Association between intrathoracic adipose tissue mass and metabolic parameters

ANASTASIIA DRIUCHINA

Turun yliopisto
University of Turku

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Supervisors:
1: Pirjo Nuutila, MD, PhD, Professor

2: Jarna Hannukainen, MSc, PhD, Adj. Professor

Examiners:
1: Pirjo Nuutila, MD, PhD, Professor

2: Pekka Hänninen, PhD, Professor

Passed:

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TURUN YLIOPISTO
Faculty of Medicine, Institute of Biomedicine
Turku PET Centre

ANASTASIIA DRIUCHINA:

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Abstract

Overweight or obesity, along with type 2 diabetes mellitus, is one of the major causes of cardiovascular morbidity and mortality. In the heart, fat is deposited inside – myocardial triglyceride content, and outside the myocardium, so called intrathoracic fat which is divided by pericardium to epicardial and pericardial adipose tissue.
The aim of this project was to establish the association between intrathoracic adipose tissue distribution and metabolic profile of patients with type 2 diabetes mellitus.
The study group consisted of 31 patients (78 % male, mean age 62 ± 8, mean BMI 32 kg/m^2) with type 2 diabetes mellitus. Magnetic resonance imaging was used to assess cardiac fat volumes. Additional laboratory and anthropometric measurements were performed.
The study showed that men tend to have higher cardiac adipose tissue mass than women (epicardial 263 and 234 g, $p = .168$; pericardial 272 and 176 g, $p = .013$; intrathoracic 535 and 410 g, $p = .018$). Subjects with larger waist circumference (more than 102 cm in men, and more than 88 cm in women) showed higher cardiac adipose tissue mass than subjects with smaller waist circumference; however, there were no statistically significant differences (epicardial 261 and 240 g; pericardial 251 and 243 g). There was a positive correlation between cardiac fat mass and waist circumference (epicardial $r_s = .398$, $p = .027$; intrathoracic $r_s = .378$, $p = .036$).
Study results showed that cardiac fat mass measurement by MRI might provide important information about the metabolic and cardiovascular risk. In the future, it would be interesting to investigate the association between cardiac fat depots and the amount of visceral adipose tissue along with lipid profile.

KEYWORDS: Intrathoracic adipose tissue, Cardiovascular, Type 2 diabetes mellitus, Metabolic syndrome, Magnetic resonance imaging

ABBREVIATIONS

AACE - American Association of Clinical Endocrinologists

ATP III - Adult Treatment Panel III

BMI – body mass index

BP – blood pressure

CT – computed tomography

CV – cardiovascular

EMA - European Medicines Agency

FFA – free fatty acid

FPG – fasting plasma glucose

GLP-1 – glucagon-like peptide 1

Hb1Ac – glycosylated hemoglobin, type 1Ac

HDL-C – high-density lipoprotein cholesterol

HGP – hepatic glucose production

HOMA – homeostatic model assessment

IDF – International Diabetes Federation

IGT – impaired glucose tolerance

LDL-C – low-density lipoprotein cholesterol

MTC – myocardial triglyceride content

MRI – magnetic resonance imaging

NMR – nuclear magnetic resonance

PET – positron emission tomography

RF – radio frequency

ROI – region of interest

SGLT2 – sodium glucose cotransporter 2

SD – standard deviation

T2DM – type 2 diabetes mellitus

US FDA – US Food and Drug Administration

VLDL-C – very low-density lipoprotein cholesterol

VOI – volume of interest

WHO – World Health Organization

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1 INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a growing public health concern worldwide. Over the past few decades, the incidence of diabetes has been steadily increasing in countries of all income levels, especially in low- and middle-income countries. Sedentary lifestyle, dietary preferences, urbanization, and many other factors account for the increasing risk of developing T2DM all over the world (International Diabetes Federation, 2015).

T2DM is a major cause of cardiovascular morbidity and mortality (Martín-Timón et al., 2014). If diabetes incidence continues to increase as it has been predicted, cardiovascular (CV) disease epidemic will become inevitable which will lead to the immense global healthcare burden (Chiha et al., 2012). However, CV risk is increased long before a patient is diagnosed with diabetes. Metabolic syndrome is a group of risk factors leading to CV diseases and diabetes. These factors include abdominal obesity, increased blood pressure (BP), increased cholesterol, insulin resistance (IR) and increased fasting plasma glucose (FPG) (National Cholesterol Education Program, 2002; Alberti and Zimmet, 1998; American Diabetes Association, 2016). Overweight or obesity is considered as a main risk factor for CV diseases (American Diabetes Association, 2016).

In the heart, fat is deposited inside as triglycerides increasing myocardial triglyceride content (MTC). Fat is also located outside the myocardium. This so called intrathoracic fat is divided by pericardium to epicardial and pericardial adipose tissue (Honkala et al., 2017). Cardiac adipose tissue plays an important role in the development of CV diseases due to three main reasons: (1) Intrathoracic adipose tissue produces inflammatory cytokines, chemokines and hormones which leads to inflammation in coronary vessels (Mazurek et al., 2003; Guzzardi and Iozzo, 2011); (2) Increased intrathoracic fat forms a mechanical restraint affecting cardiac function (Iacobellis et al., 2005); (3) Increased MTC is associated with fatty acid oversupply to the heart, mitochondrial dysfunction and increased oxidative stress (Nyman et al., 2013; Lim and Meigs, 2014; McGavock et al., 2007). Heart adiposity has been attracting a lot of interest since recent developments in the field of noninvasive imaging which allows to quite accurately quantify fat depots.

The study aims to establish the relationship between intrathoracic adipose tissue mass and metabolic parameters.

2 LITERATURE REVIEW

2.1 Heart adiposity

Cardiac adipose tissue includes three distinct fat depots: (1) MTC; (2) epicardial adipose tissue; (3) pericardial adipose tissue (Honkala et al., 2017). Myocardial fat is defined as the depots of triglyceride within cardiomyocytes. Normally, MTC is regulated by the FFA oxidation. Defect in the FFA oxidation increases accumulation of triglycerides in cardiomyocytes (Szczepaniak et al., 2003; Kankaanpää et al., 2006).

Adipose tissue surrounding the heart, so called intrathoracic fat, consists of epicardial and pericardial fat (Fig.1). Epicardial and pericardial adipose tissue have clearly distinctive characteristics – anatomical, physiological, embryological, biomolecular, and clinical (Iacobellis et al., 2005).

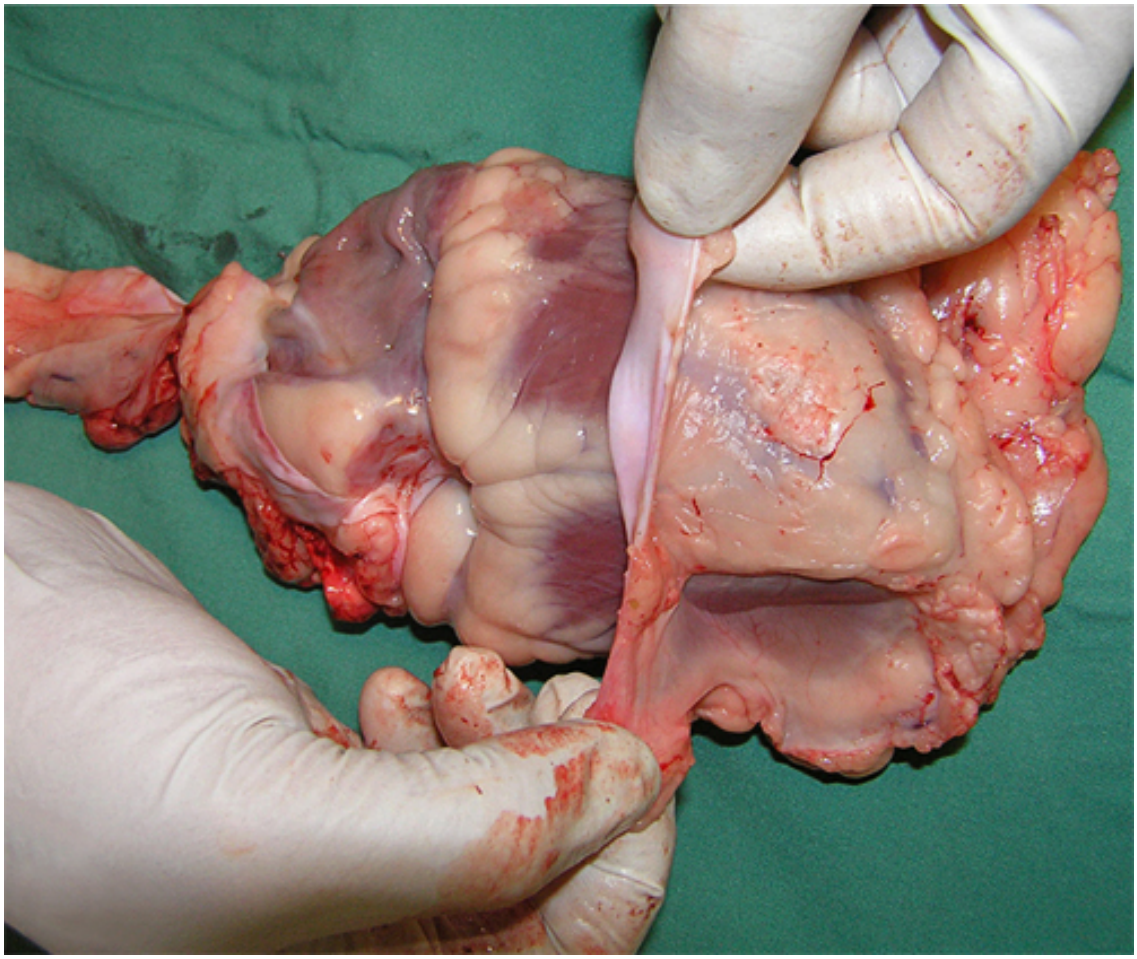


Figure 1. Epicardial and pericardial fat, from (Nelson et al., 2009).

Figure 2 shows the localization of two fat depots outside the myocardium. Epicardial fat refers to the layer of adipose tissue between myocardium and a visceral layer of pericardium. It does not extensively cover the heart – its volume depends on location, with highest concentration alongside the coronary arteries and in the atrioventricular and interventricular grooves. Epicardial fat is not separated from the heart muscle by a fascia and it shares the same blood supply from the coronary arteries (Iacobellis, 2009). Pericardial fat is located between visceral and parietal pericardium and also surrounds pericardium (Iacobellis et al., 2005). It receives blood from non-coronary sources, including the pericardiophrenic artery, a branch of the internal mammary artery (Iozzo, 2011; Noyes et al., 2014).

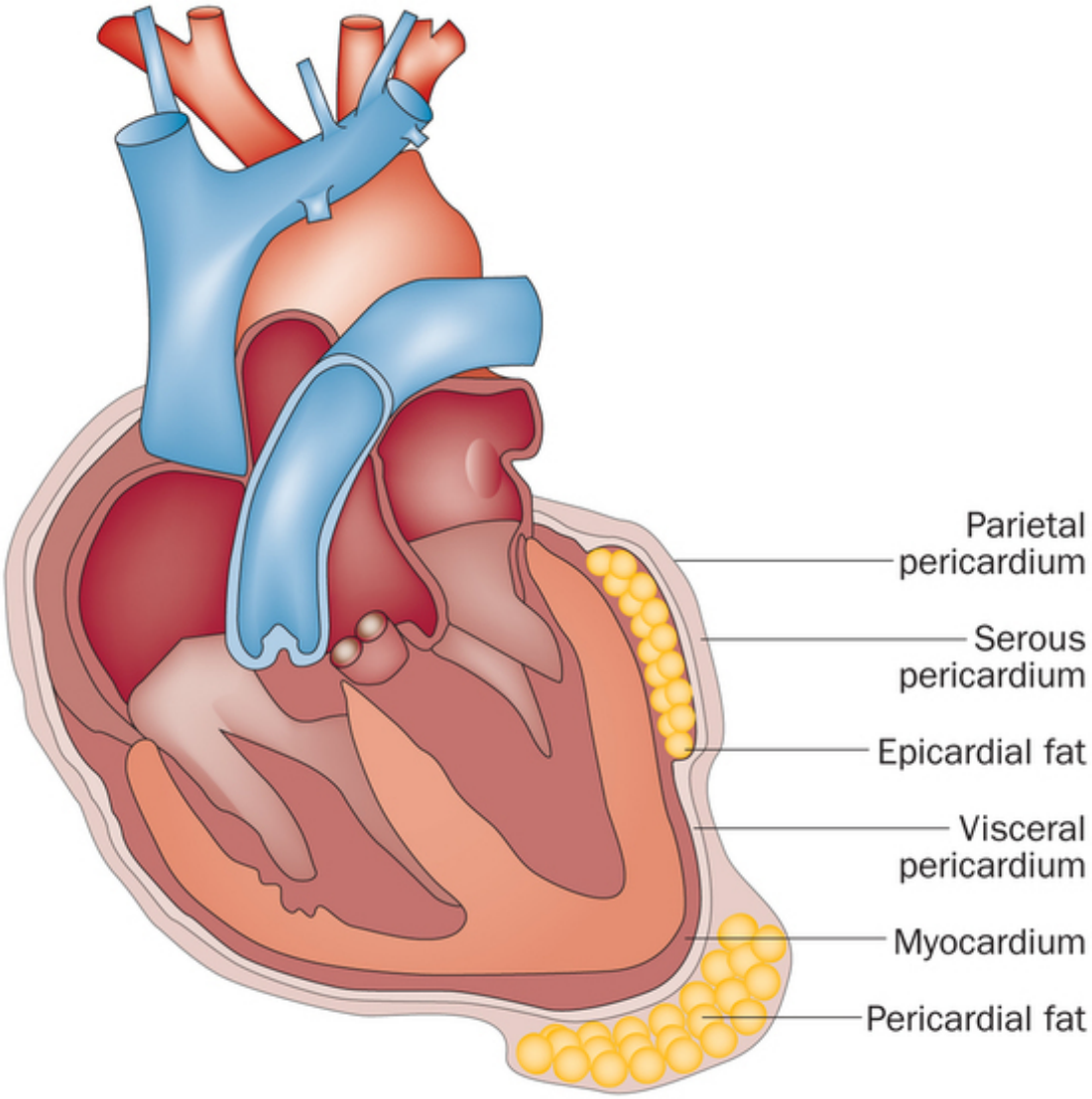


Figure 2. Localization of epicardial and pericardial fat, from (Iacobellis, 2015).

