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NON-INVASIVE STUDY OF CHANGES IN THE BODY COMPOSITION OF GROWING RABBITS USING X-RAY COMPUTER TOMOGRAPHY

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ABSTRACT

X-ray computer tomography (CT) was used to determine the changes in the body composition of 208 Pannon White growing rabbits between 6 and 16 weeks of age. A total of 21 scans per animal were taken and evaluated in the body region from the scapular arch to the end of the femur. To demonstrate the body composition at 6, 8, 10, 12, 14 and 16 weeks of age three dimensional histograms (3D) were constructed. These histograms, plotted with the density values of muscle and fat in the range of -200 to +200 on the Hounsfield scale, clearly represented the tissue composition of the rabbits' bodies, with different peaks at the scapular, renal and pelvic regions. Using the data from slaughter and then those obtained from the CT pictures a nearly linear increase of muscle and an exponential deposition of fat were observed in the period examined. The amount of muscle nearly doubled and that of the fat more than tripled during the experimental period. It was established that the weight of fat and muscle could be estimated with a medium or high accuracy (r=0.59-0.98). It was also concluded that CT is a useful tool to follow the changes in the body composition of living animals.

INTRODUCTION

In animal breeding and nutrition experiments exact knowledge of body composition is indispensable in determining the chemical maturity, nutritional requirements and body condition of animals. For this reason several previous experiments were carried out to determine the body composition at different developmental stages, mostly using experimental slaughter and chemical analysis, which are invasive methods. These techniques are very reliable and accurate in the determination of the amount and ratio of body tissues and different chemical components, but they are not able to provide any precise information about changes in these during the experimental period. To solve this problem and avoid the slaughter of animals different types of non-invasive methods have been developed (FEKETE, 1992), which allow a fairly good estimation of the body composition (KEMPSTER *et al.*, 1982; KÖVÉR *et al.*, 1996; ROMVÁRI *et al.*, 1996a; MILISITS *et al.*, 1999a) and also the periodical control of developmental (ROMVÁRI *et al.*, 1996c) and reproductive (MILISITS *et al.*, 1999b) alterations in the same animal.

One of these possible techniques is X-ray computer tomography (CT), which was developed as an aid for human clinical diagnostics and has been used for research in animal science since the early 1980s. This method is suitable for the individual examination of tissue layers by means of X-rays, by virtue of recently developed three-dimensional image formation and the non-overlapping density values of the different tissues of animals (SKJERVOLD *et al.*, 1981).

Based on the results of the previous experiments, the application of CT for rabbit research began at the end of 1991, this technique first beeing used for the *in vivo* estimation of dressing percentage (ROMVÁRI *et al.*, 1995) and later for the determination of different body constituents (ROMVÁRI *et al*., 1996a). In the present work it was applied for the examination of changes in the body composition of growing rabbits, to follow the development of muscle tissues and the deposition of fat between 6 and 16 weeks of age.

MATERIAL AND METHODS

Animals and experimental procedure

The experiment was carried out with 208 growing Pannon White rabbits, weaned at the age of 6 weeks and housed in a closed building, in groups of 5 or 6 per cage (800x500mm). The animals were kept under artificial lighting conditions (16 hours per day) and at a room temperature of 15-20°C prior to the scanning and slaughter procedure. For the *ad libitum* feeding of the young a commercial pelleted diet (DE 10.30 MJ/kg, crude protein 17.5%, crude fat 3.6%, crude fibre 12.4%) was used. Drinking water was available continuously from self-drinkers.

CT scanning procedures

CT scanning of the young was performed at 6, 8, 10, 12, 14 and 16 weeks of age (n=24, 31, 44, 40, 29 and 40). Rabbits of both sexes were chosen randomly from the experimental stock of the university and fasted for 12 hours before the scanning. The CT procedures were performed by means of a SIEMENS SOMATOM DRG third-generation X-ray tomograph, in every case during the night.

The animals chosen for the scannings were fixed with belts in a lying position in a special plexi-glass container. In this position their movement was restricted and the legs were well separated from the rump. Three rabbits were scanned simultaneously in the same container.

