Plasminogen Activator Inhibitor-1 and Pericardial Fat in Individuals With Type 2 Diabetes Mellitus

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Abstract

Background

Plasminogen activator inhibitor-1 (PAI-1) is implicated in the pathophysiology of cardiovascular disease (CVD) and is increased in individuals with type 2 diabetes mellitus (T2DM). Adipose tissue produces PAI-1, and pericardial fat is a CVD risk factor. We sought to determine the relationship between PAI-1 and pericardial fat in males and females with well-controlled T2DM.

Methods

The study population consisted of 32 males and 19 females, ages 35-70 years with T2DM, without clinical evidence of CVD or other active medical problems except for hypertension. Subjects were studied under good cardiometabolic control. Study procedures included fasting blood work and cardiovascular imaging. Cardiac magnetic resonance imaging of the heart was used to identify and quantify pericardial fat from the bifurcation of the pulmonary trunk to the last slice containing cardiac tissue.

Results

PAI-1 was positively correlated with pericardial fat ($\beta = 0.72$, $r = 0.72$, $p < 0.001$) as well as with homeostatic model assessment of insulin resistance (HOMA-IR) ($r = 0.31$, $p = 0.03$) and serum triglycerides ($r = 0.27$, $p = 0.05$). In a multivariable regression model, controlling for insulin sensitivity, triglycerides and body mass index, pericardial fat was independently associated with PAI-1 ($\beta = 0.80$, $p < 0.001$).

Conclusions

PAI-1 is positively associated with pericardial fat in individuals with T2DM.

Introduction

Type 2 diabetes mellitus (T2DM) is associated with increased risk of cardiovascular disease (CVD) (1). Individuals with T2DM, as compared to age-matched controls, have elevated levels of plasminogen activator inhibitor-1 (PAI-1), which appears to be implicated in the pathophysiology of CVD (2, 3). PAI-1 is a serine proteinase inhibitor (4) and is the main physiological inhibitor of the endogenous fibrinolytic system primarily through inhibition of tissue plasminogen activator (5). Circulating PAI-1 concentrations are positively associated with incident cardiovascular events in at-risk individuals (6). Further, in individuals with no prior history of CVD, PAI-1 is a predictor of CVD independent of conventional risk factors (7).

PAI-1 is produced by multiple tissues, including vascular endothelium and adipose tissue (5). Visceral adipose tissue is metabolically more active than subcutaneous adipose tissue (8) and produces more PAI-1 (9). Pericardial fat, which includes epicardial and paracardial fat, is an independent predictor of CVD (10) and is associated with calcified coronary plaque (11). One study showed an association between epicardial fat, a form of visceral adipose tissue (12), and PAI-1 in 27 females with morbid obesity without diabetes (13). To our knowledge, the association between PAI-1 and pericardial fat, or its components, has not been studied in males and females with T2DM.

The main purpose of this study was to determine the relationship between the two cardiovascular risk factors – PAI-1 and pericardial fat – in individuals with T2DM and no prior history of CVD. In addition, we evaluated the relationship between PAI-1 and additional cardiometabolic risk factors in T2DM, including body mass index (BMI), lipids, blood pressure, insulin sensitivity measures and two early CVD markers, coronary flow

reserve (CFR) and myocardial extracellular volume (ECV), a marker of extracellular matrix expansion. Reductions of CFR and increases in ECV are known to be associated with increased cardiovascular morbidity and mortality in individuals with T2DM (14, 15).

Methods

Study Population

This is a post-hoc analysis of baseline data obtained prior to drug randomization from a previously published study (ClinicalTrials.gov identifier: NCT00865124) (16). The study included males and females with T2DM without clinical evidence of coronary artery disease or other active medical problems except for hypertension. Exclusion criteria were clinical evidence of coronary, cerebrovascular, or peripheral vascular disease; asthma; estimated glomerular filtration rate <60 ml/min/1.73m^2; cigarette smoking; pregnancy; illicit drug use; contraindications to cardiac magnetic resonance or adenosine; uncontrolled hypertension (systolic blood pressure >160 mm Hg); and active major medical illness. Fifty-one participants (32 males and 19 females) with T2DM had PAI-1 levels and pericardial fat assessed at baseline and were included in the primary analysis. Partners HealthCare Institutional Review Board approved the protocol, and all participants provided written informed consent.

