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Original Contribution

MORPHOLOGY AND ULTRASONOGRAPHY OF THE PERICARDIAL AND EPICARDIAL ADIPOSE TISSUE IN HEALTHY RABBITS *(Oryctolagus cuniculus)*

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ABSTRACT
Rabbits develop a visceral type obesity and therefore is exceptionally suitable as a model for lipid metabolism investigation. Ten clinically healthy New Zealand White rabbits from both genders, weighing from 3.5 to 3.7 kg were used. The blood plasma concentrations of total cholesterol and triglycerides were 1.45 ± 0.15 mmol/L and 0.63 ± 0.01 mmol/L, respectively. The highest amount of pericardial adipose tissue was observed around the free surface of the right ventricle and the apex of the heart. The average mass of pericardial adipose tissue was 3.72 ± 0.24 g (0.1% of body weight and 47% of heart weight).
By ultrasonography, pericardia adipose tissue was visualized as a moderately echoic homogeneous structure against the hyperechoic myocardium of the right ventricle. Its thickness was 3.2 ± 0.26 mm.
The results of the present study showed that the rabbit was a suitable model for monitoring of quantitative changes in visceral fat depots in mammals, allowing their study in vivo by ultrasonography instead of utilizing dissection.

Key words: rabbits, pericardial adipose tissue, epicardial adipose tissue, ultrasonography

INTRODUCTION

The New Zealand White rabbit belongs to average rabbit breeds (2–5 kg) bred for meat production. It is characterized with a rapid growth rate and is prone to deposition of large amounts of fat. Rabbits are small and relatively inexpensive models for research purposes (1, 2).

The blood lipid profile in rabbits is similar to that in men. In this animal species, a central (visceral) type of obesity is developing, thus making it extremely appropriate for investigations on human lipid metabolism (3).

The reference values of total blood cholesterol in rabbits according to (4) are between 0.14 and 1.86 mmol/L, and those of triglycerides: from 0.90 to 1.55 mmol/L. (3) have established total cholesterol level of 1.99 mmol/L and triglycerides of 0.49 mmol/L in healthy male rabbits, and cholesterol values reported by (5) were between 0.90 and 1.4 mmol/L.

In the thorax, the visceral adipose tissue is localized in the mediastinum and around the heart (6). The pericardial adipose tissue (PAT) is located on the parietal layer of the pericardium (7) covers about 80% of the heart

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and amounts to 20–50% of its weight (8). A relatively abundant PAT is observed in wild animals, guinea pigs, rabbits, domestic mammals and men (9,8). The increased amount of PAT results to both mechanical occlusion of the coronary blood circulation (10), and to increased influx of inflammatory mediators in coronary arteries, provoking the formation of atherosclerotic lesions (11).

Epicardial adipose tissue (EAT) lies on the myocardium and is covered by the visceral pericardium (12). Because of its close connection to the myocardium, (13) consider EAT to be the true visceral adipose depot. It is placed in the atrioventricular and interventricular grooves along the large coronary branches, around the right ventricular free wall and both atria (8,14).

EAT is a metabolically active structure that synthesizes a large number of bioactive molecules influencing the contractile properties of coronary arteries from one part, and a system of protection against the toxic effect of fatty acids on the myocardium and the local blood circulation bed (8,14)

In men, the thickness of epicardial and pericardial fat correlates positively to body weight, the fatty infiltration of the right ventricle and the increased left ventricular mass (10, 15).
(16) have observed many signs of human obesity in female New Zealand White rabbits that, after a 12-week high-fat diet, exhibited higher body weight by 46%, higher left ventricular weight by 52% (heart hypertrophy) and increased blood triglyceride concentrations.

Transthoracic ultrasonography is a non-invasive, inexpensive *in vivo* technique for visualization and measurement of PAT and EAT as parameters of human visceral obesity. The measurement of EAT and PAT thickness is done on the right ventricular free wall, where the absolute thickness of the adipose tissue is the highest, while the parasternal views along the long and short heart axes permit their most precise determination. EAT appears more frequently as a hypoechoic space, but if it massive, is hyperechoic and its dimensions are influenced by the heart cycle. PAT is a hypoechoic area anterior to EAT and its dimensions are not significantly altered throughout the heart cycle (8,7).

The purpose of the present investigation was to describe the topography of pericardial and epicardial adipose tissue in rabbits and to determine in vivo their thickness.

MATERIAL AND METHODS

In this study, ten clinically healthy 4-month-old New Zealand White rabbits from both genders, weighing 3.5–3.7 kg were used. Animals were reared in cages at ambient temperature of 20 ^{o}C, air humidity 65–70% and 12-hour light day. Rabbits received ad libitum pelleted feed (18.3% crude protein, 12.5% crude fibre 1.2% fat) and water.