The origin of two fat depots also differs. Epicardial fat, similar to mesenteric and omental fat, emerges from mesothelial cells, and pericardial fat evolves from thoracic mesenchyme (Iacobellis et al., 2005). As it was mentioned above, epicardial fat receives blood from coronary circulation. On the contrary, pericardial fat is provided with blood by the pericardiophrenic artery, a branch of the internal thoracic artery, thus it has non-coronary sources of blood supply (Bertaso et al., 2013).

In health, cardiac fat depots play protective and regulative role. In the absence of fascia separating epicardial adipose tissue and myocardium, potential interactions between these tissues can take place. Acting as a buffer, epicardial fat accumulates and releases free fatty acids (FFAs) when needed in order to prevent lipotoxicity or to provide myocardium with energy (Gaborit et al., 2013; Talman et al., 2014; Guzzardi and Iozzo, 2011). As an endocrine organ, epicardial adipose tissue produces pro- and anti-inflammatory adipocytokines (Baker et al., 2006). In comparison to subcutaneous adipose tissue, it expresses considerably higher amount of monocyte chemoattractant protein-1 (MCP-1), IL-1β, IL-6, soluble IL-6 receptor and TNF-α (Guzzardi and Iozzo, 2011). Much uncertainty still exists about the physiological impact of pericardial fat (Mazurek et al., 2003).

There is no general agreement about the normal thicknesses and volumes of epicardial and pericardial fat. It is established from the variety of studies that cardiac fat depots correlate with gender, age and ethnicity. Thus, men tend to have larger amount of cardiac fat than women. It increases with ageing. Moreover, cardiac fat volumes seem to be larger in people of Caucasian race (Iozzo et al., 2009; Alexopoulos et al., 2010).

The different methods used for the assessment of intrathoracic adipose tissue volume also explain part of the variability between studies. Noninvasive imaging is typically done with ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI). Echocardiographic measurement of epicardial adipose tissue thickness was proposed as a reliable and simple method in clinical practice with no extra cost since patients with high CV risk are regularly referred to echocardiography (Iacobellis et al., 2003). However, it is not always possible to distinguish with echocardiography epicardial fat from pericardial fat due to poor visualization of pericardium. Usually, echocardiography gives higher epicardial fat volumes, whereas the amount of pericardial fat might be underestimated (Bertaso et al., 2013).

Computed tomography (CT) is considered as an accurate imaging technique for measurement of cardiac fat (Dey et al., 2010). Its high spatial and temporal resolution provides precise quantification of epicardial and pericardial adipose tissue volumes separately (Marwan and Achenbach, 2013). However, this method has a number of limitations such as the radiation exposure and relatively high cost.

Magnetic resonance imaging (MRI) does not use ionizing radiation, but it suffers from other serious drawbacks. High price and time-consuming manual image analysis of adipose tissue are some of the problems (Dey et al., 2012). With use of whole body MRI, it is also technically challenging to accurately differentiate between visceral and parietal pericardium which can lead to incorrect measurement of epicardial and pericardial fat depots, whereas cardiac MRI can make this distinction (Kessels et al., 2006).

Although some research has been carried out on the *in vivo* quantification of intrathoracic adipose tissue, still there is no "gold standard" method (Rosito et al., 2008). Role of CT and MRI is increasing due to the possibility of volumetric measurement. Table 1 shows the average amount of epicardial and/or pericardial adipose tissue from various studies.

	Author	Number of subjects	Amount of intrathoracic fat (mean)
CT	(Sarin et al., 2008)	151	EAT 118 ± 43 g (range 23 to 252 g)
	(Rosito, G.A, Massaro, J.M, Hoffmann, 2008)	1155	PAT and intrathoracic fat 101 and 178 g in women and 126 and 262 g in men, respectively
	(Mahabadi et al., 2009)	1267	PAT 114 ± 46 g, intrathoracic fat 106 ± 58 g
	(Iwasaki et al., 2011)	197	EAT 91± 37 g (range 10,6 to 242,6 g)
	(Alexopoulos et al., 2010)	214	EAT 73 ± 39 g (range 9 to 230 g)
	(Wang et al., 2010)	224	EAT 102 ± 40 g
MRI	(Flüchter et al., 2007)	28 (healthy)	EAT 64,6 ± 21.2 g (range 28.2 to 112.8 g)
	(Gaborit et al., 2012)	30 (healthy)	EAT 51 ± 24 g (range 17 to 116 g)
	(Wong et al., 2011)	20 (healthy)	PAT 155 g (range 120 to 174 g)
	(Teme et al., 2014)	20	EAT 87 ± 42 g

Table 1. Intrathoracic adipose tissue measurements from various studies.
EAT – epicardial adipose tissue, PAT – pericardial adipose tissue

2.2 Metabolic syndrome and type 2 diabetes mellitus

2.2.1 Clinical criteria for diagnosis of metabolic syndrome

Metabolic syndrome is a group of risk factors leading to CVDs and diabetes. It was described by Reaven in 1988 as a cluster of several risk factors, such as hyperglycemia, hypertension, dyslipidemia, which cause heart disease. He used the term Syndrome X, which was later renamed as metabolic syndrome (Reaven, 1988).

Currently, there are 6 main metabolic components which can be related to CV diseases (Fig. 3).

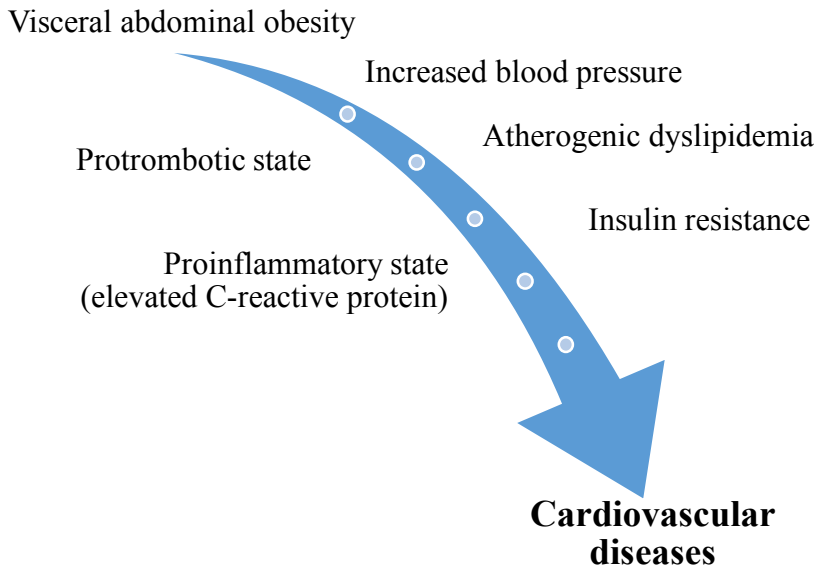


Figure 3. Metabolic components related to CV diseases

At the moment, there are several clinical criteria recommended by different organizations. During National Cholesterol Education Program Expert Panel in 2002, Adult Treatment Panel III (ATP III) proposed 5 parameters associated with metabolic syndrome. Three of five risk factors should be presented in order to make a diagnosis of metabolic syndrome. Abdominal obesity is considered as the predominant cause of metabolic syndrome (National Cholesterol Education Program, 2002). In 2006, International Diabetes Federation (IDF) updated these values. Table 2 shows an overview of updated clinical criteria provided by IDF. According to the new worldwide definition of metabolic syndrome, waist circumference should be more than 94 cm for men and more than 80 cm

for women with some variations between different ethnic groups. Thus, in the USA guidelines ATP III values are still in use; for Asian ethnicities defining levels are smaller than those for populations of European origin (International Diabetes Federation, 2006).

Risk factor	Defining level	
	men	women
Abdominal obesity (waist circumference)	> 94 cm (ATP III > 102 cm)	> 80 cm (ATP III > 88 cm)
Raised triglycerides	≥ 150 mg/dL, or specific treatment	
Reduced HDL cholesterol	< 40 mg/dL	< 50 mg/dL
Elevated blood pressure	≥ 130/ ≥ 85 mm Hg	
Increased fasting glucose	≥ 100 mg/dL (ATP III 110 mg/dL)	

Table 2. IDF clinical criteria (International Diabetes Federation, 2006)

IDF has proposed a number of additional parameters associated with metabolic syndrome, such as abnormal body fat distribution, dysglycemia, proinflammatory and prothrombotic state, etc. Further research in this field could also be conducted to determine the impact of these risk factors for diagnosis (International Diabetes Federation, 2006).

According to World Health Organization (WHO) clinical criteria, insulin resistance is a mandatory risk factor for diagnosis of metabolic syndrome. Apart from insulin resistance, two other risk factors should be presented (Alberti and Zimmet, 1998).

American Association of Clinical Endocrinologists (AACE) clinical criteria combine criteria of ATP III and WHO definition of metabolic syndrome. Although, there is no required number of risk factors, a physician diagnoses insulin resistance in accordance with clinical perception. When a patient's condition transforms to T2DM, the term insulin resistance is not relevant anymore (Einhorn et al., 2003).

Cardiovascular diseases are the major clinical outcomes of metabolic syndrome. Insulin resistance is the second most common clinical outcome. Majority of people with metabolic syndrome have increased risk for T2DM (Reaven, 1988). Apart from cardiovascular diseases and type 2 diabetes mellitus, patients with this syndrome suffer from other conditions, such as fatty liver, asthma, polycystic ovary syndrome (Grundy et al., 2004).

2.2.2 Visceral adipose tissue

Energy is stored into subcutaneous adipose tissue and visceral adipose tissue. In adults, subcutaneous fat constitutes the largest part of the total body fat. Visceral adipose tissue refers to the fat surrounding internal organs. To date, research has shown that visceral fat is metabolically more active than subcutaneous fat and is significantly correlated with cardiovascular risk factors and the metabolic syndrome (Wajchenburg, 2014). In abdomen, visceral adipose tissue can be approximately partitioned into intraperitoneal and extraperitoneal compartments by delineation along ascending and descending colons and kidneys. The reason for this separation is that blood from intraperitoneal compartment drains into the portal vain while blood from retroperitoneal compartment drains into inferior vena cava (Abate et al., 1994). Likely, metabolism of these two compartments differ, which is an important issue for future studies.

There are several methods of measuring visceral fat: anthropometric measurements and imaging techniques. Waist circumference is the simplest but less precise way of visceral fat estimation. This anthropometric technique is also considered more reliable in quantification of subcutaneous fat rather than visceral abdominal fat (Bonora et al., 1995). Imaging techniques, such as MRI or CT, provide precise measurements; however, these methods have several disadvantages. MRI cannot provide adequate calculation of visceral fat in patient with obesity. A potential problem of CT scans is the exposure to ionizing radiation. Moreover, both MRI and CT are expensive techniques. Recent evidence suggests abdominal ultrasonography as a reliable imaging technique for visceral adipose tissue assessment due to its low price, accuracy and easy application (Ribeiro-Filho et al., 2000; Iacobellis et al., 2003).