The examinations began with the taking of a so-termed overall topogram, which resembles a conventional two-dimensional X-ray image. In this image the anatomical levels of the later scans could be marked with horizontal lines as markers.

In this experiment a total of 21 scans were taken from each animal, with 8 mm thickness and with different distances between the scans, depending on the length of the vertebral column. In this way scans with the same serial number represent the body composition at the same anatomical points, and so animals of different sizes could be compared. The scanning range extended from the scapular arch to the end of the femur in each case.

Image evaluation

The evaluation of the images obtained was performed in accordance with ROMVÁRI *et al*. (1996b), using only the density values corresponding to muscle, water and fat. The extreme values (i. e., air and bone) were excluded from the evaluation.

The analysis of the images began with the determination of the frequency of pixels (elements of images) at every density value between -200 and +200 on the Hounsfield scale. These 400 values were then reduced to 40 so-termed Hounsfield variables (HUv) by totalling the number of pixels corresponding to 10 consecutive density values on the scale (HUv1=∑(-200)-(-191), HUv2=∑(-190)-(-181), … HUv40=∑(+190)-(+199)). These variables were then used to estimate the amount of fat and muscle and to plot three-dimensional histograms to illustrate the body composition of the animals.

Estimation of the mass of fat and muscle

Because scans taken with 8mm thickness have a certain width in this case, each pixel of the images corresponds to a prism. Therefore, the amount of fat and muscle could be estimated by counting the prisms corresponding to the density of fat and muscle respectively.

Three-dimensional histograms

To demonstrate the body composition of the growing rabbits at different developmental stages so-termed three-dimensional histograms were created by the method of negative exponential interpolation. To show the changes in the body composition between two examined time points, so-termed differential histograms were plotted by subtracting the latter from the former. In both cases the SYSTAT statistics software package (SYSTAT, 1990) was used for the creation of the histograms.

Slaughter procedure

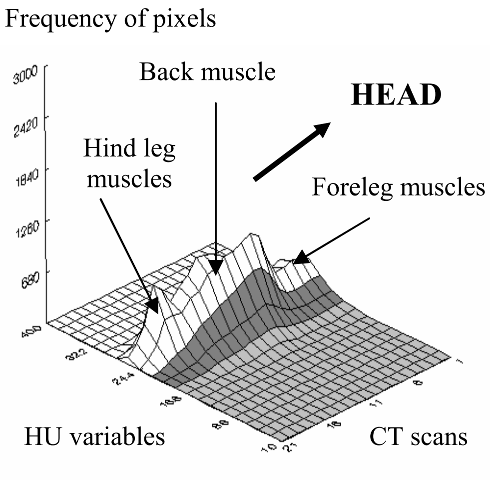
After the CT examinations the rabbits were fasted for another 12 hours and then slaughtered by the method of BLASCO *et al.* (1993). In accordance with the specification of this method the hot carcass was cut into pieces between the 7th and 8th dorsal and between the 6th and 7th lumbar vertebrae. The weight of the fore, intermediate and hind parts obtained was measured immediately, after which that of the scapular and kidney fat was recorded.

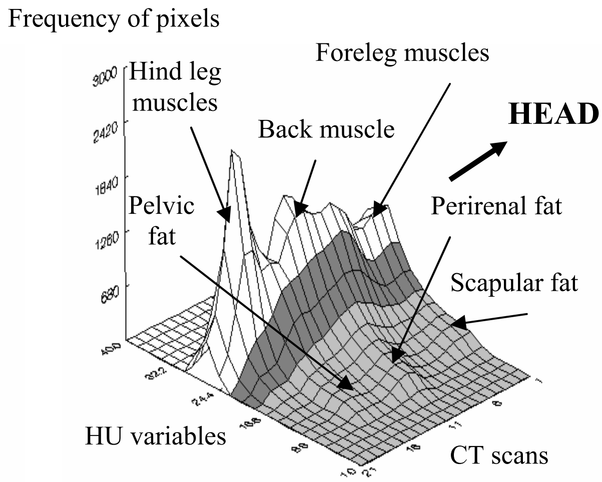
Statistical procedures

To test the predictive value of the amount of pixels corresponding to fat and muscle, linear regressions were carried out between the estimated weight of fat and muscle and the values recorded at slaughter. For the statistical procedure the SPSS statistics software package, version 8.0, was used (SPSS FOR WINDOWS 8.0, 1997).

