TÜBİTAK

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Ultrasound imaging, biochemical blood analyses, and weight investigations of dissectible fat depots in New Zealand white rabbits

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Abstract: The purpose of the present study was to evaluate changes in real-time ultrasound imaging traits and weight of dissectible fat depots (inguinal, interscapular, and perirenal) in rabbits with different body weights and to monitor the changes in blood glucose and constituents of lipid profiles. In this study, 18 clinically healthy male New Zealand white rabbits were used. The rabbits were fed with standard diet and were divided into 3 groups according to their body weight: group 1 at 1.06 ± 0.03 kg, group 2 at 2.1 ± 0.05 kg, and group 3 at 3.06 ± 0.03 kg. Examined by ultrasonography, the inguinal and interscapular fat depots appeared as bands with weak to moderate echogenicity, whereas perirenal fat was moderate to hyperechoic. The thickness of subcutaneous fat depots measured by ultrasound increased along with body weight and differences between the groups were found to be statistically significant ($P < 0.001$). The differences in perirenal fat thickness between rabbits from group 1 and group 2 were not found to be statistically significant. Perirenal fat thickness in the rabbits from group 3 was higher ($P < 0.001$) than that of the other groups. Perirenal fat weight in group 3 correlated positively ($r = 0.82$; $P < 0.05$) to body weight. Blood biochemical analysis showed that blood glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were in the reference range for all groups regardless of the fact that TG and HDL-C in groups 2 and 3 were significantly higher than those in rabbits from group 1. The in vivo ultrasound screening of adipose tissue, together with blood lipid profile, is an indicator of good health and proper energy balance in rabbits bred for meat or as companion animals.

Key words: Rabbits, dissectible fat depots, ultrasonography, fat depots weight, lipid profile

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Introduction

During the last years, real-time imaging techniques for in vivo investigations of body structures in animals have been more and more widely used instead of dissections (1). Ultrasonography (US) (2), computed tomography (3,4), and magnetic resonance imaging (5) have been successfully applied for in vivo assessment of body structures in rabbits (6).

US imaging is a noninvasive, low-cost, and useful method for evaluating subcutaneous and visceral adipose tissue, which have different anatomic locations and metabolic activities (6,7).

Data from US monitoring of back fat thickness have been used to evaluate the body weight, body composition, energy balance, and meat quality in pigs (8), cows (9), and lambs (10). US measurements of subcutaneous fat have been used to assess fat stores and nutritional status in free-ranging animals (11).

According to new definitions in rabbit meat research, dissectible fat depots are inguinal (IgFD), interscapular (IsFD), and perirenal (PFD). The percentage of perirenal fat is a predictor of the percentage of dissectible fat in the whole rabbit carcass (12). Changes in the IgFD and the IsFD depend on the diet and the different postpartum periods of does (13). The IsFD increases by 24% in rabbits fed a high-fat diet (14). US is a simple and fast method for proving the effect of lipolytic substances injected into the IsFD in New Zealand white rabbits as a new experimental model in aesthetic surgery (15,16).

The PFD in rabbits is the most sensitive to diet variations and its mass increases by 40% in rabbits fed with high-fat diets (14). The amount of perirenal fat is significantly higher in castrated male rabbits than in noncastrated rabbits (17). The use of US to assess the PFD thickness is a good practical method of estimating perirenal fat weight, energy balance, and body condition changes of rabbit does at different points in their reproductive cycles (2,13,18,19).

The nutritional state of rabbits can also be assessed using the blood glucose levels and the constituents of blood lipid profiles (6,10,20).

In available references (13–17), fat depots and lipid metabolism in rabbits were mainly investigated to assess the effect of various factors upon them.

Consequently, the aim of the present study was to investigate the US and weight traits of dissectible fat depots and to monitor the changes in blood glucose and lipid profiles in clinically healthy male rabbits with different body weights fed with standard diet.

Materials and methods

The experiments were performed under approval of the Animal Ethics Committee at the Faculty of Veterinary Medicine, Trakia University, Bulgaria.

Experimental animals

In this study, 18 clinically healthy male New Zealand white rabbits were used. Animals were weaned at 30 days of age. They were fed a standard commercial diet (18.3% crude protein, 12.5% crude fiber, 1.2% fat) given as pellets twice daily. Water was provided ad libitum. Rabbits were housed in metal cages with dimensions of 80 × 60 × 40 cm. The environmental temperature ranged from 20 to 22 °C, relative humidity ranged from 65% to 70%, and 12 h of light was provided per day.

Rabbits were divided into 3 experimental groups containing 6 animals each according to their average body weight: group 1 at 1.06 ± 0.03 kg, group 2 at 2.1 ± 0.05 kg, and group 3 at 3.06 ± 0.03 kg.