There is strong evidence that amount of visceral fat increases with age, both in men and women (Enzi et al., 1986). It was also shown that, since visceral fat is metabolically more active, weight loss affects visceral fat more than subcutaneous fat due to higher level of lipolysis (Bjorntorp, 1992).

As it was previously mentioned, visceral adipose tissue has the highest lipolytic activity which leads to the increased free fatty acids (FFA) flux to the liver. The flux tends to intensify gluconeogenesis and production of very low-density lipoproteins (VLDL) which results in ectopic triglyceride accumulation. Above all, the flux leads to reduced hepatic insulin extraction, which in return increase level of insulin; finally, insulin inhibits hepatic glucose production (HGP) (Wajchenburg, 2014). Apart from preferential oxidation of FFA over glucose, various mechanisms explaining insulin resistance has been suggested (Delarue and Magnan, 2007). These are oxidative stress, inflammation, lipid metabolites in skeletal muscle and liver which alter insulin signaling pathway by activating serine kinase cascade (Petersen and Shulman, 2006; Boden, 2011). Thus, excessive levels of FFA will certainly lead to failure of ß-cells and the development of T2DM (Wajchenburg, 2014). As shown in Figure 4, this process demonstrates a distinct relationship between the amount of visceral fat and insulin resistance through the increased levels of FFA.

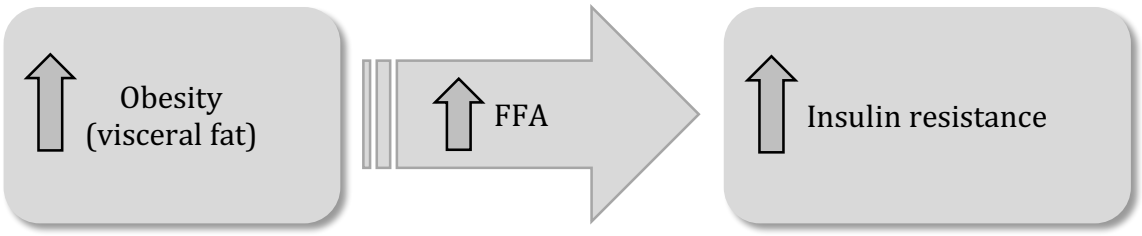


Figure 4. Relationship between the amount of visceral fat and insulin resistance

2.2.3 Type 2 diabetes mellitus

Type 2 diabetes mellitus has been known as non–insulin-dependent diabetes and makes up about 90% of all diabetes cases. It occurs due to a relative insulin deficiency on the background of insulin resistance. This key feature contrasts with type 1 diabetes mellitus when absolute insulin deficiency leads to ketoacidosis and death in case insulin is not replaced. Due to quite slow development of hyperglycemia the signs of T2DM can initially go unnoticed for years, which leads to increased risk of diabetes complications in undiagnosed patients (Walker, 2013).

Over the past few decades, the incidence of diabetes has been steadily increasing in countries of all income levels, especially in low- and middle-income countries. T2DM is a major cause of cardiovascular morbidity and mortality. It is associated both with microvascular complications, including nephropathy, retinopathy and neuropathy, as well as with macrovascular complications such as CV diseases, cerebrovascular disease and peripheral artery disease. Overweight or obese, being related to T2DM, is a further risk factor for hypertension which is a common co-morbidity in both CV diseases and T2DM (American Diabetes Association, 2016).

Glucose homeostasis is normally maintained by several coordinated actions: gluconeogenesis inhibition, glycolysis activation and stimulation of glucose uptake into peripheral insulin-dependent tissues (skeletal muscles and adipose tissue) (DeFronzo and Tripathy, 2009). Insulin, being released from β-endocrine cells of Islets of Langerhans in pancreas, is the main regulator not only glucose metabolism but also lipid metabolism. It increases triglyceride synthesis, FFAs synthesis, decreases lipolysis, ketogenesis and fatty acid oxidation. FFA oxidation provides energy for further gluconeogenesis and leads to ketone bodies production.

Insulin resistance, a key feature in T2DM, is progressively increasing over time. In the early, asymptomatic stage, insulin resistance is well compensated by increased release of insulin from pancreatic cells (hyperinsulinemia). Development of β-cell defect leads to impaired glucose tolerance (IGT). In the clinical stage, with a greater loss of β-cell function, hyperglycemia becomes manifest, hepatic glucose production increases along with decreased glycogen synthesis and increased fat synthesis (American Diabetes Association, 2016).

The mechanism of insulin resistance in T2DM remains unclear. Apart from genetic factor, the most common theory is based on T2DM association with overweight or obesity. In obese, bigger amount of metabolically active visceral adipose tissue produces more FFAs due to increased rate of lipolysis. Circulated FFAs can become a fuel supply for oxidation in peripheral tissue replacing glucose, thus affecting insulin resistance. In addition, excess visceral adiposity leads to greater release of adipokines, regulatory hormones which may have an influence on insulin sensitivity. Finally, adipose tissue tends to accumulate a considerable amount of macrophages causing chronic inflammation. Increased inflammation is thought to be another reason of insulin sensitivity in obese individuals (Guilherme et al., 2008).

2.2.4 Therapeutic interventions

Standards of medical care in T2DM include pharmacological therapy (oral agents, non-insulin injectables, insulin replacement), medical nutrition therapy, exercise therapy (physical activity promotes glucose uptake in skeletal muscles), weight management (weight loss improves insulin sensitivity) and bariatric surgery (in terms of weight loss) (American Diabetes Association, 2016).

1. Pharmacological therapy

Glycemic control is the main goal of diabetes management. Chronic hyperglycemia causes glycation of proteins as well as other changes in chemicals. Glycosylated hemoglobin (HbA1c) is the most accurate and objective method to measure glycemic control since hemoglobin can be easily obtained from a blood sample (Walker, 2013) A wide range of pharmacotherapy is available, the most common oral antihyperglycemic drugs are biguanides (metformin), sulphonylureas, thiazolidinediones, GLP-1 analogs, α-glucosidase inhibitors etc.

Insulin resistance can be another prospective target of drug intervention (Alberti and Zimmet, 1998). At the moment, there are two classes of approved drugs on the market – metformin and thiazolidinedione pioglitazone, which reduce insulin resistance. Further investigation in the field of drug discovery is going on (Bailey, 2005).

The majority of recently available diabetes drugs have several limitations such as cost, dosing adjustments and schedules, adverse effects, etc.; thus, alternative therapies have been developed. Recently, researchers have shown an increased interest in sodium glucose cotransporter 2 inhibitors (SGLT 2), novel oral antihyperglycemic drugs, which decrease glucose reabsorption in the kidney, thus increasing urinary glucose excretion. They improve glycemic control with a low risk of hypoglycemia. Due to their original insulin-independent mode of action specifically targeting the kidney, they can be used in patients with insufficient insulin release (Vasilakou et al., 2013). Beyond glycemic control, these drugs have other beneficial effects on CV risk factors. These factors include decrease in blood pressure, weight, visceral adiposity, hyperinsulinemia, arterial stiffness, albuminuria, circulating uric acid levels and oxidative stress (Inzucchi et al., 2015). At the moment, there are 4 drugs approved by US Food and Drug Administration (FDA) and European Medicines Agency (EMA): canagliflozin, dapagliflozin, empagliflozin and ertugliflozin (Inzucchi et al., 2015; MSD, 2017). Another antidiabetic drug, liraglutide (an analog of GLP-1) has also shown significant cardioprotective effects, including weight loss (Nauck et al., 2009; Marso et al., 2013). Recently, it has been found that liraglutide reduced epicardial adipose tissue volume which can be associated with its cardioprotective effects (Iacobellis et al., 2017).

As for metabolic syndrome, drug therapy is particularly important as a secondary management strategy. However, very little is known about pathophysiological mechanisms of metabolic syndrome, and no specific treatment has been discovered. Various drugs can be used to target individual metabolic risk factors. For example, atherogenic dyslipidemia can be treated with use of statins or fibrates, high BP – with use of angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and other antihypertensive drugs recommended by current guidelines etc. (International Diabetes Federation, 2006).

2. Diet

According to IDF and ATP III, obesity is the predominant cause of metabolic syndrome and, therefore. it is suggested that overweight should be the main target of therapy. Recommendations focus on weight reduction together with increased physical activity. Weight reduction can be achieved by calorie limitation and change in dietary habits. Such clinical management has an influence on different risk factors – weight loss contributes

to serum cholesterol, triglycerides, glucose and BP reduction, reduces insulin resistance, and it increases HDL cholesterol (National Cholesterol Education Program, 2002; International Diabetes Federation, 2006).

3. Exercise therapy

Different kinds of exercise, such as aerobic exercise, resistance exercise, endurance and passive exercise has been shown to have a great impact on T2DM and metabolic syndrome. Although the mechanism of the impact of exercise therapy on T2DM is unclear, it is known that physical activity promotes glucose uptake in skeletal muscles via glucose transporter 4. During the exercise it leads to reducing plasma glucose level in diabetic patients (Thent et al., 2013).

It has conclusively been shown that large amount of cardiac fat correlates with cardiovascular risk factors. Data from several sources have identified the decreased epicardial adipose tissue volume and thickness associated with weight loss, either through low-calorie diet or bariatric surgery (Willens et al., 2007; Iacobellis et al., 2008b). Collectively, these studies outline a critical role for the management of cardiac adipose tissue. Further research should be undertaken to investigate the possible ways of controlling the size of cardiac fat.

2.3 Role of epicardial and pericardial fat in T2DM

Most studies show the associations between cardiac adipose tissue depots (epicardial, pericardial fat and MTC) and metabolic syndrome (Iacobellis and Willens, 2009; Calabuig et al., 2017). Increased low-density lipoprotein (LDL) cholesterol and arterial blood pressure, decreased adiponectin and fasting insulin, as well as other components of metabolic syndrome have shown a correlation with excessive amount of epicardial adipose tissue (Iacobellis et al., 2003). According to some studies, pericardial fat depots is also correlated with metabolic components (Sironi et al., 2012; Rosito et al., 2008; Iacobellis et al., 2005; Sacks and Fain, 2007). In addition, it has been found that epicardial adipose tissue is related to unstable angina, myocardial infarction and coronary plague vulnerability (Homsi et al., 2016; Zhou et al., 2016; Ito et al., 2012).

There are few theories which can explain high risk of CV diseases. One of them is a mechanical theory. Large amount of pericardial adipose tissue can affect cardiac structure and function – ventricles should pump blood with more effort due to mechanical restraint.

Since epicardial adipose tissue has the same source of microcirculation as myocardium and due to its close proximity to the heart, it has been suggested that tissue inflammation and vasocrine effects can play a role in high risk of CV diseases when fat depots are increased (Mazurek et al., 2003). Epicardial adipose tissue, similar to other visceral depots, produces inflammatory cytokines, chemokines and hormones. This can lead to amplification of existing inflammation in coronary vessels. There are two possible mechanisms of this influence – paracrine signaling and vasocrine signaling (Sacks and Fain, 2007). Previous research has indicated that high release of epicardial adipokines could lead to a coronary atherogenesis (Lee et al., 2009). However, some consequences of adipocyte-derived inflammation might be favourable. Thus, inflammatory mediators released from epicardial adipose tissue can cause collateral vascularization in patients with obstructive coronary artery disease by stimulation of angiogenesis (Mazurek et al., 2003).

However, the evidence for this relationship is inconclusive as some authors tend to explain it by strong correlation between epicardial and visceral abdominal adipose tissue (Iacobellis et al., 2003). Moreover, in light of recent research, it has conclusively been shown that the amount of epicardial fat does not necessarily correlate with overall obesity.

In a small study conducted by Iacobellis et al. (2003), two men underwent physical examination and MRI for the assessment of body fat disposal. Whereas they were of the same age and had the same BMI, MRI revealed a representative difference. The first patient showed a severe visceral obesity along with significant epicardial adipose tissue thickness. Examination of the second patient found a large amount of subcutaneous fat along with normal epicardial fat thickness values. This study provided important suggestion about epicardial adipose tissue as a sign of visceral obesity independent of BMI.

Cardiac adipose tissue plays a crucial role in the development of diabetes mellitus (Iacobellis and Malavazos, 2010). Increased epicardial fat depots produce excessive amount of inflammatory mediators, FFAs, adiponectin, which results in impaired glucose metabolism and diabetes mellitus (Noyes et al., 2014). Previous research conducted in non-diabetic patients has shown that increased cardiac adiposity, particularly increased epicardial fat depots, is related to impaired fasting glucose (Iacobellis et al., 2008a).

