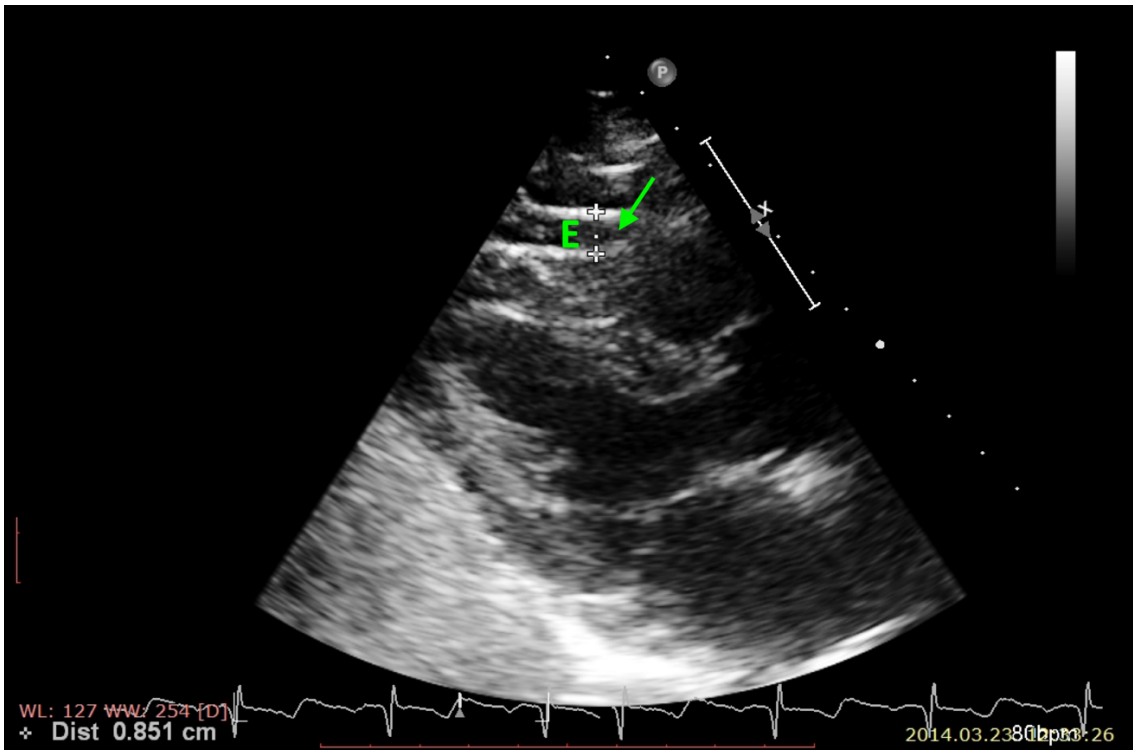

Figure 1.

Quantification of epicardial adipose tissue by echocardiography (parasternal view). The thickness of the area between the myocardium and the visceral layer of the pericardium is 0.85 cm indicating epicardial adipose tissue (E, green arrow).

In contrast to echocardiography, MRI provides accurate area measurements and, in this way, EAT volume can be calculated **(Figure 2)**. Area measurements with MRI correspond well with fat thickness determination with echocardiography, although a systemic bias through overestimation of EAT with echocardiography might occur (19). Although the lack of ionizing radiation is preferable, there are disadvantages of this modality; it is less available in routine clinical practice, it is more expensive and has worse spatial resolution compared to CT.

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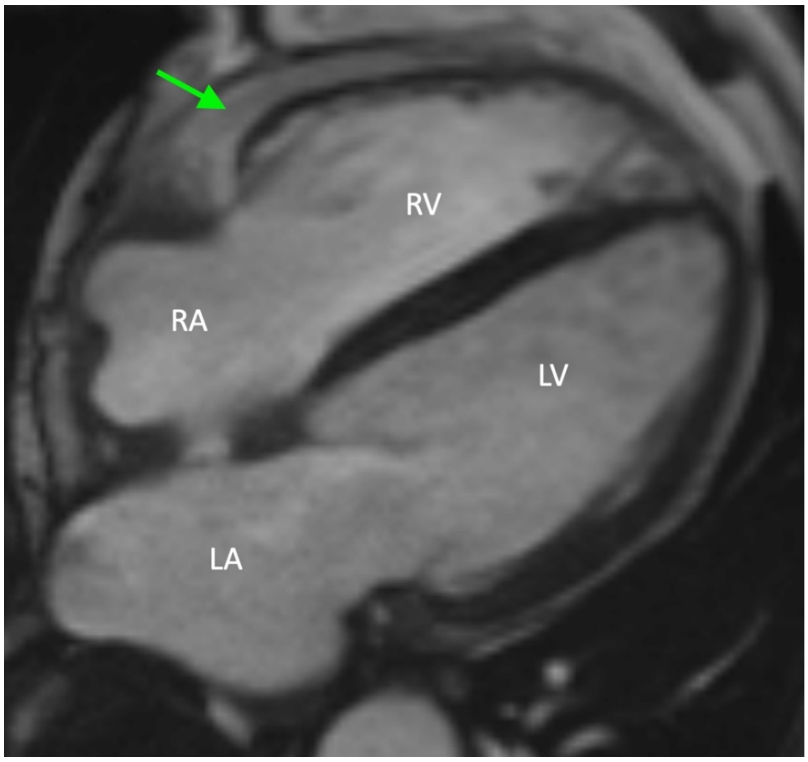

Figure 2.

Epicardial adipose tissue (green arrow) demonstrated using magnetic resonance imaging (MRI) technique.

RA: right atrium, LA: left atrium, RV: right ventricle, LV: left ventricle

True volume assessment of EAT is feasible using multidetector-row CT (MDCT), which has superior spatial resolution among the imaging modalities **(Figure 3)**. It is of note that the specificity and sensitivity of epicardial fat measurements with MDCT are the best comparing to alternative imaging methods. Epicardial fat quantification is performed in a standardized fashion on prospectively ECG triggered non-contrast CT scans which extend from the pulmonary artery bifurcation to the diaphragm. The identification of EAT is based on thresholds of fat attenuation. Typically, lower thresholds of attenuation range from -250 to -190 Hounsfield units (HU) and upper thresholds are set between -50 and -30 HU. In contrast to area and thickness measurements, volume quantification provides the most accurate way for assessing the true epicardial fat quantity which can be performed on volume rendered image reconstructions (20). In addition, non-enhanced cardiac CT scans can be used for the quantification of coronary artery calcification

resulting in more reliable cardiovascular risk assessment (21). Importantly, native CT results in a very small (~0.5 mSv) radiation dose. *Maurovich-Horvat et al* found in a former collaborative work that the measurement of pericoronary adipose tissue proved to be highly reproducible by using MDCT (22).

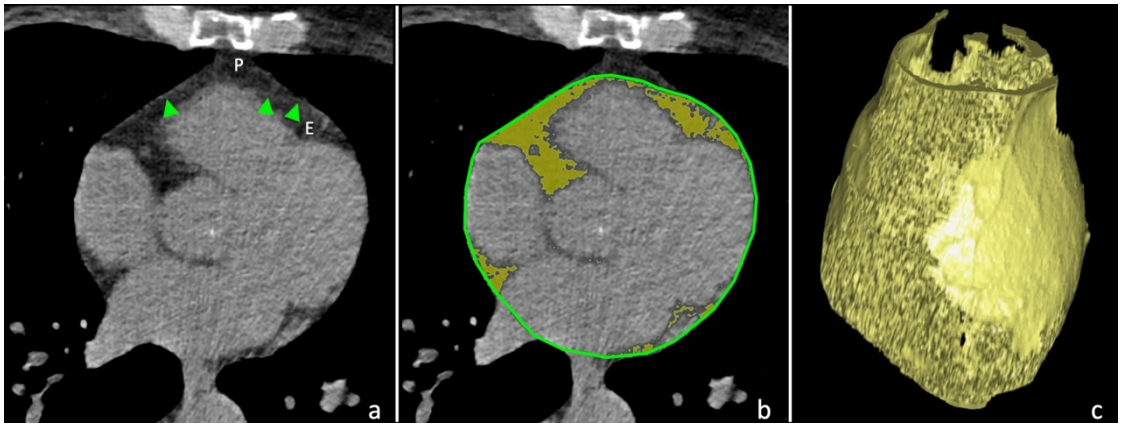

Figure 3.

Measuring epicardial adipose tissue quantity by cardiac computed tomography (CT)

a) Axial section at the aortic root. The green arrows indicate the visceral layer of the pericardium. Epicardial fat (E) is located inside while pericardial fat (P) outside of the visceral layer.
b) Epicardial adipose tissue (yellow) at the corresponding section
c) Volume rendered reconstruction of the total epicardial fat compartment (yellow).

The volume of epicardial adipose tissue was 112 cm^3.

1.1.3. Anatomical characteristics of EAT

In physiological circumstances the epicardial fat covers nearly 80% of the heart surface. According to earlier observations this fat compartment contributes with 20% to the whole heart quantity (23). The EAT-covered heart region includes the heart basis and the apex, the atrioventricular sulci, the entire surface of the right ventricle, and the great coronary vessels with their origins. The distribution of EAT is mostly inhomogeneous, the biggest mass is localized on the lateral and anterior walls of the right atrium, but in normal circumstances it covers also the atrioventricular and the interventricular sulci and the

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main coronary arteries as well. In case of extremely enlarged EAT, it can accumulate also on the surface of the left atrium and along the vessel's adventitia with spreading into the myocardium. It is of note, that there is no separating fascia layer between the epicardial fat and the myocardium providing a close proximity of these two different tissues (24). In histological investigations it has been earlier established that adipocytes in the EAT are smaller than those in the abdominal or the subcutaneous fat compartments (25). Beyond adipocytes, EAT includes nerves, ganglions, vessels, inflammatory cells and fibrocytes as well (**Figure 4**).

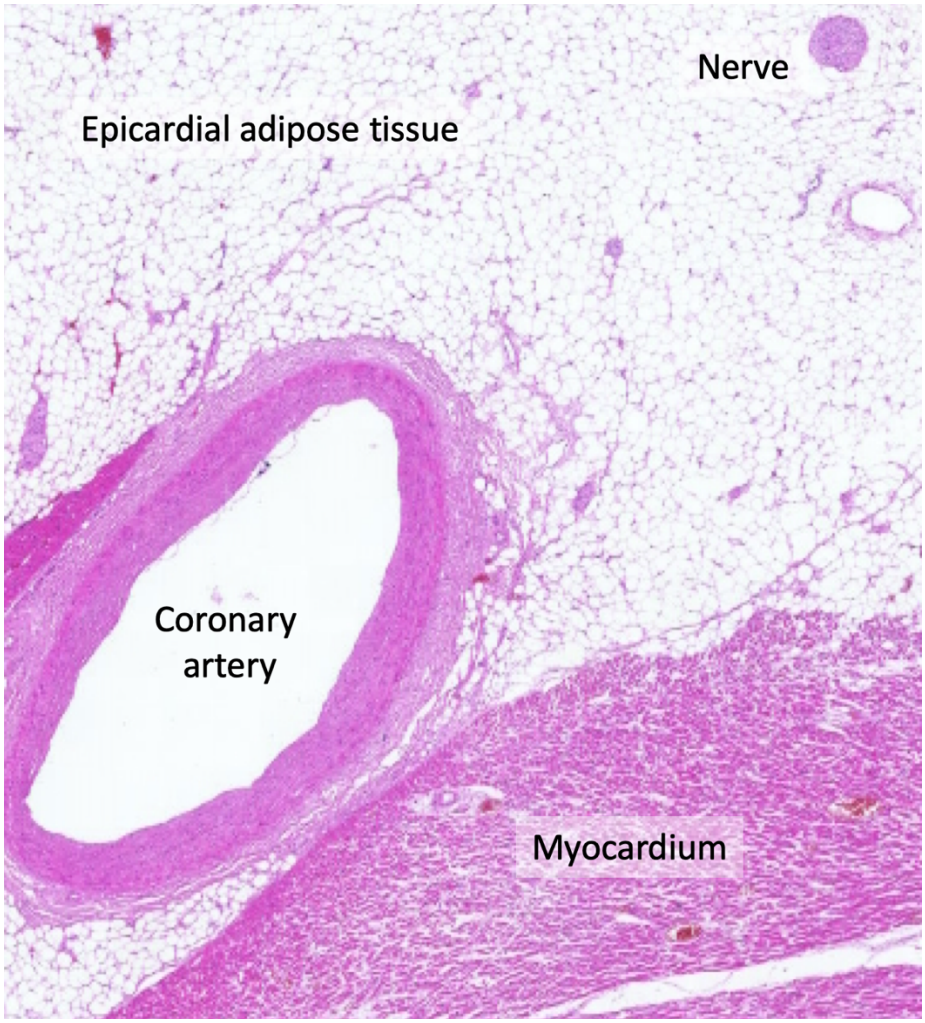

Figure 4.

Microscopic view of the epicardial adipose tissue. It is of note that there is no separating fascia layer between the epicardial fat and the myocardium.

(Histological image is courtesy of Zoltán Sápi, MD, DSc, Semmelweis University, Institute of Pathology, Budapest. In: Nagy E, Jermendy ÁL et al: Arch Med Sci 2017.)

Age, gender, body weight and ethnicity should be taken into consideration among physiological determinants of EAT (26-29). EAT seems to increase with age (30). The quantity of EAT depends on gender and body mass index (BMI). For example, pericardial fat was reported to be 137±54 cm^3 among men and 108±41 cm^3 among women of the Framingham offspring cohort (31). In patients with high BMI (>27 kg/m^2) EAT volume was more than two times higher compared to those with a BMI <27 kg/m^2 (155±15 cm^3 vs 67±12 cm^3) (20). Some ethnic differences in epicardial and pericardial fat thickness may also occur; non-Hispanic White men have more epicardial and pericardial fat than do African Americans (32).

The biochemical features of small adipocytes in EAT may also differ from those of other fat compartments. In experimental studies EAT had higher rate of free fatty acid (FFA) release than adipose tissue elsewhere in the body suggesting that EAT might play a role in local energy supply for the myocardium. In addition, a lower oxidative capacity and a lower rate of glucose utilization were also documented (33). On the other hand, a 5-fold higher expression of uncoupled protein-1 (UCP-1) was found in EAT comparing to other fat depots (34). The UCP-1 is a specific protein in brown fat which is necessary to its energy production, and doesn't appear in other type of fat tissues. This latter feature is in line with the fact that epicardial fat evolves from the brown adipose tissue during embryogenesis.

1.1.4. Physiological function of EAT

Several physiological functions of EAT are already known from different studies or supposed from its biochemical or anatomical features. Unfortunately, experimental evidences supporting these observations are limited due to the very small amount of EAT in experimental animals (rodents).

It is suggested that functions of EAT may include protection of the myocardium against hypothermia (34). In addition, EAT can provide a mechanical protective role for coronary circulation. It can attenuate the torsion developed by the myocardium contraction or the

arterial pulse wave but it has a permissive role as well in positive remodeling of coronary arteries (35).

Besides this, EAT has a substantial role in energy supply to the myocardium and should be considered as a provider of energy source during period of high energy demand (13). On the other hand, EAT may protect the myocardium from cardiotoxic effect of large amount of FFA due to its capacity of fast FFA utilization (36). Taken together EAT may serve as a unique energy buffering pool in the homeostasis of the myocardium.

In addition, adiponectin secretion from epicardial adipocytes may promote the coronary circulation. Adiponectin improves the endothelial function through stimulation of the nitrogen monoxide synthase, reduces the oxidative stress, and indirectly decreases the level of interleukin-6 (IL-6) and C-reactive protein (CRP) by reducing tumor necrosis factor-α (TNFα) production (37, 38). Adiponectin also has some extracardiac effect such as increased glucose utilization in the hepatocytes and muscle cells which may result in improving insulin sensitivity (39).

1.1.5. EAT in the pathomechanism of atherosclerosis

Some years ago a hypothesis about the direct role of EAT in the development and progression of coronary atherosclerosis has been raised and paracrine and vasocrine effects of EAT due to close proximity of epicardial fat to coronary arteries were supposed (40). The hypothesis was indirectly supported by a pathological study in subjects with myocardial bridge. Namely, no atherosclerosis was observed in coronary segments at myocardial bridge where surrounding fat on the coronary arteries was lacking (41).

In a landmark study, *Mazurek et al* analyzed epicardial and subcutaneous fat from the lower extremity in obese patients referred for coronary artery bypass grafting. They found increased level of inflammatory mediators (IL-6, TNF-α, interleukin-1β [IL-1β], monocyte chemoattractant protein-1 [MCP-1]), macrophages, lymphocytes and basophils in epicardial fat as compared to subcutaneous fat compartments (42). Others found that

epicardial and omental fat exhibited a broadly comparable pathogenic messenger ribonucleotide acid (mRNA) profile indicating macrophage infiltration into the epicardial fat (43). In another study, mediators of the nuclear factor-kappaB (NFkappaB) and c-Jun N-terminal kinase (JNK) pathways were suggested to involve in the inflammatory profile of EAT highlighting the role of the macrophages in the inflammation within this tissue (44). These studies indicate that chronic inflammation occurs locally as well as systemically potentially contributing further to the pathogenesis of CAD.

It was documented that the epicardial adipocytes had impaired adiponectin secretion and increased leptin production in obese patients with hypertension, metabolic syndrome and CAD (45, 46). This shift in the adiponectin/leptin ratio enhances the development of atherosclerosis. Namely, the decreased adiponectin expression attenuates endothelial function and leads to increased TNF-α production triggering systematic inflammation and oxidative stress. The altered leptin level promotes atherogenic changes in endothelial cells such as increased adhesion of monocytes, higher level of macrophage-to-foam cell transformation, unfavorable changes in lipid levels, and elevation of CRP and inflammatory cytokine levels. All these alterations may lead to development and destabilization of atherosclerotic plaques in coronary arteries (5).

Based on several studies it became widely accepted that EAT should be considered as a source of inflammatory mediators that might directly influence the myocardium and coronary arteries (**Figure 5**) (47, 48). Two mechanisms of influence (paracrine and vasocrine) were suggested (40). Paracrine way of influence means that adipokines released from pericoronary fat may diffuse across the arterial wall (adventitia, media, and intima) and finally can interact with endothelial cells in the intima and with vascular smooth cells in the media. The alternative vasocrine way of effect can be realized by release of adipocytokines and FFAs from EAT directly into vasa vasorum of the coronary arterial wall (49). It was suggested that vasocrine way of influence may be predominant over paracrine effect in case of more advanced atherosclerotic lesions where inflammatory mediators may diffuse only with difficulties (43).