Ultrasonography

US measurements were obtained using ultrasound unit 600 VET (CHISON, China) fitted with a 7-MHz convex transducer. Images were printed on a thermoprinter Mitsubishi P93 (Japan).

US examinations of the rabbits were performed under anesthesia using 5 mg/kg Zoletil$^{(R)}$ 50 (Virbac), administrated intramuscularly. The fur in the inguinal, interscapular, and right and left soft abdominal walls regions was removed with an electric shaver. EKO gel (Lessa, Spain) was applied to the scanning areas.

For determination of the IgFD, rabbits were fixed in dorsal recumbency with flexed stifle joints. The transducer was placed longitudinally, first in the left and then in the right inguinal region at the stifle joint level. The distance between the subcutis and the contact surface with the medial femoral fascia was accepted as the thickness of the IgFD.

Animals were fixed in ventral recumbency with cranially stretched humeral joints for IsFD measurements. The transducer was placed longitudinally between the scapulae, first left and then right, to the median plane. In the interscapular region, the distance between the subcutis and the contact surface with the superficial fascia was accepted as the thickness of the IsFD.

The left and right PFDs were observed after fixation of rabbits in the respective lateral recumbency. The transducer was placed in a parallel position toward the transverse processes of the lumbar vertebrae in order to identify the kidneys. The distance between the convex border of the kidney at hilus level and the contact surface of the depot with the transversal fascia was accepted as the PFD thickness.

The results of US measurements (in millimeters) are average values of measurements of the left and right parts of the 3 fat depots.

Postmortem examinations

After the US, rabbits were euthanized by an intravenous application of 0.5 g of thiopental (Sandoz GmbH, Austria).

The IgFDs, IsFDs, and PFDs were removed. The weights of all fat depots (in grams) were determined with an electronic balance (ADAM AQT-200, Adam Equipment Co. Ltd., United Kingdom).

Biochemical analyses

On the day of US, from each animal, samples of venous blood from the external jugular vein were collected. Blood glucose concentrations were assayed on the basis of the glucose oxidase method with a glucometer (Prestige LX, Home Diagnostics, Inc., USA). Plasma total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were assayed colorimetrically using commercial kits (Giesse Diagnostics, Italy). Low-density lipoprotein cholesterol (LDL-C) was calculated by the following equation: LDL-C = TC – HDL-C – (TG/2.2). All blood parameters are presented in mmol/L.

Statistical analysis

All data are presented as mean values ± standard error of the mean (mean ± SEM) and were tested for normality with the Kolmogorov–Smirnov test. The statistical processing of data was performed by ANOVA (Statistica v. 6.1, StatSoft Inc., USA). The statistical significance of differences between groups was determined by the post hoc least significance difference test. Three levels of significance were used: $P < 0.05$, $P < 0.01$, and $P < 0.001$.

Results

The US imaging of the IgFDs and IsFDs showed that they were visualized quickly and easily because they were superficially located and covered with a thin common integument only. Both subcutaneous depots were visualized as bands with weak to moderate echogenicity on the background of more hyperechoic images of the subcutis and underlying muscular fascia.

The PFD in rabbits was seen as a moderate to hyperechoic band (white arrows) against the echoic renal parenchyma and the abdominal muscles (Figures 1 and 2). The observation of the right PFD in the 3 groups of animals allowed a clear visualization of the contact between the caudate lobe of the liver (L) and the right kidney (RK) (Figure 2).

The subcutaneous IgFD and IsFD thickness measured by US increased along with body weight. Differences between groups 1 and 2 ($P < 0.001$), groups 1 and 3 ($P < 0.001$), and groups 2 and 3 ($P < 0.01$) were found to be statistically significant. PFD thickness measurements showed no statistically significant differences between groups 1 and 2. In rabbits from group 3, the PFD was higher than in the other 2 groups and differences were found to be statistically significant ($P < 0.001$) (Table 1).

The weight of all investigated fat depots in rabbits also increased along with the body weight. Results of our measurements clearly showed that in all groups, IgFD weight was higher than IsFD weight. The differences between groups 1 and 3 ($P < 0.001$) and groups 2 and 3 ($P < 0.001$) were statistically significant. The PFD weight in group 3 was significantly higher than in groups 1 and 2 and was correlated positively with the body weight. However, this correlation was statistically significant only in group 3 ($r = 0.82$; $P < 0.05$) (Table 2).

Blood biochemical analyses showed that blood glucose, TC, TG, LDL-C, and HDL-C were within the reference range in the rabbits of all groups.