2.4 Magnetic resonance imaging

Since the development of nuclear magnetic resonance (NMR) in 1946 by Bloch and Purcell, the possible use of the finding has been attracting a lot of interest (Bloch et al., 1946; Purcell et al., 1946). In 1970s, the idea of MRI as a new imaging technique captured the interest of researchers (Lauterbur, 1973). Today, MRI is a commonly used imaging modality which allows to assess both morphology and physiological processes of the body. The great advantage of MRI over other imaging modalities such as CT or positron emission tomography (PET) is that it does not use ionizing radiation. However, there are some limitations. MRI cannot be performed in patients with metal implants and heart pacemakers due to potential risks. Applications of the technique vary from visualization the vascular system by magnetic resonance angiography or brain function by functional MRI to chemical and physical assessment of individual molecules with use of magnetic resonance spectroscopy.

The main idea behind MRI is the imaging of a proton located in the nucleus of a hydrogen atom. A proton has two main properties – a spin and a positive charge. An electrical charge in motion is called an electrical current, which in addition is followed by a magnetic field (Westbrook et al., 2011). When a proton is placed in an external magnetic field, the spin rotates in two directions – with or against the direction of the magnetic field (Fig. 5).

An MRI mainly comprises of a super conducting magnet made of super conducting wire, gradient coils and RF coils. In order to generate magnetic field, current is introduced in the super conducting wire. The gradient coils are required for spatial encoding of magnetic signal while the RF coils are necessary for detections of electron spin from within the body as well as for producing the B_1 magnetic field. This magnetic field helps in rotating the spins. The spin of the electrons detected by the RF coils is the MRI signal which is digitized using a control circuit digitizer and is transferred to a computer where an inverse Fourier transform is used to convert the digitized signal into image (McRobbie et al., 2006).

As said before, MRI signals are mainly generated by the quantum property termed ‘spin’ of a hydrogen proton which is abundant in humans as they are typically comprised of 70% of water. The spin of this hydrogen nucleus gives a transition dipole moment which,

in a strong magnetic field like that of an MRI resides in two energy levels. They can either be parallel or anti-parallel to the magnetic field, the later having a slightly higher energy. Majority of these 'spins' reside in the lower energy state. The difference between the higher and lower energy state is determines the strength of the MR signal. The difference and hence the strength of the signal can be enhanced by using a stronger external magnet. This magnet also makes the hydrogen protons undergo a phenomenon called precession. Precession refers to the 'wobble' that the top of the spinning proton undergoes because of gravity. This precession frequency of the spinning proton is also known as Larmor frequency. This is also dependent on the strength of the magnetic field (Hashemi et al., 2012; Westbrook et al., 2011).

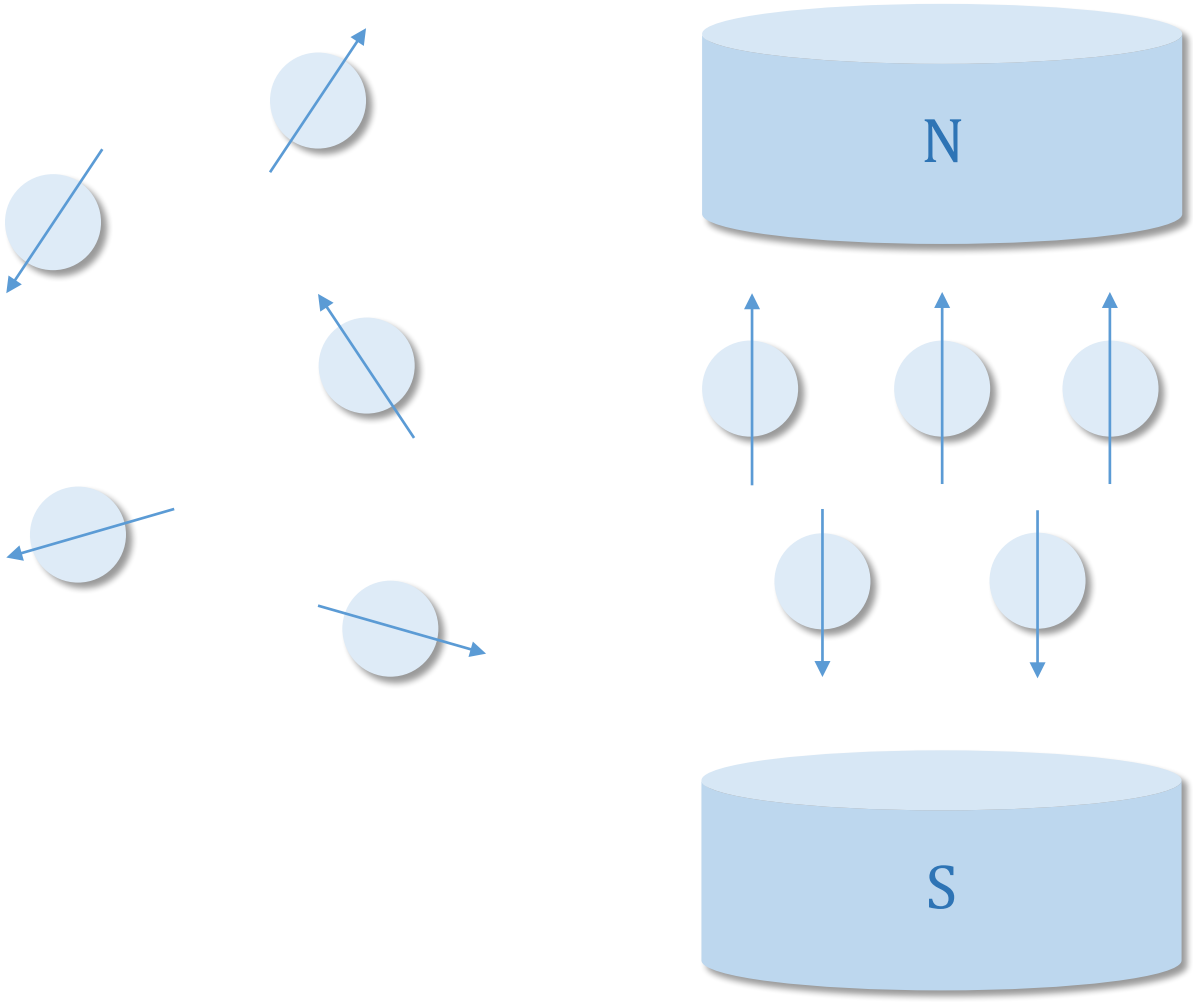


Figure 5. Without external magnetic field, protons are randomly aligned. In presence of a strong magnetic field, they are aligned in 2 directions, either parallel or antiparallel to the magnetic field.
Modified from (Schilds, 1991)

After a patient is placed in the scanner, the protons align parallel or antiparallel to the magnetic field. The magnetic field emits radio frequency (RF) pulse which leads to energy exchange with the protons. It is important that the RF pulse and the protons have the same frequency – a phenomenon called "resonance". As a result, some protons exchange their energy, a new transversal magnetization appears, thus the longitudinal magnetization is decreased (Schilds, 1991). When the RF pulse is stopped, the protons return to their original states. The process is called longitudinal and transversal relaxation.

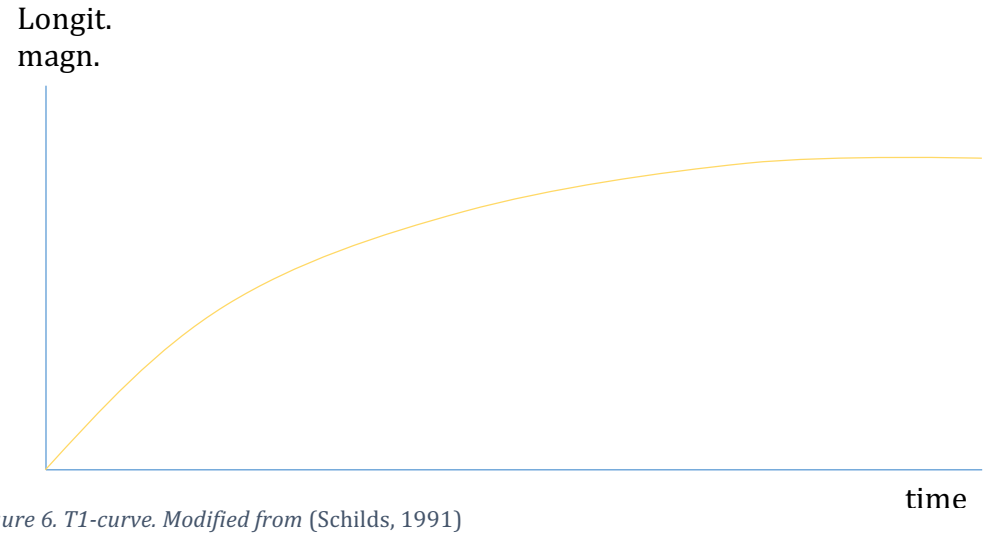


Figure 6. T1-curve. Modified from (Schilds, 1991)

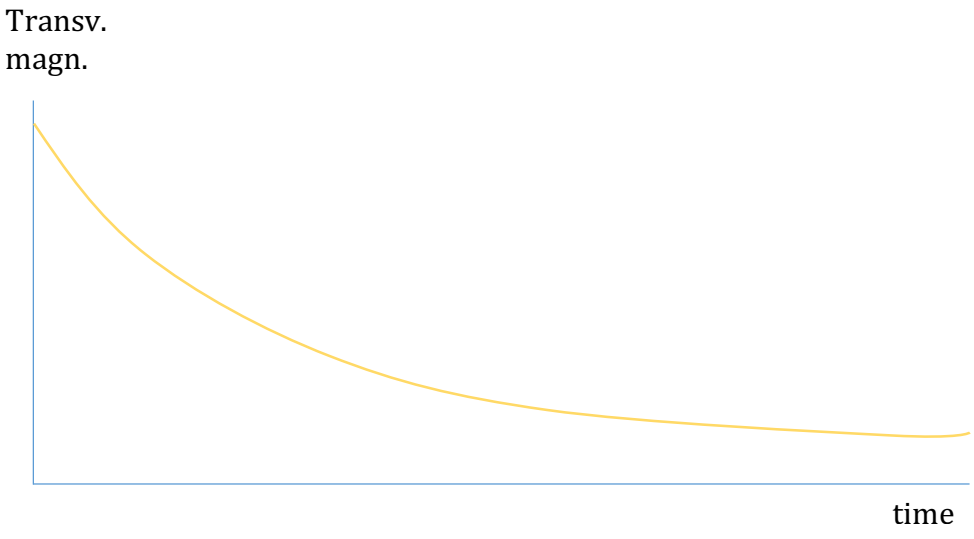


Figure 7. T2-curve. Modified from (Schilds, 1991)

Longitudinal relaxation, or spin-lattice relaxation, (T1) is a time constant describing the process of longitudinal magnetization recovery. In a similar way, transverse relaxation, or spin-spin relaxation, (T2) is a time constant describing the speed of the transversal

magnetization reduction (Kullberg, 2007). T1 relaxation time is normally longer than T2 relaxation time – in absolute values T1 is about 300-2000 ms, and T2 is about 30-150 ms (Schilds, 1991). The resulting curves are shown in figures 6 and 7.

The RF excitation and MRI signal detection by the scanner is repeated multiple times, resulting in image composition. T1 and T2 relaxation time vary with the magnetic field strength and in different tissues. Thus, T1 is longer in liquids and shorter in fat tissue. T2 is longer in water than in other liquids containing macromolecules (Schilds, 1991).

Perfect soft tissue contrast is one of the many reasons why MRI technique is so commonly used in practice. Together with good spatial resolution and lack of ionizing radiation, MRI is considered as a "gold standard" for the assessment of body fat and body composition.

3 AIMS AND HYPOTHESES

The objective of the study is:

- To evaluate the association between intrathoracic adipose tissue mass and metabolic components in patients with type 2 diabetes mellitus

It was hypothesized that intrathoracic fat depots correlate with main components of metabolic syndrome.

4 MATERIALS AND METHODS

4.1 Study population and design

I analyzed cross-sectional T2DM patients who had previously participated in a clinical trial (clinicaltrials.gov identifier: NCT02426541) A group of 31 female and male type 2 diabetes mellitus patients aged 35-70 years old with HbA1c ≥ 6.5% and ≤10.5% participated in this study. Females were of non-childbearing potential. The study was performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with guidelines of the International Conference on Harmonization, Good Clinical Practice and applicable regulatory requirements. Participants were fully informed of the possible risks and benefits associated with this study, and their consent was obtained prior to performing any study-related activity.

