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Original article

DYNAMICS OF THE DEVELOPMENT OF SUBCUTANEOUS FAT DEPOTS IN RABBITS – A GROSS ANATOMICAL AND MICROSCOPIC STUDY

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Summary

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In this study, gross anatomical and microscopic features of interscapular (IsFD) and inguinal (InFD) fat depots of 24 New Zealand White rabbits were evaluated. Rabbits were equally distributed into 4 groups: 1st – newborns, 2nd – 1 month old, 3rd – 2 months old and 4th – 3 months old. The cranial subcutaneous fat pad in newborns covered dorsal and ventral cervical and interscapular regions. As age advanced, cervical lobes underwent a rapid reduction but the development of interscapular lobes continued. IsFD in rabbits from 1st and 2nd group was composed of both white and brown adipocytes, while in 3rd and 4th groups it consisted of white adipocytes only. InFD in rabbits from all tested groups occupied respective inguinal region and no age-dependent changes in shape and topography were observed. In all groups InFD was composed of white adipocytes only. The highest growth rate of interscapular and inguinal adipocytes was established in one-month-old rabbits. Differences in anatomy and histology of interscapular and inguinal fat depots in rabbits could be successfully used for comparison in other experiments in the field of adipobiology and autologous transplantation, where fat depots undergo significant morphological changes.

Key words: gross anatomy, histology, interscapular and inguinal fat depots, rabbits

INTRODUCTION

The clustering of a large number of adipocytes in a certain anatomical region, together with stromal vascular cells, blood vessels, lymph vessels, lymph nodes and nerves, is defined by Hausman *et al.* (2001) and Cinti (2012) as a fat depot. The sum of fat depots in the body and the combination of two types of adipose tissue (white and brown) is called adipose organ (Hausman *et al*., 2001; Cinti, 2005).

Despite the increased interest and intensive study of adipose organ in the recent years, the anatomical and histological nomenclatures (Nomina Histologica, 1992; Nomina Anatomica Veterinaria, 2017) do not propose a classification of fat depots in animals. Such is suggested by Tran & Kahn (2010), according to which the superficial subcutaneous fat depots in mammals are composed of both white and brown adipose tissue, while the deep visceral ones comprised only white fat tissue. Other classifications are proposed by Cinti (2005; 2007; 2012) for rodents, by Shen *et al*. (2003) and Kwok *et al*. (2016) for humans. The authors divide fat tissue in the body into two main compartments – subcutaneous and visceral, the latter being localised in the thoracic and abdominal cavities.

Unlike other domestic mammals and humans, subcutaneous adipose tissue in rabbits is concentrated primarily in the interscapular and inguinal region as a clearly demarcated fat pad, allowing it easy dissection and separation from the underlying fasciae and muscles. This peculiarity made Blasco & Ouhayoun (1993) to designate interscapular (IsFD) and inguinal (InFD) depots in rabbits as dissectable fat.

The amount of subcutaneous fat in newborn rabbits (Fernández & Fraga, 1996; Castelini, 2006), as well as in other animals (Schoonderwoerd *et al*., 1986) is crucial not only for body weight but also for the survival of animals in conditions of malnutrition. According to Ovcharov & Takeva (2001), Miranville (2006) and Sheldon (2011), the development of adipose tissue in the early postnatal period is due to the hyperplastic and hypertrophic growth of adipocytes, and the subsequent development is mainly due to hypertrophy. This however depends on the greatest extent on the animal species. In this regard, Atanassova *et al*. (2012) have established that gluteal subcutaneous depot in neonatal rats was composed of well-developed unilocular adipocytes, but Hausman (1982) did not detect typical differentiated unilocular adipocytes in the subcutaneous fat from pig foetuses to the end of prenatal development.

Adipocytes vary greatly in size after the body has completed its physical development. As a result, the mass of the adipose organ as a whole undergoes significant changes (Bailey *et al*., 1993; Hausman *et al*., 2001). On the other hand, adipocytes from different body fat depots grow at different rates, which causes differences in their size. Reyne *et al*. (1985) and Bailey *et al*. (1993) have found that interscapular and inguinal fat cells' diameter was smaller compared to that of perirenal cells in rabbits and rats. Opposite to them, Ahmakov (1988), Eurell & Van Sickle (1998) and Cinti (2007) have described that subcutaneous mammary adipocytes exceed in size perirenal adipocytes.