Study Procedures

Prior to the baseline assessment, all participants completed a 3-month run-in period to establish good metabolic control. Each participant was placed on enalapril 20 mg daily with tapering of all other anti-hypertensive agents except amlodipine, which was added if systolic blood pressure ≥ 140 mm Hg; was started on simvastatin if low-density lipoprotein was > 100 mg/dL; and had anti-diabetic medications adjusted to target a glycosylated hemoglobin (HbA1c) level ≤ 7%, as previously described (16). Study procedures were conducted after admitting the study participants to the inpatient Center for Clinical Investigation at Brigham and Women's Hospital.

Assessment Protocol

Four days prior to and during the 2-day in-patient admission, participants consumed a caffeine-free, isocaloric diet (250 mmol/day of sodium, 100 mmol/day of potassium, 1,000 mg/day of calcium, 300 mg/day of magnesium, and at least 30% carbohydrate by calories). During the diet, doses of anti-diabetic medications were reduced as needed to avoid hypoglycemia. After an overnight fast, blood samples were collected and supine systolic and diastolic blood pressures were measured every 5 minutes for 30 minutes by automated blood pressure cuff (GE Healthcare, Marlborough, MA), and the average blood pressures (systolic and diastolic, respectively) were used for analysis. Subjects underwent cardiac positron emission tomography (PET) imaging for determination of CFR (ratio of adenosine-stimulated to rest myocardial blood flow corrected for the baseline pulse-pressure product), and cardiac magnetic resonance imaging (MRI) for determination of myocardial ECV allowing us to quantify extracellular matrix expansion, as previously described (16). Cardiac MRI was also used to determine pericardial fat (see below).

Serum PAI-1 was analyzed using an ELISA assay (R&D Systems, Minneapolis, MN; intra-assay variation 5.8-7.4%, inter-assay variation 5.6-7.2%). Insulin was measured using an Access Chemiluminescent Immunoassay (Beckman Coulter, Fullerton, CA; intra-assay variation 2.0-4.2%, inter-assay variation 3.1-5.6%). Blood levels of electrolytes, HbA1c and lipids were assessed using routine clinical assays from the Laboratory Corporation of America (Raritan, NJ).

Pericardial Adipose Tissue Quantification

Cardiac MRI was performed using a 3-T magnet (Magnetom Tim Trio or Verio; Siemens Healthcare, Erlangen, Germany) with an 8-element phased-array coil. Axial T1 turbo spin echo images were used for pericardial adipose tissue quantification. QMass MR Version 7.6 software (Medis Medical Imaging Systems, Raleigh, NC) was used to trace and quantify adipose tissue on a dedicated offline workstation. Axial slices (8 mm thickness) were analyzed from the bifurcation of the pulmonary trunk (superior border) to the last slice containing cardiac tissue (inferior border). The anterior and posterior borders of analysis were the anterior edge of the thoracic cavity and the posterior segment of the descending aorta and periaortic fat, respectively (see Figure 1). We traced an outer border containing all pericardial adipose tissue (consisting of both epicardial and paracardial adipose tissues) and an inner border within the myocardium to exclude non-adipose tissue. Periaortic fat is contiguous with pericardial fat at multiple axial levels of the heart. Accordingly, periaortic fat was included across all slices in the analysis for consistency. Near the inferior border of analysis, abdominal structures and the diaphragm become apparent. Thus, to assure the tissue analyzed was cardiac in nature, sagittal cross-section images were used simultaneously with axial images to identify the tissue in two planes and accurately trace it. A reference region was drawn within posterior skeletal muscle for each imaging slice. Pericardial adipose tissue was defined as tissue within the two borders traced with pixel intensity more than 4 standard deviations above the mean pixel intensity for the reference posterior skeletal muscle.

The QMass MR Version 7.6 software quantified pericardial adipose tissue across all analyzed slices for a given participant and output a total volume. Intra-reader and inter-reader reproducibility assessed on a random sample of 20% of participants were excellent: intraclass correlation coefficients were 0.99 and 0.98 for intra-reader and inter-reader analyses, respectively.