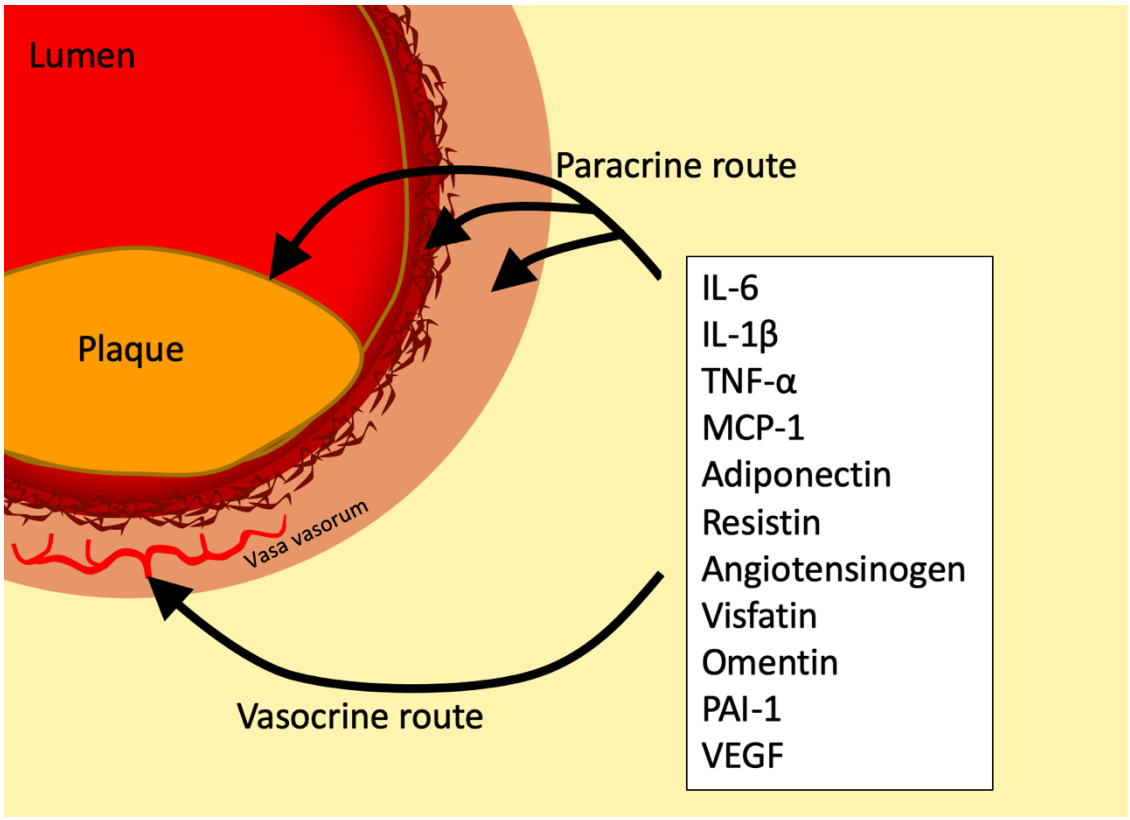

Figure 5.

Routes for paracrine and vasocrine effects of epicardial adipose tissue on coronary arteries and plaque formation.

IL: interleukin, TNF-α: tumor necrosis factor-α, MCP-1: monocyte chemoattractant protein-1, PAI-1: plasminogen activator inhibitor-1, VEGF: vascular endothelial growth factor

The relationship of EAT with CAD were analyzed by several clinical studies (50-55). In the Framingham and the MESA (Multiethnic Study of Atherosclerosis) epidemiological studies a significant association of epicardial fat with coronary artery calcification was found which remained significant after adjustment for traditional cardiovascular risk factors (56, 57). The increased epicardial fat proved to be associated with more advanced atherosclerosis in another study (58). Epicardial fat was associated with non-calcified coronary plaques as well (59, 60). A significant relationship of increased epicardial fat volume (>130.7 cm^3) with vulnerable plaques was also documented (61). The relationship of morphological features of vulnerable plaques (positive remodeling, spotty

calcifications, and low CT attenuation in necrotic core) to the pericardial fat was also studied and the volume of pericardial fat proved to be nearly twice as high in patients with vulnerable plaques as compared to those without CAD (62, 63). Pericardial fat was associated with myocardial ischemia detected by single photon emission computed tomography (SPECT) in patients without known CAD (64). EAT correlated with the degree of coronary atheromatosis suggesting that its excessive accumulation might contribute to the development of acute coronary syndrome and coronary total occlusions (65, 66). In another study, EAT thickness was independently associated with the thrombolysis in myocardial infarction (TIMI) risk score in patients with non-ST-elevation myocardial infarction (NSTEMI) and unstable angina pectoris (67). In patients with the metabolic syndrome increased EAT was associated with impaired coronary flow reserve (68). In a different patient population (in women with chest pain and angiographically normal coronary arteries) EAT thickness was correlated with reduced coronary flow reserve (69). Different surrogate parameters of atherosclerosis were also investigated by others and an association between EAT thickness and carotid intima-media thickness in type 2 diabetic patients as well as in children and adolescents with obesity was found (70, 71). Moreover, EAT showed an independent association with arterial stiffness in an asymptomatic Korean cohort (72). In a recent study, *Maurovich-Horvat et al* investigated the relationship of different thoracic fat depots with coronary atherosclerosis and found an independent association between pericoronary fat and CAD. In addition, pericoronary fat correlated with inflammatory biomarkers as well suggesting that while systemic inflammation plays a role in the pathogenesis of CAD, there are additional local effects that may exist (73). In a systematic review and meta-analysis an association between the elevated location-specific thickness of EAT at the left atrioventricular groove and the obstructive CAD was found (55).

In the majority of studies increase of EAT volume was associated with stenosis of the coronary arteries (74, 75). Since these studies are cross-sectional it is uncertain whether adipose tissue plays a causal role in the development of atherosclerosis. Importantly, two longitudinal studies have reported results that support the hypothesis of ‘outside to inside signaling’ as a cause of atherosclerosis (56, 76). In these studies, intrathoracic fat and EAT volume were measured and an increase of their quantity was associated with

incident coronary heart disease and with major adverse cardiac events. Associations were independent from BMI and other risk factors, suggesting that EAT is one of the factors contributing to CAD.

1.1.6. Role of EAT in other cardiac and non-cardiac abnormalities

The relationship of EAT with atrial fibrillation was analyzed in several clinical studies (77-79). A strong association between EAT and atrial fibrillation (both paroxysmal and persistent) was documented by *Al Chekakie et al*; the relationship proved to be independent of traditional risk factors and atrial enlargement (80). In another study, EAT thickness was verified as an independent predictor for post-ablational recurrence of atrial fibrillation (81). In patients with peritoneal dialysis the increased EAT was associated with impaired left ventricle diastolic capacity independently of CRP level, a marker of systemic inflammation (82).

Epicardial fat necrosis is a rare clinical condition, 26 cases were reported till 2011 (83). It should be considered among differential diagnoses of chest pain. The etiology is obscure, but the prognosis is good. In general, the presenting symptom is left-sided chest pain in a previously healthy individual with an associated juxtacardiac mass seen in chest radiography. CT or MRI may confirm the correct diagnosis resulting in the avoidance of surgical intervention.

Typically, type 2 diabetes is preceded by prediabetes but insulin resistance syndrome due to obesity may be the first pathological stage in the long-lasting asymptomatic period of diabetes. The insulin resistance syndrome (called also as the metabolic syndrome) includes insulin resistance and different metabolic abnormalities (elevated serum triglycerides, lower HDL-cholesterol, hyperglycemia) as well as elevated blood pressure. Obesity, especially abdominal visceral fat accumulation plays a central role in this syndrome. Although the use of term and the suggested pathomechanism of the metabolic syndrome became debatable some years ago, the association between the enlarged

abdominal visceral fat compartment and the increased cardiovascular risk remained unquestionable (84).

Several clinical investigations were dedicated to assess the characteristics of EAT in the metabolic syndrome, prediabetes and type 2 diabetes. In a meta-analysis, EAT was 7.5 ± 0.1 mm in thickness in the metabolic syndrome (n=427) compared to 4.0 ± 0.1 mm in controls (n=301) and EAT correlated significantly with the components of the metabolic syndrome (85). EAT volume was significantly higher in patients with type 2 diabetes than in nondiabetic subjects and EAT volume was significantly associated with components of the metabolic syndrome (58). In asymptomatic type 2 diabetic patients the thickness of EAT proved to be an independent risk factor for significant coronary artery stenosis but not for silent myocardial ischemia (86). A strong correlation was found between fasting plasma glucose and EAT measured with CT or echocardiography (87, 88). EAT quantity was higher in patients with type 2 diabetes mellitus compared to lean subjects or obese patients without diabetes. In addition, the difference in EAT volume between men and women was more pronounced in subjects with impaired fasting glucose or diabetes mellitus (89). A clear relationship of epicardial fat and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, surrogate markers of fatty liver, were documented in a cross-sectional, observational study (90). Taken together, the insulin resistance syndrome (the metabolic syndrome), type 2 diabetes, non-alcoholic fatty liver disease (NAFLD) and CAD are associated with increased amount of epicardial fat (91).

The role of EAT was also investigated in type 1 diabetes. Interestingly, higher epicardial fat and serum leptin levels were found in subjects with type 1 diabetes than in non-diabetic controls. The epicardial fat thickness and serum leptin levels proved to be the best independent correlates of each other in patients with type 1 diabetes independently of BMI, glycemic control and daily insulin requirement (92). Recently, patients with type 1 diabetes (n=100) from the Diabetes Control and Complications Trial / Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study were investigated. In this pilot study, the accumulation of adipose tissue in epicardial and intra-thoracic spaces were highly associated with greater body mass index (BMI), bigger waist to hip ratio,

greater weighted glycated hemoglobin values, elevated triglycerides and a history of elevated albumin excretion rate or end-stage renal disease (93).

1.1.7. Treatment options for modifying EAT volume

Lifestyle changes, bariatric surgery and different drugs may apply. Reduction in weight (BMI) by using very-low calorie diet resulted in a decrease of EAT volume in severely obese subjects (n=20; echocardiographic EAT thickness at baseline: 12.3±1.8 mm, at 6 months follow-up: 8.3±1.0 mm; p=0.001) (94). Similarly, beneficial effect was observed as a result of regular exercise training in a small group of patients (n=24; echocardiographic EAT volume at baseline: 8.11±1.64 mm, at 12 weeks follow-up: 7.39±1.54; p<0.001) (95). In addition, EAT volume decreased after bariatric surgery as well, however one study found that myocardial triglyceride content did not change significantly (96, 97). In a meta-analysis, diet or bariatric surgery proved to be more beneficial than exercise training in reducing EAT volume (98). The effect of drugs on EAT is controversial (99-101). Atorvastatin resulted in a more pronounced decrease of EAT than simvastatin/ezetimibe (102). Pioglitazone compared with metformin increased pericardial fat volume in patients with type 2 diabetes (103). Short term (3 months) use of glucagon-like-receptor agonists (exenatide, liraglutide) decreased the volume of EAT in type 2 diabetic patients (104). In a longer (26 weeks) randomized controlled trial exenatide twice daily (versus standard antidiabetic treatment) proved to be effective in reducing both epicardial and liver fat content in obese patients with type 2 diabetes (n=44). In this study EAT volume was measured by MRI and expressed as ml; the initial EAT value changed by -8.8±2.1% in the exenatide group whereas minimal change was observed in patients with standard treatment (-1.2±1.6%; p=0.0003). The beneficial effect was mainly weight loss dependent (105). In another pilot study, sitagliptin, a dipeptidyl-peptidase-4-inhibitor, also decreased the volume of EAT in a 24-week long study with obese type 2 diabetic patients (n=26); echocardiographic EAT thickness decreased from 9.98±2.63 mm to 8.10±2.11 mm; p<0.001) (106). Lately, SGLT2-inhibitors are emerging as potential new therapeutic options as multiple studies reported a significant decrease in EAT volume after treatment (107-109). For example, a small study from Japan (108)

documented that EAT volume (measured by CT) significantly decreased by the end of 6 months follow-up in type 2 diabetic patients (n=40) with dapagliflozin treatment vs. conventional antidiabetic therapy (-16.4±8.3 cm^3 vs. 4.7±8.8 cm^3; p=0.01). Clearly, EAT should be considered as a novel therapeutic target and statins, as well as antidiabetic drugs are the best candidates so far (110, 111).

1.2. Relationship of EAT to other fat compartments

Association between EAT and other fat compartments was investigated by several authors and was a topic of a recent position statement (57, 112, 113). Generally, EAT volume and obesity parameters (weight, BMI and waist circumference) are closely related (85, 114). Nevertheless, the clinical significance of abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) differ. While abdominal SAT should be considered as a manifestation of enlarged fat depot, abdominal VAT is involved in the pathomechanism of the metabolic syndrome and may increase the cardiovascular risk through production of different cytokines (4). Particular abdominal fat depots carry specific clinical consequences. The enlarged visceral fat depot is characterized primarily with increased lipolysis leading to hepatic steatosis. Non-alcoholic fatty liver disease is often regarded as the hepatic manifestation of the insulin resistance (115) and is considered as a novel predictor of cardiovascular disease (116, 117). Importantly, NAFLD may progress from steatosis to non-alcoholic steatohepatitis (NASH) and even to cirrhosis and liver cancer (118). There are some publications indicating that EAT volume is associated with increasing hepatic lipid accumulation (119). Interestingly, fat deposition in the pancreas (fatty pancreas), termed non-alcoholic fatty pancreas disease (NAFPD) has gained much attention only in the last years (120). Lipid accumulation in pancreas may promote the development of chronic pancreatitis and exacerbate the clinical picture of acute pancreatitis (121). The potential relationship of pancreatic lipid accumulation with β-cell dysfunction is debated (122). NAFLD may often coexist with NAFPD (123).

1.3. Contribution of genetic and environmental factors on fat compartments

As fat compartments differ in embryogenetic origin, physiological and pathophysiological functions (124) it is plausible that their accumulation leads to local or systemic adiposity and increase of their quantity is influenced by genetic and environmental factors. Classical twin studies compare monozygotic (MZ) and dizygotic (DZ) same-gender twin pairs to help evaluate the degree of genetic and environmental influences on body composition (125).

As for obesity (BMI) in general, earlier studies demonstrated a predominant genetic effect on BMI. It seems that anthropometric parameters (weight, height and, consequently BMI) are highly dependent on genetics. In a clinical study with twins from Hungary, phenotype of weight proved to be dependent on genetic factors by 88% (126). An international twin study documented that heritability contributed by 82% for weight and by 79% for BMI phenotypes (127). In a twin study from the United States, 63.6% of the total variance of BMI was explained by genetic components (128). In a review paper, authors reported heritability estimates of BMI between 0.57 and 0.86 in twins from early adulthood to late middle age (129).

As for abdominal obesity (waist circumference), genetic and environmental influences were also investigated, and authors found in an earlier study performed by dual-energy x-ray absorptiometry that genetics highly predominated over environmental factors in contribution to phenotype (130). In the already mentioned Hungarian twin study heritability for waist circumference was calculated as high as 71% (126). This number was 74% in the international twin study (127).

Abdominal SAT and VAT quantities were evaluated in the Framingham Heart Study Offspring and Third-Generation Study cohorts. It has been shown by CT imaging that abdominal SAT and VAT quantities have a heritability of 57% and 36%, respectively (131). However, the methodology for evaluating heritability in this study differed from that of classical twin studies.

Intraabdominal adipose tissue compartments were investigated in early family studies as well, where estimates for VAT ranged between 42% and 56% whereas that of SAT proved to be 42% (132, 133). Taken together, data regarding the heritability of abdominal adipose tissue compartment sizes are scarce and the findings are based on family studies and on measurement methods with limited accuracy.

Regarding EAT, we did not find former data available whether EAT compartment quantity depends predominantly on genetic or environmental factors.

1.4. Summarizing data from the literature

The epicardial fat is a unique fat compartment localized between the myocardial surface and the visceral layer of the pericardium. The EAT can be quantified by non-invasive cardiac imaging techniques such as echocardiography, MRI or cardiac CT.

Among physiological determinants of EAT age, gender, body weight and ethnicity should be considered. Physiological functions of EAT may include protection of the myocardium against hypothermia and a mechanical protective role for coronary circulation. In addition, EAT may serve as a unique energy buffering pool in the homeostasis of the myocardium.

As for pathophysiological functions it is widely accepted that EAT should be considered as a source of inflammatory mediators that might directly influence the myocardium and coronary arteries. In line with these observations clinical studies suggested that EAT - through paracrine and vasocrine effects - may have an impact on the development and progression of coronary atherosclerosis. In addition, an association between increased EAT and atrial fibrillation was also documented. The insulin resistance syndrome (the metabolic syndrome), type 2 diabetes, NAFLD and CAD proved to be associated with increased amount of epicardial fat. Interestingly, an accumulation of EAT was observed also in patients with type 1 diabetes.

Treatment options for modifying EAT volume include lifestyle changes, bariatric surgery and using different drugs. Weight reduction in obese subjects may lead to a decrease in EAT volume while effects of different drugs on EAT are controversial. Nevertheless, EAT should be considered as a new cardiovascular therapeutic target.

No data are available whether EAT compartment quantity depends predominantly on genetic or environmental factors. Furthermore, data regarding the heritability of abdominal adipose tissue compartment sizes are scarce and the findings are based on family studies and on measurement methods with limited accuracy.

DOI:10.14753/SE.2020.2396

2. Aims

After adopting a proper and reliable method for evaluating the quantity of EAT by using cardiac CT scan in our department, we designed a study to evaluate the heritability of EAT quantity in comparison to that of abdominal SAT and VAT volumes. Then, we assessed the association between EAT volume and the presence of CAD in order to evaluate to potential role of EAT in the development of CAD.