As can be seen from the above facts, the topography, size and micromorphology of the subcutaneous part of the adipose organ show large species- and age-related variations. Data on fat depots in rabbits are mainly related to their morphological changes in the course of various experiments.

The main aim of this study was to establish the dynamics in the development of interscapular and ingunal fat depots in healthy rabbits during their normal growth at both macroscopic and microscopic level.

MATERIALS AND METHODS

Animals

Twenty-four rabbits of the White New Zealand breed were used to conduct this investigation. According to their age, rabbits were divided into four groups with 6 rabbits, (3 males and 3 females) in each group. The 1st group included newborns with mean body weight of 58.22±1.03 g (the newborns were obtained 2 to 12 h after their death for reasons of traumatic injury or suffocation, without compromising the body integrity); the 2nd group – 1 month old rabbits with mean body weight of 1.07±0.05 kg, the 3rd group – 2 months old with mean body weight of 2.09±0.04 kg and the rabbits from the 4th group were 3 months of age with mean body weight of 3.08 ±0.06 kg.

The animals were provided by Institute of Agriculture, Stara Zagora, where they were reared according to the required zootechnical parameters for the respective category of rabbits. The rabbits were weaned between the 30th and 35th day. They received twice daily a pelleted diet containing 18.3% crude protein, 12.5% crude fibre, 1.2% fat. Water was given *ad libitum*.

Rabbits from the 2nd, 3rd and 4th groups were euthanised in a slaughterhouse according to the requirements and approval of the Animal Ethics Committee at the Faculty of Veterinary Medicine, Trakia University, Bulgaria (permits 27/2010 and 39/2010 and 52/2012).

Gross anatomy

After skinning, the topography and gross anatomical features of the IsFD and InFD fat depots on each carcass were determined.

Light microscopy

From each of investigated depots, tissue samples were obtained and fixed in a 10% aqueous solution of formalin (Merck KGaA, Darmstadt, Germany), dehydrated in graded ethanol, cleared in xylene and embedded in paraffin. Histological slices with 5 μm thickness were obtained using LEICA RM2235 rotary microtome. The slices were deparaffinised in xylene, hydrated in graded ethanol, stained with haematoxylin and eosin, dehydrated in ethanol and cleared in xylene (Krastev & Kovachev, 1973; Vitanov *et al*., 1995). Using light microscope Primo Star (ZEISS, Germany) and the software for analysis (Soft Imaging System GmbH, Germany), cross-sectional areas (in μm^2) of 100 selected adipocytes with preserved nuclei and cell membranes from each depot were measured.

RESULTS

Gross anatomy

- *Interscapular fat depot*

Results from gross anatomical observations in newborn rabbits after skin removal showed that dorsal and ventral cervical and interscapular regions were covered by massive subcutaneous adipose layer. The fat depot was dark yellow or beige in colour with macroscopically well visible lobation and lobulation, allowing the easy division of the complexly structured adipose tissue into lobes. IsFD comprised the most dorsal part of the complex and was composed of two parallel bands occupying the entire interscapular region. The other part of the complex comprised the cervical lobes. They were better developed in newborns and located craniolaterally to IsFD, on the left and right part of the neck, like a collar. Cervical lobes covered completely the lateral surfaces of trapezius muscle (cervical part) and cleidocephalic muscle. Cranially, the lobes extended to the base of the ear and their caudal border was almost parallel to the cranial edge of the scapula, to the level of the shoulder joint (Fig. 1A). Without any anatomical landmarks, the cervical lobes continued in a ventral direction, where they touched each other on the