4.1.1 Inclusion criteria and exclusion criteria

Inclusion criteria:

1) Signed informed consent
2) Female or male patients aged 35-70 years old
3) Type 2 diabetes mellitus defined as HbA1c of ≥ 6.5% and ≤10.5%
4) Stable T2DM treatment (more than 3 months)
5) BMI ≤ 40 kg/m^2
6) Non-childbearing potential female patients (hysterectomized or postmenopausal women)

Exclusion criteria:

1) Involvement in the study planning or assessment
2) Previous participation in this study or another clinical study during the last 3 months
3) Metal implants, a pacemaker or other contraindications to MRI
4) Clinically significant disorder or disease which might affect the results of the current study

5) Volume depleted patients
6) Recent cardiovascular events (within 2 months prior the study)
7) Congestive heart failure defined as New York Heart Association (NYHA) class IV, unstable or acute congestive heart failure
8) BP ≥ 165/100 mm Hg
9) Recent history of drug abuse or alcohol abuse
10) Weight loss more than 5% within 3 months prior to study

Subjects underwent:

- Brief physical examination and vital signs (blood pressure and pulse measurements)
- Collection and measurements of body weight and waist circumference
- MRI scan
- Blood sampling
- Euglycemic hyperinsulinemic clamp
- Collection of adverse events and use of concominant medications

4.2 Study assessments

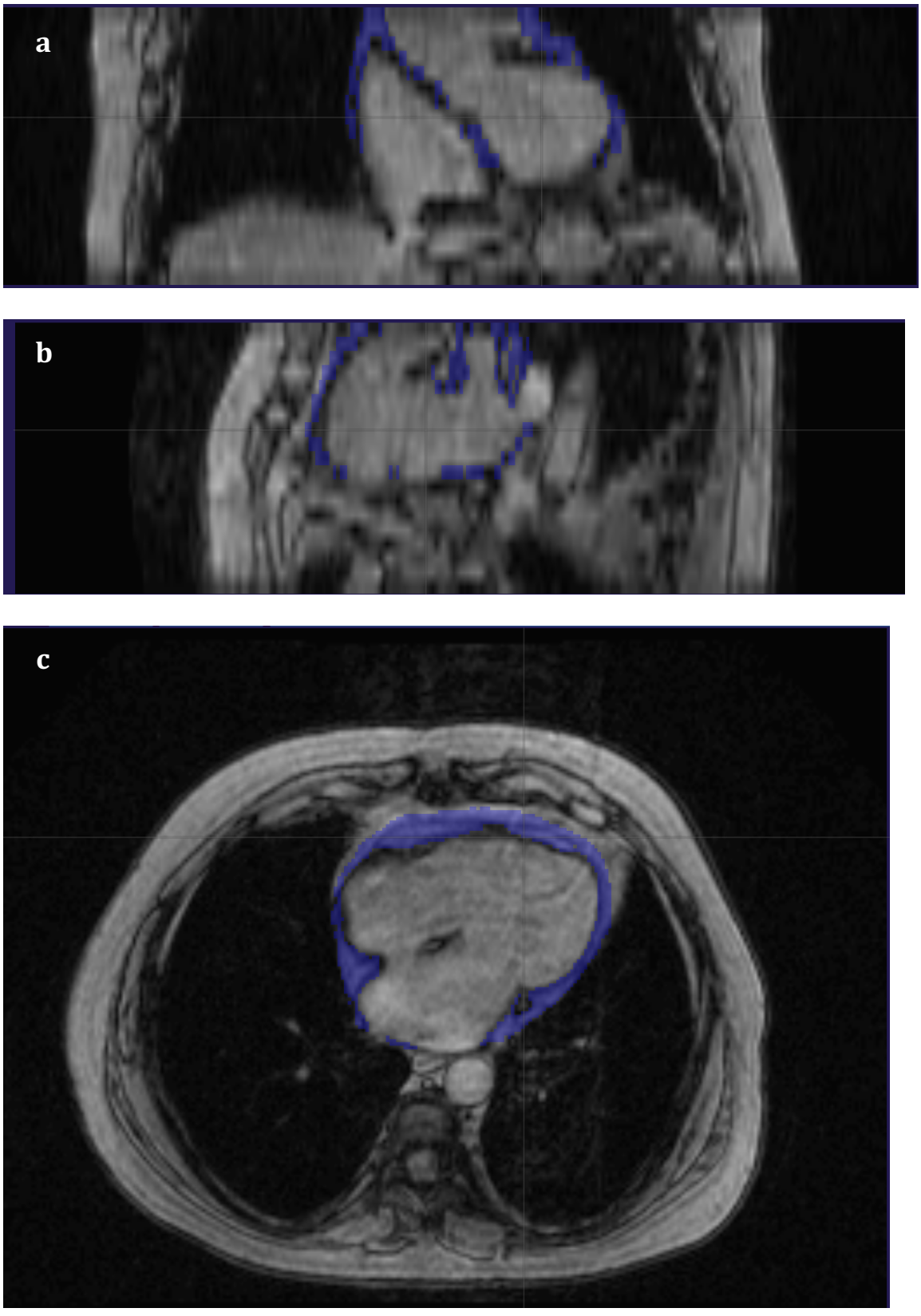
4.2.1 Magnetic resonance imaging

Magnetic resonance imaging was performed to assess fat depots and body composition. The MRI scans were performed using the PET MRI scanner at Turku PET Centre, Turku, Finland.

MRI data evaluation was performed by Carimas (version 2.9; Turku PET Centre, Turku, Finland). With use of the previously validated basic analysis protocol for measuring volume of cardiac fat, three-dimensional volumes of interest (VOIs) was manually drawn in the epicardial and pericardial adipose tissue compartments while avoiding bone, muscle and skin. Separate region of interest (ROI) segments were drawn from the highest edge of pericardium to its lowest edge paying attention to the anatomical structures as it gets close to the ends (towards the liver or towards the pulmonary artery). Within the volumes of interest, fat was defined as voxels within a window of different Hounsfield units for each case. The analysis continued with ROI drawing of all the fat around

pericardium (intrathoracic fat). At the top of the heart, the intrathoracic fat ROI segments should be drawn from the same slice as in the pericardium analysis, but at the bottom drawing can be continued as long as intrathoracic fat continues. The volume of pericardial fat was quantified by subtraction of epicardial fat from intrathoracic fat. To convert the measured volumes into weight, an adipose tissue density of 0,9196 g/ml was used.

A representative example of cardiac fat depot quantification by MRI is demonstrated in Fig. 8.


a
b
c

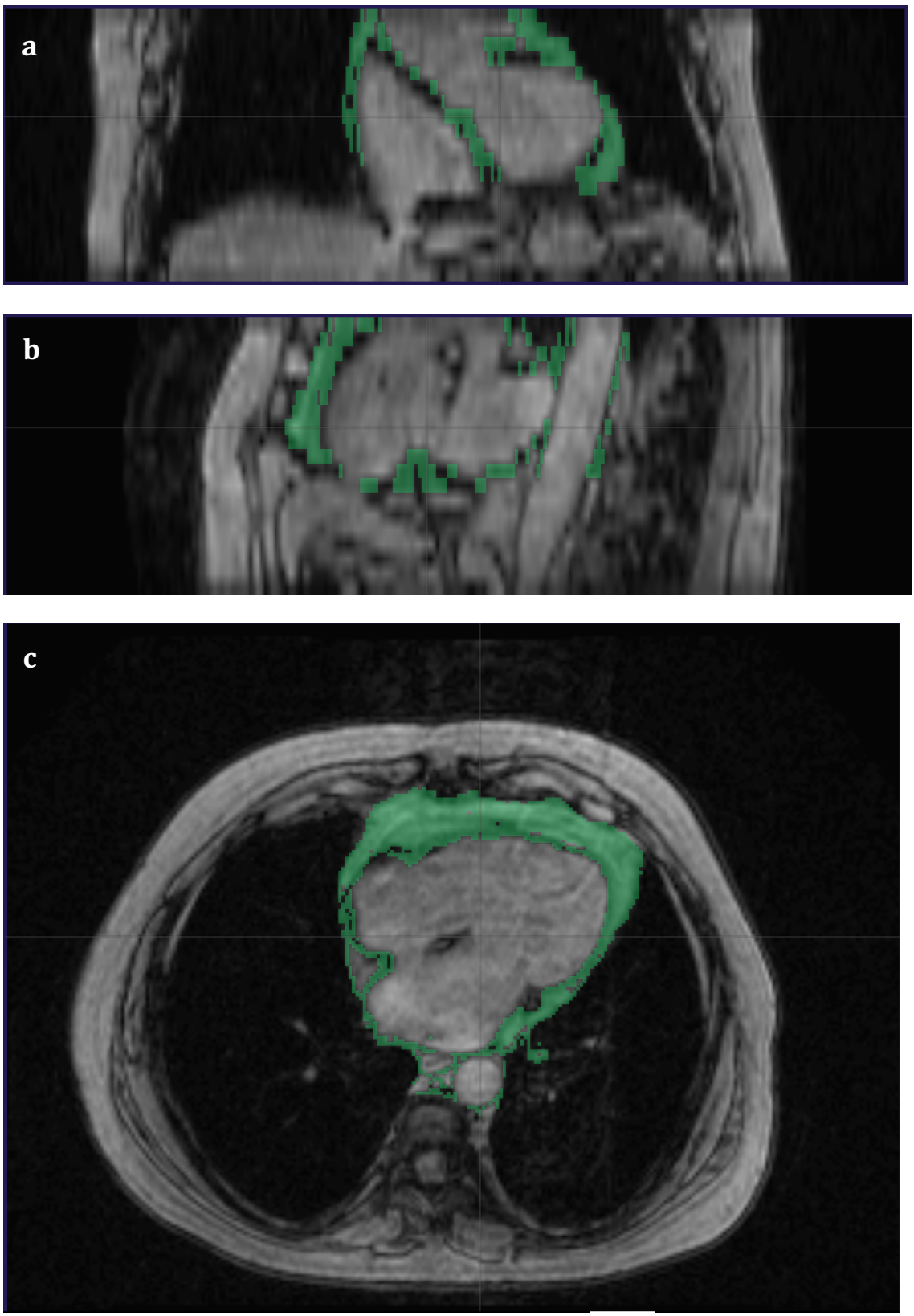


Figure 8. Representative example of epicardial and intrathoracic fat volume quantification by MRI; epicardial fat is highlighted in blue, intrathoracic fat – in green;, a: axial, b. coronal, c: sagittal view

4.2.2 Euglycemic-hyperinsulinemic insulin clamp

Euglycemic-hyperinsulinemic clamp is the gold-standard method to assess insulin sensitivity in humans. The procedure was performed on fasting subjects based on the original protocol by Defronzo et al. The insulin level was raised to 100 muU/ml and maintained by a continuous insulin infusion by insulin, while the glucose concentration was maintained at a normal level. Under a condition of steady euglycemia (the final 40 min of study), the amount of infused glucose equals the amount of the whole body glucose uptake (M-value) (DeFronzo et al., 1979).

4.2.3 Other measurements

The laboratory parameters (HbA1c, fasting plasma glucose, insulin, glucagon, GLP-1) were measured to assess glycemic efficacy. During eugycemic insulin clamp blood samples were taken to analyze FFAs, insulin and glucose. The local hospital laboratory at Turku PET Centre, Turku, Finland was used for the analyses.

The subject's weight was recorded in kilogram (kg), with light clothes and no shoes; the subject's height was recorded in centimeters, with no shoes; body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. The subject's waist circumference was measured in centimeters midway between the lowest rib and the iliac crest.

Vital sign measurements included sitting systolic and diastolic blood pressure and heart rate. Vital signs were measured after the patient rests for approximately 5 minutes and with the patient in a sitting position. The blood pressure measurement was repeated after at least 30 seconds and the average of the two readings recorded.

The OGTT was performed on fasting subjects. Liquid containing 75 g of glucose was given, and blood samples were taken at the zero time (baseline), and at 15, 30, 60, 90, 120 min during the test to measure glucose and insulin levels.

The Homeostatic Model Assessment (HOMA) calculator (The University of Oxford, 2013) was used to calculate ß-cell function, insulin sensitivity and insulin resistance (Matthews et al., 1985). As it can be seen from the figure 9, ß-cell function and insulin

sensitivity are estimated as percentages of a normal reference population while insulin resistance is calculated using standard units.