Statistical Methods

Normally distributed data are expressed as mean ± standard deviation. Data not normally distributed are expressed as median [interquartile range]. Our primary goal was to test the association between PAI-1 levels and pericardial fat volume. Secondary analyses included the relationships between PAI-1 and biochemical markers of diabetes as well as CFR and ECV. Pearson's correlations were used to assess relationships between PAI-1 and CVD risk factors. Power calculations were performed for the parent study (16). For this post-hoc analysis, a sample size of 51 provides at least 80% power with 5% alpha for a Pearson correlation coefficient of 0.40 or greater. Data not normally distributed were log-transformed for Pearson's correlations. Below and above-median comparisons for demographic and other variables were performed using Wilcoxon signed-rank tests. Categorical data analyses were performed using Fisher's exact test. Given pericardial fat was our main exposure, we constructed multivariable regression models to assess the association between PAI-1 and pericardial fat while adjusting for other factors (age, sex, BMI and any other variable with a $p \leq 0.05$ on univariate correlation with PAI-1). A value of $p \leq 0.05$ was deemed statistically significant. Data more than four standard deviations from the mean of all participants were removed for

analysis. All statistical analyses were performed with SPSS version 24 (IBM Corporation, Armonk, NY).

Results

Subject Characteristics

We studied 51 individuals (19 females and 32 males) ages 54.5 ± 7.5 years and BMI 32.2 ± 4.8 kg/m^2, with no differences in age and BMI between sexes. Mean pericardial fat volume was significantly higher in males versus females (345.6 ± 136.7 vs. 262.1 ± 86.9 cm^3, $p = 0.02$) and in Caucasian participants versus non-Caucasian participants (358.1 ± 124.7 vs. 241 ± 92.1 cm^3, $p < 0.001$).

The baseline characteristics of the 51 individuals are stratified by below and above-median pericardial fat and are displayed in Table 1. Individuals with below-median pericardial fat had significantly higher HbA1c values than individuals with above-median pericardial fat ($p = 0.04$, see Table 1). However, the use of anti-diabetic medications, including use of insulin and/or insulin secretagogues did not differ between below and above-median pericardial fat categories. The most common anti-diabetic medications used in this cohort were metformin (84.3% of participants), sulfonylureas (31.4%), and insulin (17.6%). All other classes of anti-diabetic medications were used in less than 10% of the cohort and were in combination with a class of anti-diabetic medication listed above.

PAI-1, Pericardial Fat and Cardiometabolic Risk Factors

PAI-1 levels were similar between males and females (4.4 ± 2.5 vs. 3.7 ± 1.7 ng/mL, $p = 0.32$), and between Caucasians and non-Caucasians (4.5 ± 2.4 vs. 3.6 ± 1.8 ng/mL, $p = 0.16$). Subjects with above-median pericardial fat had significantly higher PAI-1 levels ($p < 0.001$) and lifetime atherosclerotic cardiovascular disease (ASCVD) risk scores ($p < 0.01$) as compared to those with below-median pericardial fat. However, there were no differences between below and above-median pericardial fat in other

cardiovascular risk factors including blood pressure, lipid levels, C-reactive protein (CRP), HOMA-IR, CFR or myocardial ECV. Additionally, there was no difference between below and above-median pericardial fat with amlodipine or statin medication use.

PAI-1 was positively correlated with pericardial fat ($\beta = 0.72$, $r = 0.72$, $p < 0.001$) (Figure 2), homeostatic model assessment of insulin resistance (HOMA-IR) ($r = 0.31$, $p = 0.03$) and triglycerides ($r = 0.27$, $p = 0.05$). The correlation between PAI-1 and BMI was not statistically significant ($r = 0.25$, $p = 0.08$). Additionally, PAI-1 did not correlate with CFR and myocardial ECV ($r = 0.25$, $p = 0.08$; $r = 0.02$, $p = 0.91$, respectively), ASCVD, or the other factors, including CRP, displayed in Table 2.

In a multivariable regression model including age, sex, BMI, HOMA-IR and triglycerides, pericardial fat was independently associated with PAI-1 ($\beta = 0.80$, $p < 0.001$) (displayed in Table 3). The removal of five participants without clinical evidence of CVD, but with evidence of coronary artery disease on imaging analysis did not affect the significance of the results.

To assess whether anti-diabetic medications had an impact on PAI-1 levels in our participants, we performed an exploratory analysis focused on the most frequently used anti-diabetic medications in the study. PAI-1 levels were similar in those on and off metformin (4.2 ± 2.3 vs. 3.8 ± 1.7 ng/mL; $p = 0.68$), on and off sulfonylureas (4.8 ± 2.9 vs. 3.8 ± 1.7 ng/mL; $p = 0.13$), and on and off insulin (3.2 ± 0.9 vs. 4.3 ± 2.4 ng/mL; $p = 0.19$).