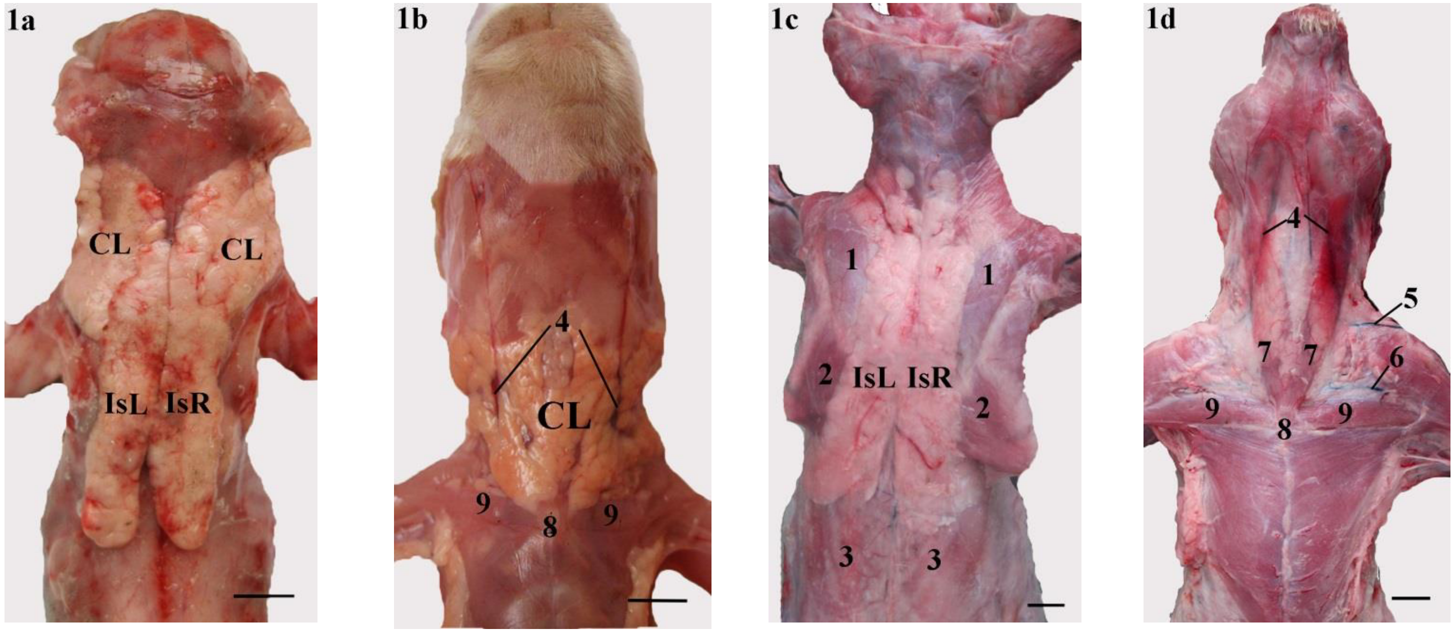

Fig. 1. Intrascapular fat dept (IsFD) in a female newborn (A, B) and a female rabbit from group 3 (C, D). The skin and superficial fascia of the trunk are removed. CL – cervical lobes; IsR, IsL – right and left lobes of IsFD; 1– *m. supraspinatus*; 2 – *m. trapezius*; 3 – *m. latissimus dorsi*; 4 – *v. jugularis externa;* 5 – *v. omobrachialis;* 6 – *v. cephalica;* 7 – *m. sternomastoideus;* 8 – *manubrium sterni*; 9 – *m. pectoralis primus*. Dorsal (A, C) and ventral (B, D) views. Bar=1 cm.

median line of the neck. The cranial edge of pectoral primus muscle appeared as a caudal border of cervical lobes and the transverse line at the level of mandibular angle as a cranial margin (Fig. 1B). Ventrally, the adipose tissue covered the ventral surface of sternomastoid and sternohyoid muscles, as well as the external jugular vein. The cervical lobes were packaged by the superficial fascia of the neck. As age advanced, the cervical lobes underwent a rapid reduction but the development of interscapular lobes continued.

The gross anatomy of IsFD in the other three age groups showed that cervical lobes appeared as a thin adipose layer. On the other hand, the development of the intercapular lobes continued, but they retained their relative topographic position with respect to the other anatomical structures. Cranially, the lobes reached the level of 5th cervical vertebra, caudally ended to the plane through the 8th – 9th thoracic vertebra, and ventrally bordered *m. trapezius*, *m. supraspinatus* and *m. latissimus dorsi.* Interscapular lobes were covered by the superficial fascia of the trunk, which allowed them to remain attached to the underlying musculature, when the skin has been removed.

The colour of IsFD ranged from dark yellow to beige in newborns and white in other groups of rabbits. The lobulation of the depot was macroscopically visible (Fig. 1A–D).