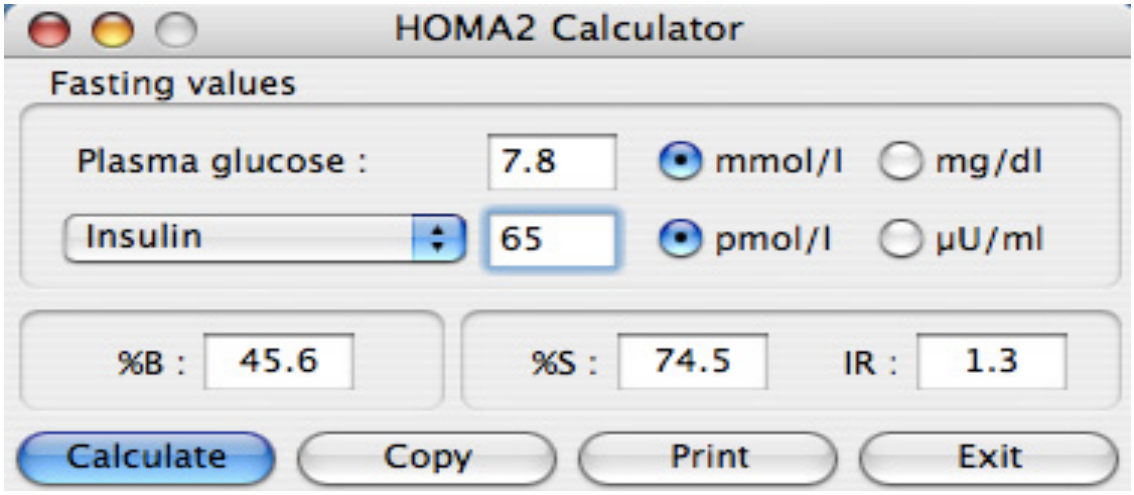


Figure 9. HOMA calculator

4.3 Statistical analysis

Data analysis was performed using SPSS (version 23; SPSS, Chicago, IL). Continuous data was reported as mean ± SD of the mean, and categorical data was reported as percentages. For justification of analyses, the normality of data distribution was checked using Shapiro-Wilk test. Logarithmic transformation was used to achieve the normal distribution assumption when necessary. Transformed model based means were translated back to the original scale. Comparison of variables between two groups was performed with independent samples T test, or non-parametric Mann-Whitney U test. Comparison of variables between 3 groups was performed using Kruskal-Wallis test. Correlations of cardiac fat volumes and anthropometric and clinical variables were performed using Pearson´s correlation (or Spearman's rank-order correlation analysis for non-normally distributed variables). Multiple regression analysis was performed to calculate the contribution of different covariates to the prediction of epicardial fat volume. All tests are two sided, and a P-value of <0.05 is considered statistically significant.

5 RESULTS

5.1 Subjects characteristics

Table 3 presents an overview of anthropometric and clinical characteristics of study subjects.

Age, years	62 ± 8
Male, %	77,4 %
Anthropometric measurements and vital signs	
Height, cm	172 ± 9
Weight, kg	95 ± 14
Waist, cm	110 ± 10
BMI, kg/m^2	32 ± 4
BP syst, mm Hg	149 ± 14
BP diast, mm Hg	85 ± 8
Heart rate, /min	69 ± 10
Laboratory measurements	
FFA, mmol/l	0,67 ± 0,17
FPG, mmol/l	9,1 ± 1,8
HbA1c, mmol/mol	52 ± 6
GHb-A1c, %	6,9 ± 0,6
HOMA	
ß-cell function, %	61.6 ± 29.6
Insulin sensitivity, %	46.9 ± 25.1
Insulin resistance	2.82 ± 1.55
Cytokines	
IL-6, pg/ml	5,14 ± 6,11
TNF-α, pg/ml	4,23 ± 1,86
MCP-1, pg/ml	418,82 ± 214,11

Table 3. Basic characteristics of study subjects

5.2 Epicardial and pericardial fat depots

Cardiac fat and gender

Figure 10 shows the distribution of cardiac fat between males (n = 24) and females (n = 7). From the chart it can be seen that males tend to show higher cardiac adipose tissue mass than females. Difference in pericardial fat and total intrathoracic fat is statistically significant (pericardial adipose tissue 272 and 176 g, p = .013; intrathoracic – 535 and 410 g, p = .018). No statistically significant difference in epicardial adipose tissue occured (263 and 234 g, p = .168).

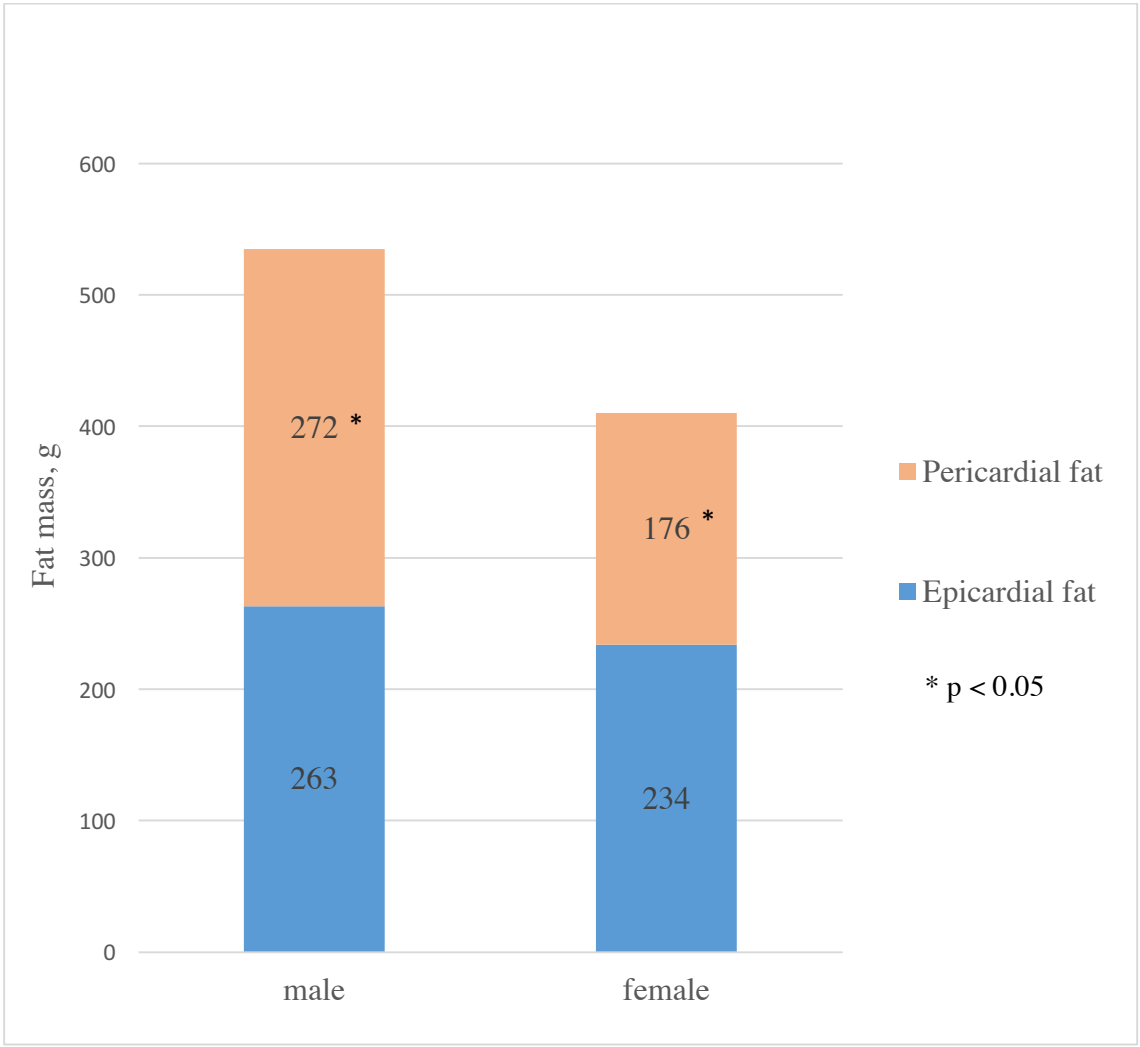


Figure 10. Epicardial and intrathoracic adipose tissue volume in males and females.

Cardiac fat and age

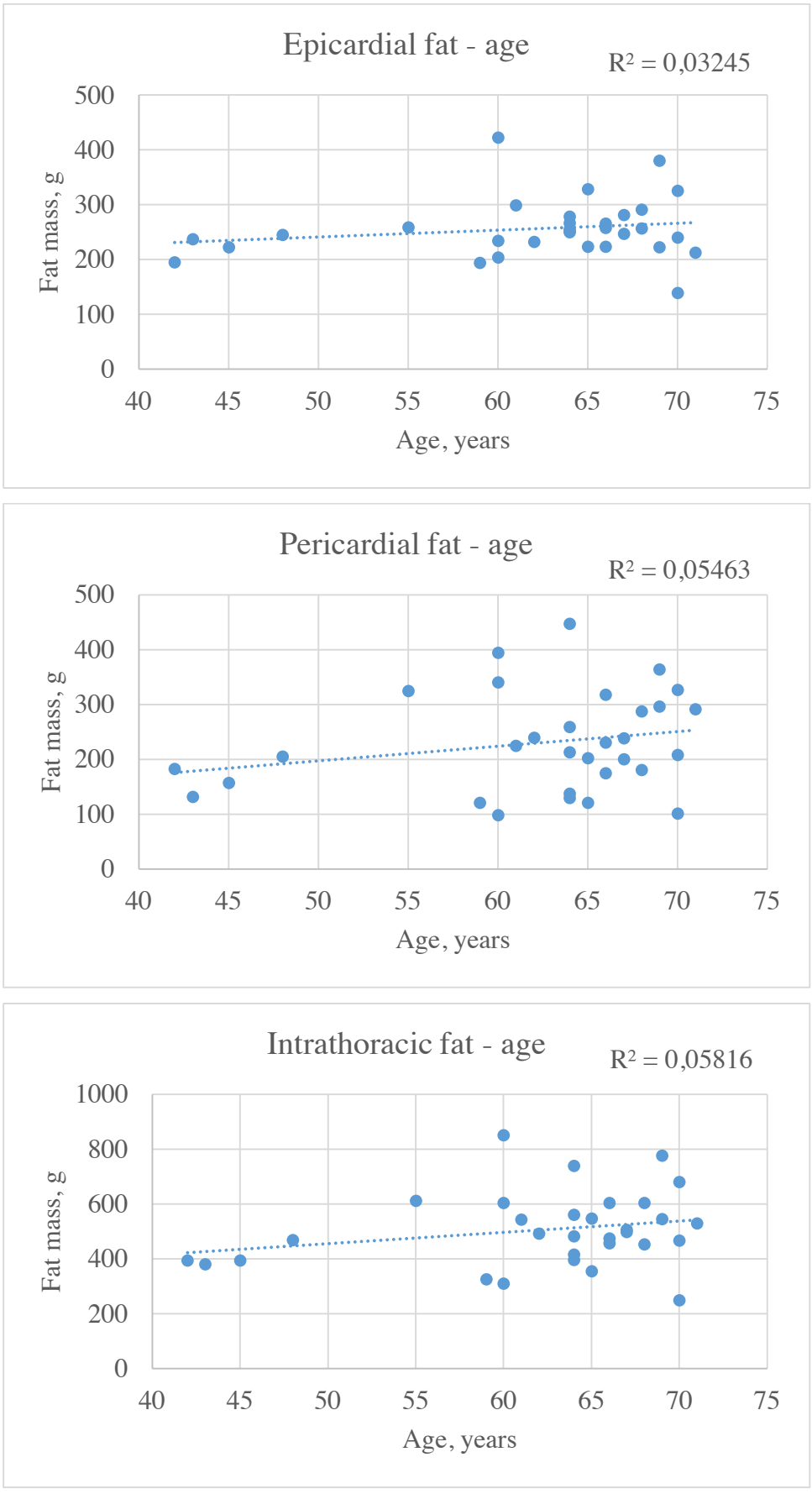


Figure 11. Correlation between cardiac fat depots and age

Figure 11 shows the correlation between cardiac fat depots and age. From the charts it can be seen that there was no evidence that age has an influence on heart adiposity.

Cardiac fat and waist circumference

Although the scatterplot showed weak positive correlation between epicardial adipose tissue mass and waist circumference (Fig. 12), statistical analysis of the data confirmed the significance of these findings (Table 4). Also there was the same strength positive correlation between total intrathoracic fat mass and waist circumference which was confirmed with statistical analysis (Fig. 13, Table 5).