The aims of the study were

2.1. to evaluate the heritability of EAT quantity - for this reason a classical twin study was performed and genetic and environmental influences on EAT volumes were estimated; in addition, a special attention was paid to evaluating heritability of EAT in comparison to that of abdominal SAT and VAT volumes;

2.2. to assess the relationship of EAT volume to the presence of CAD - for this reason the association between EAT quantity and radiomorphological signs of CAD was evaluated.

DOI:10.14753/SE.2020.2396

3. Methods

3.1. Classical twin study

3.1.1. Patients

The study was conducted as a part of the BUDAPEST-GLOBAL (Burden of atherosclerotic plaques study in twins - Genetic Loci and the Burden of Atherosclerotic Lesions) clinical study; the participants had been co-enrolled with the large, international, multicenter Genetic Loci and the Burden of Atherosclerotic Lesions (GLOBAL) clinical study (http//:www.ClinicalTrials.gov: NCT01738828) (134, 135).

The primary aim of the BUDAPEST-GLOBAL clinical study was to evaluate the influence of genetic and environmental factors on the burden of coronary artery disease. We hypothesized that the correlation of coronary plaque volume would be stronger between the MZ twins as compared to DZ twins, which might suggest that this CAD phenotype could be mainly driven by genetic factors. The secondary aims of the study were to quantify the heritability of coronary artery geometry, furthermore to assess the association between CAD heritability and the heritability of hepatic lipid accumulation, epicardial and abdominal adipose tissue quantity, carotid intima-media thickness and hemodynamic parameters. Classical and new cardiovascular risk factors were measured and potential associations with coronary artery disease and adipose tissue compartments were analyzed. In this PhD work, results of measurements of EAT and abdominal fat quantities and those of a clinical study performing to assess the potential association between EAT quantity and CAD are summarized.

In the BUDAPEST-GLOBAL clinical study we searched the Hungarian Twin Registry's database (136) to identify adult MZ and same-sex DZ twins whose previously registered disease history meets the inclusion criteria of the study. The aim was to balance the overall participation for 50% females and at least 50% DZ twins. These twins were contacted by phone or email and the study protocols were described in detail. Thereafter, detailed study description was sent by email or mail to twins, which included inclusion

and exclusion criteria as well. The majority (90%) of the contacted twin pairs were willing to participate. Inclusion and exclusion criteria are listed in **Table 3.** Of note, subjects with pregnancy, regular alcohol consumption (more than 2 units daily), conditions possibly interfering with compliance during CT scanning and acute infection within three weeks were excluded from the study.

Table 3. Enrollment criteria

Inclusion criteria
1. Monozygotic (MZ) twins and same-sex dizygotic (DZ) twins
2. Age: females 40-75 years, males 35-75 years
3. The participant has signed the institutional review board/ethics committee-approved informed consent form
Exclusion criteria
1. Subjects for whom coronary computed tomography angiography is contraindicated per institutional standard of care (history of severe and/or anaphylactic contrast reaction, inability to cooperate with scan acquisition and/or breath-hold instructions, pregnancy, clinical instability, and renal insufficiency).
2. Subjects with previous coronary arterial revascularization (percutaneous coronary intervention or coronary artery bypass grafting)
3. Subjects with atrial fibrillation/flutter or frequent irregular or rapid heart rhythms, which occurred within the past 3 months
4. Subjects with a pacemaker or implantable cardioverter-defibrillator implant
5. Active congestive heart failure or the presence of known non-ischemic cardiomyopathy
6. Known genetic disorders of atherosclerosis, lipid, or lipoprotein metabolism

All subjects were asked not to smoke and not to eat three hours, not to drink alcohol and coffee ten hours prior to the examinations. During the enrolment, the zygosity was assessed using a standardized questionnaire based on seven self-reported responses (137). The timeline of study procedures is described in **Table 4.**

Table 4. Timeline of study procedures

	Procedure	Assessed parameters
Day 1	Physical examination	Anthropometric parameters
	Questionnaire	Past medical history and current lifestyle
	Blood draw	Laboratory parameters and panomics data
	Non-contrast enhanced CT	Agatston-score, epicardial fat, hepatic lipid accumulation, abdominal fat
	Contrast-enhanced CT	Coronary plaque and geometry
Day 2	Echocardiography	Standard analysis and speckle tracking
	Vascular ultrasonography	Both carotid and femoral arteries
	Hemodynamic measurements	Brachial and central blood pressures, pulse wave velocity values, augmentation indices

In the BUDAPEST-GLOBAL study we enrolled prospectively a total of 202 twin subjects (101 twin pairs) between April 2013 and July 2014. We summarize the main clinical characteristics of the patients here in **Table 5**. As in some patients we recognized inadequate image quality for the respective analysis, the number of patients in a particular clinical study differed from that of the total cohort. Therefore, the patients' characteristics of the specific clinical study are incorporated into the relevant results.

The national ethics committee approved the BUDAPEST-GLOBAL study (ETT TUKEB: 58401/2012/EKU [828/PI/12]; Amendment: 12292/2013/EKU). All patients provided written, informed consent before the investigations. The study was carried out according to the principles stated in the Declaration of Helsinki.

Table 5. Demographics, twin characteristics in the BUDAPEST-GLOBAL study

* Data are mean values plus or minus standard deviation.

** Difference between MZ and DZ twins: t-test or Chi-square test as appropriate.

Characteristics	Full cohort (n=202)	MZ (n=122)	DZ (n=80)	p**
Women (%)	130 (64.4%)	74 (60.7%)	56 (70.0%)	0.18
Age (years)*	56.2 ± 9.4	54.9 ± 9.7	58.3 ± 8.4	0.01
Height (cm)*	166.3 ± 9.6	166.2 ± 10.0	166.4 ± 9.0	0.87
Weight (kg)*	77.3 ± 17.2	77.4 ± 17.7	77.1 ± 16.4	0.92
Body mass index ($kg \times m^{-2}$)*	27.8 ± 5.3	27.8 ± 5.0	27.9 ± 5.8	0.94
Waist circumference (cm)*	97.1 ± 14.0	96.8 ± 14.2	97.5 ± 13.7	0.72
Hypertension (%)	86 (42.6%)	49 (40.2%)	37 (46.3%)	0.39
Diabetes mellitus (%)	18 (8.9%)	12 (9.8%)	6 (7.5%)	0.57
Dyslipidemia (%)	87 (43.1%)	48 (39.3%)	39 (48.8%)	0.19
Current smoker (%)	31 (15.3%)	19 (15.6%)	12 (15.0%)	0.91

3.1.2. Anthropometric data and medical history

Complete physical examination was performed and anthropometric parameters (weight, height and waist circumference) were recorded. Weight was measured with calibrated digital scale while height was recorded with a wall-mounted stadiometer. Body mass index (BMI - kg/m^2) was calculated from weight and height values (weight [kg] was divided by height [m] on square meter). We measured waist circumference by a standard method at the midpoint between the lowest rib and the iliac crest at the end of expiration, placing the tape horizontally.

Brachial blood pressure was measured prior the CT. A 12-lead ECG and echocardiographic evaluation were performed in each twin subject.

Smoking habit was assessed and smoking years were recorded and alcohol consumption was evaluated as units per week. Physical activity, diet and socio-economic status were assessed by using questionnaires. Prevalence of hypertension, diabetes mellitus, dyslipidemia and cerebrovascular disease was documented based on the medical history of the participants.

3.1.3. Laboratory parameters

Enrolled twins underwent a peripheral blood draw, and blood was aliquoted and stored as whole blood, plasma, serum, and buffy coat. All subjects underwent whole genome sequencing according the protocol described in the GLOBAL study (134). This part of the study is still ongoing and does not belong to the current PhD work. Conventional biomarker testing was performed at Health Diagnostic Laboratory, Inc (Richmond, VA; United States of America). Fasting lipid profile was measured on an auto-analyzer using standard clinical methods (Beckman-Coulter). Hemoglobin A1c was measured using Trinity Biotech reagents (Trinity Biotech USA Inc, Jamestown, NY).

3.1.4. Epicardial fat volumetric assessment

Every subject underwent a non-contrast enhanced CT scan of the heart using a 256-slice CT scanner (Philips Brilliance iCT, Philips Healthcare, Best, The Netherlands; 120 kVp with tube current of 20 to 50 mAs depending on BMI, gantry rotation time 270 ms). The pericardial space was manually traced in each CT-slice in the native cardiac CT datasets. The adipose tissue was defined as tissue in the attenuation range of -45 to -195 HU. EAT was defined as any adipose tissue within the visceral pericardium from the level of the right pulmonary artery to the diaphragm (57, 113). The EAT segmentation was automatically interpolated within the manually traced region of interest (ROI), and the volume was calculated by using an offline workstation (Extended Brilliance Workspace, Philips Healthcare, Best, The Netherlands). Representative cases from the twin study can be seen in **Figure 6**.

DOI:10.14753/SE.2020.2396

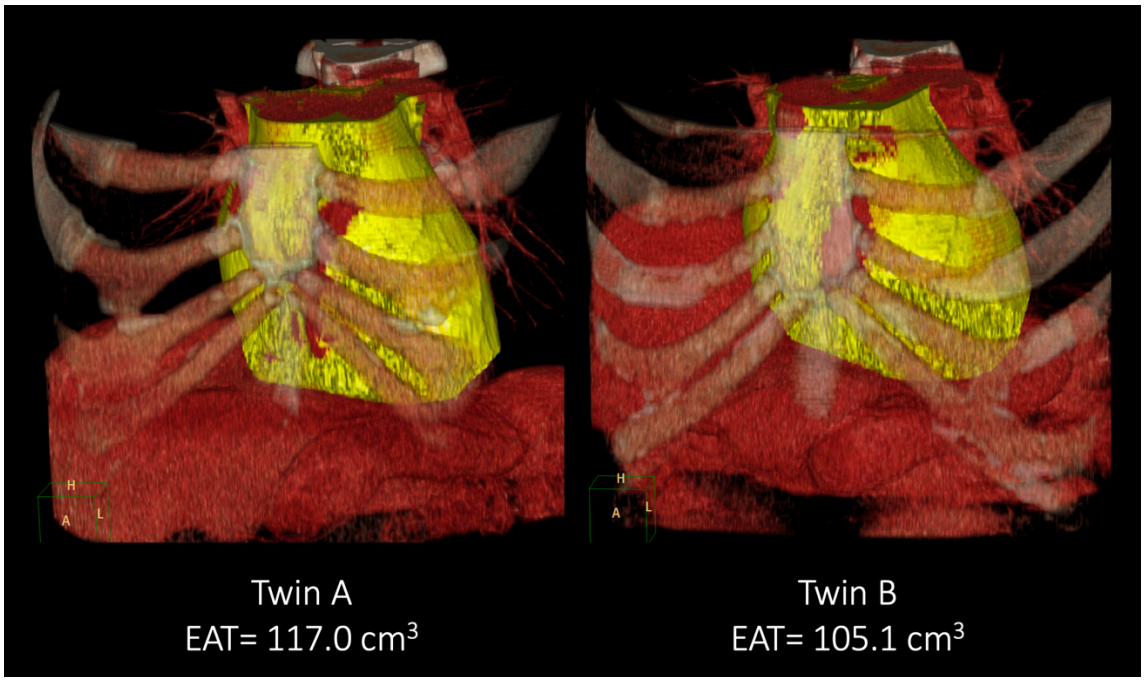

Figure 6. Epicardial adipose tissue (EAT) quantity

Representative cases from the study: epicardial fat volume in a monozygotic twin pair. Volume rendered reconstructions are shown of the mediastinal region; epicardial fat volume is marked with yellow.

3.1.5. Assessment of abdominal SAT and VAT

Subsequently after the non-enhanced cardiac CT a single 5 mm thick slice (120 kVp; 200 mA; gantry rotation time, 270 ms) was acquired at the level of L3-L4 vertebrae. The single CT slice was loaded onto an offline workstation and the subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) areas (cm^2) were measured using a dedicated offline workstation (Extended Brilliance Workspace, Philips Health Care, Best, The Netherlands). A semi-automated software tool identified the abdominal muscular wall separating the SAT and VAT compartments with the possibility of manual adjustment when needed. To identify pixels containing adipose tissue an attenuation range of -45 to -195 HU was defined (138).

Importantly, the native CT of the heart and abdomen resulted in a small (0.70 ± 0.16 mSv) radiation dose.

Representative cases from the twin study can be seen in **Figure 7.**

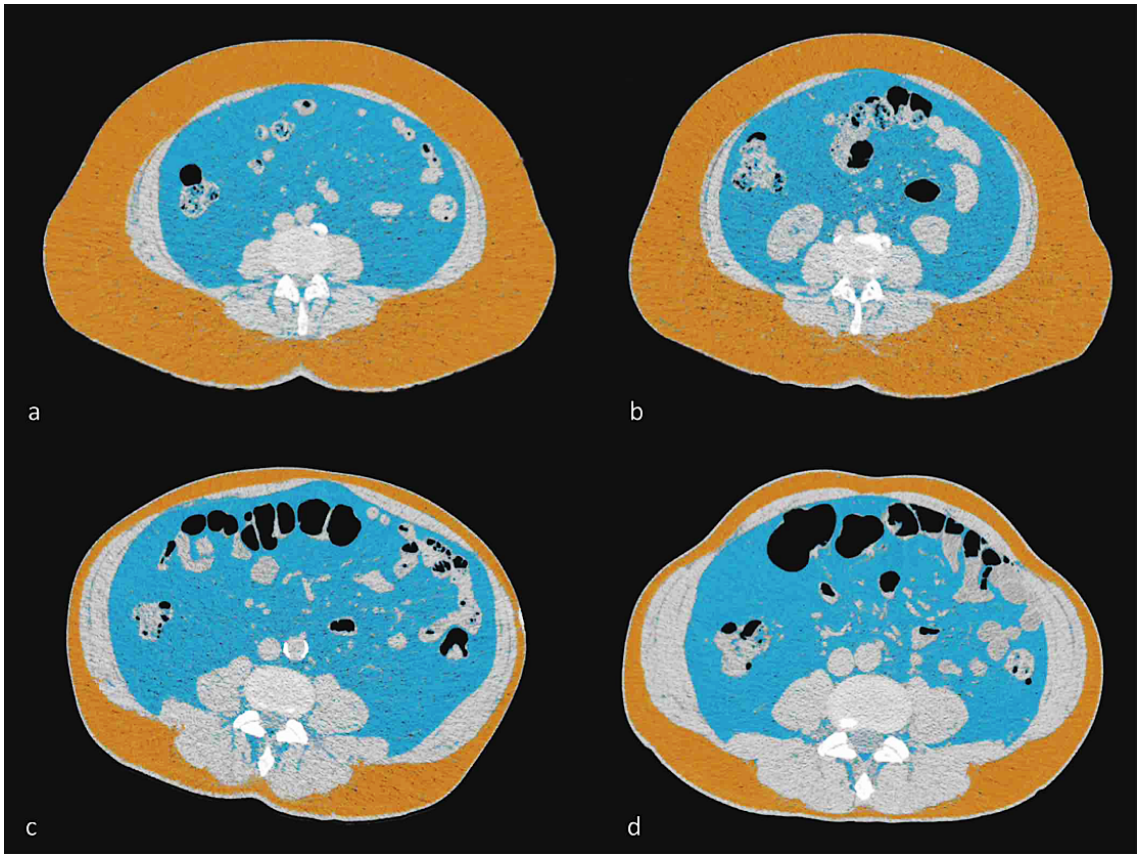

Figure 7. Abdominal subcutaneous and visceral adipose tissue compartments (SAT and VAT) in monozygotic twin pairs - representative cases from the study

a-b) Axial images of the abdomen at the level of the L3/L4 vertebrae. Subcutaneous fat (orange color) is predominant in this monozygotic twin pair.

c-d) Axial images of the abdomen at the level of the L3/L4 vertebrae. Visceral fat (blue color) is more prominent in this monozygotic twin pair.

3.1.6. Cardiac computed tomography

ECG triggered coronary CT angiography (CTA) was performed using a 256-slice multidetector CT (Brilliance iCT, Philips Health Care, Best, The Netherlands). We administered per os β-blockers (metoprolol, maximum dose 100 mg) 1 hour before the CT scan if the heart rate was >65 beat per minute. Intravenous β-blocker (metoprolol) was administered (maximum cumulative dose 20 mg) on the table if the heart rate was still higher than 65 beat per minute. Sublingual nitroglycerin (0.8 mg) was administered on the table, maximum 2 minutes before the image acquisition. Images were acquired during a single inspiratory breath hold in axial mode with 270 ms rotation time, 128×0.625 mm collimation, tube voltage of 100-120 kVp, maximum effective tube current-time product of 200-300 mAs at 78% of the R-R interval. Triphasic contrast injection protocol was used with 80 mL of iodinated contrast agent in average (Iomeprol 400 g/cm^3, Iomeron, Bracco Imaging S.p.A., Milano, Italy); mixture of contrast agent and saline (10 mL contrast agent and 30 mL saline); and 40 mL saline solution, all injected at a rate of 4.5-5.5 ml/s. We have reconstructed the minimum slice thickness (0.8 mm) available in prospective ECG triggered image acquisition with an increment of 0.4 mm, which resulted in an approximately 0.6 mm isotropic resolution. The mean effective radiation dose of the coronary CTA scans was 3.64±1.04 mSv (dose length product: 260.1±74.5 mGy×cm). All image analyses were performed offline on dedicated cardiac workstations (Intellispace Portal, Philips Healthcare, Best, The Netherlands).

3.1.7. Coronary plaque assessment

The coronary CTA datasets were analyzed on a qualitative and quantitative basis. Coronary segments with a minimum diameter of 2.0 mm are included in the analysis. Each coronary segment is assessed for presence of plaque, plaque type, degree of stenosis, plaque features and plaque attenuation pattern. Coronary plaque is classified as non-calcified plaque, partially calcified plaque or calcified plaque (139, 140). Stenosis is graded as none, minimal (<25%), mild (25%-49%), moderate (50%-69%), severe (70%-99%), or occlusion (100%), based on visual estimation of percent diameter stenosis (139).

Segment involvement score and segment involvement score index is used to provide a semi-quantitative measurement of plaque burden (141). In the clinical study for evaluating the relationship of EAT volume to the CAD, coronary CTA was evaluated on subject-to-subject basis and subjects were classified into groups with and without CAD (CAD-positive and CAD-negative subjects).