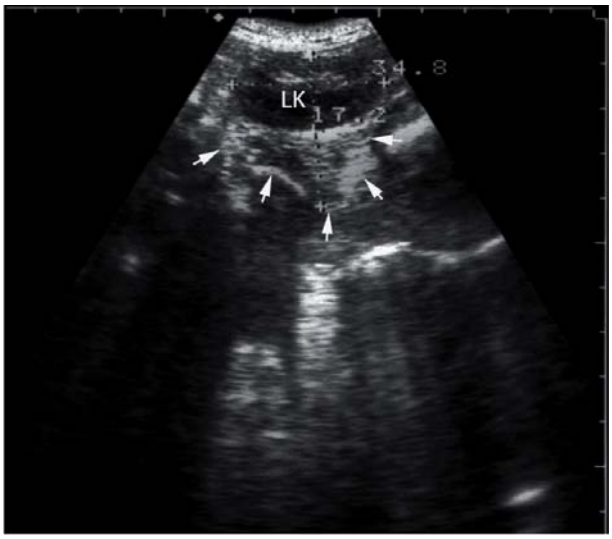

Figure 1. Longitudinal ultrasound imaging of the left perirenal fat depot (white arrows) in a New Zealand white rabbit with body weight of 3.0 kg. LK: left kidney.

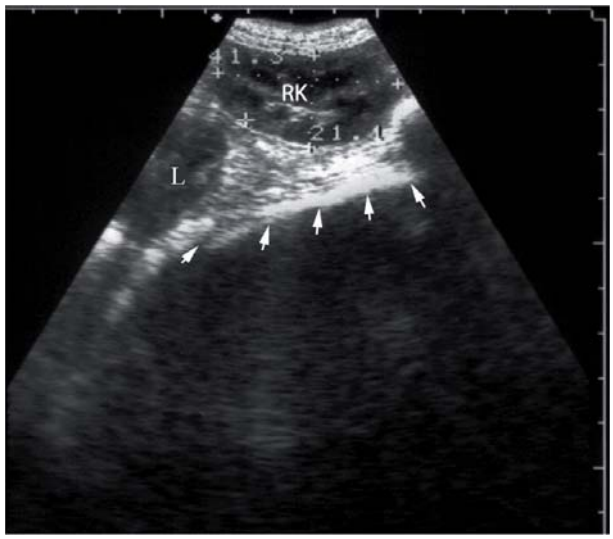

Figure 2. Longitudinal ultrasound image of the right perirenal fat depot (white arrows) in a New Zealand white rabbit with body weight of 3.0 kg. RK: right kidney, L: caudate lobe of the liver.

Table 1. Thickness of fat depots measured by US in 3 groups of rabbits.

Parameters	Group 1 (n = 6) Mean ± SEM	Group 2 (n = 6) Mean ± SEM	Group 3 (n = 6) Mean ± SEM
Inguinal fat thickness (mm)	3.87 ± 0.08	5.42 ± 0.19 ***	6.17 ± 0.17 c, E
Interscapular fat thickness (mm)	3.22 ± 0.08	4.72 ± 0.24 ***	5.80 ± 0.19 c, E
Perirenal fat thickness (mm)	4.35 ± 0.34	4.82 ± 0.29	14.49 ± 1.08 c, F

Different superscripts in the same row indicate significant differences ($P < 0.05$) between the groups as follow: group 1 and group 2, ***$P < 0.001$; group 1 and group 3, $^{c}P < 0.001$; group 2 and group 3, $^{E}P < 0.01$ and $^{F}P < 0.001$.

Table 2. Weight measurements of fat depots in 3 groups of rabbits.

Parameters	Group 1 (n = 6) Mean ± SEM	Group 2 (n = 6) Mean ± SEM	Group 3 (n = 6) Mean ± SEM
Inguinal fat weight (g)	13.54 ± 0.62	16.75 ± 1.00	26.67 ± 1.52 c, F
Interscapular fat weight (g)	5.20 ± 0.33	7.24 ± 0.37	18.43 ± 1.10 c, F
Perirenal fat weight (g)	10.54 ± 0.40	22.83 ± 2.00 ***	76.07 ± 2.55 c, F

Different superscripts in the same row indicate significant differences ($P < 0.05$) between the groups as follow: group 1 and group 2, ***$P < 0.001$; group 1 and group 3, $^{c}P < 0.001$; group 2 and group 3, $^{F}P < 0.001$.

Nevertheless, plasma TG and HDL-C concentrations in group 1 were significantly lower than in groups 2 and 3 ($P < 0.001$), while LDL-C values were higher in group 1 than group 3 ($P < 0.05$). There were no significant differences in blood glucose and TC between groups (Figure *3)*.