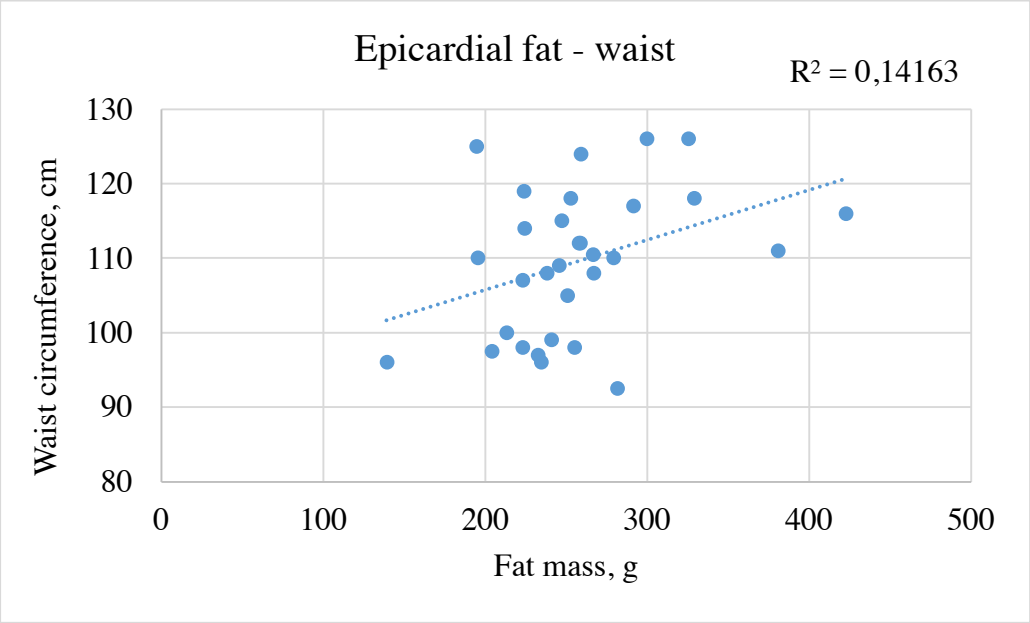


Figure 12. Correlation between epicardial fat depot and waist circumference

		Epicardial fat volume	Waist, cm
Epicardial fat volume	Pearson Correlation	1	.378*
	Sig. (2-tailed)		.036
	N	31	31
Waist, cm	Pearson Correlation	.378*	1
	Sig. (2-tailed)	.036	
	N	31	31
* Correlation is significant at the 0.05 level (2-tailed).			

Table 4. Statistical orrelation between epicardial fat depot and waist circumference

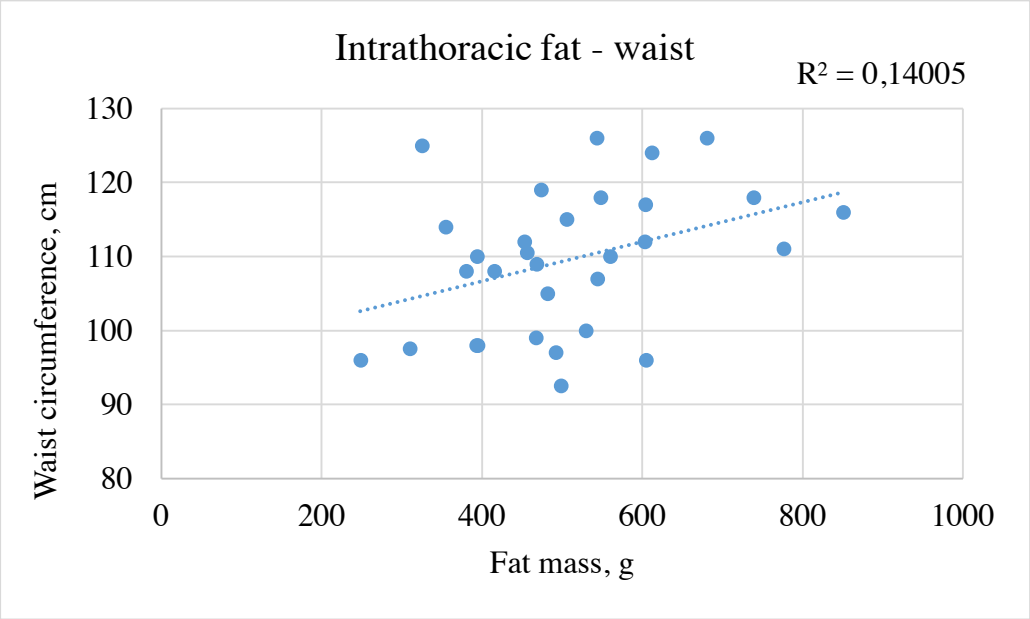


Figure 13. Correlation between intrathoracic fat depot and waist circumference

		Thoracic fat volume	Waist, cm
Thoracic fat volume	Pearson Correlation	1	.373*
	Sig. (2-tailed)		.039
	N	31	31
Waist, cm	Pearson Correlation	.373*	1
	Sig. (2-tailed)	.039	
	N	31	31
* Correlation is significant at the 0.05 level (2-tailed).			

Table 5. Statistical orrelation between epicardial fat depot and waist circumference

Cardiac fat and body fat distribution

Table 6 shows the comparison of epicardial and pericardial fat mass depending on different conditions of body fat distribution. Predominant visceral fat accumulation is represented by waist circumference > 102 cm in men and > 88 cm in women. Predominant peripheral fat accumulation is represented by waist circumference < 102 cm in men and < 88 cm in women.

Subjects with predominant visceral fat accumulation showed higher cardiac adipose tissue mass than subjects with predominant peripheral fat distribution, however, there were no statistically significant differences. There was a significant difference in BMI

among subjects with predominant visceral fat accumulation and subjects with peripheral fat. No significant differences in age occurred.

	All (n = 31)	Visceral fat accumulation (n = 24)	Peripheral fat accumulation (n = 7)	p
Age, years	62 ± 8	62 ± 8	63 ± 9	NS
BMI, kg/m^2	32 ± 4	33.6 ± 3.4	26.0 ± 1.5	< .001
Epicardial fat mass, g	257 ± 54	261 ± 61	240 ± 22	NS
Pericardial fat mass, g	250 ± 98	251 ± 105	243 ± 80	NS
Intrathoracic fat mass, g	507 ± 136	513 ± 150	483 ± 74	NS

Table 6. Cardiac adipose tissue in different conditions of fat tissue distribution. Data is presented as mean ± SD. Statistical analysis was performed using the medians of values (Mann-Whitney test). NS – not significant.

Cardiac fat and degree of obesity

The bar chart below illustrates the association between cardiac adipose tissue mass and degree of obesity. The subjects were divided into 3 groups according to their BMI values: lean (BMI < 24.9), overweight (BMI between 25.0 and 29.9) and obese (BMI > 30.0). Further statistical test revealed that no significant correlation between these groups was evident (epicardial fat p = .842, intrathoracic fat p = .950, total thoracic fat p = .830).

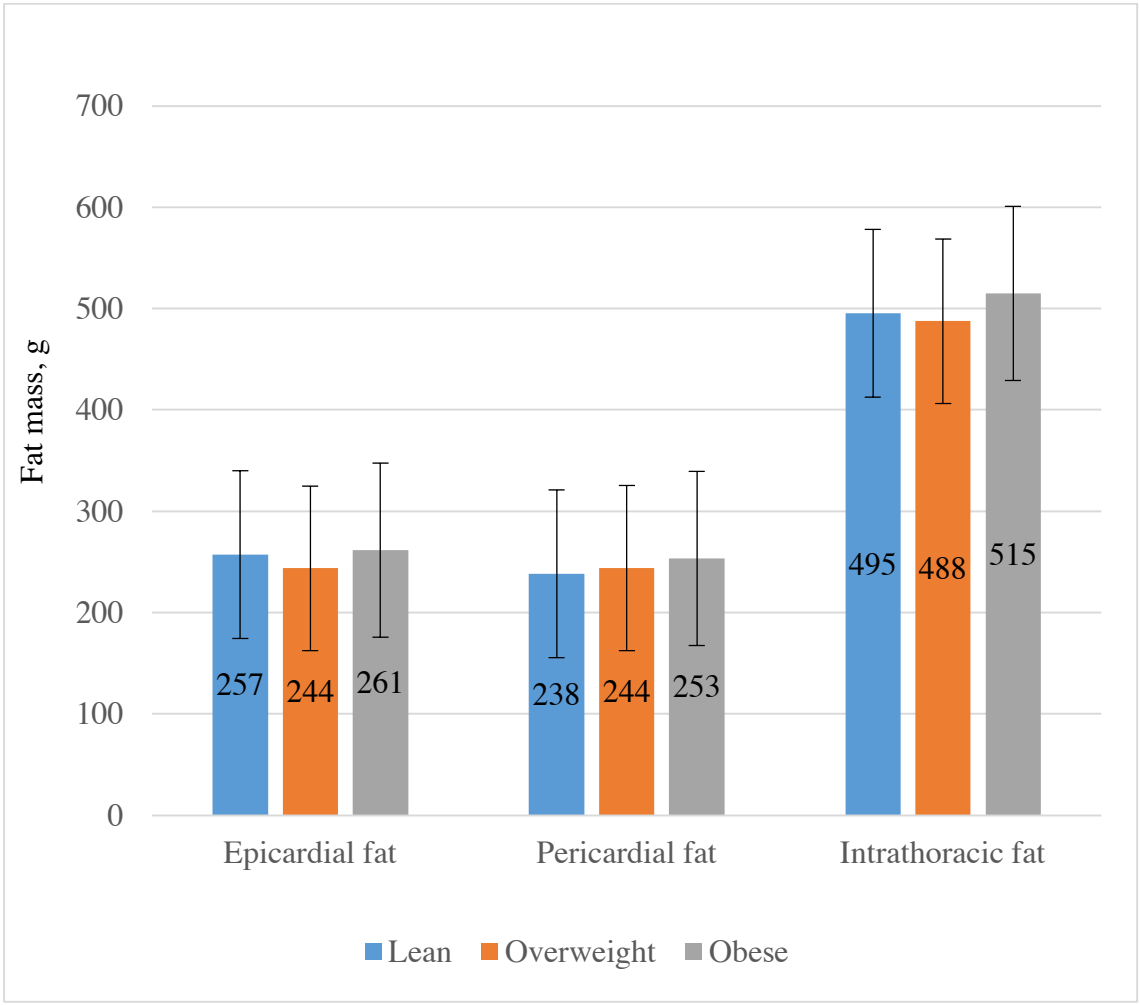


Figure 14. Association between cardiac fat depots and degree of obesity

Cardiac fat and arterial blood pressure

It can be seen from the data in Table 7 that no evidence was found for associations between cardiac fat depots and arterial blood pressure.

		BP systolic (mm Hg)	BP diastolic (mm Hg)
Epicardial fat mass	Correlation Coefficient	-.253	-.079
	Sig. (2-tailed)	.169	.672
	N	31	31
Pericardial fat mass	Correlation Coefficient	.077	-.027
	Sig. (2-tailed)	.680	.885
	N	31	31
Intrathoracic fat mass	Correlation Coefficient	-.001	-.012
	Sig. (2-tailed)	.997	.948
	N	31	31

Table 7. Correlations between cardiac fat depots and arterial blood pressure.
** Correlation is significant at the 0.05 level (2-tailed).*
*** Correlation is significant at the 0.01 level (2-tailed).*

Cardiac fat and smoking

As shown in Fig. 15, smoking subjects reported a larger mass of all cardiac fat depots. However, no statistical evidence was found for non-linear associations between smoking and non-smoking subjects (epicardial fat 250 and 289 g, p = .629; pericardial fat 240 and 301 g, p = .307; intrathoracic fat 491 and 590 g, p = .452, respectively).