RESULTS

The body composition of the rabbits at 6 and 16 weeks of age is presented in *Figs. 1 and 2.*





☐ region of muscle ■ region of water density ■ region of fat

Fig. 1 Body composition of the rabbits at 6 weeks of age

Fig. 2 Body composition of the rabbits at 16 weeks of age

In these figures the serial numbers of CT scans (1-21), the HU variables (1-40) and the frequency of pixels at the different density values are shown on axes X, Y and Z, respectively. In these histograms the region of fat tissue can be seen between HUv1 and HUv18 and that of muscle between HUv23 and HUv40. These two regions are separated by the region of water density between HUv19 and HUv22. The tissue composition of the rabbits of different ages is demonstrated by the elevations in the histograms.

In *Fig. 1*, which represents the body composition at 6 weeks of age, 3 distinct peaks can be observed in the region of the muscle. These show the anatomical location and also the amount of foreleg, back and hind leg muscles, separated by the less muscular thoracal region (scans 4-5) and pelvic region (scans 15-16), respectively.

In contrast to muscle, the amount of fat is very low at the age of 6 weeks and, therefore, peaks corresponding to this tissue cannot be observed in the histogram in *Fig. 1*. The very intensive increase in fat deposition begins in the second half of the examined period only, resulted in a clearly visible peaks at the main depot regions of the body at 16 weeks of age (*Fig. 2*).

The intensity of deposition can be followed more precisely from the differential histograms in *Figs. 3-7*. A very early deposition can be seen in the renal region between 6 and 8 weeks of age (*Fig. 3*), while a later start in deposition is visible in the scapular and pelvic regions of the body in *Figs. 4 and 5*.

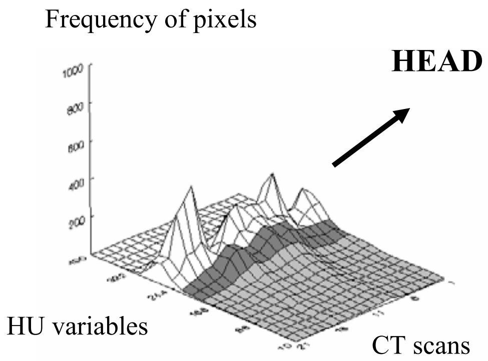


Fig. 3 Changes in body composition between 6 and 8 weeks of age

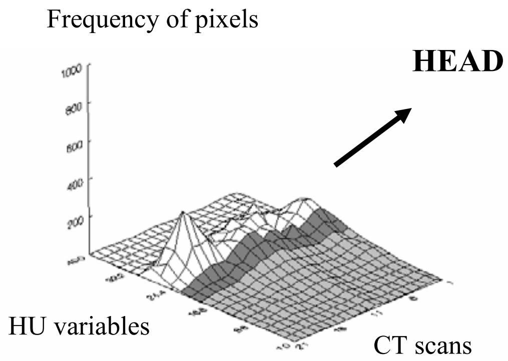


Fig. 4 Changes in body composition between 8 and 10 weeks of age

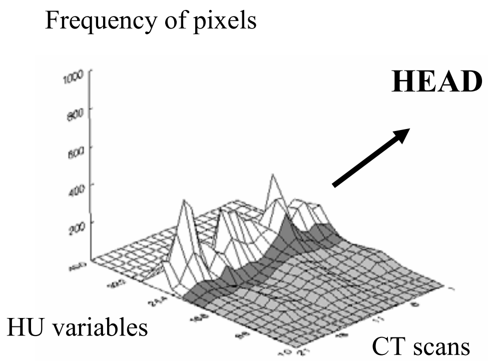


Fig. 5 Changes in body composition between 10 and 12 weeks of age

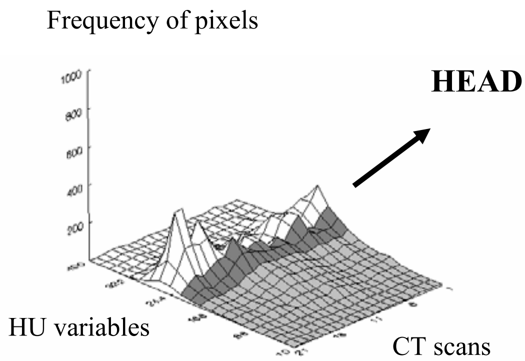


Fig. 6 Changes in body composition between 12 and 14 weeks of age

The most intensive deposition in all the regions can be observed in the last two weeks of the period examined (*Fig. 7*).