Discussion

Our investigations allowed us, similarly to Fortun-Lamothe (6), to assume that US is a low-cost, quick, and easy method for examination of subcutaneous and visceral fat depots in rabbits. The drawbacks of the method are the strong variability of sonograms depending on patients' movements, the respiratory rate, and the pressure applied with the transducer on the area of interest by the investigator (7). Unlike Pascual et al. (19), we anesthetized the rabbits in order to minimize movements and to obtain more accurate results. The investigator did not apply excessive pressure with the transducer to avoid lower fat depot dimensions.

Literature data with regard to US of subcutaneous fat depots in rabbits (which has recently become a new experimental animal model in plastic surgery) are mainly aimed at the IsFD (15,16). Our results showed that the thickness of the IsFD determined by US was lower than that of the IgFD. The thickness of both fat depots increased along with the body weight of rabbits fed a standard diet.

US measurement of PFD thickness in rabbits is the best predictor of PFD weight and meat quality (2), unlike in other animal species where the back fat thickness is considered to be the best trait (8–10). According to Pascual et al. (2), the most appropriate site for left and right PFD thickness measurement is 3 cm cranial to the seventh lumbar vertebra. In contrast, similarly to Diniz et al. (7), we chose the convex border of the respective kidney as a point of reference. The reason was that the adipose tissue is situated in close vicinity to the kidneys and its increased amount could result in both mechanical compression of parenchyma and blood vessels and in increased release of adipokines.

The weight measurements showed that the IgFD was heavier than the IsFD, and our results were similar to those of Fernández and Fraga and of Salles et al. (14,15). Both increased along with body weight increase, and in rabbits from group 1 (1.06 ± 0.03 kg) the IgFD was even heavier than the PFD. The changes in IgFD in rabbits are often underestimated and Pascual et al. (2) even added its weight to that of the PFD. In our opinion, these should be measured separately because of their different locations and various microscopic and metabolic characteristics. Data from the statistical analysis were similar to the findings of Pascual et al. (2,19) and confirmed that the weight and US imaging measurements of the PFD correlated positively to body weight, unlike subcutaneous depots, where the relationship was not statistically significant.

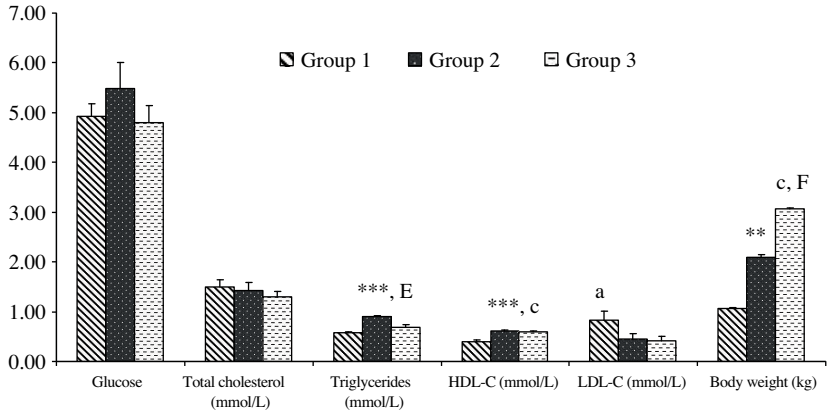

Figure 3. Blood parameters in clinically healthy rabbits with different body weight fed with standard diet (n = 18; mean ± SEM). Different superscripts in the same row indicate significant differences ($P < 0.05$) between the groups as follow: group 1 and group 2, $^{***}P < 0.001$; group 1 and group 3, $^{a}P < 0.05$ and $^{c}P < 0.001$; group 2 and group 3, $^{E}P < 0.01$ and $^{F}P < 0.001$.

Blood biochemical results showed that all parameters studied were within the reference range for the 3 groups of rabbits. The data about TC were very close to those reported by Vachkova et al. (21) about rabbits fed a standard diet on the 14th postweaning day. The statistically significant differences in plasma TG and HDL-C concentrations in animals with higher body weight correlate to some extent with the data obtained by Georgiev et al. (17) and Picone et al. (20) after castration and feeding of a high-calorie diet. However, the induced obesity was accompanied by both increased cholesterol and blood glucose levels, whereas rabbits fed a standard diet did not exhibit such deviations.

In conclusion, the in vivo US screening of dissectible fat depots is an indicator of good health and proper energy balance in rabbits bred for meat or as companion animals. In clinically healthy rabbits fed with standard diet, the weight of subcutaneous and visceral fat depots increases along with body weight growth, but blood parameters of the lipid profile and glucose are within the reference range no matter what the body weight is. The results of our study could be considered as a base for future investigations in rabbit gross anatomy, imaging anatomy, and various metabolic disorders in this animal as a new experimental model for human obesity.

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