3.1.8. Reproducibility of measuring EAT, SAT and VAT quantities

For assessing the reproducibility of EAT, SAT and VAT quantity measurements, two readers (Adam L. Jermendy, Zsofia D. Drobni) performed repeated measurements on 10 randomly selected MZ twin pairs and 10 randomly selected DZ twin pairs images in order to determine the intra-class correlation coefficient (ICC).

3.1.9. Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD), whereas categorical variables are expressed as numbers and percentages. MZ and DZ twins were compared using Student's t-tests and Chi-square tests. Correlations were calculated using Pearson correlation coefficients. Coefficient values are interpreted as: 1.00 - 0.81: excellent; 0.80 - 0.61: good; 0.60 - 0.41: moderate; 0.40 - 0.21: fair; 0.20 - 0.00: poor (142). Descriptive statistics, correlations and reproducibility measurements were calculated using IBM SPSS Statistics version 23 (IBM, Armonk, NY, USA).

Heritability was assessed in two steps; first, co-twin correlations between the siblings were analyzed in MZ and DZ pairs separately. Next, genetic structural equation models were used to model the magnitude of genetic and environmental factors influencing the different fat compartments.

All phenotypes are caused by genetic and environmental factors. MZ twins share nearly 100% of their genome, while DZ twins only share half. Genetic similarity is caused by additive genetic components (A). While MZ twins share almost 100% of A, DZ twins only share 50% of A. Environmental components are grouped as common factors (C)

i.e. same early childhood, education in the same school, living in the same town, etc. which equally effect the siblings and unique factors (E) such as specific eating and drinking habits, different physical activity and life-style, etc. which cause differences within families. In our study, both MZ and DZ twins shared 100% of their C factors and none of their E factors. Covariance between the siblings can be decomposed into A, C and E latent variables using genetic structural equation models (143). The likelihood ratio test was used to assess the fit of submodels compared to the full model. If the fit did not decrease significantly by removing one of the parameters, then the more parsimonious submodel was selected. Furthermore, multivariate genetic models can be used to further decompose the results of the heritability estimates into common and unique genetic and environmental factors. Common genetic factors refer to genes that are driving the heritability of all three fat components simultaneously (A_c), while common (C_c) and unique (E_c) environmental factors refer to circumstantial factors that affect the heritability of all three phenotypes. The remaining variance then can be attributed to genetic (A_s), common (C_s) and unique (E_s) environmental factors specific of a given phenotype, which are independent of the other phenotypes. Therefore, the heritability of the fat compartments was decomposed to common (A_c, C_c, E_c) and specific (A_s, C_s, E_s) genetic and environmental factors. Independent and common pathway models were used to find the most parsimonious model best describing our data. All calculations were adjusted for age and sex. Log likelihood-based 95% confidence intervals (CI) were calculated for all estimated parameters. All calculations were performed using R version 3.2.5. (144). Twin modelling was performed using OpenMx version 2.5.2 (145). A *p* value lower than 0.05 was considered significant.

3.2. Assessing the relationship of EAT volume to CAD

3.2.1. Patients and methods

We included 195 subjects (age: 56.1±9.4 years, female 64.1%) from the BUDAPEST-GLOBAL study. All subjects underwent coronary CT angiography (CTA) and were classified into groups with and without CAD (CAD-pos: n=106 and CAD-neg: n=89,

respectively), based on the presence or absence of any plaque in coronary CTA. In addition, we measured the EAT volume on a native cardiac scan and the abdominal adipose tissue areas on a single CT-slice acquired at the L3/L4 level. Details of methods are given in the previous sections.

3.2.2. Statistical analysis

We used Student's unpaired t-test for assessing the statistical difference between CAD-pos and CAD-neg groups and a robust maximum likelihood estimation for correcting the potential bias from set of twins. We estimated the association between CAD and risk factors (including EAT, SAT and VAT values) using a logistic regression analysis. We used female gender, age, hypertension, dyslipidemia, diabetes mellitus, BMI, EAT, SAT and VAT in the model.

4. Results

4.1. Assessing genetic and environmental influences on EAT quantity in comparison to abdominal SAT and VAT volumes

Overall, 180 twins (57 MZ twin pairs, 33 DZ twin pairs) were included from the BUDAPEST-GLOBAL study. Our study population represents a middle-aged, slightly overweight Caucasian population (**Table 6**).

Intra-reader agreement showed excellent reproducibility for all CT based fat measurements as intra-class correlations (ICC) proved to be higher than 0.98 (ICC_{EAT} = 0.99; ICC_{SAT} = 0.98; ICC_{VAT} = 0.99). We also found excellent reproducibility regarding inter-reader variability (ICC_{EAT} = 0.98; ICC_{SAT} = 0.99; ICC_{VAT} = 0.99).

Co-twin correlations between the siblings showed that for all three parameters, MZ twins have stronger correlations than DZ twins, suggesting prominent genetic effects (EAT: r_{MZ} = 0.81, r_{DZ} = 0.32; SAT: r_{MZ} = 0.80, r_{DZ} = 0.68; VAT: r_{MZ} = 0.79, r_{DZ} = 0.48).

For all three fat compartments AE model excluding common environmental factors proved to be best fitting [EAT: A: 73% (95% CI = 56%-83%), E: 27% (95% CI = 16-44%); SAT: A: 77% (95% CI = 64%-85%), E: 23% (95% CI = 15%-35%); VAT: A: 56% (95% CI = 35%-71%), E: 44% (95% CI = 29%-65%)]. Detailed results can be found in **Table 7.**

In multi-trait model fitting analysis overall contribution of genetic factors to EAT, SAT and VAT was 80%, 78% and 70%, whereas that of environmental factors was 20%, 22% and 30%, respectively (**Table 8**). We began with multi-trait model fitting by running a Cholesky decomposition of our data (Model 1, Cholesky ACE). All further models were compared to this full model. We dropped all C-s in the 2. model (Model 2, Cholesky AE) which did not decrease fit significantly (p = 0.85, AIC = 6.47) indicating the insignificance of common environmental factors, thus later models only assuming A and E factors were considered. Independent pathway model calculating with common and specific A and E factors (Model 3, Independent pathway AE) showed slightly worse fit

than model 2 (p = 0.85, AIC = 6.54). We calculated a common pathway model (Model 4, Common pathway AE 1) where common A and E factors were mediated through a latent phenotype, while the residual variance was decomposed to specific A and E factors which showed better fit based on information criteria measures (p = 0.78, AIC = 4.57). A model similar to the previous one (Model 5, Common pathway AE 2) but dropping the specific A of VAT proved to be the best fitting model (p = 0.85, AIC = 2.57). Detailed contribution of common and specific genetic and environmental factors for all three fat compartments can be found in **Table 8**, while the path diagram of the model can be found in **Figure 8.**

Results of the multi-variate analysis suggest that a common latent phenotype is associated with the tissue compartments investigated. Based on our results, 98% (95% CI = 77%-100%) of VAT heritability can be accounted by this common latent phenotype which also effects SAT and EAT heritability. This common latent phenotype accounts for 26% (95% CI = 13%-42%) of SAT and 49% (95% CI = 32%-72%) of EAT heritability. This common latent phenotype is influenced by genetics in 71% (95% CI = 54%-81%) and environmental effects in 29% (95% CI = 19%-46%). Accordingly, the proportion of common and specific genetic and environmental factors contributing to the adipose tissue quantities may differ from each other, for example in case of EAT heritability is caused by 35% common genetic, 45% specific genetic, 14% common environmental, and 6% specific environmental factors (**Figure 8**).

We also assessed whether the heritability of one of the parameters was independent of the remaining two phenotypes. To answer these questions, we ran common pathway models where the EAT did not have any common factors to SAT and VAT (Model 6, Common pathway AE SAT-VAT), but this showed significantly decreased fit as compared to the full model ($p = 5.61*10^{-26}$, AIC = 139.06). A model suggesting SAT was independent of VAT and EAT (Model 7, Common pathway AE VAT-EAT) also showed significantly decreased fit ($p = 3.94*10^{-10}$, AIC = 60.53). The last model where we assumed VAT to be independent of SAT and EAT (Model 8, Common pathway AE SAT-EAT) showed the worse fit ($p = 2.17*10^{-32}$, AIC = 169.95). These results all suggest that none of the phenotypes is independent of the other two, thus the heritability of EAT or

DOI:10.14753/SE.2020.2396

SAT or VAT phenotype is associated with the remaining two phenotypes. Detailed model fit results can be found in **Table 9.**

Table 6. Demographics, clinical-laboratory data and quantity of fat compartments measured in twins

Continuous variables are presented as mean ± SD, while categorical as n (%). P values represent two-sided p values for independent t-tests and those of Chi-square tests done between the monozygotic (MZ) and dizygotic (DZ) twin groups. BMI: body mass index; CRP: C-reactive protein; HbA1c: hemoglobinA1c; HDL: high-density lipoprotein; LDL: low-density lipoprotein

Variable	Total (n = 180)	MZ (n = 114)	DZ (n = 66)	p
Demographic, basic hemodynamic characteristics and medical history				
Female (n, %)	114 (63.3%)	68 (59.6%)	46 (69.7%)	0.52
Age (years)	55.8 ± 9.6	54.3 ± 9.7	58.4 ± 8.6	<0.01
Height (cm)	166.4 ± 9.6	166.7 ± 10.1	165.9 ± 8.8	0.63
Weight (kg)	77.2 ± 17.5	77.6 ± 18.3	76.4 ± 16.2	0.67
BMI (kg/m^2)	27.7 ± 5.2	27.7 ± 5.1	27.8 ± 5.4	0.98
Waist (cm)	96.9 ± 14.2	96.8 ± 14.6	96.9 ± 13.6	0.96
Hypertension (n, %)	76 (42.2%)	42 (36.8%)	34 (51.5%)	0.84
Diabetes mellitus (n, %)	15 (8.3%)	9 (7.9%)	6 (9.1%)	0.89
Dyslipidemia (n, %)	80 (44.4%)	46 (40.4%)	34 (51.5%)	0.48
Current smoker (n, %)	28 (15.6%)	17 (14.9%)	11 (16.7%)	0.88
Laboratory parameters				
Fasting blood glucose (mmol/l)	5.35 ± 1.34	5.31 ± 1.48	5.41 ± 1.06	0.66
HbA1c (%)	5.5 ± 0.9	5.5 ± 0.9	5.3 ± 0.9	0.13
Serum total cholesterol (mmol/l)	5.56 ± 1.09	5.63 ± 1.11	5.42 ± 1.07	0.21
Serum LDL-cholesterol (mmol/l)	3.47 ± 0.99	3.52 ± 1.04	3.37 ± 0.89	0.32
Serum HDL-cholesterol (mmol/l)	1.62 ± 0.39	1.61 ± 0.41	1.65 ± 0.35	0.56
Triglycerides (mmol/l)	1.57 ± 1.09	1.62 ± 1.23	1.47 ± 0.77	0.36
Serum creatinine (µmol/l)	80.0 ± 9.0	80.0 ± 9.0	80.0 ± 9.0	0.41
Serum CRP (mg/l)	2.9 ± 4.5	2.7 ± 2.9	3.3 ± 6.5	0.37
Serum leptin (ng/ml)	18.4 ± 17.9	16.2 ± 13.5	22.4 ± 23.6	0.06
CT-based fat measurements				
Epicardial fat (mm^3)	97.1 ± 45.4	94.9 ± 43.2	101.0 ± 49.2	0.38
Subcutaneous fat (mm^2)	217.9 ± 97.4	218.6 ± 90.1	216.7 ± 109.4	0.90
Visceral fat (mm^2)	156.6 ± 87.9	158.9 ± 89.2	152.6 ± 86.0	0.64

DOI:10.14753/SE.2020.2396

Table 7. Detailed model information regarding single trait classical twin models of CT-based fat measurements

Detailed results of calculated single trait ACE structure equation models. Log likelihood-based confidence intervals are represented in parenthesis. * indicates the most parsimonious full model based on AIC and BIC values. ** indicate the most parsimonious submodel based on likelihood difference test. A: additive genetic factors; C: common environment; E: unique environmental factors; -2LL: minus 2 log-likelihood value; AIC: Akaike information criterion; BIC: Bayesian information criterion

Variable	Full model	Estimated parameters	A	CI	C	CI	E	CI	Model *-2LL*	AIC	BIC	Difference to Saturated model *-2LL*	Difference to Saturated model *p*	Difference to Full model *-2LL*	Difference to Full model *p*
Epicardial fat	ACE*	ACE	0.73	[0.53-0.83]	0.00	[0.00-0.14]	0.27	[0.16-0.44]	410.50	418.50	428.50	11.89	0.06		
		AE**	0.73	[0.56-0.83]			0.27	[0.16-0.44]	410.50	416.50	424.00	11.89	0.10	0.00	1.00
		CE			0.38	[0.19-0.55]	0.62	[0.45-0.81]	428.89	434.89	442.39	30.29	<0.001	18.39	<0.001
		E					1.00	[1.00-1.00]	443.18	447.18	452.18	44.57	<0.001	32.679	<0.001
Subcutaneous fat	ACE*	ACE	0.53	[0.12-0.84]	0.23	[0.00-0.59]	0.24	[0.15-0.37]	429.32	437.32	447.32	3.81	0.70		
		AE**	0.77	[0.64-0.85]			0.23	[0.15-0.35]	430.22	436.22	443.72	4.71	0.70	0.90	0.34
		CE			0.65	[0.51-0.75]	0.35	[0.25-0.49]	436.01	442.01	449.51	10.50	0.16	6.69	<0.01
		E					1.00	[1.00-1.00]	484.54	442.01	449.51	59.03	<0.001	55.22	<0.001
Visceral fat	ACE*	ACE	0.56	[0.14-0.71]	0.00	[0.00-0.32]	0.44	[0.29-0.65]	370.27	378.27	388.27	6.69	0.34		
		AE**	0.56	[0.35-0.71]			0.44	[0.29-0.65]	370.27	376.27	383.77	6.69	0.46	0.00	1.00
		CE			0.38	[0.19-0.54]	0.62	[0.46-0.81]	376.18	382.18	389.68	12.60	0.08	5.91	0.02
		E					1.00	[1.00-1.00]	390.38	394.38	399.38	26.80	<0.001	20.11	<0.001

DOI:10.14753/SE.2020.2396

DOI:10.14753/SE.2020.2396

Table 8. Proportion of common and specific genetic and environmental factors contributing to the phenotypic quantity of CT based fat measurements

Variable	Epicardial fat	Subcutaneous fat	Visceral fat
Common genetic and environmental factors			
genetic factors (A_C)	35%	18%	70%
environmental factors (E_C)	14%	8%	28%
Specific genetic and environmental factors			
genetic factors (A_S)	45%	60%	0%
environmental factors (E_S)	6%	14%	2%
Overall contribution of genetic and environmental factors			
genetic factors (A)	80%	78%	70%
environmental factors (E)	20%	22%	30%

Table 9. Detailed model information regarding multi-trait classical twin models of CT-based fat measurements

Detailed results of calculated multi-trait structure equation models. -2LL: minus 2 log-likelihood value; AIC: Akaike information criterion; BIC: Bayesian information criterion; df: degrees of freedom; A: additive genetic factors; C: common environment; E: unique environmental factors; SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue; EAT: epicardial adipose tissue

Model number	Model name	Estimated parameters	Model *-2LL*	Model *df*	AIC	BIC	Difference to Saturated model *-2LL*	Difference to Saturated model *df*	Difference to Saturated model *p*	Difference to Full model *-2LL*	Difference to Full model *-df*	Difference to Full model *p*
1	Cholesky ACE	24	1047.78	516	15.78	-1274.12	31.38	30	0.40			
2	Cholesky AE	18	1050.47	522	6.47	-1298.43	34.08	36	0.56	2.69	6	0.85
3	Independent pathway AE	18	1050.54	522	6.54	-1298.36	34.15	36	0.56	2.76	6	0.84
4	Common pathway AE 1	17	1052.57	524	4.57	-1305.33	36.18	38	0.55	4.79	8	0.78
5	Common pathway AE 2	16	1052.57	525	2.57	-1309.83	36.18	39	0.60	4.79	9	0.85
6	Common pathway AE SAT-VAT	16	1189.06	525	139.06	-1173.34	172.67	39	$9.66*10^{-19}$	141.28	9	$5.61*10^{-26}$
7	Common pathway AE VAT-EAT	16	1110.53	525	60.53	-1251.86	94.14	39	$1.86*10^{-6}$	62.75	9	$3.94*10^{-10}$
8	Common pathway AE SAT-EAT	16	1219.96	525	169.95	-1142.44	203.57	39	$3.81*10^{-24}$	172.18	9	$2.17*10^{-32}$