Discussion

To our knowledge, this study is the first to investigate the association between PAI-1 and pericardial fat in individuals with diabetes. We demonstrate that PAI-1 levels positively correlate with pericardial fat volume in males and females with T2DM, no prior history of CVD and good cardiometabolic control. The association between PAI-1 and pericardial fat was independent of other factors known to be associated with PAI-1 (e.g. BMI, triglycerides and insulin sensitivity).

Despite ongoing efforts to improve control of glycemia, blood pressure and lipids, individuals with T2DM have increased risk of CVD (17). PAI-1 is a cardiovascular risk factor and is thought to have a role in thrombotic vascular disease (18). Increased PAI-1 gene expression has been observed in atherosclerotic plaque (19). Relative to individuals without diabetes, individuals with T2DM have increased PAI-1 in comparably obstructive excised segments of diseased coronary arteries (20). Further, PAI-1 levels are increased in diabetes (21). Consistent with this finding, PAI-1 levels were elevated in our study population compared with published values of PAI-1 in healthy subjects (~1.5 ng/mL) assessed at a similar time of day (22).

Prior studies have shown that PAI-1 levels are positively associated with BMI (23), triglycerides (24), and measures of insulin sensitivity (2). We found similar associations in our cohort of individuals with T2DM. However, these associations were no longer apparent when pericardial fat was included in the multivariable regression model. This raises the possibility that pericardial fat may have unique characteristics. Pericardial fat is composed of epicardial fat (fat present between the myoepicardium and the visceral pericardium) and paracardial fat (fat situated externally to the parietal pericardium) (25). Epicardial fat is supplied by branches of the coronary arteries and shares a

microcirculation with the myocardium. Thus, it has been suggested that there may be direct influence of epicardial fat on coronary vasculature and myocardium, perhaps through paracrine or vasocrine secretion of bioactive molecules (26). Indeed, epicardial fat is associated with fatal and nonfatal coronary events in the general population independent of traditional cardiovascular risk factors (27).

When primary adult rat cardiomyocytes are incubated in conditioned media generated from explants of epicardial adipose tissue biopsies obtained from humans with T2DM, contractile function is markedly impaired and insulin-stimulated signaling is significantly blunted (28). The authors found that the secretory profile (PAI-1 was not assessed) of epicardial fat was considerably different from paracardial fat, and that the secretion patterns differed between individuals with and without diabetes. Additionally, they showed that the increased secretory profile from epicardial fat in individuals with T2DM (compared to individuals without diabetes) contributed to cardiomyocyte dysfunction in rat cells. In total, the above evidence suggests that diabetes-specific secretory changes from epicardial fat could contribute to the pathogenesis of T2DM-related cardiovascular dysfunction.

In the current study, CFR and ECV did not significantly correlate with PAI-1 on univariate analysis. CFR is reduced in individuals with diabetes versus non-diabetic controls (29). CFR in our study population was consistent with previously published values for individuals with T2DM (30). Myocardial ECV for our study population was elevated compared with published values of myocardial ECV in healthy subjects (31), consistent with studies showing increased myocardial ECV in individuals with diabetes versus non-diabetic controls (15). The lack of significant correlation between PAI-1 and

CFR and ECV may suggest that these factors, all of which are affected in diabetes, may be regulated through distinct mechanisms.

Our study has some important limitations to address. The study was cross-sectional and does not demonstrate cause and effect. The number of participants studied may not allow us to determine whether specific anti-diabetic medications affect PAI-1 levels. The sensitivity of our imaging was not sufficient to reliably identify the pericardial lining with each axial slice, likely due to the potential temporal motion artifact inherent to the cardiovascular MRI technique (12). Consequently, we quantified pericardial adipose tissue in total and did not separate the volume into its components of epicardial and paracardial adipose tissues. We did not assess abdominal visceral adipose tissue, which is known to significantly correlate with PAI-1 (32), so we cannot determine the relative importance of pericardial and abdominal visceral fat in predicting PAI-1 levels. The one study examining the relationship between epicardial fat and inflammatory markers reported – in 27 females with morbid obesity but not diabetes – that epicardial fat thickness correlated with PAI-1 independent of abdominal visceral adipose tissue (13). This suggests a unique relationship between epicardial fat and PAI-1 levels.