- *Inguinal fat depot*

In rabbits from all tested groups, the subcutaneous InFD was observed in the respective topographic region – *regio inguinalis dextra et sinistra*. It consisted of a right and a left part, which converged in caudal direction and merged at the level of *tendo praepubicus,* so in a ventral view, the inguinal depot resembled the latin letter "V". The merged portion bordered caudally the testicles in the male rabbits or the vulva in females. Craniodorsal extremities of both parts touched the cranial edge of *m. tensor fasciae latae* and ended at the level of *regio tuberis coxae*. Laterally, the depot was in contact with the proximal parts of *m. gracilis* and *m. sartorius*, and craniomedially: with the lateral surface of *m. obliquus externus abdominis*. The InFD was covered by superficial fascia of the trunk and skin. It is noteworthy that with advancing of age of rabbits, the topographic boundaries of

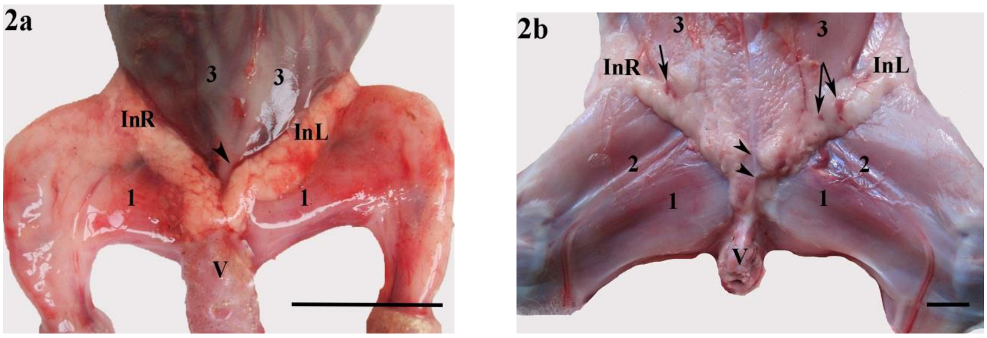

Fig. 2. Ventral view of inguinal fat depot (InFD) in a female newborn (A) and a female rabbit from group 3 (B). The skin and superficial fascia of the trunk are removed. InR, InL – right and left parts of InFD; arrows – *lnn. subiliaci;* arrowheads – *tendo praepubicus*; 1– *m. gracilis*; 2 – *m. sartorius*; 3 – *m. obliquus externus abdominis*; v – *vulva*. Bar=1 cm.

the depot did not change their topography (Fig. 2A, B).

The inguinal fat was white in colour with macroscopically well-defined lobation in all age groups. In the middle third of this fat depot, *lnn. subiliaci* were seen, two or three in a package.

Light microscopy

- *Interscapular fat depot*

Microscopic observations showed that the main cellular elements of the cranial subcutaneous depot in newborn rabbits were both brown or multilocular adipocytes (MA) and white or unilocular adipocytes (UA). Around predominating brown areas, white fat cells were scattered into groups or singly. From the thin connective tissue capsule in the periphery to the interior of the depot, connective tissue septa dividing it into lobules and sublobules of different size were inserted. The blood vessels and nerves followed septal branches (Fig. 3).

In the rabbits of the 2nd group, the interscapular fat was also made up of both adipocyte types, but brown adipocytes were concentrated in groups as islets in between prevailing zones of white fat cells. The islets had different location in lobules, so some of them occupied the central lobular parts and others were eccentrically located. The predominant shape of UA cross section was oval. In the multilocular areas, blood vessels were more densely arranged than in the unilocular zones (Fig. 3).

IsFD in the rabbits of 3rd and 4th groups was built only by UA with pentagonal or hexagonal shape of the transverse cross section and nuclei compressed at some of angles (Fig. 3).

The highest intensity of interscapular adipocytes growth was shown in one-month-old rabbits where the size of fat cells' cross sectional area was 5.3 times higher than that in the 1st group. The area increase in the 3rd group was 1.3 times and in the 4th group: 2.8 times (Table 1).

- *Inguinal fat depot*

The inguinal depot in newborn rabbits was composed of UA only. The shape of cross-sectional surface of the majority of cells was oval or round. The fat lobules were demarcated by thick connective tissue septae where, together with the abundance of blood vessels and nerves, groups of small adipocytes were found. It was established that fat cells in this groups had a different phenotype, as they showed the presence of one or two larger fat droplets lying centrally, around which there were many small ones. The microscopic observations on the inguinal depot in the 2nd group showed that it was made of unilocular fat cells with oval cross-sectional surface and in the 3rd and 4th age groups, the cross-section shape was polygonal (Fig. 3).