The experiment was carried out under the strict observance of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, the ***European Convention*** for the ***Protection*** of Pet Animals, and Law on Animal Protection in the Republic of Bulgaria (part IV: Experiments with animals, art 26, 27, 28, 24.01.2008; promulgated in Official Gazette 13/2008).

For accurate performance of ultrasonography and avoiding artefacts due to involuntary movements of animals, rabbits were anesthetized with 5 mg/kg Zoletil 50 (Virbac) IM. Each animal was restrained in dorsal recumbency. Ultrasound coupling gel (EKO gel, Lessa, Espana) was used for achieving appropriate contact. Ultrasonography equipment 600 VET (CHISON, China) was used, equipped with 7 MHz multifrequency convex transducer. Images were recorded on a thermal printer P93 (Mitsubishi, Japan).

Blood samples were obtained from all rabbits for analysis of blood cholesterol and triglyceride concentrations on a semi-auto chemistry analyzer BA-88A with commercial kits (Giesse Diagnostics, Italy).

After the end of the experiments rabbits were slaughtered in a licensed slaughterhouse (Euro Top, Stara Zagora) with concordance with requirements for humane treatment of animals. The thoracic cavity was open by incisions through the rib cartilages and removal of the sternum. After determining the positions of the pericardium and the heart, the latter were extirpated from the thoracic cavity. By means of a circular incision at the base of large blood vessels, the pericardium with the PAT was removed and weighed on analytical balance AQT-200 (ADAM Equipment Inc., Danbury

USA). Separately, the heart with the EAT was also weighed.

Blood parameters, morphology and ultrasonography data were statistically processed (STATISTICA v 6.1, StatSoft Inc. 2002). Data are presented as mean ± standard error of the mean (SEM).

RESULTS

The results from blood analysis showed that plasma concentrations of total cholesterol in rabbits (1.45 ± 0.15 mmol/L) and triglycerides (0.63 ± 0.01 mmol/L) were normal.

It is shown that in rabbits, the base of the heart and the pericardium are cranially situated in the transverse plane through the 3rd rib, and the apex of the heart and the pericardium with PAT reach the transverse plane through the 6th intercostal space or the 7th rib in a caudal direction. The long axis of the heart is parallel to the sternum, whereas the pericardium with the PAT are located on the dorsal surface of the transverse thoracic muscle at the level between the 2nd and the 5th sternebrae. The sternopericardial ligament is a very weak connective tissue band connecting the pericardium and the sternum.

After dissecting the thorax, the mediastinal adipose tissue is visible from the point of entry into the thorax to the base of the heart, whereas in caudal direction PAT covers almost completely the heart **(Fig. 1).**

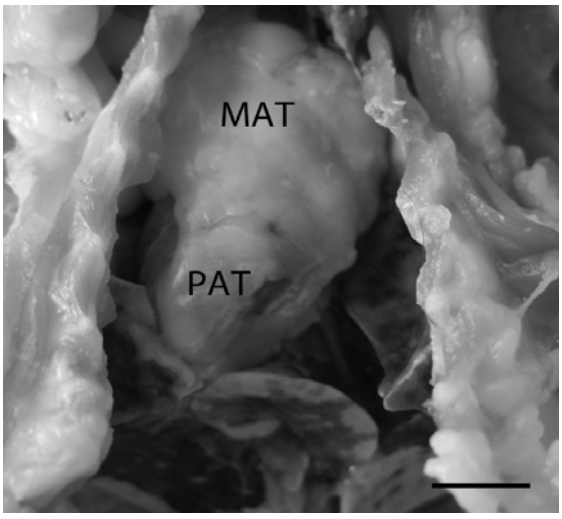

Figure 1. Rabbit thorax after removal of the sternum – ventral view. MAT – mediastinal adipose tissue; PAT – pericardial adipose tissue. Bar 1,5cm.

The largest amount of PAT is observed around the right ventricular free wall and particularly around the caudal end of the right ventricle and the apex of the heart **(Fig. 2).**

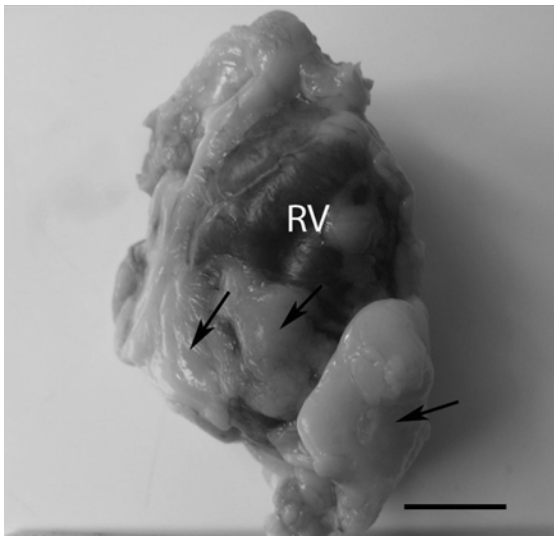

Figure 2. Rabbit heart and pericardium after their removal from the thoracic cavity. RV – right ventricle. Pericardial adipose tissue around the right ventricle and heart's apex (arrows). Bar 1,5cm.