Our study has multiple advantages. MRI technique is non-invasive, without radiation, and able to calculate a total fat volume. We performed our assessments in participants under good cardiometabolic control in our Center for Clinical Investigation in order to control for and accurately assess factors that have been associated with PAI-1 such as BMI (23), triglycerides (24), aldosterone (33), glycemia (2), circadian rhythm (22), and blood pressure (34). Given our control of cardiometabolic factors, our population may not be representative of the average population with T2DM, and this

may explain why our PAI-1 levels, although elevated compared to healthy controls, were not as high as some reports of individuals with T2DM (21).

Conclusion

Our study suggests that in individuals with well-controlled diabetes, PAI-1 is positively associated with pericardial fat. Future studies are needed to determine whether, in T2DM, pericardial fat affects the coronary arterial system, whether the effects of its components (epicardial and paracardial fat) differ, and whether these effects impact cardiac function in humans with T2DM.

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Table 1. Study Sample Characteristics* Stratification by Pericardial Fat	≤ Median Pericardial Fat (n = 26)	> Median Pericardial Fat (n = 25)
Age (years)	54 ± 8.3	55 ± 6.7
Sex		
Male (n, %total)	13, 25.5%	19, 37.2%
Female (n, %total)	13, 25.5%	6, 11.8%
Body Mass Index (kg/m^2)	31.4 ± 4	32.9 ± 5.4
Race ‡		
Non-Caucasian (n, %total)	12, 23.5%	5, 9.8%
Caucasian (n, %total)	14, 27.5%	20, 39.2%
Pericardial Fat (cm^3)	218.6 ± 51.4	414.2 ± 100.9 ‡
Plasminogen Activator Inhibitor-1 (ng/mL)	2.8 [1.1]	5.4 [3.3] ‡
C-Reactive Protein (mg/L)	2 [2.4]	1.9 [2]
Duration of Diabetes (years)	7.5 [12.3]	6 [8]
Diabetic Medications		
Diet-Controlled (n, % total)	2, 3.9%	3, 5.9%
Monotherapy (n, % total)	11, 21.6%	9, 17.6%
≥ Two Medications (n, % total)	13, 25.5%	13, 25.5%
Insulin Use (n, % total)	7, 13.7%	2, 3.9%
Hemoglobin A1c (%)	7 [1.1]	6.6 [1.1] †
HOMA-IR [(Glucose (mg/dL) x Insulin (μIU/mL)/405)]	3 ± 2.3	2.7 ± 1.8
Blood Pressure		
Systolic Blood Pressure (mm Hg)	123.5 ± 12.1	126.2 ± 14.2
Diastolic Blood Pressure (mm Hg)	72.2 [11.7]	74.3 [8.7]
Amlodipine Use (n, % total)	8, 15.7%	9, 17.6%
Lipids		
Total Cholesterol (mg/dL)	142.5 [34]	149 [35.5]
Triglycerides (mg/dL)	102.5 [86]	110 [54.5]
High-Density Lipoprotein (mg/dL)	43.5 ± 10.1	42.3 ± 14.5
Low-Density Lipoprotein (mg/dL)	79 ± 29.2	80 ± 23
Statin Use (n, % total)	20, 39.2%	20, 39.2%
24-Hour Urine Creatinine (mg/total volume)	1435.5 ± 446	1667 ± 337.6 †
24-Hour Urine Sodium (mEq/total volume)	272.5 [98]	275 [85.3]
24-Hour Urine Aldosterone (μg/total volume)	4 [6.8]	7.5 [8]
Aldosterone – baseline (ng/dL)	2.5 [0.7]	2.5 [1.6]
Aldosterone – stimulated (ng/dL)	8.3 [6]	9.7 [3.9]
Myocardial Extracellular Volume	0.4 ± 0.1	0.4 ± 0.1
Coronary Flow Reserve	2.32 [0.8]	2.3 [1]
Atherosclerotic Cardiovascular Disease 10-year Risk Score (%)	7 [7]	11.1 [10.9]
Atherosclerotic Cardiovascular Disease Lifetime Risk Score (%)	50 [19]	69 [9.5] ‡

*Data are presented as mean ± standard deviation or as median [interquartile range]
† $p \leq 0.05$ between groups; ‡ $p \leq 0.01$ between groups
HOMA-IR, homeostatic model assessment of insulin resistance