DOI:10.14753/SE.2020.2396

DOI:10.14753/SE.2020.2396

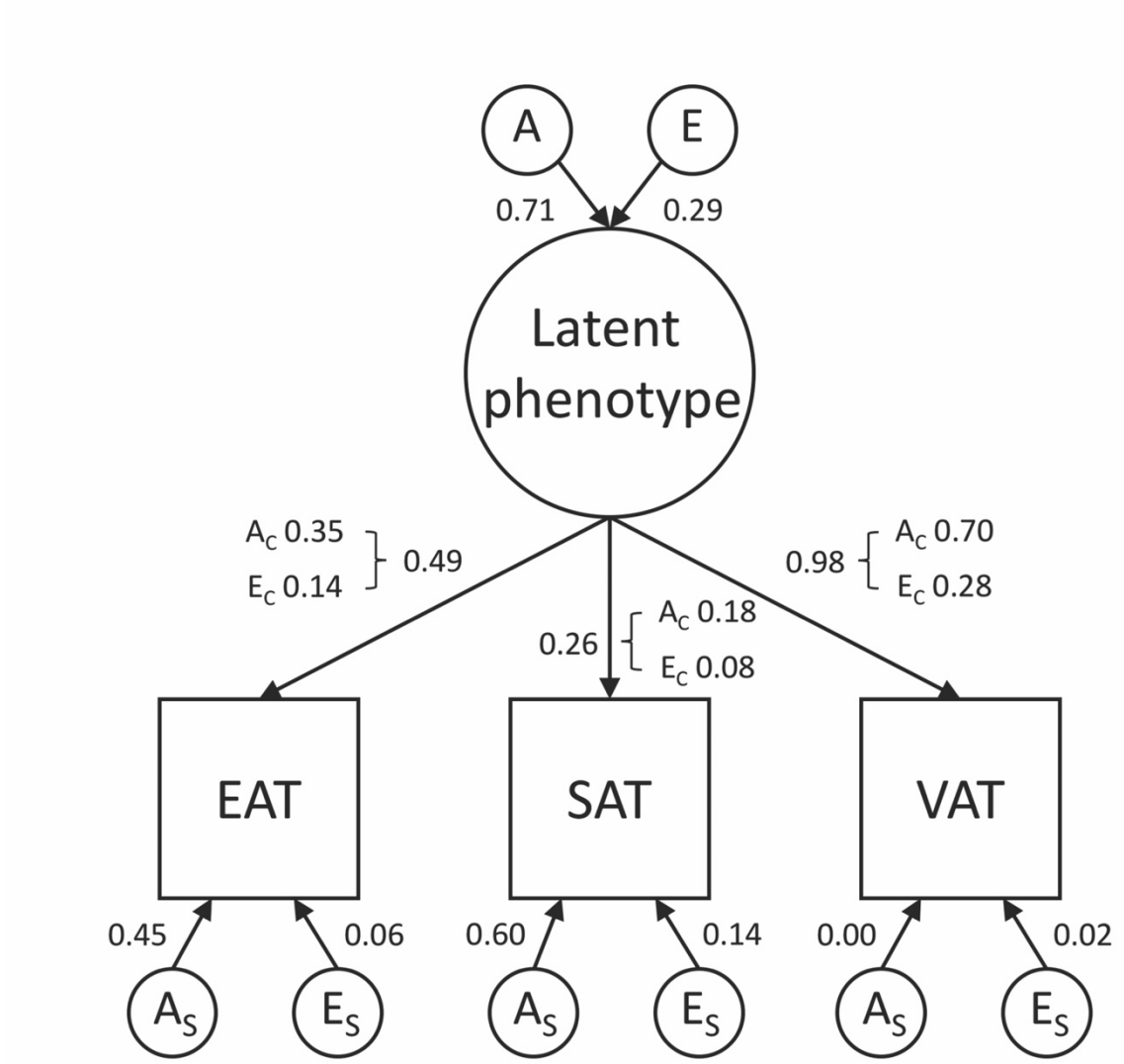

Figure 8. Proportion of phenotypic variance of CT-based fat measurements

The image shows squared standardized path coefficients of best fitting model 5. The common pathway model calculating with only common genetic and environmental factors proved to be the best. Residual variances were decomposed to specific genetic and environmental factors. In case of VAT only specific environmental factors were considered. A: additive genetic factors; E: unique environmental factors; A_c: common additive genetic factor; A_s: specific additive genetic factor; E_c: common environmental factor; E_s: specific environmental factor; EAT: epicardial adipose tissue; SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue

DOI:10.14753/SE.2020.2396

4.2. Evaluating the association between EAT volume and the presence of CAD

The patients' characteristics are given in **Table 10**. Subjects from the CAD-pos group were older, had a higher BMI, weight, waist circumference, EAT, abdominal SAT and VAT volumes than subjects from the CAD-neg group. The ratio of EAT/SAT and EAT/BMI were higher in CAD-pos vs. CAD-neg patients. There were less female in the CAD-pos group, and in this group the presence of hypertension, dyslipidemia and diabetes were more frequent. Considering the lipid and glucose levels, we observed a significant difference only in the serum triglyceride levels favoring CAD-negative patients.

Table 10. Clinical characteristics and main laboratory findings in CAD-negative and CAD-positive patients

Continuous variables are presented as mean ± SD, while categorical as n (%).

BMI: body mass index; CAD: coronary artery disease; EAT: epicardial adipose tissue; SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue; LDL: low-density lipoprotein; HDL: high-density lipoprotein

Variable	CAD-negative patients (n=89)	CAD-positive patients (n=106)	p
age (years)	51.9 ± 9.3	59.7 ± 8.0	<0.001
women	70 (78.6%)	55 (51.9%)	<0.001
hypertension	20 (22.5%)	62 (58.5%)	<0.001
dyslipidemia	30 (33.7%)	55 (51.9%)	0.014
diabetes mellitus	4 (4.5%)	14 (13.2%)	0.046
BMI (kg/m^2)	26.4 ± 4.2	28.8 ± 5.6	0.015
weight (kg)	72.4 ± 13.5	81.0 ± 18.5	0.002
waist circumference (cm)	92.8 ± 11.2	100.4 ± 14.9	<0.001
EAT (cm^3)	73.9 ± 27.3	117.2 ± 46.8	<0.001
SAT (cm^2)	202.0 ± 83.5	230.3 ± 102.6	<0.001
VAT (cm^2)	115.5 ± 60.1	190.7 ± 89.9	<0.001
EAT/SAT (cm^3/cm^2)	0.41 ± 0.21	0.64 ± 0.51	<0.001
EAT/VAT (cm^3/cm^2)	0.75 ± 0.38	0.75 ± 0.51	0.982
EAT/BMI ($cm^3/kg \times m^{-2}$)	2.80 ± 0.90	4.00 ± 1.50	<0.001
total cholesterol (mmol/l)	5.6 ± 1.0	5.5 ± 1.1	0.598
triglycerides (mmol/l)	1.3 ± 0.9	1.7 ± 1.1	0.021
LDL-cholesterol (mmol/l)	3.5 ± 1.0	3.5 ± 1.0	0.847
HDL-cholesterol (mmol/l)	1.7 ± 0.3	1.6 ± 0.4	0.070
fasting blood glucose (mmol/l)	5.2 ± 0.9	5.5 ± 1.6	0.056

Age (odds ratio [OR]: 1.100 p<0.001), hypertension (OR: 3.265 p<0.05), female sex (OR: 0.117 p<0.001) and the volume of EAT in 10 cm^3 clusters (OR: 1.315 p=0.001) were independent predictors for CAD. A 10 cm^3 increment in the volume of EAT increased the risk of CAD with 31%, independently from BMI values. Female sex was a protective factor, therefore male sex should be considered a positive predictive factor (**Table 11**).

DOI:10.14753/SE.2020.2396

Table 11. Association between CAD (coronary artery disease) and clinical/laboratory parameters (risk factors) - results of the logistic regression analysis

BMI: body mass index; EAT: epicardial adipose tissue; SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue

Variable	Odds ratio	p
age	1.100	<0.001
female gender	0.117	<0.001
BMI	0.841	0.043
hypertension	3.265	0.029
dyslipidemia	1.763	0.208
diabetes mellitus	1.489	0.638
EAT (10 cm^3)	1.315	0.001
SAT (cm^2)	1.007	0.057
VAT (cm^2)	0.999	0.803

DOI:10.14753/SE.2020.2396

5. Discussion

5.1. Heritability of EAT volume

In a classical twin study, we showed that EAT, SAT and VAT quantities can be measured reliably by CT. We demonstrated that genetics have substantial, while environmental factors have only a modest influence on EAT, SAT and VAT volumes. Furthermore, our findings show that common and specific genetic effects both play an important role in developing these phenotypes. None of the phenotypic appearance of EAT, SAT and VAT proved to be completely independent of the other two. To the best of our knowledge, this is the first clinical study to evaluate the genetic and environmental dependence of EAT quantity and assessed simultaneously the joint heritability of EAT, SAT and VAT in twin pairs.

In the total cohort, SAT mean quantity was higher (217.9 mm^2) than that of VAT (156.6 mm^2), the ratio of the quantities was nearly similar to other observations in a different population (138). The mean volume of EAT (97.1 cm^3) was in the range of middle-aged healthy subjects (146). It is of note, that SAT and VAT was planimetrically but EAT was volumetrically measured in our cohort. Importantly, there was no significant difference in the assessed fat volumes comparing MZ to DZ subjects.

We used advanced statistical methods to decipher the ratio of genetic and environmental effects on EAT, SAT and VAT quantities. In addition to single trait analysis, we performed multi-trait models to explore the complex interactions of multiple quantitative traits. This method has been recently used to dissect genetic mechanisms underlying complex diseases such as obesity (147, 148). We demonstrated that common genetic effects predominated over common environmental influences on the latent phenotype (71% versus 29%). On the other hand, while the latent phenotype markedly influenced VAT (98%), its effect was minimal on SAT (26%) and its impact on EAT was intermediate (49%). Our results also suggest a stronger phenotypic relationship of VAT to EAT than VAT to SAT. Latent phenotype could be related to BMI, obesity or total fat depot but this was not specifically investigated in our analysis. Regarding the whole distribution of variance of CT-based fat measurements it seems that the phenotypic

appearance of EAT, SAT and VAT quantities are driven by common and specific genetic and environmental factors (**Figure 8, Table 8**). Finally, in Model 6-8 analyses (**Table 9**) we found that none of the fat compartments' heritability was independent of the other two. Taken together, an interplay between common and specific genetic effects and environmental influences may be hypothesized, but the magnitude of their relative impact on different adipose tissue compartments varies.

We demonstrated a relatively strong genetic dependence of EAT, which has not been described previously. The genetic dependence of anthropometric parameters (weight, height, BMI) has been well documented in former twin studies (126, 149, 150). Heritability of different ectopic fat compartments (hepatic lipid accumulation) was also investigated in twins, and in this case environmental factors predominated over genetic influences (151). Hence, heritability of different adipose tissue compartments and that of ectopic fats may vary.

The presence of strong genetic predisposition does not automatically translate to the development of clinical disease phenotype. Considering this fact, early and continuous preventive efforts should be implemented. In case of obesity, intervention should be initiated as early as possible and all modifiable risk factors should be addressed with diet, physical activity and behavioral interventions starting as early as preschool age (152, 153). Importantly, weight loss and exercise training may reduce EAT and abdominal adipose tissue volumes in adult subjects with obesity (94, 95).

In our study, abdominal SAT and VAT were planimetrically assessed using a single 5 mm thick slice at the level of L3-L4 vertebrae. This method was chosen in order to minimize the radiation dose. Moreover, it was documented in the Framingham heart study that planimetric area based measurements of abdominal SAT and VAT are strongly associated with abdominal SAT and VAT volumes (154).

Non-contrast enhanced CT scan was used to evaluate quantities of various fat compartments, although other non-invasive methods (echocardiography, magnetic resonance imaging [MRI]) have been used previously. Echocardiography has several disadvantages including poor reproducibility and high dependence of investigator's experience (17). MRI provides accurate area measurements but is not as widely available

in routine clinical practice as CT. Furthermore, it is more expensive and has poorer spatial resolution compared to CT (19). The CT-based volumetric measurements in our study were highly reproducible. In addition, it is important to note that to the best of our knowledge, our study represents the first investigation using CT phenotyping of fat compartments in twins.

Our results have to be interpreted within the context of their limitations. The sample size was modest but comparable to that of other classical twin studies (155). The zygosity in our twin cohort was classified according to validated questionnaires. Nevertheless, this method is widely accepted in clinical studies (137). Our results were derived from a healthy twin Caucasian population; therefore, the generalizability of our findings is limited. There was a small albeit significant difference in age of MZ versus DZ pairs but in our genetic analyses all parameters were age- and gender-adjusted. Nevertheless, an over-estimation of heritability might occur if twin-twin correlations would decline with age in DZ pairs but regarding the mean age of MZ and DZ pairs, a substantial effect should not be considered in our study.

The strengths of our study are worth mentioning. The study was performed at an institution with vast experience in cardiac CT imaging and in conducting twin studies. Furthermore, all CT scans were performed by the same trained investigators. The reliability of CT scan measurements proved to be excellent. The use of structural equation model for evaluating heritability was not restricted to univariate analysis only. The predominant genetic effect on EAT, SAT and VAT was demonstrated not only in single trait but in multi-trait analyses; the latter is considered a more robust method.

Taken together, genetic factors have substantial influence, while environmental factors have only a modest impact on EAT volume, abdominal SAT and VAT quantities. There is a considerable amount of common genetic background influencing the quantities of all three adipose tissue compartments.

DOI:10.14753/SE.2020.2396

5.2. Relationship of EAT volume to the presence of CAD

We found that the quantity of EAT is associated - among others - with the presence of CAD as a 10 cm^3 increment in the volume of EAT increased the risk of CAD with 31%, independently from the BMI values. Importantly, we do not want to interpret this result as a causative relationship, but we feel that our results are in accordance with former publications suggesting that EAT may contribute to the pathomechanism of CAD. For example, *Ishii et al* in an early investigation observed that in patients with myocardial bridge syndrome coronary arteries covered only by myocardium did not exhibit signs of atherosclerosis, indicating that EAT might play an important role in developing coronary atherosclerosis (41). From the early publication of *Mazurek et al* it became widely accepted that EAT - through vasocrine and paracrine mechanisms - might contribute to the pathomechanism of CAD (42). Furthermore, as we pointed out in the introduction (1.1.5. section), several cross-sectional studies, like ours, documented an association between EAT quantity and the presence of CAD. Obviously, from cross-sectional studies, either smaller or larger ones, one cannot conclude to causality. Notably, numbers of prospective clinical studies in this field are limited but follow-up observations published so far supported the causative relationship of EAT volume to the presence of CAD (56, 76).

At the moment, EAT is not used in cardiovascular risk assessment models. Nevertheless, in the Heinz Nixdorf Recall Study *Mahabadi et al* documented that EAT volume has a predictive value on major adverse cardiovascular events (HR: 1.15 [95% CI: 1.01–1.30]), and improved the combined predictive value of Framingham risk score and Ca-scoring (AUC=0.749 vs. 0.764; p=0.011) (156). In addition, *Cheng et al* found that adding pericardial fat volume (≥125 cm^3) to Framingham risk score and calcium score (≥400 Agatston score) resulted in a trend toward improved prediction compared to the latter two only (ROC analysis, area under curve 0.73 vs. 0.68; p=0.058) (76). Further studies are needed to assess whether adding EAT quantity to other risk factors may really improve the accuracy of CAD prediction.

The limitations of our study should be mentioned. The study design was cross-sectional and, therefore, we cannot infer causality. The patients were asymptomatic and had negative medical history from cardiological point of view. Therefore, the generalizability of our results to other patients' groups remains questionable. It is also of note that the morphological distinction of CAD-pos and CAD-neg cases may differ from that of clinical judgement.

Taken together, our results are in line with former observations documenting that EAT quantity are associated with the presence of CAD and suggesting that EAT may have a role in the development of atherosclerosis in the coronary arteries. If the causal relationship could definitively be proven, then, interventions (lifestyle-modification or using drugs) should be considered reasonable to initiate for reducing EAT volume which may finally lead to a decrease in development and progression of CAD.

DOI:10.14753/SE.2020.2396

6. Conclusions

We demonstrated that genetic effects have substantial, while environmental factors have only a modest influence on EAT, SAT and VAT volumes. Our findings show that common and specific genetic effects both play an important role in developing these phenotypes. None of the phenotypic appearance of EAT, SAT and VAT proved to be completely independent of the other two.

We documented a relatively strong genetic dependence of EAT, which has not been described previously. The genetic dependence of anthropometric parameters (weight, height, BMI) has been well documented in former studies. Heritability of different ectopic fat compartments (hepatic lipid accumulation) was also investigated previously, and in this case environmental factors predominated over genetic influences. Hence, heritability of different adipose tissue compartments and that of ectopic fats may vary.

The presence of strong genetic predisposition does not automatically translate to the development of clinical disease phenotype. Considering this fact, early and continuous preventive efforts should be implemented. In case of obesity, intervention should be initiated as early as possible and all modifiable risk factors should be addressed with diet, physical activity and behavioral interventions starting even in childhood.

We found that the quantity of EAT is associated - among others - with the presence of CAD. These results suggest a potential role of EAT in the development of atherosclerosis in the coronary arteries. Accordingly, it is reasonable to consider the quantity of EAT in risk assessment, so as to improve the accuracy of CAD risk prediction.

DOI:10.14753/SE.2020.2396

7. Summary

Various adipose tissue compartments play an important role in the development of cardiometabolic diseases. The quantity of different fat compartments is influenced by genetic and environmental factors. The epicardial fat is a unique fat compartment localized between the myocardial surface and the visceral layer of the pericardium. Epicardial adipose tissue (EAT) can be quantified by non-invasive cardiac imaging techniques such as cardiac CT. Recently, experimental and clinical studies suggested that EAT may have an impact on the development and progression of coronary atherosclerosis. After adopting a proper and reliable method for evaluating the quantity of EAT by using cardiac CT scan in our department, we designed a study to evaluate the heritability of EAT quantity in comparison to that of abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) volumes. In addition, we assessed the relationship of EAT volume to the presence of coronary artery disease (CAD).

We demonstrated in a classical twin study that genetics have substantial, while environmental factors have only a modest influence on EAT, SAT and VAT volumes. Our findings show that common and specific genetic effects both play an important role in developing these phenotypes. None of the phenotypic appearance of EAT, SAT and VAT proved to be completely independent of the other two. As presence of strong genetic predisposition does not automatically translate to the development of clinical disease phenotype, early and continuous preventive efforts should be implemented in order to prevent obesity. Intervention should be initiated as early as possible and all modifiable risk factors should be addressed with diet, physical activity and behavioral interventions starting even in the childhood.

In our study, the quantity of EAT was associated with the presence of CAD supporting former concepts that EAT might have a role in the development of CAD. It seems reasonable to involve EAT quantity into cardiovascular risk assessment, but the validity of this new method should be investigated in prospective clinical studies.

DOI:10.14753/SE.2020.2396

8. Összefoglalás

A kardiometabolikus kórképek patomechanizmusában a különböző zsírszöveti kompartmentek jelentős szerepet kapnak. A zsírszöveti raktárak, illetve a zsírszövet mennyiségének kialakulását genetikai és környezeti tényezők határozzák meg. Az epicardialis zsírszövet (EAT: epicardial adipose tissue) sajátos lokalizációjú és szerepű zsírszöveti kompartment, amely a myocardium felszíne és a pericardium visceralis lemeze között helyezkedik el. Az epicardialis zsírszövet mennyisége nem-invazív módon, kardiológiai képalkotó módszerekkel, viszonylag könnyen és pontosan a szív CT-vizsgálatával kvantifikálható. Újabb kísérletes és klinikai tanulmányok eredményei arra utalnak, hogy az epicardialis zsírszövet befolyással lehet a koronária-ateroszklerózis kialakulására és progressziójára. Vizsgálatunk során az EAT mennyiségének öröklődését vizsgáltuk, összefüggésben a hasi subcutan zsírszövet (SAT: subcutaneous adipose tissue) és visceralis zsírszövet (VAT: visceral adipose tissue) alakulásával. Továbbá, vizsgáltuk az EAT mennyisége és a koronária-betegség (CAD: coronary artery disease) radiomorfológiai jeleinek együttes előfordulását.