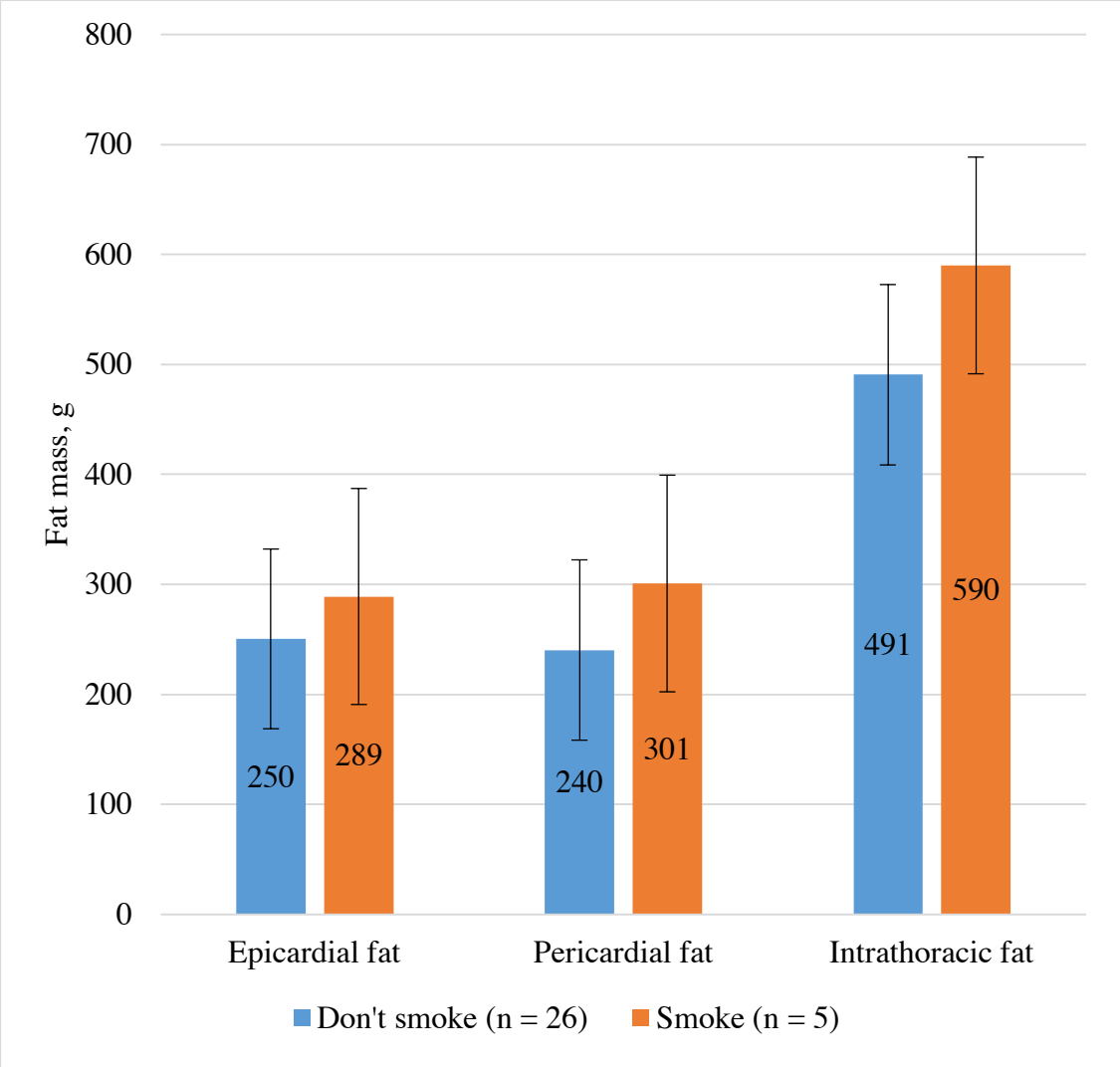


Figure 15. Association between cardiac fat depots and smoking

Cardiac fat and alcohol consumption

The analysis of relationship between cardiac fat and alcohol consumption showed that there is no significant difference between two groups (Fig. 16).

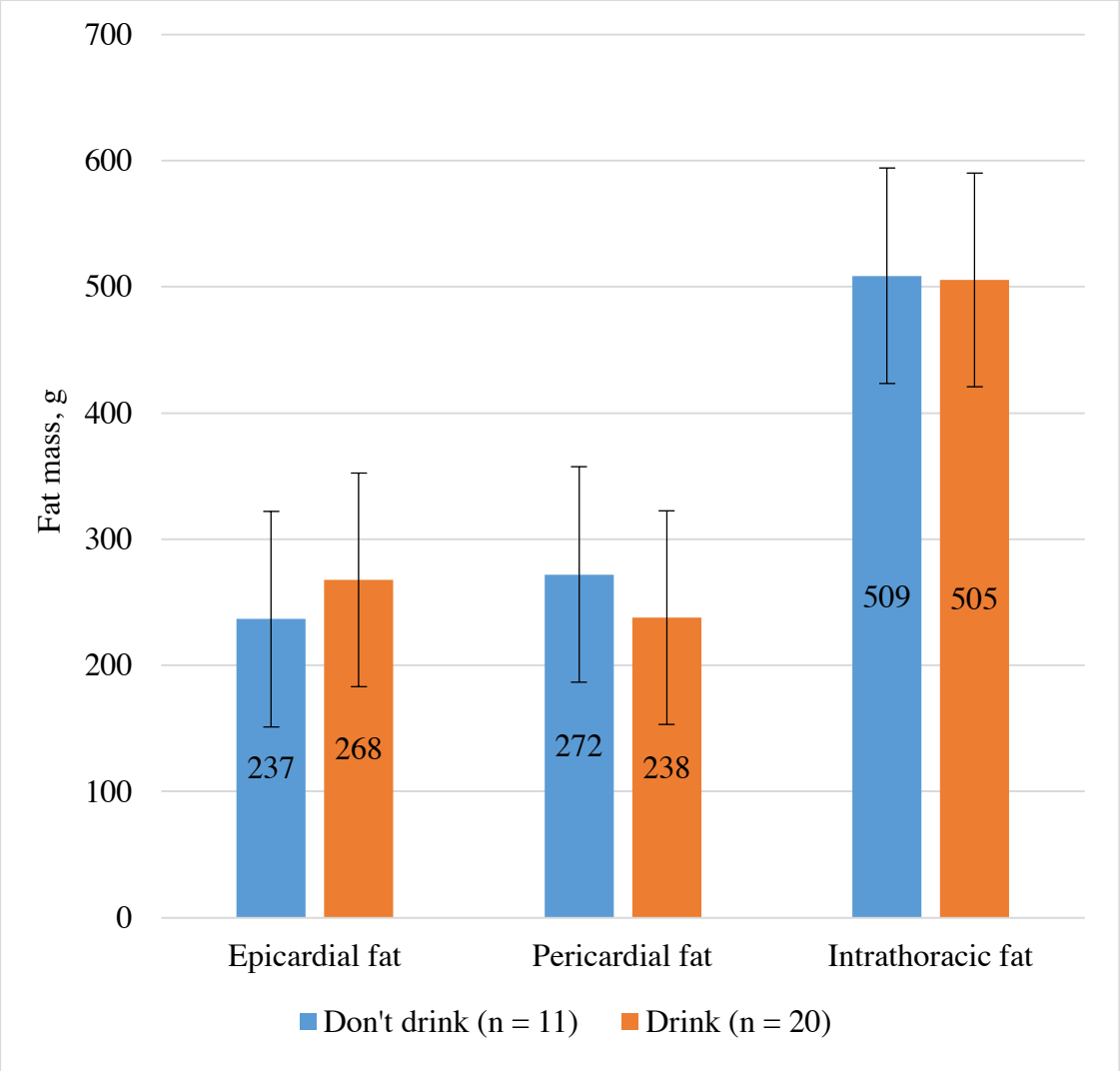


Figure 16. Association between cardiac fat depots and alcohol consumption

A Spearman's rank-order correlation was run to assess the relationship between the cardiac fat measurements and a range of anthropometric and clinical characteristics of study subjects (Table 8).

There was no significant correlation regarding weight, BMI, BP and heart rate, diabetes duration, different laboratory measurements, measurements from the clamp and the results obtained from HOMA. There was a positive correlation between epicardial fat mass and waist circumference, which was statistically significant, $r_s = .398$, $p = .027$. Also, there was a strong positive correlation between epicardial and total thoracic fat mass, which was statistically significant, $r_s = .663$, $p < .001$, as well as between epicardial and intrathoracic fat mass, $r_s = .444$, $p = .012$, and between intrathoracic and thoracic fat

mass, $r_s = .950$ $p < .001$. In addition, a positive correlation was present between thoracic fat mass and waist circumference, which was statistically significant, $r_s = .378$, $p = .036$.

		Epicardial fat volume	Intrathoracic fat volume	Total thoracic fat volume	Waist, cm
Epicardial fat mass	Correlation Coefficient	1.000	.444*	.663**	.398*
	Sig. (2-tailed)	.	.012	.000	.027
	N	31	31	31	31
Intrathoracic fat mass	Correlation Coefficient	.444*	1.000	.950**	.306
	Sig. (2-tailed)	.012	.	.000	.095
	N	31	31	31	31
Total thoracic fat mass	Correlation Coefficient	.663**	.950**	1.000	.378*
	Sig. (2-tailed)	.000	.000	.	.036
	N	31	31	31	31
Waist, cm	Correlation Coefficient	.398*	.306	.378*	1.000
	Sig. (2-tailed)	.027	.095	.036	.
	N	31	31	31	31

Table 8. Relationships between cardiac fat depots and waist circumference
**.Correlation is significant at the 0.05 level (2-tailed*
***. Correlation is significant at the 0.01 level (2-tailed).*

6 DISCUSSION

The present cross-sectional study was designed to study the association between cardiac adipose tissue distribution and metabolic profile of 31 patients with type 2 diabetes mellitus.

The investigation has demonstrated that epicardial and pericardial adipose tissue masses are different between genders. Men tend to have larger amount of cardiac fat than women. These findings complement those of earlier studies. (Iozzo et al., 2009; Alexopoulos et al., 2010). However, we did not found the increase in cardiac fat with aging as shown before.

Many studies have shown the association between cardiac adiposity and metabolic syndrome components (Sironi et al., 2012; Rosito et al., 2008; Iacobellis et al., 2005; Sacks and Fain, 2007). Abdominal obesity is considered as the predominant cause of metabolic syndrome (National Cholesterol Education Program, 2002). Although, in the present study there was a significant difference in BMI among subjects with predominant visceral fat accumulation and subjects with peripheral fat, the study conducted by Iacobellis (2003) provided important suggestion about epicardial adipose tissue as a sign of visceral obesity independent of BMI. The present study showed that subjects with predominant visceral fat accumulation (waist circumference > 102 cm in men and > 88 cm in women) showed higher cardiac adipose tissue mass than subjects with predominant peripheral fat distribution (waist circumference < 102 cm in men and < 88 cm in women), however there were no statistically significant differences. There was a definite positive correlation between epicardial adipose tissue mass and waist circumference, as well as between intrathoracic fat mass and waist circumference. The association between cardiac adipose tissue mass and degree of obesity according to BMI showed no difference between subject groups.

Hypertension, being a component of metabolic syndrome, has shown a correlation with excessive amount of epicardial adipose tissue (Iacobellis et al., 2003). This study did not find significant associations between cardiac fat depots and arterial blood pressure. A possible explanation can be that most of the subjects were using antihypertensive medications which led to inconclusive results.

In addition, such cardiovascular risk factors as smoking and alcohol consumption, were tested as possible predictors of heart adiposity. The analysis of relationship between cardiac fat depot mass and smoking, as well as between cardiac fat and alcohol consumption showed no significant difference.

There were few limitations in the study. As it is well known, metabolic syndrome is a group of risk factors leading to CVDs and diabetes (Reaven, 1988). The study group was consisted of patients with pre-existing type 2 diabetes mellitus admitted for the treatment. Thus, it was impossible to see the real picture of metabolic syndrome. Also, the number of participants was not adequate enough to see the associations with metabolic components. These results therefore need to be interpreted with caution. Secondly, the image quality was not optimal due to poor quality of MRI scans. Whole body MRI scans did not aim to assess cardiac depots. During the image analysis, I had to put together 2 slices in order to get a complete heart image. This could lead to inadequate quantification of VOI voxels. Moreover, since ROIs were drawn by hand there was a significant risk of manual errors in quantification. Finally, there was lack of visceral fat data and lipid profile. Research has shown that visceral fat is metabolically more active than subcutaneous fat and is significantly correlated with cardiovascular risk factors and the metabolic syndrome (Wajchenburg, 2014). As it was previously mentioned, visceral adipose tissue has the highest lipolytic activity which leads to the increased free fatty acid flux to the liver. Excessive levels of FFA lead to failure of ß-cells and the development of T2DM (Wajchenburg, 2014). Thus, there is a distinct relationship between the amount of visceral fat and insulin resistance. Due to absent information about lipid profile of the subjects, this study was unable to investigate the association between such metabolic components as triglycerides and HDL cholesterol levels and cardiac fat depot volumes.

In conclusion, study results showed that cardiac fat mass measurement by MRI can provide important information about the metabolic and cardiovascular risk, especially in those people who have normal BMI and/or waist circumference values. However, these findings do not suggest that epicardial and pericardial adipose tissue can be used as an indicator for metabolic syndrome. It would be interesting to investigate the association between cardiac fat depots and the amount of visceral adipose tissue along with lipid profile. Future research should therefore concentrate on the investigation of prognostic significance of cardiac adipose tissue and shed light on intrinsic pathophysiological mechanisms underlying the relationship between cardiac fat and metabolic syndrome.

7 ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor Pirjo Nuutila for providing me with an opportunity to work with her. She helped me come up with the thesis topic, and has been a guiding force throughout the process of doing the analysis as well as writing of this manuscript. This work would have been so much worse if it hadn't been for her input and guidance every step of the way.

My second supervisor Jarna Hannukainen has been extremely helpful while I was working in Turku PET center. She was always there to help me in every way possible despite her busy schedule and for that I'm truly grateful. Most of all, I feel honored to work with both of my supervisors because of their excellent research and tremendous work ethic, something that I hope I can incorporate in my life as well.

I thank the entire PET Centre and BIMA staff for giving me the opportunity to come and work with some of the most amazing people I've met in my life. It has widened my horizon and has set a path which will definitely lead me to my eventual goals.

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8 REFERENCES

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