Table 2. Pearson's Correlations with PAI-1*

Variable	r	p	n
Age	0.10	0.50	51
Body Mass Index	0.25	0.08	51
Pericardial Fat ‡	0.72	<0.001	51
C-Reactive Protein*	0.00	0.98	50
Duration of Diabetes*	0.10	0.49	51
Hemoglobin A1c*	0.03	0.86	50
HOMA-IR [(Glucose x Insulin/405)]*†	0.31	0.03	51
Systolic Blood Pressure	0.22	0.12	51
Diastolic Blood Pressure *	0.05	0.74	51
Triglycerides*†	0.27	0.05	51
Cholesterol*	0.09	0.55	51
High-Density Lipoprotein*	0.16	0.25	51
24-Hour Urine Creatinine	0.17	0.22	51
24-Hour Urine Sodium	0.07	0.62	51
24-Hour Urine Aldosterone*	0.25	0.07	51
Aldosterone baseline*	0.10	0.49	51
Angiotensin II-Stimulated Aldosterone*	0.01	0.95	44
Myocardial Extracellular Volume	0.02	0.91	51
Coronary Flow Reserve*	0.25	0.08	50
ASCVD 10-Year Risk*	0.16	0.26	51
ASCVD Lifetime Risk*	0.24	0.09	51

*Represents a variable log-transformed for Pearson's correlation
† $p \leq 0.05$
‡ $p \leq 0.01$
HOMA-IR, homeostatic model assessment of insulin resistance
ASCVD, atherosclerotic cardiovascular disease

Table 3. Multivariable Regression Analysis of Predictors of Plasminogen Activator Inhibitor-1

Model	R-squared (r^2)	Effect Estimate†	P value
1[a]	0.52	0.72	<0.001
2[b]	0.57	0.79	<0.001
3[c]	0.58	0.83	<0.001
4[d]	0.59	0.81	<0.001
5[e]	0.60	0.81	<0.001
6[f]	0.60	0.80	<0.001