Klasszikus ikervizsgálat során igazoltuk, hogy a genetikai tényezőknek meghatározó, a környezeti tényezőknek kevésbé jelentős szerepe van az EAT, SAT és VAT mennyiségének alakulásában. A zsírszöveti kompartmentek fenotípusát közös és specifikus genetikai tényezők határozzák meg. Az EAT, VAT és SAT öröklődése nem bizonyult egymástól teljesen függetlennek. A genetikai meghatározottságot figyelembe véve fontos, hogy az elhízás elleni prevenciós tevékenység - az egészséges táplálkozásra, a rendszeres sportolásra való nevelés - már korán, gyermekkorban megkezdődjön.

Adataink szerint az EAT mennyisége összefüggést mutat a CAD jelenlétével, ez a megfigyelés erősíti azokat a korábbi elképzeléseket, amelyek szerint az EAT szerepet kaphat a CAD kialakulásában. Az EAT meghatározása és értékének kockázatbecslő rendszerekbe történő beépítése elősegítheti a kardiovaszkuláris kockázat pontosabb, személyre szabott becslését, a részletek tisztázása azonban még további vizsgálatokat igényel.

9. Bibliography

1. Zimmet P, Alberti KGMM, Shaw J. (2001) Global and societal implications of the diabetes epidemic. Nature, 414: 782-787.

2. Despres JP, Lemieux I. (2006) Abdominal obesity and metabolic syndrome. Nature, 444: 881-887.

3. Poirier P, Despres JP. (2003) Waist circumference, visceral obesity, and cardiovascular risk. J Cardiopulm Rehabil, 23: 161-169.

4. Lim S, Meigs JB. (2014) Links between ectopic fat and vascular disease in humans. Arterioscler Thromb Vasc Biol, 34: 1820-1826.

5. Iacobellis G, Malavazos AE, Corsi MM. (2011) Epicardial fat: From the biomolecular aspects to the clinical practice. Int J Biochem Cell B, 43: 1651-1654.

6. Ansaldo AM, Montecucco F, Sahebkar A, Dallegri F, Carbone F. (2019) Epicardial adipose tissue and cardiovascular diseases. Int J Cardiol, 278: 254-260.

7. Antonopoulos AS, Antoniades C. (2017) The role of epicardial adipose tissue in cardiac biology: classic concepts and emerging roles. J Physiol, 595: 3907-3917.

8. Bedford E. (1972) The story of fatty heart. A disease of Victorian times. Br Heart J, 34: 23-28.

9. Despres JP, Cartier A, Cote M, Arsenault BJ. (2008) The concept of cardiometabolic risk: Bridging the fields of diabetology and cardiology. Ann Med, 40: 514-523.

10. Iacobellis G, Ribaudo MC, Assael F, Vecci E, Tiberti C, Zappaterreno A, Di Mario U, Leonetti F. (2003) Echocardiographic epicardial adipose tissue is related to

anthropometric and clinical parameters of metabolic syndrome: A new indicator of cardiovascular risk. J Clin Endocr Metab, 88: 5163-5168.

11. Douglass E, Greif S, Frishman WH. (2017) Epicardial fat: pathophysiology and clinical significance. Cardiol Rev, 25: 230-235.

12. Iacobellis G, Barbaro G. (2019) Epicardial adipose tissue feeding and overfeeding the heart. Nutrition, 59: 1-6.

13. Iacobellis G, Bianco AC. (2011) Epicardial adipose tissue: emerging physiological, pathophysiological and clinical features. Trends Endocrin Met, 22: 450-457.

14. Iacobellis G. (2009) Epicardial and pericardial fat: close, but very different. Obesity, 17: 625-625.

15. Hirata Y, Yamada H, Sata M. (2018) Epicardial fat and pericardial fat surrounding the heart have different characteristics. Circ J, 82: 2475-2476.

16. Iacobellis G, Willens HJ. (2009) Echocardiographic epicardial fat: a review of research and clinical applications. J Am Soc Echocardiog, 22: 1311-1319.

17. Saura D, Oliva MJ, Rodriguez D, Pascual-Figal DA, Hurtado JA, Pinar E, de la Morena G, Valdes M. (2010) Reproducibility of echocardiographic measurements of epicardial fat thickness. Int J Cardiol, 141: 311-313.

18. Kim BJ, Kang JG, Lee SH, Lee JY, Sung KC, Kim BS, Kang JH. (2017) Relationship of echocardiographic epicardial fat thickness and epicardial fat volume by computed tomography with coronary artery calcification: data from the CAESAR study. Arch Med Res, 48: 352-359.

19. Sicari R, Sironi AM, Petz R, Frassi F, Chubuchny V, De Marchi D, Positano V, Lombardi M, Picano E, Gastaldelli A. (2011) Pericardial rather than epicardial fat is a

cardiometabolic risk marker: An MRI vs echo study. J Am Soc Echocardiog, 24: 1156-1162.

20. Gorter PM, van Lindert ASR, de Vos AM, Meijs MSFL, van der Graaf Y, Doevendans PA, Prokop M, Visseren FLJ. (2008) Quantification of epicardial and peri-coronary fat using cardiac computed tomography; reproducibility and relation with obesity and metabolic syndrome in patients suspected of coronary artery disease. Atherosclerosis, 197: 896-903.

21. Madaj P, Budoff MJ. (2012) Risk stratification of non-contrast CT beyond the coronary calcium scan. J Cardiovasc Comput, 6: 301-307.

22. Maurovich-Horvat P, Kallianos K, Engel LC, Szymonifka J, Fox CS, Hoffmann U, Truong QA. (2011) Influence of pericoronary adipose tissue on local coronary atherosclerosis as assessed by a novel MDCT volumetric method. Atherosclerosis, 219: 151-157.

23. Rabkin SW. (2007) Epicardial fat: properties, function and relationship to obesity. Obes Rev, 8: 253-261.

24. Iacobellis G, Corradi D, Sharma AM. (2005) Epicardial adipose tissue: anatomic, biomolecular and clinical relationships with the heart. Nat Clin Pract Card, 2: 536-543.

25. Bambace C, Telesca M, Zoico E, Sepe A, Olioso D, Rossi A, Corzato F, Di Francesco V, Mazzucco A, Santini F, Zamboni M. (2011) Adiponectin gene expression and adipocyte diameter: a comparison between epicardial and subcutaneous adipose tissue in men. Cardiovascular Pathology, 20: e153-e156.

26. Adams DB, Narayan O, Munnur RK, Cameron JD, Wong DT, Talman AH, Harper RW, Seneviratne SK, Meredith IT, Ko BS. (2017) Ethnic differences in coronary plaque and epicardial fat volume quantified using computed tomography. Int J Cardiovasc Imaging, 33: 241-249.

27. Hanley C, Matthews KA, Brooks MM, Janssen I, Budoff MJ, Sekikawa A, Mulukutla S, El Khoudary SR. (2018) Cardiovascular fat in women at midlife: effects of race, overall adiposity, and central adiposity. The SWAN Cardiovascular Fat Study. Menopause, 25: 38-45.

28. Kim SA, Kim MN, Shim WJ, Park SM. (2017) Epicardial adipose tissue is related to cardiac function in elderly women, but not in men. Nutr Metab Cardiovasc Dis, 27: 41-47.

29. Mancio J, Pinheiro M, Ferreira W, Carvalho M, Barros A, Ferreira N, Vouga L, Ribeiro VG, Leite-Moreira A, Falcao-Pires I, Bettencourt N. (2017) Gender differences in the association of epicardial adipose tissue and coronary artery calcification: EPICHEART study: EAT and coronary calcification by gender. Int J Cardiol, 249: 419-425.

30. Bertaso AG, Bertol D, Duncan BB, Foppa M. (2013) Epicardial fat: definition, measurements and systematic review of main outcomes. Arq Bras Cardiol, 101: E18-E28.

31. Fox CS, Gona P, Hoffmann U, Porter SA, Salton CJ, Massaro JM, Levy D, Larson MG, D'Agostino RB, O'Donnell CJ, Manning WJ. (2009) Pericardial fat, intrathoracic fat, and measures of left ventricular structure and function the Framingham Heart Study. Circulation, 119: 1586-1591.

32. Willens HJ, Gomez-Marin O, Chirinos JA, Goldberg R, Lowery MH, Iacobellis G. (2008) Comparison of epicardial and pericardial fat thickness assessed by echocardiography in african american and non-hispanic white men: a pilot study. Ethnic Dis, 18: 311-316.

33. Marchington JM, Mattacks CA, Pond CM. (1989) Adipose tissue in the mammalian heart and pericardium: structure, foetal development and biochemical properties. Comp Biochem Physiol B, 94: 225-232.

34. Sacks HS, Fain JN, Holman B, Cheema P, Chary A, Parks F, Karas J, Optican R, Bahouth SW, Garrett E, Wolf RY, Carter RA, Robbins T, Wolford D, Samaha J. (2009) Uncoupling protein-1 and related messenger ribonucleic acids in human epicardial and other adipose tissues: epicardial fat functioning as brown fat. J Clin Endocrinol Metab, 94: 3611-3615.

35. Prati F, Arbustini E, Labellarte A, Sommariva L, Pawlowski T, Manzoli A, Pagano A, Motolese M, Boccanelli A. (2003) Eccentric atherosclerotic plaques with positive remodelling have a pericardial distribution: a permissive role of epicardial fat? A three-dimensional intravascular ultrasound study of left anterior descending artery lesions. Eur Heart J, 24: 329-336.

36. Iozzo P. (2010) Metabolic toxicity of the heart: Insights from molecular imaging. Nutr Metab Cardiovas, 20: 147-156.

37. Deng G, Long Y, Yu YR, Li MR. (2010) Adiponectin directly improves endothelial dysfunction in obese rats through the AMPK-eNOS Pathway. Int J Obesity, 34: 165-171.

38. Li R, Wang WQ, Zhang H, Yang X, Fan Q, Christopher TA, Lopez BL, Tao L, Goldstein BJ, Gao F, Ma XL. (2007) Adiponectin improves endothelial function in hyperlipidemic rats by reducing oxidative/nitrative stress and differential regulation of eNOS/iNOS activity. Am J Physiol-Endoc M, 293: E1703-E1708.

39. Payne GA, Kohr MC, Tune JD. (2012) Epicardial perivascular adipose tissue as a therapeutic target in obesity-related coronary artery disease. Brit J Pharmacol, 165: 659-669.

40. Sacks HS, Fain JN. (2007) Human epicardial adipose tissue: A review. Am Heart J, 153: 907-917.

41. Ishii T, Asuwa N, Masuda S, Ishikawa Y. (1998) The effects of a myocardial bridge on coronary atherosclerosis and ischaemia. J Pathol, 185: 4-9.

42. Mazurek T, Zhang LF, Zalewski A, Mannion JD, Diehl JT, Arafat H, Sarov-Blat L, O'Brien S, Keiper EA, Johnson AG, Martin J, Goldstein BJ, Shi Y. (2003) Human epicardial adipose tissue is a source of inflammatory mediators. Circulation, 108: 2460-2466.

43. Baker AR, da Silva NF, Quinn DW, Harte AL, Pagano D, Bonser RS, Kumar S, McTernan PG. (2006) Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease. Cardiovasc Diabetol, 5.

44. Baker AR, Harte AL, Howell N, Pritlove DC, Ranasinghe AM, da Silva NF, Youssef EM, Khunti K, Davies MJ, Bonser RS, Kumar S, Pagano D, McTernan PG. (2009) Epicardial adipose tissue as a source of nuclear factor-kappa B and c-Jun N-terminal kinase mediated inflammation in patients with coronary artery disease. J Clin Endocr Metab, 94: 261-267.

45. Eiras S, Teijeira-Fernandez E, Shamagian LG, Fernandez AL, Vazquez-Boquete A, Gonzalez-Juanatey JR. (2008) Extension of coronary artery disease is associated with increased IL-6 and decreased adiponectin gene expression in epicardial adipose tissue. Cytokine, 43: 174-180.

46. Iacobellis G, Pistilli D, Gucciardo M, Leonetti F, Miraldi F, Brancaccio G, Gallo P, di Gioia CRT. (2005) Adiponectin expression in human epicardial adipose tissue in vivo is lower in patients with coronary artery disease. Cytokine, 29: 251-255.

47. Matloch Z, Cinkajzlova A, Mraz M, Haluzik M. (2018) The role of inflammation in epicardial adipose tissue in heart diseases. Curr Pharm Des, 24: 297-309.

48. Packer M. (2018) Epicardial adipose tissue may mediate deleterious effects of obesity and inflammation on the myocardium. J Am Coll Cardiol, 71: 2360-2372.

49. Yudkin JS, Eringa E, Stehouwer CDA. (2005) "Vasocrine" signalling from perivascular fat: a mechanism linking insulin resistance to vascular disease. Lancet, 365: 1817-1820.

50. Wang TD, Lee WJ, Shih FY, Huang CH, Chen WJ, Lee YT, Shih TTF, Chen MF. (2010) Association of epicardial adipose tissue with coronary atherosclerosis is region-specific and independent of conventional risk factors and intra-abdominal adiposity. Atherosclerosis, 213: 279-287.

51. Yerramasu A, Dey D, Venuraju S, Anand DV, Atwal S, Corder R, Berman DS, Lahiri A. (2012) Increased volume of epicardial fat is an independent risk factor for accelerated progression of sub-clinical coronary atherosclerosis. Atherosclerosis, 220: 223-230.

52. Acele A, Baykan AO, Yuksel Kalkan G, Celiker E, Gur M. (2017) Epicardial fat thickness is associated with aortic intima-media thickness in patients without clinical manifestation of atherosclerotic cardiovascular disease. Echocardiography, 34: 1146-1151.

53. Mancio J, Azevedo D, Saraiva F, Azevedo AI, Pires-Morais G, Leite-Moreira A, Falcao-Pires I, Lunet N, Bettencourt N. (2018) Epicardial adipose tissue volume assessed by computed tomography and coronary artery disease: a systematic review and meta-analysis. Eur Heart J Cardiovasc Imaging, 19: 490-497.

54. Patel VB, Shah S, Verma S, Oudit GY. (2017) Epicardial adipose tissue as a metabolic transducer: role in heart failure and coronary artery disease. Heart Fail Rev, 22: 889-902.

55. Wu FZ, Chou KJ, Huang YL, Wu MT. (2014) The relation of location-specific epicardial adipose tissue thickness and obstructive coronary artery disease: systemic review and meta-analysis of observational studies. BMC Cardiovasc Disord, 14: 62.

56. Ding JZ, Hsu FC, Harris TB, Liu YM, Kritchevsky SB, Szklo M, Ouyang P, Espeland MA, Lohman KK, Criqui MH, Allison M, Bluemke DA, Carr JJ. (2009) The association of pericardial fat with incident coronary heart disease: the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Clin Nutr, 90: 499-504.

57. Rosito GA, Massaro JM, Hoffmann U, Ruberg FL, Mahabadi AA, Vasan RS, O'Donnell CJ, Fox CS. (2008) Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample - The framingham heart study. Circulation, 117: 605-613.

58. Wang CP, Hsu HL, Hung WC, Yu TH, Chen YH, Chiu CA, Lu LF, Chung FM, Shin SJ, Lee YJ. (2009) Increased epicardial adipose tissue (EAT) volume in type 2 diabetes mellitus and association with metabolic syndrome and severity of coronary atherosclerosis. Clin Endocrinol, 70: 876-882.

59. Alexopoulos N, McLean DS, Janik M, Arepalli CD, Stillman AE, Raggi P. (2010) Epicardial adipose tissue and coronary artery plaque characteristics. Atherosclerosis, 210: 150-154.

60. Konishi M, Sugiyama S, Sugamura K, Nozaki T, Ohba K, Matsubara J, Matsuzawa Y, Sumida H, Nagayoshi Y, Nakaura T, Awai K, Yamashita Y, Jinnouchi H, Matsui K, Kimura K, Umemura S, Ogawa H. (2010) Association of pericardial fat accumulation rather than abdominal obesity with coronary atherosclerotic plaque formation in patients with suspected coronary artery disease. Atherosclerosis, 209: 573-578.

61. Ito T, Nasu K, Terashima M, Ehara M, Kinoshita Y, Ito T, Kimura M, Tanaka N, Habara M, Tsuchikane E, Suzuki T. (2012) The impact of epicardial fat volume on coronary plaque vulnerability: insight from optical coherence tomography analysis. Eur Heart J-Card Img, 13: 408-415.

62. Schlett CL, Ferencik M, Kriegel MF, Bamberg F, Ghoshhajra BB, Joshi SB, Nagurney JT, Fox CS, Truong QA, Hoffmann U. (2012) Association of pericardial fat and coronary high-risk lesions as determined by cardiac CT. Atherosclerosis, 222: 129-134.

63. Nerlekar N, Brown AJ, Muthalaly RG, Talman A, Hettige T, Cameron JD, Wong DTL. (2017) Association of epicardial adipose tissue and high-risk plaque characteristics: a systematic review and meta-analysis. J Am Heart Assoc, 6: e006379.

64. Tamarappoo B, Dey D, Shmilovich H, Nakazato R, Gransar H, Cheng VY, Friedman JD, Hayes SW, Thomson LEJ, Slomka PJ, Rozanski A, Berman DS. (2010) Increased pericardial fat volume measured from noncontrast CT predicts myocardial ischemia by SPECT. JACC Cardiovasc Imaging, 3: 1104-1112.

65. Ueno K, Anzai T, Jinzaki M, Yamada M, Jo Y, Maekawa Y, Kawamura A, Yoshikawa T, Tanami Y, Sato K, Kuribayashi S, Ogawa S. (2009) Increased epicardial fat volume quantified by 64-multidetector computed tomography is associated with coronary atherosclerosis and totally occlusive lesions. Circ J, 73: 1927-1933.