The most significant epicardial adipose tissue was found out in the coronary groove, while a small amount of EAT, even none, was observed in interventricular grooves and on the right ventricular free wall **(Fig. 3).**

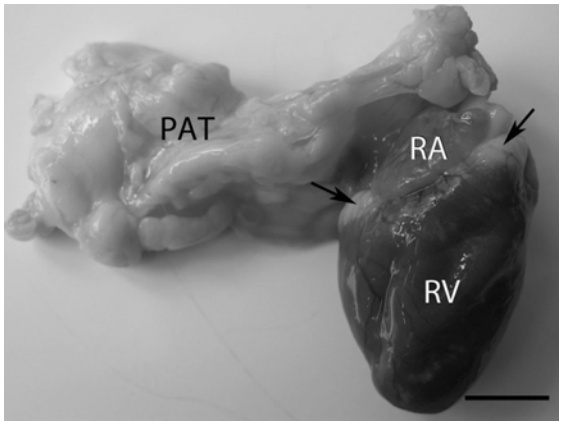

Figure 3. Rabbit heart after separation of the pericardial adipose tissue (PAT). RV –right ventricle; RA – right atrium; epicardial adipose tissue (arrows). Bar 1,5cm.

Because of the lack of a structure connecting the myocardium and EAT, its precise separation and weighing was not possible, and therefore the heart was weighed together with EAT.

The results from weight measurements showed that PAT weight was on the average 3.72 ± 0.24 g that was 0.1% of body weight. The heart weighed 7.89 ± 0.33 g i.e. 0.2% of body weight, therefore, PAT represents 47% of heart weight.

Rabbit heart and pericardium were identified ultrasonographically on the basis of their topographic anatomy features. The right ventricular wall appeared as a hyperechoic linear finding. PAT was observed on the caudal part of the right ventricle in the direction of heart's apex. In this area, it was visualized as moderately echoic homogeneous structure against the hyperechoic myocardium. The thickness of PAT as determined by ultrasonography was 3.2 ± 0.26 mm **(Fig. 4)**.

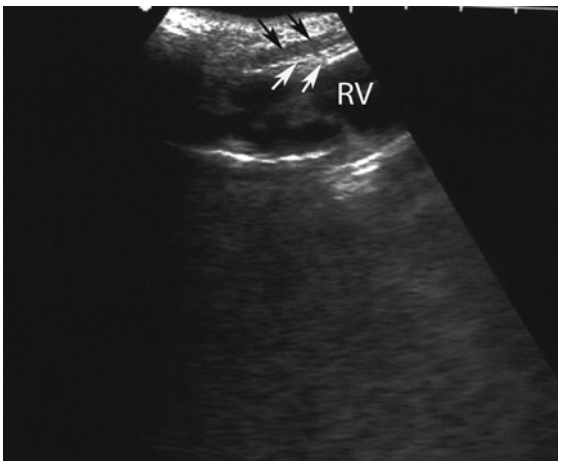

Figure 4. Ultrasonography along the long axis of the heart. RV – right ventricle. The hyperechoic wall of right ventricular myocardium (white arrows); pericardial adipose tissue (black arrows)

DISCUSSION

In this research, we have used clinically healthy New Zealand White rabbits having reached slaughter weight. Even when fed normal diets, rabbits are prone to deposit significant amount of visceral fat. The observed localization of a large visceral adipose depot in the mediastinum and around the heart corresponds to findings of (6) in rodents. Although a profuse pericardial adipose tissue was present, the blood plasma cholesterol and triglyceride concentrations were within the reference range for this species as reported by (4,3,5).

In rabbits, the volume of pericardial adipose tissue is especially big in accordance to data of others (9,8) in wild animals, guinea pigs, domestic mammals and men. Unlike (9) and (8) in this study we did not find out a large amount of epicardial adipose tissue. EAT is constantly present only in the groove around coronary blood vessels, while in interventricular groove and around the right ventricular wall is considerable less or absent. This is in contrast with the findings of (13) for fatty infiltration of the right ventricle even with people with normal weight.

Similarly to (17) and (7), we believe that transthoracic ultrasonography is a non-invasive and adequacy accurate method for visualization of cardiac structures and the adipose tissue.

In rabbits, we did not observe EAT, in contrast to ultrasonography studies of EAT and PAT in men (8) and sheep (14). Therefore, by means of ultrasonography, only PAT was visualized and quantitatively measured.

The weight measurements showed that PAT weight in rabbits was about 47% of the heart mass, just as in humans (8).

The present study allowed us to conclude that in pericardial adipose tissue in rabbits was abundant irrespective of the normal plasma concentrations of total cholesterol and triglycerides. Ultrasonography permitted its measurement in vivo and thus, the evaluation of the extent of visceral obesity.

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