Adipocytes dimensions from the inguinal depot of rabbits increased most intensively up to 1 month of age – 6.1 times than these in newborns. The inguinal adipocytes area in the 3rd group was 1.5 times larger than the area in the 2nd group and in the 4th group – 1.7 times larger compared to the 3rd group (Table 1).

DISCUSSION

In this study, the main topographic, macroscopic and microscopic characteristics of interscapular and inguinal subcutaneous fat depots in New Zealand White rabbits were established. The rabbits were divided into 4 age categories - from newborns to 3 months of age, when they reach slaughter weight, but have not yet reached sexual maturity. No statistically signifi-

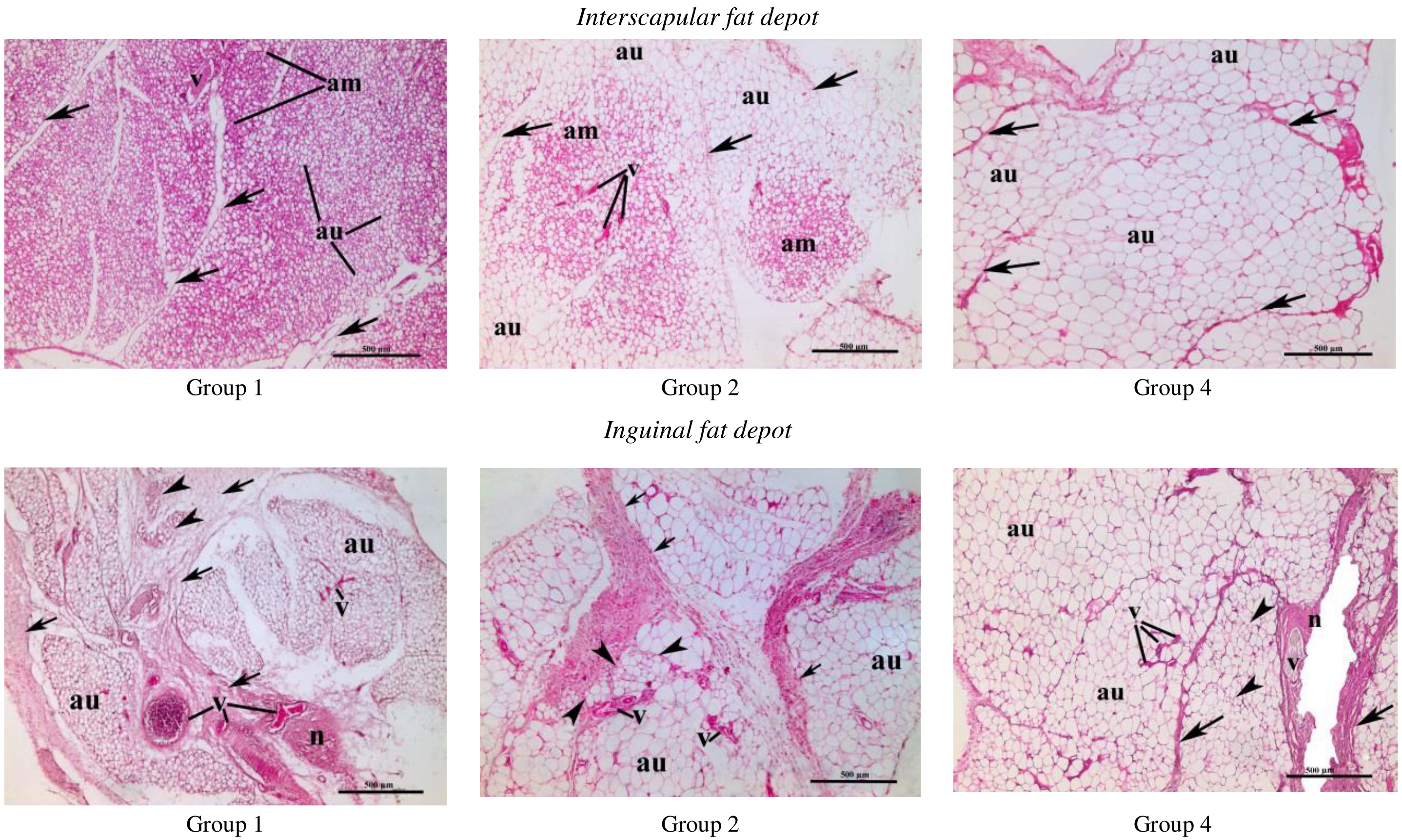

Fig. 3. Light microscopy of IsFD and InFD in male rabbits from the 1st, 2nd and 4th group; *au* – zones of unilocular (white) adipocytes, *am* – zones of multilocular (brown) adipocytes; arrows – connective tissue septae; arrowheads – groups of small adipocytes; *n* – nerves; *v* – vessels. Optical magnification ×50; scale bar = 500 µm.