66. Nasri A, Najafian J, Derakhshandeh SM, Madjlesi F. (2018) Epicardial fat thickness and severity of coronary heart disease in patients with diabetes mellitus type II. ARYA Atheroscler, 14: 32-37.

67. Ozcan F, Turak O, Canpolat U, Kanat S, Kadife I, Avci S, Isleyen A, Cebeci M, Tok D, Basar FN, Aras D, Topaloglu S, Aydogdu S. (2014) Association of epicardial fat thickness with TIMI risk score in NSTEMI/USAP patients. Herz, 39: 755-760.

68. Tok D, Cagli K, Kadife I, Turak O, Ozcan F, Basar FN, Golbasi Z, Aydogdu S. (2013) Impaired coronary flow reserve is associated with increased echocardiographic epicardial fat thickness in metabolic syndrome patients. Coronary Artery Dis, 24: 191-195.

69. Sade LE, Eroglu S, Bozbas H, Ozbicer S, Hayran M, Haberal A, Muderrisoglu H. (2009) Relation between epicardial fat thickness and coronary flow reserve in women with chest pain and angiographically normal coronary arteries. Atherosclerosis, 204: 580-585.

70. Cabrera-Rego JO, Iacobellis G, Castillo-Herrera JA, Valiente-Mustelier J, Gandarilla-Sarmientos JC, Marin-Julia SM, Navarrete-Cabrera J. (2014) Epicardial fat thickness correlates with carotid intima-media thickness, arterial stiffness, and cardiac geometry in children and adolescents. Pediatr Cardiol, 35: 450-456.

71. Cetin M, Cakici M, Polat M, Suner A, Zencir C, Ardic I. (2013) Relation of epicardial fat thickness with carotid intima-media thickness in patients with type 2 diabetes mellitus. Int J Endocrinol, 2013: 769175.

72. Park HE, Choi SY, Kim HS, Kim MK, Cho SH, Oh BH. (2012) Epicardial fat reflects arterial stiffness: assessment using 256-slice multidetector coronary computed tomography and cardio-ankle vascular index. J Atheroscler Thromb, 19: 570-576.

73. Maurovich-Horvat P, Kallianos K, Engel LC, Szymonifka J, Schlett CL, Koenig W, Hoffmann U, Truong QA. (2015) Relationship of thoracic fat depots with coronary atherosclerosis and circulating inflammatory biomarkers. Obesity, 23: 1178-1184.

74. Picard FA, Gueret P, Laissy JP, Champagne S, Leclercq F, Carrie D, Juliard JM, Henry P, Niarra R, Chatellier G, Steg PG. (2014) Epicardial adipose tissue thickness correlates with the presence and severity of angiographic coronary artery disease in stable patients with chest pain. PLoS One, 9: e110005.

75. Sinha SK, Thakur R, Jha MJ, Goel A, Kumar V, Kumar A, Mishra V, Varma CM, Krishna V, Singh AK, Sachan M. (2016) Epicardial adipose tissue thickness and its association with the presence and severity of coronary artery disease in clinical setting: a cross-sectional observational study. J Clin Med Res, 8: 410-419.

76. Cheng VY, Dey D, Tamarappoo B, Nakazato R, Gransar H, Miranda-Peats R, Ramesh A, Wong ND, Shaw LJ, Slomka PJ, Berman DS. (2010) Pericardial fat burden on ECG-gated noncontrast CT in asymptomatic patients who subsequently experience adverse cardiovascular events. JACC Cardiovasc Imaging, 3: 352-360.

77. Gaeta M, Bandera F, Tassinari F, Capasso L, Cargnelutti M, Pelissero G, Malavazos AE, Ricci C. (2017) Is epicardial fat depot associated with atrial fibrillation? A systematic review and meta-analysis. Europace, 19: 747-752.

78. Nakamori S, Nezafat M, Ngo LH, Manning WJ, Nezafat R. (2018) Left Atrial Epicardial Fat Volume Is Associated With Atrial Fibrillation: A Prospective Cardiovascular Magnetic Resonance 3D Dixon Study. J Am Heart Assoc, 7: e008232.

79. Zhu W, Zhang H, Guo L, Hong K. (2016) Relationship between epicardial adipose tissue volume and atrial fibrillation: A systematic review and meta-analysis. Herz, 41: 421-427.

80. Al Chekakie MO, Welles CC, Metoyer R, Ibrahim A, Shapira AR, Cytron J, Santucci P, Wilber DJ, Akar JG. (2010) Pericardial fat is independently associated with human atrial fibrillation. J Am Coll Cardiol, 56: 784-788.

81. Chao TF, Hung CL, Tsao HM, Lin YJ, Yun CH, Lai YH, Chang SL, Lo LW, Hu YF, Tuan TC, Chang HY, Kuo JY, Yeh HI, Wu TJ, Hsieh MH, Yu WC, Chen SA. (2013) Epicardial adipose tissue thickness and ablation outcome of atrial fibrillation. PLoS One, 8: e74926.

82. Lin HH, Lee JK, Yang CY, Lien YC, Huang JW, Wu CK. (2013) Accumulation of epicardial fat rather than visceral fat is an independent risk factor for left ventricular diastolic dysfunction in patients undergoing peritoneal dialysis. Cardiovasc Diabetol, 12: 127.

83. Baig A, Campbell B, Russell M, Singh J, Borra S. (2012) Epicardial fat necrosis: an uncommon etiology of chest pain. Cardiol J, 19: 424-428.

84. Borch-Johnsen K, Wareham N. (2010) The rise and fall of the metabolic syndrome. Diabetologia, 53: 597-599.

85. Rabkin SW. (2014) The relationship between epicardial fat and indices of obesity and the metabolic syndrome: a systematic review and meta-analysis. Metab Syndr Relat Disord, 12: 31-42.

86. Kim HM, Kim KJ, Lee HJ, Yu HT, Moon JH, Kang ES, Cha BS, Lee HC, Lee BW, Kim YJ. (2012) Epicardial adipose tissue thickness is an indicator for coronary artery stenosis in asymptomatic type 2 diabetic patients: its assessment by cardiac magnetic resonance. Cardiovasc Diabetol, 11: 83.

87. Wang TD, Lee WJ, Shih FY, Huang CH, Chang YC, Chen WJ, Lee YT, Chen MF. (2009) Relations of epicardial adipose tissue measured by multidetector computed tomography to components of the metabolic syndrome are region-specific and independent of anthropometric indexes and intraabdominal visceral fat. J Clin Endocrinol Metab, 94: 662-669.

88. Iacobellis G, Barbaro G, Gerstein HC. (2008) Relationship of epicardial fat thickness and fasting glucose. Int J Cardiol, 128: 424-426.

89. Iozzo P, Lautamaki R, Borra R, Lehto HR, Bucci M, Viljanen A, Parkka J, Lepomaki V, Maggio R, Parkkola R, Knuuti J, Nuutila P. (2009) Contribution of glucose tolerance and gender to cardiac adiposity. J Clin Endocrinol Metab, 94: 4472-4482.

90. Iacobellis G, Pellicelli AM, Grisorio B, Barbarini G, Leonetti F, Sharma AM, Barbaro G. (2008) Relation of epicardial fat and alanine aminotransferase in subjects with increased visceral fat. Obesity (Silver Spring), 16: 179-183.

91. Iacobellis G. (2015) Local and systemic effects of the multifaceted epicardial adipose tissue depot. Nat Rev Endocrinol, 11: 363-371.

92. Iacobellis G, Diaz S, Mendez A, Goldberg R. (2014) Increased epicardial fat and plasma leptin in type 1 diabetes independently of obesity. Nutr Metab Cardiovasc Dis, 24: 725-729.

93. Darabian S, Backlund JY, Cleary PA, Sheidaee N, Bebu I, Lachin JM, Budoff MJ, Group DER. (2016) Significance of epicardial and intrathoracic adipose tissue volume among type 1 diabetes patients in the DCCT/EDIC: a pilot study. PLoS One, 11: e0159958.

94. Iacobellis G, Singh N, Wharton S, Sharma AM. (2008) Substantial changes in epicardial fat thickness after weight loss in severely obese subjects. Obesity (Silver Spring), 16: 1693-1697.

95. Kim MK, Tomita T, Kim MJ, Sasai H, Maeda S, Tanaka K. (2009) Aerobic exercise training reduces epicardial fat in obese men. J Appl Physiol (1985), 106: 5-11.

96. Gaborit B, Jacquier A, Kober F, Abdesselam I, Cuisset T, Boullu-Ciocca S, Emungania O, Alessi MC, Clement K, Bernard M, Dutour A. (2012) Effects of bariatric surgery on cardiac ectopic fat: lesser decrease in epicardial fat compared to visceral fat loss and no change in myocardial triglyceride content. J Am Coll Cardiol, 60: 1381-1389.

97. Altin C, Erol V, Aydin E, Yilmaz M, Tekindal MA, Sade LE, Gulay H, Muderrisoglu H. (2018) Impact of weight loss on epicardial fat and carotid intima media thickness after laparoscopic sleeve gastrectomy: A prospective study. Nutr Metab Cardiovasc Dis, 28: 501-509.

98. Rabkin SW, Campbell H. (2015) Comparison of reducing epicardial fat by exercise, diet or bariatric surgery weight loss strategies: a systematic review and meta-analysis. Obes Rev, 16: 406-415.

99. Raggi P, Gadiyaram V, Zhang C, Chen Z, Lopaschuk G, Stillman AE. (2019) Statins reduce epicardial adipose tissue attenuation independent of lipid lowering: a potential pleiotropic effect. J Am Heart Assoc, 8: e013104.

100. Xourgia E, Papazafiropoulou A, Melidonis A. (2018) Effects of antidiabetic drugs on epicardial fat. World J Diabetes, 9: 141-148.

101. Zsori G, Illes D, Ivany E, Kosar K, Holzinger G, Tajti M, Palinkas E, Szabovik G, Nagy A, Palko A, Czako L. (2019) In new-onset diabetes mellitus, metformin reduces fat accumulation in the liver, but not in the pancreas or pericardium. Metab Syndr Relat Disord, 17: 289-295.

102. Park JH, Park YS, Kim YJ, Lee IS, Kim JH, Lee JH, Choi SW, Jeong JO, Seong IW. (2010) Effects of statins on the epicardial fat thickness in patients with coronary artery stenosis underwent percutaneous coronary intervention: comparison of atorvastatin with simvastatin/ezetimibe. J Cardiovasc Ultrasound, 18: 121-126.

103. Jonker JT, Lamb HJ, van der Meer RW, Rijzewijk LJ, Menting LJ, Diamant M, Bax JJ, de Roos A, Romijn JA, Smit JW. (2010) Pioglitazone compared with metformin increases pericardial fat volume in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab, 95: 456-460.

104. Morano S, Romagnoli E, Filardi T, Nieddu L, Mandosi E, Fallarino M, Turinese I, Dagostino MP, Lenzi A, Carnevale V. (2015) Short-term effects of glucagon-like peptide 1 (GLP-1) receptor agonists on fat distribution in patients with type 2 diabetes mellitus: an ultrasonography study. Acta Diabetol, 52: 727-732.

105. Dutour A, Abdesselam I, Ancel P, Kober F, Mrad G, Darmon P, Ronsin O, Pradel V, Lesavre N, Martin JC, Jacquier A, Lefur Y, Bernard M, Gaborit B. (2016) Exenatide decreases liver fat content and epicardial adipose tissue in patients with obesity and type 2 diabetes: a prospective randomized clinical trial using magnetic resonance imaging and spectroscopy. Diabetes Obes Metab, 18: 882-891.

106. Lima-Martinez MM, Paoli M, Rodney M, Balladares N, Contreras M, D'Marco L, Iacobellis G. (2016) Effect of sitagliptin on epicardial fat thickness in subjects with type 2 diabetes and obesity: a pilot study. Endocrine, 51: 448-455.

107. Diaz-Rodriguez E, Agra RM, Fernandez AL, Adrio B, Garcia-Caballero T, Gonzalez-Juanatey JR, Eiras S. (2018) Effects of dapagliflozin on human epicardial

adipose tissue: modulation of insulin resistance, inflammatory chemokine production, and differentiation ability. Cardiovasc Res, 114: 336-346.

108. Sato T, Aizawa Y, Yuasa S, Kishi S, Fuse K, Fujita S, Ikeda Y, Kitazawa H, Takahashi M, Sato M, Okabe M. (2018) The effect of dapagliflozin treatment on epicardial adipose tissue volume. Cardiovasc Diabetol, 17: 6.

109. Yagi S, Hirata Y, Ise T, Kusunose K, Yamada H, Fukuda D, Salim HM, Maimaituxun G, Nishio S, Takagawa Y, Hama S, Matsuura T, Yamaguchi K, Tobiume T, Soeki T, Wakatsuki T, Aihara KI, Akaike M, Shimabukuro M, Sata M. (2017) Canagliflozin reduces epicardial fat in patients with type 2 diabetes mellitus. Diabetol Metab Syndr, 9: 78.

110. Mazurek T, Opolski G. (2015) Pericoronary adipose tissue: a novel therapeutic target in obesity-related coronary atherosclerosis. J Am Coll Nutr, 34: 244-254.

111. Iacobellis G. (2016) Epicardial fat: a new cardiovascular therapeutic target. Curr Opin Pharmacol, 27: 13-18.

112. Neeland IJ, Ross R, Despres JP, Matsuzawa Y, Yamashita S, Shai I, Seidell J, Magni P, Santos RD, Arsenault B, Cuevas A, Hu FB, Griffin B, Zambon A, Barter P, Fruchart JC, Eckel RH, International Atherosclerosis S, International Chair on Cardiometabolic Risk Working Group on Visceral O. (2019) Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. Lancet Diabetes Endocrinol, 2019 July, doi: 2010.1016/S2213-8587(2019)30084-30081 [Epub ahead of print].

113. Mahabadi AA, Massaro JM, Rosito GA, Levy D, Murabito JM, Wolf PA, O'Donnell CJ, Fox CS, Hoffmann U. (2009) Association of pericardial fat, intrathoracic fat, and visceral abdominal fat with cardiovascular disease burden: the Framingham Heart Study. Eur Heart J, 30: 850-856.

114. Guglielmi V, Sbraccia P. (2017) Epicardial adipose tissue: at the heart of the obesity complications. Acta Diabetol, 54: 805-812.

115. Abdelmalek MF, Diehl AM. (2007) Nonalcoholic fatty liver disease as a complication of insulin resistance. Med Clin North Am, 91: 1125-1149.

116. Hamaguchi M, Kojima T, Takeda N, Nagata C, Takeda J, Sarui H, Kawahito Y, Yoshida N, Suetsugu A, Kato T, Okuda J, Ida K, Yoshikawa T. (2007) Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. World J Gastroenterol, 13: 1579-1584.

117. Schindhelm RK, Diamant M, Heine RJ. (2007) Nonalcoholic fatty liver disease and cardiovascular disease risk. Curr Diab Rep, 7: 181-187.

118. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. (2018) Mechanisms of NAFLD development and therapeutic strategies. Nat Med, 24: 908-922.

119. Brouha SS, Nguyen P, Bettencourt R, Sirlin CB, Loomba R. (2018) Increased severity of liver fat content and liver fibrosis in non-alcoholic fatty liver disease correlate with epicardial fat volume in type 2 diabetes: A prospective study. Eur Radiol, 28: 1345-1355.

120. Singh RG, Yoon HD, Wu LM, Lu J, Plank LD, Petrov MS. (2017) Ectopic fat accumulation in the pancreas and its clinical relevance: A systematic review, meta-analysis, and meta-regression. Metabolism, 69: 1-13.

121. Acharya C, Navina S, Singh VP. (2014) Role of pancreatic fat in the outcomes of pancreatitis. Pancreatology, 14: 403-408.

122. Yu TY, Wang CY. (2017) Impact of non-alcoholic fatty pancreas disease on glucose metabolism. J Diabetes Investig, 8: 735-747.

123. Della Corte C, Mosca A, Majo F, Lucidi V, Panera N, Giglioni E, Monti L, Stronati L, Alisi A, Nobili V. (2015) Nonalcoholic fatty pancreas disease and nonalcoholic fatty liver disease: more than ectopic fat. Clin Endocrinol (Oxf), 83: 656-662.

124. Despres JP. (2012) Body fat distribution and risk of cardiovascular disease: an update. Circulation, 126: 1301-1313.

125. Ramachandrappa S, Farooqi IS. (2011) Genetic approaches to understanding human obesity. J Clin Invest, 121: 2080-2086.

126. Jermendy G, Horvath T, Littvay L, Steinbach R, Jermendy AL, Tarnoki AD, Tarnoki DL, Metneki J, Osztovits J. (2011) Effect of genetic and environmental influences on cardiometabolic risk factors: a twin study. Cardiovasc Diabetol, 10: 96.

127. Tarnoki AD, Tarnoki DL, Medda E, Cotichini R, Stazi MA, Fagnani C, Nistic AL, Lucatelli P, Boatta E, Zini C, Fanelli F, Baracchini C, Meneghetti G, Schillaci G, Osztovits J, Jermendy G, Kiss RB, Pr AdIN, Karlinger K, Lannert A, Metneki J, Molnar AA, Garami Z, Berczi V, Halasz I, Baffy G. (2014) Bioimpedance analysis of body composition in an international twin cohort. Obes Res Clin Pract, 8: e201-298.

128. Segal NL, Feng R, McGuire SA, Allison DB, Miller S. (2009) Genetic and environmental contributions to body mass index: comparative analysis of monozygotic twins, dizygotic twins and same-age unrelated siblings. Int J Obes (Lond), 33: 37-41.

129. Silventoinen K, Kaprio J. (2009) Genetics of tracking of body mass index from birth to late middle age: evidence from twin and family studies. Obes Facts, 2: 196-202.

130. Carey DG, Nguyen TV, Campbell LV, Chisholm DJ, Kelly P. (1996) Genetic influences on central abdominal fat: a twin study. Int J Obes Relat Metab Disord, 20: 722-726.

131. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, Vasan RS, Murabito JM, Meigs JB, Cupples LA, D'Agostino RB, Sr., O'Donnell CJ. (2007) Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation, 116: 39-48.

132. Perusse L, Despres JP, Lemieux S, Rice T, Rao DC, Bouchard C. (1996) Familial aggregation of abdominal visceral fat level: results from the Quebec family study. Metabolism, 45: 378-382.