† Effect estimate (β coefficient) for pericardial fat in each model

[a] Pericardial fat unadjusted

[b] Pericardial fat adjusted for age and sex

[c] Pericardial fat adjusted for age, sex and BMI

[d] Pericardial fat adjusted for age, sex, BMI, and triglycerides

[e] Pericardial fat adjusted for age, sex, BMI, and HOMA-IR

[f] Pericardial fat adjusted for age, sex, BMI, triglycerides, and HOMA-IR

BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance

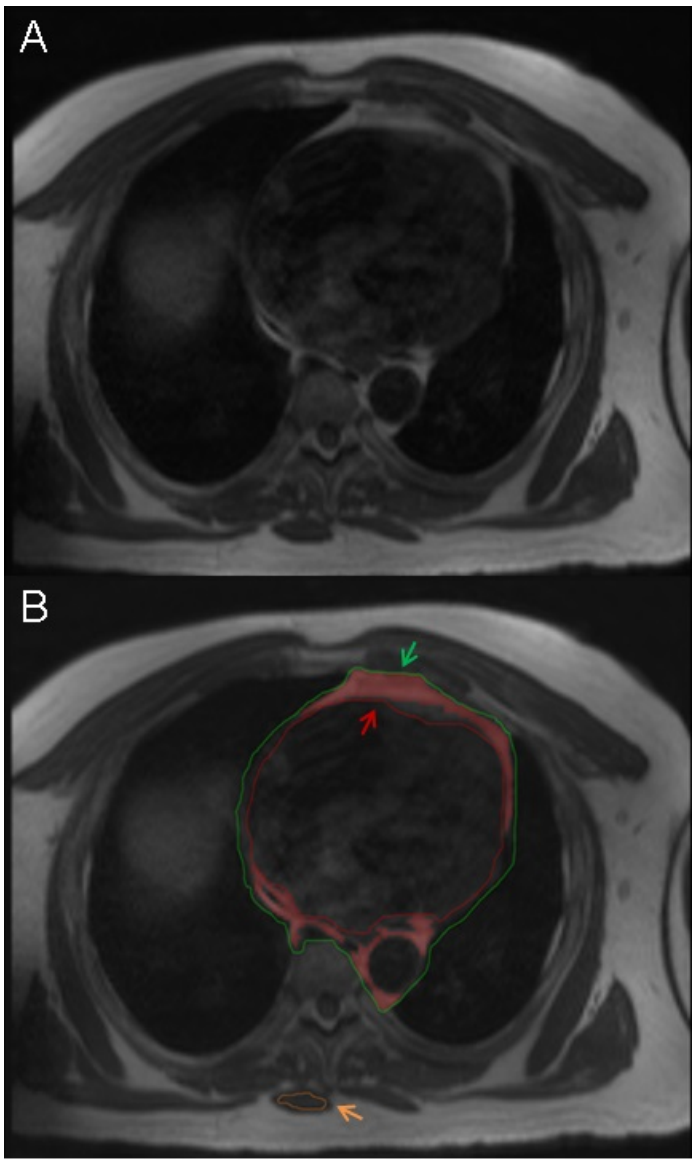

Figure 1. Example of axial T1 turbo spin echo slice.

A) Unmarked axial slice. B) Axial slice after cardiac contours drawn. The pericardial fat is highlighted in red and is located between the green (outer paracardial fat and posterior periaortic fat borders) and red (myoepicardial border) lines as indicated by the green and red arrows. Fat is defined as tissue with pixel intensity more than 4 standard deviations above the mean pixel intensity for the reference posterior skeletal muscle (orange circle and orange arrow).

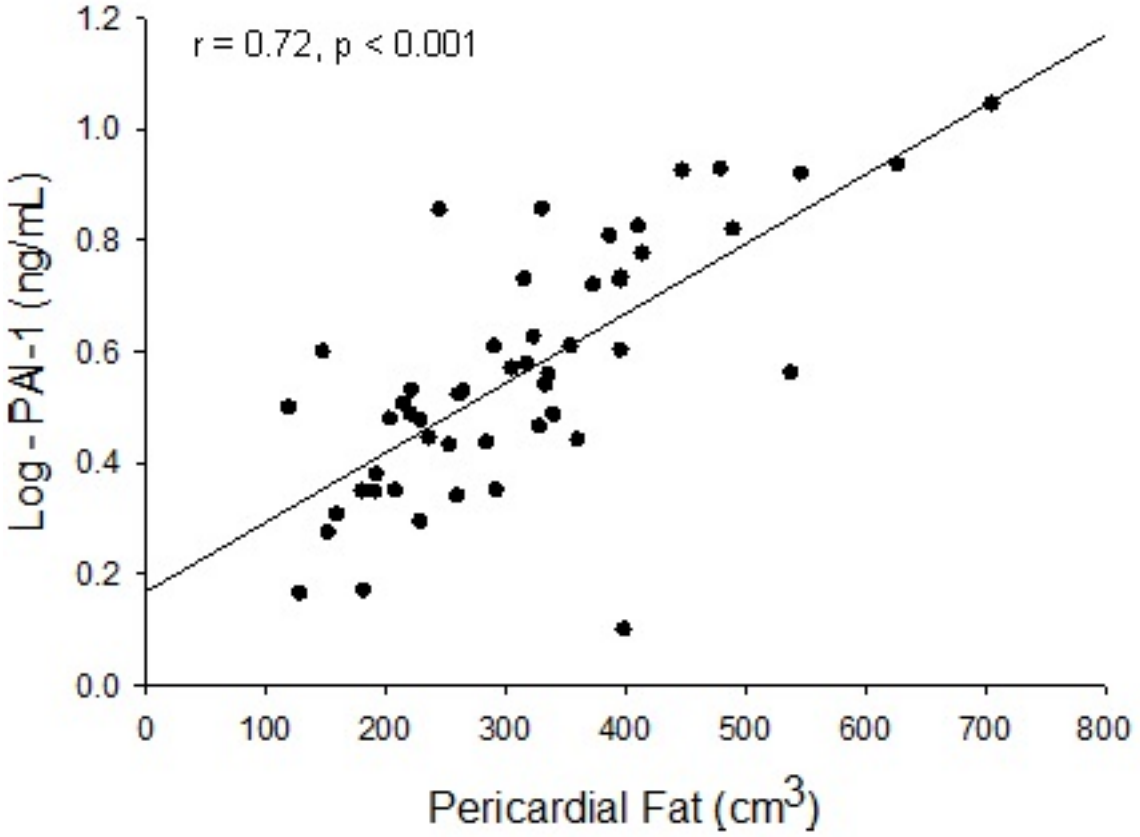

Figure 2. Pericardial fat is positively correlated with plasminogen activator inhibitor-1 (PAI-1); (n = 51; equation of regression line: y = 0.0013x + 0.1675).