Table 1. Cross sectional area (µm^2) of subcutaneous adypocytes in rabbits at different ages. Data are mean ± SEM of 6 rabbits (3 male and 3 female)

Fat depot	1st group (newborn)	2nd group (1 month old)	3rd group (2 months old)	4th group (3 months old)
Interscapular	427.32 ±9.62	2265.99 ±56.62 ^{A}	3004.25 ±78.87 ^{B}	8619.61 ±215.2 ^{C}
Inguinal	544.03 ±10.60	3324.92 ±79.21 ^{A1}	5098.19 ±105.18 ^{B1}	8854.78 ±163.42 ^{C1}

Statistically significant differences (P<0.001) in cross sectional areas of interscapular and inguinal adypocytes are indicated by superscripts as follows: A, A1: between 1st and 2nd group; B, B1: between 2nd and 3rd group; C, C1: between 3rd and 4th group.

cant differences in the development and mass of subcutaneous depots between males and females were found in this age range. Therefore, an equal number of male and female rabbits were included in the experimental groups.

Unlike other domestic mammals and humans (Fawcett & Jensh, 2002; Shen *et al*., 2003; Reese, 2004), in which the subcutaneous adipose tissue forms a complete layer of different thickness in the individual body parts, fat tissue in the rabbits was found to accumulate predominantly around the neck, interscapular and inguinal areas.

The gross anatomy of subcutaneous depots in newborn rabbits differed from that of the other age groups mainly into two aspects. On one hand, they were better developed than the visceral fat depots, in line with findings of Vezinhet & Prud'hon (1975). The authors have shown that subcutaneous fat in newborn rabbits had an earlier development in contrast to the lambs, whose internal fat percentage at birth was higher. On the other hand, observations in the newborn group showed that the subcutaneous fat in the cervical and interscapular region was not only larger than the inguinal depot, but was best developed throughout the body. This was also confirmed by results of weight measurements performed by Yonkova (2014).

Hudson & Hull (1975) have defined complexly structured fat formation in the cervical and interscapular region in newborn rabbits as cranial subcutaneous fat depot. IsFD constitutes only one, the most dorsal part of this formation. While the cervical lobes from the complex undergo a very rapid reduction with the growth of rabbits, the interscapular ones continued their progressive development in rabbits of groups 2, 3 and 4. In the process of growth and development, interscapular lobes retained their relative topographic position with respect to the other anatomical structures. These anatomical features are similar with the findings of Hull and Segal (1965), according to which after 2nd day after birth, the weight of cranial adipose depot diminished sharply due to the rapid release of lipid and non-lipid components from the tissue, affecting mainly the cervical lobes. Perhaps for this reason, subcutaneous depots are of interest in newborns, while growing rabbits are better suited for studies related to visceral fat.

The inguinal fat had a much simpler anatomical structure. In all experimental groups fat tissue was found as accumulation in the form of stripes covering the ventrolateral surface of the respective inguinal region. The shape and topography of the InFD in all experimental groups were similar to those described by Cinti (2005; 2007; 2012) in laboratory rodents and by Rauch *et al.* (1969) in the deer-like mouse.

The present data about subcutaneous fat development in rabbits kept in normal zoohygienic conditions provide a good background for comparison with experimental rabbits (Bailey *et al*., 1993; Fernández & Fraga, 1996; Castelini, 2006), subjected to different diets and physical exertion.

From the point of view of meat production, IsFD and InFD remain with the carcass after skinning. This has led Blasco *et al.* (1993) and Blasco & Ouhayoun (1993) to label them as dissectable fat depots. The amount of dissectable fat is crucial for the dietary qualities of rabbit meat due to the higher content of saturated fatty acids, higher atherogenic and saturation index (Yonkova *et al*., 2017).

Good knowledge of the macroanatomical and topographic features of subcutaneous adipose tissue makes possible its *in vivo* practical estimation by computed tomography and ultrasonography (McEvoy *et al*., 2009; Yonkova *et al.* 2012).