133. Hong Y, Rice T, Gagnon J, Despres JP, Nadeau A, Perusse L, Bouchard C, Leon AS, Skinner JS, Wilmore JH, Rao DC. (1998) Familial clustering of insulin and abdominal visceral fat: the HERITAGE Family Study. J Clin Endocrinol Metab, 83: 4239-4245.

134. Voros S, Maurovich-Horvat P, Marvasty IB, Bansal AT, Barnes MR, Vazquez G, Murray SS, Voros V, Merkely B, Brown BO, Warnick GR. (2014) Precision phenotyping, panomics, and system-level bioinformatics to delineate complex biologies of atherosclerosis: rationale and design of the "Genetic Loci and the Burden of Atherosclerotic Lesions" study. J Cardiovasc Comput Tomogr, 8: 442-451.

135. Maurovich-Horvat P, Tarnoki DL, Tarnoki AD, Horvath T, Jermendy AL, Kolossvary M, Szilveszter B, Voros V, Kovacs A, Molnar AA, Littvay L, Lamb HJ, Voros S, Jermendy G, Merkely B. (2015) Rationale, design, and methodological aspects of the BUDAPEST-GLOBAL study (Burden of Atherosclerotic Plaques Study in Twins-Genetic Loci and the Burden of Atherosclerotic Lesions). Clin Cardiol, 38: 699-707.

136. Littvay L, Metneki J, Tarnoki AD, Tarnoki DL. (2013) The Hungarian Twin Registry. Twin Res Hum Genet, 16: 185-189.

137. Heath AC, Nyholt DR, Neuman R, Madden PA, Bucholz KK, Todd RD, Nelson EC, Montgomery GW, Martin NG. (2003) Zygosity diagnosis in the absence of genotypic data: an approach using latent class analysis. Twin Res, 6: 22-26.

138. Maurovich-Horvat P, Massaro J, Fox CS, Moselewski F, O'Donnell CJ, Hoffmann U. (2007) Comparison of anthropometric, area- and volume-based assessment of abdominal subcutaneous and visceral adipose tissue volumes using multi-detector computed tomography. Int J Obes (Lond), 31: 500-506.

139. Leipsic J, Abbara S, Achenbach S, Cury R, Earls JP, Mancini GJ, Nieman K, Pontone G, Raff GL. (2014) SCCT guidelines for the interpretation and reporting of coronary CT angiography: a report of the Society of Cardiovascular Computed Tomography Guidelines Committee. J Cardiovasc Comput Tomogr, 8: 342-358.

140. Maurovich-Horvat P, Ferencik M, Bamberg F, Hoffmann U. (2009) Methods of plaque quantification and characterization by cardiac computed tomography. J Cardiovasc Comput Tomogr, 3 Suppl 2: S91-98.

141. Min JK, Shaw LJ, Devereux RB, Okin PM, Weinsaft JW, Russo DJ, Lippolis NJ, Berman DS, Callister TQ. (2007) Prognostic value of multidetector coronary computed tomographic angiography for prediction of all-cause mortality. J Am Coll Cardiol, 50: 1161-1170.

142. Altman DG. Practical statistics for medical research. Chapman and Hall, London, 1991: 611.

143. Hoyle RH. Handbook of structural equation modeling. Guilford Press, New York, 2012: 617-635.

144. R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

145. Neale MC, Hunter MD, Pritikin JN, Zahery M, Brick TR, Kirkpatrick RM, Estabrook R, Bates TC, Maes HH, Boker SM. (2016) OpenMx 2.0: extended structural equation and statistical modeling. Psychometrika, 81: 535-549.

146. Nagy E, Jermendy AL, Merkely B, Maurovich-Horvat P. (2017) Clinical importance of epicardial adipose tissue. Arch Med Sci, 13: 864-874.

147. Tayo BO, Harders R, Luke A, Zhu X, Cooper RS. (2008) Latent common genetic components of obesity traits. Int J Obes (Lond), 32: 1799-1806.

148. Li F, Zhao J, Yuan Z, Zhang X, Ji J, Xue F. (2013) A powerful latent variable method for detecting and characterizing gene-based gene-gene interaction on multiple quantitative traits. BMC Genet, 14: 89.

149. Barsh GS, Farooqi IS, O'Rahilly S. (2000) Genetics of body-weight regulation. Nature, 404: 644-651.

150. Malis C, Rasmussen EL, Poulsen P, Petersen I, Christensen K, Beck-Nielsen H, Astrup A, Vaag AA. (2005) Total and regional fat distribution is strongly influenced by genetic factors in young and elderly twins. Obes Res, 13: 2139-2145.

151. Tarnoki AD, Tarnoki DL, Bata P, Littvay L, Osztovits J, Jermendy G, Karlinger K, Lannert A, Preda I, Kiss RG, Molnar AA, Garami Z, Baffy G, Berczi V. (2012) Heritability of non-alcoholic fatty liver disease and association with abnormal vascular parameters: a twin study. Liver Int, 32: 1287-1293.

152. Orio F, Tafuri D, Ascione A, Marciano F, Savastano S, Colarieti G, Orio M, Colao A, Palomba S, Muscogiuri G. (2016) Lifestyle changes in the management of adulthood and childhood obesity. Minerva Endocrinol, 41: 509-515.

153. Colquitt JL, Loveman E, O'Malley C, Azevedo LB, Mead E, Al-Khudairy L, Ells LJ, Metzendorf MI, Rees K. (2016) Diet, physical activity, and behavioural interventions for the treatment of overweight or obesity in preschool children up to the age of 6 years. Cochrane Database Syst Rev, 3: CD012105.

154. Irlbeck T, Massaro JM, Bamberg F, O'Donnell CJ, Hoffmann U, Fox CS. (2010) Association between single-slice measurements of visceral and abdominal subcutaneous

adipose tissue with volumetric measurements: the Framingham Heart Study. Int J Obesity, 34: 781-787.

155. Nilsson SE, Read S, Berg S, Johansson B. (2009) Heritabilities for fifteen routine biochemical values: findings in 215 Swedish twin pairs 82 years of age or older. Scand J Clin Lab Invest, 69: 562-569.

156. Mahabadi AA, Lehmann N, Mohlenkamp S, Pundt N, Dykun I, Roggenbuck U, Moebus S, Jockel KH, Erbel R, Kalsch H, Groups HNI. (2016) Noncoronary measures enhance the predictive value of cardiac CT above traditional risk factors and CAC score in the general population. JACC Cardiovasc Imaging, 9: 1177-1185.

DOI:10.14753/SE.2020.2396

10. Bibliography of the candidate's publications

10.1. Publications closely related to the present thesis

1. **Jermendy AL**, Kolossvary M, Drobni ZD, Tarnoki AD, Tarnoki DL, Karady J, Voros S, Lamb HJ, Merkely B, Jermendy G, Maurovich-Horvat P. (2018) Assessing genetic and environmental influences on epicardial and abdominal adipose tissue quantities: a classical twin study. Int J Obes, 42:163-168. **IF:4.514**

2. Nagy E*, **Jermendy AL***, Merkely B, Maurovich-Horvat P. (2017) Clinical importance of epicardial adipose tissue. Arch Med Sci, 13: 864-874.
   *the authors contributed equally to the manuscript
   (review article)

3. Maurovich-Horvat P, Tárnoki DL, Tárnoki ÁD, Horváth T, **Jermendy ÁL**, Kolossváry M, Szilveszter B, Voros V, Kovács A, Molnár AÁ, Littvay L, Lamb HJ, Voros S, Jermendy G, Merkely B. (2015) Rationale, Design, and Methodological Aspects of the BUDAPEST-GLOBAL Study (Burden of Atherosclerotic Plaques Study in Twins-Genetic Loci and the Burden of Atherosclerotic Lesions). Clin Cardiol, 38:699-707. **IF:2.431**

   Articles in Hungarian

4. Drobni Zs D, Kolossváry M, Karády J, **Jermendy ÁL**, Littvay L, Tárnoki ÁD, Tárnoki DL, Voros Sz, Jermendy Gy, Merkely B, Maurovich-Horvat P. (2017) Van-e összefüggés az epikardiális zsírszövet és a koszorúér-betegség között? Cardiologia Hungarica, 47: 25-29.

10.2. Publications not related to the present thesis

1. Szilveszter B, Oren D, Molnár L, Apor A, Nagy AI, Molnár A, Vattay B, Kolossváry M, Karády J, Bartykowszki A, **Jermendy ÁL,** Suhai FI, Panajotu A, Maurovich-Horvat P, Merkely B. Subclinical leaflet thrombosis is associated with impaired reverse remodelling after transcatheter aortic valve implantation. Eur Heart J Cardiovasc Imaging. 2019 Oct 30. doi: 10.1093/ehjci/jez256. **IF:5.260**

2. Tarnoki AD, Szalontai L, Fagnani C, Tarnoki DL, Lucatelli P, Maurovich-Horvat P, **Jermendy AL,** Kovacs A, Molnar AA, Godor E, Fejer B, Hernyes A, Cirelli C, Fanelli F, Farina F, Baracchini C, Meneghetti G, Gyarmathy AV, Jermendy G, Merkely B, Pucci G, Schillaci G, Stazi MA, Medda E. (2019) Genetic and environmental factors on heart rate, mean arterial pressure and carotid intima media thickness: a longitudinal twin study. Cardiol J. 2019 Sep 6. doi: 10.5603/CJ.a2019.0089. **IF:1.743**

3. Kiss LZ, Bagyura Z, Csobay-Novák C, Lux Á, Polgár L, **Jermendy Á,** Soós P, Szelid Z, Maurovich-Horvat P, Becker D, Merkely B. (2019) Serum Uric Acid Is Independently Associated with Coronary Calcification in an Asymptomatic Population. J Cardiovasc Transl Res, 12: 204-210. **IF:2.756**

4. Bikov A, Kolossváry M, **Jermendy AL,** Drobni ZD, Tarnoki AD, Tarnoki DL, Forgó B, Kovacs DT, Losonczy G, Kunos L, Voros S, Merkely B, Maurovich-Horvat P. (2019) Comprehensive coronary plaque assessment in patients with obstructive sleep apnea. J Sleep Res, 6: e12828. **IF:3.432**

5. Pucci G, Tarnoki AD, Medda E, Tarnoki DL, Littvay L, Maurovich-Horvat P, **Jermendy AL,** Godor E, Fejer B, Hernyes A, Lucatelli P, Fanelli F, Farina F, Baracchini C, Meneghetti G, Jermendy G, Merkely B, Schillaci G, Fagnani C, Stazi MA. (2018) Genetic and environmental determinants of longitudinal stability of arterial stiffness and wave reflection: a twin study. J Hypertens, 36: 2316-2323. **IF:4.209**

6. Lucatelli P, Fagnani C, Tarnoki AD, Tarnoki DL, Sacconi B, Fejer B, Stazi MA, Salemi M, Cirelli C, d'Adamo A, Fanelli F, Catalano C, Maurovich-Horvat P, **Jermendy AL**, Jermendy G, Merkely B, Molnar AA, Pucci G, Schillaci G, Farina F, Meneghetti G, Baracchini C, Medda E. (2018) Genetic influence on femoral plaque and its relationship with carotid plaque: an international twin study. Int J Cardiovasc Imaging, 34: 531-541. **IF:1.860**

7. Bartykowszki A, Kolossváry M, **Jermendy ÁL**, Karády J, Szilveszter B, Károlyi M, Balogh O, Sax B, Merkely B, Maurovich-Horvat P. (2018) Image Quality of Prospectively ECG-Triggered Coronary CT Angiography in Heart Transplant Recipients. Am J Roentgenol, 210: 314-319. **IF:3.161**

8. Vecsey-Nagy M, Simon J, Szilveszter B, Karady J, Jermendy A, Merkely B, Maurovich-Horvat P. (2018) Role of Multidetector Computed Tomography in Transcatheter Aortic Valve Implantation - from Pre-procedural Planning to Detection of Post-procedural Complications. Journal of Cardiovascular Emergencies, 4: 178-186.

9. Szilveszter B, Kolossváry M, Karády J, **Jermendy ÁL**, Károlyi M, Panajotu A, Bagyura Z, Vecsey-Nagy M, Cury RC, Leipsic JA, Merkely B, Maurovich-Horvat P. (2017) Structured reporting platform improves CAD-RADS assessment. J Cardiovasc Comput Tomogr, 11: 449-454. **IF:3.095**

10. Karády J, Panajotu A, Kolossváry M, Szilveszter B, **Jermendy ÁL**, Bartykowszki A, Károlyi M, Celeng C, Merkely B, Maurovich-Horvat P. (2017) The effect of four-phasic versus three-phasic contrast media injection protocols on extravasation rate in coronary CT angiography: a randomized controlled trial. Eur Radiol, 27: 4538-4543. **IF:4.027**

11. Fejer B, Tarnoki AD, Tarnoki DL, Lucatelli P, Littvay L, Maurovich-Horvat P, **Jermendy AL**, Kovacs A, Godor E, Fagnani C, Stazi MA, Molnar AA, Fanelli F,

Cirelli C, Farina F, Baracchini C, Meneghetti G, Pucci G, Jermendy G, Merkely B, Schillaci G, Medda E. (2017) Heritability of the femoral intima media thickness. Eur J Intern Med, 41: 44-48. **IF:3.282**

12. Celeng C, Kolossváry M, Kovács A, Molnár AÁ, Szilveszter B, Horváth T, Károlyi M, **Jermendy ÁL**, Tárnoki ÁD, Tárnoki DL, Karády J, Voros S, Jermendy G, Merkely B, Maurovich-Horvat P. (2017) Aortic root dimensions are predominantly determined by genetic factors: a classical twin study. Eur Radiol, 27: 2419-2425. **IF:4.027**

13. Károlyi M, Szilveszter B, Kolossváry M, Takx RA, Celeng C, Bartykowszki A, **Jermendy ÁL**, Panajotu A, Karády J, Raaijmakers R, Giepmans W, Merkely B, Maurovich-Horvat P. (2017) Iterative model reconstruction reduces calcified plaque volume in coronary CT angiography. Eur J Radiol, 87: 83-89. **IF:2.843**

14. Maurovich-Horvat P, Károlyi M, Horváth T, Szilveszter B, Bartykowszki A, **Jermendy ÁL**, Panajotu A, Celeng C, Suhai FI, Major GP, Csobay-Novák C, Hüttl K, Merkely B. (2015) Esmolol is noninferior to metoprolol in achieving a target heart rate of 65 beats/min in patients referred to coronary CT angiography: A randomized controlled clinical trial. J Cardiovasc Comput Tomogr, 9: 139-45. **IF:2.472**

15. Horváth T, Osztovits J, Pintér A, Littvay L, Cseh D, Tárnoki AD, Tárnoki DL, **Jermendy AL**, Steinbach R, Métneki J, Schillaci G, Kollai M, Jermendy G. (2014) Genetic impact dominates over environmental effects in development of carotid artery stiffness: a twin study. Hypertens Res, 37: 88-93. **IF:2.658**

16. Jermendy G, Horváth T, Littvay L, Steinbach R, **Jermendy ÁL**, Tárnoki ÁD, Tárnoki DL, Métneki J, Osztovits J. (2011) Effect of genetic and environmental influences on cardiometabolic risk factors: a twin study. Cardiovasc Diabetol, 10: 96. **IF:3.346**

17. Osztovits J, Horváth T, Littvay L, Steinbach R, **Jermendy Á**, Tárnoki Á, Tárnoki D, Métneki J, Kollai M, Jermendy G. (2011) Effects of genetic vs. environmental factors on cardiovascular autonomic function: a twin study. Diabet Med, 28: 1241-1248. **IF:2.902**

Articles in Hungarian

1. Jermendy Gy, Littvay L, Steinbach R, **Jermendy Á**, Tárnoki Á, Tárnoki D, Métneki J, Osztovits J. (2011) A metabolikus szindróma összetevőinek genetikai meghatározottsága: ikervizsgálatok eredményei. Orv Hetil, 152: 1265-1271.

2. Jermendy Gy, Horváth T, Littvay L, Steinbach R, **Jermendy ÁL**, Tárnoki ÁD, Tárnoki DL, Métneki J, Osztovits J. (2011) Kardiometabolikus kockázati tényezők és öröklődés: ikervizsgálatok eredményei. Metabolizmus, 9: 304-309.

Cumulative impact factor of the candidate's publications related to the thesis: **6.945**

Cumulative impact factor of the candidate's publications not related to the thesis: **51.073**

Total cumulative impact factor of the candidate's publications: **58.018**

DOI:10.14753/SE.2020.2396

11. Acknowledgements

First of all, I would like to express my sincere gratitude to **Pál Maurovich-Horvat**, my mentor from the beginning of my clinical and scientific career. It was a privilege for me that I could join his clinical and research team in the Heart and Vascular Center of the Semmelweis University. I performed my scientific work as a member of the Cardiovascular Imaging Research Group which gained international reputation with his enthusiastic guidance. He always supported my clinical work enabling me to learn image interpretation and basic elements of clinical research.

I would like to gratefully thank the continuous support of **Professor Béla Merkely**, director of Heart and Vascular Center of Semmelweis University. Not only the optimal research environment due to state-of-the-art equipment available but the inspiring scientific atmosphere were fundamental for me during my PhD studies.

During my studies I have been fortunate to be surrounded with a great and supportive team of colleagues. All of my research projects were performed as a real team work, with so many people contributing to their successful completion. I am very grateful to **Márton Kolossváry** for his continued effort to support my projects, and for the thorough statistical analysis. In the clinical part of the twin studies I received continuous support from **Zsófia Drobni, Bálint Szilveszter, Júlia Karády, Andrea Bartykowszki, Mihály Károlyi, Alexisz Panajotu, Ferenc Imre Suhai, Csilla Celeng,** and **Dorottya Hörcsik.** I am thankful to **Eszter Nagy**, her contribution to our research projects was essential. From the Hungarian Twin Registry, I received continuous help from **Dávid Tárnoki, Ádám Tárnoki** and **Levente Littvay.** I would like to thank **György Balázs** and **Professor Kálmán Hüttl** the clinical support which they provided me from the beginning of my career. I deeply appreciate the work of devoted assistants and radiographers.

Finally, I am thankful to my parents and my sister for their tremendous support, and I am especially grateful to my wife, **Andrea** whose patience and incentive always helped me to overcome difficulties and challenges.