The established dynamic changes in the gross anatomy of IsFD and InFD are inextricably linked with their histological features. Light microscopic observations and adipocytes measurements in this study were performed on histological sections from whole tissue samples, not on isolated fat cells (Bjørndal *et al.,* 2011). This allows estimating the adipocytes in their native disposition and conjunction with vessels, nerves and connective tissue, hence a more accurate interpretation and comparison with results from pathological changes in the subcutis and other changes of cellularity in adipobiological experiments.

When describing the physiology of fat depots in newborn rabbits, Hull & Segal (1965) have identified cranial fat complex with brown adipose tissue. However, this was not confirmed by the present histological examination. The slides from all interscapular tissue samples, along with the predominant areas of brown adipocytes demonstrated loci composed of white fat cells only. Therefore, the identification of brown adipose tissue with the interscapular fat depot is inaccurate because microscopic morphology of IsFD in newborn rabbits is heterogeneous. The presence of differentiated unilocular adipocytes in subcutaneous depots in newborns is a mark of their maturity (Miranville, 2006). This fact suggested that adipocyte differentiation is complete in newborn rabbits, unlike pig foetuses (Hausman, 1982), in which typical subcutaneous unilocular adipocytes are not observed even immediately prior to their birth. This is in line with the statement by Atanassova *et al*. (2012), that differentiation of fat cells ends at different times in newborns and depends primarily on the species.

In rabbits of group 2, cross-sectional shape of white fat cells was oval or circular and the area was 5.3-fold greater compared to that of white adipocytes in newborns. The brown zones still existed, althouth with smaller dimensions. While the white adipocytes were located on the periphery of the fat lobules, the brown adipocytes occupied their central parts. The intensity of IsFD growth resulted from intensive accumulation of triglycerides in the fat drops in support of the opinion of Gonzales & Orlando (2007) that postnatal development of fat tissue is mainly due to fat cells hypertrophy. The observed transdifferentiation of a large number of multilocular into unilocular adipocytes in the early postnatal development is observed also in newborn calves and lambs (Gemmell, 1972, Alexander *et al*., 1975, Trayhurn *et al*., 1993) and confirm the theory of plasticity of the adipose organ (Cinti, 2012). In the 3rd group, multilocular adipocytes were not detected. White fat cells' cross-sectional area increased 1.3 times, compared to that in group 2. Although the differences between the 2nd and 3rd groups were statistically significant, the adipocyte growth was much slower compared to the adipocyte size in the 4th group, where the area was 2.8 times larger than that in group 3.

The microscopic structure of inguinal depot in newborn rabbits differed from that of the interscapular one by the thicker connective tissue septa between the fatty lobules. Similar lobulation of the inguinal adipose tissue is described by Atanassova *et al.* (2012) in newborn rats. The depot was built of differentiated unilocular adipocytes with a round or oval shape of the cross-section, and statistically significantly larger sizes vs interscapular adipocytes. Like the interscapular depot, the greatest rate of adipocyte growth was observed until the first month of rabbit growth and the cross-sectional area was 6.1 times larger than that in the 1st group. There was also a change in the shape of the cells' cross section – from oval to polygonal, i.e. the accumulation of lipids in inguinal adipocytes at this early stage of the body's development in rabbits was more intensive than in interscapular ones. The values for area for the 3rd and 4th group of rabbits in this study were similar to those measured for subcutaneous adipocytes in mice, rats and humans (Ahmakov *et al*., 1988; Eurell & Van Sickle, 1998; Cinti, 2007).

In all experimental groups, the connective tissue septa between the adipose lobules together with the network of capillaries and nerves in InFD were better developed than those from the IsFD. These results correlate closely with the findings of Sbarbati *et al.* (2010) which has studied ultrastructural characteristics of fat in the pubic region in humans.

The superficial position, specific cellular characteristics, rich blood supply and innervation: all of them features of the subcutaneous fat depots in rabbits, make these animals very suitable for obtaining multipotent adipose derived stem cells (Vachkova *et al.,* 2016), for the needs of regenerative medicine (Gimble *et al*., 2007), autologous transplantology (Gorecka *et al.,* 2018) as well as in the cases of lipectomy (Hausman *et al*., 2004).

The knowledge of the dynamic changes in subcutaneous adipocytes of standardised White New Zealand rabbits can serve as a basis for comparison in a variety of experimental studies that track the influence of environmental factors on the adipose tissue cellularity.

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