In contrast to fat, the growth of muscle seems to be steady in the first half of the examination period and it decreases only after the 12th or 14th week of age. The decrease of intensity begins earlier in the scapular part of the body and only two weeks later in the pelvic and renal regions.

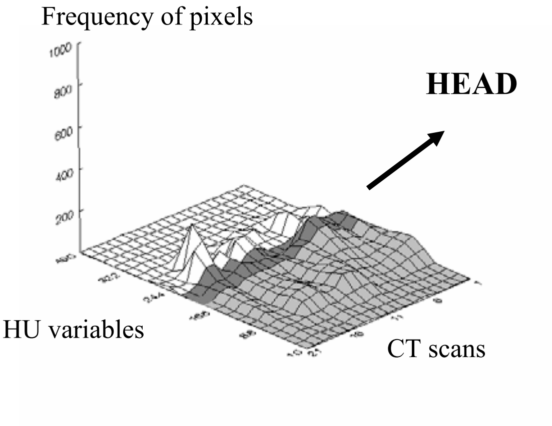


Fig. 7 Changes in body composition between 14 and 16 weeks of age

The tendencies shown by the histograms are very similar to the data recorded at slaughter (*Figs. 8 and 9*).

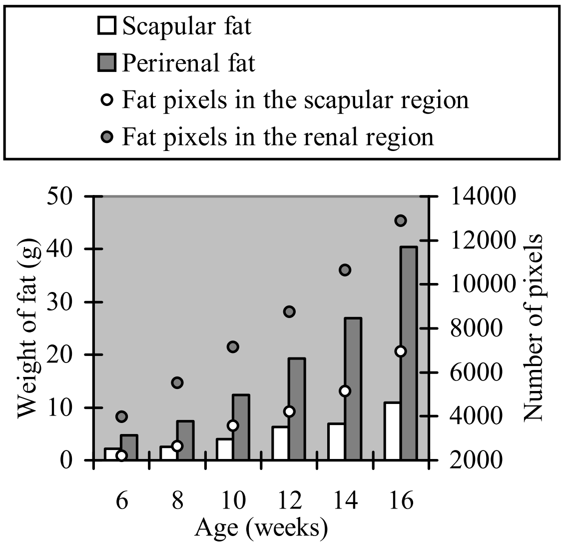


Fig. 8 Changes in the amount of fat between 6 and 16 weeks of age

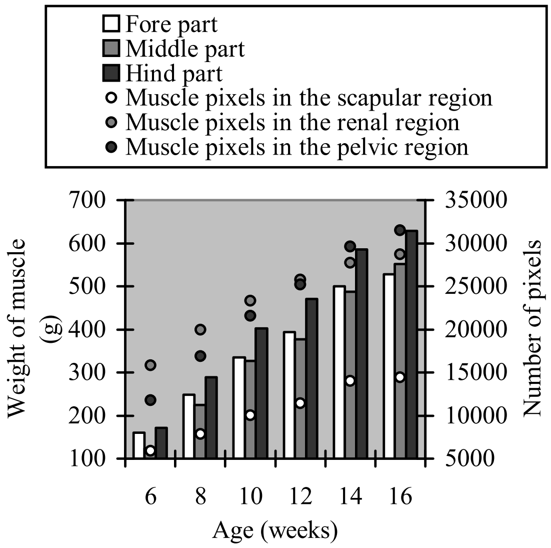


Fig. 9 Changes in the amount of muscle between 6 and 16 weeks of age

The increase in fat deposition seems to be exponential and that of muscle nearly linear in the period examined. The correlation coefficients between the estimated weights and the weights recorded at slaughter are medium or high in the case of fat and muscle (*Table 1*).

Table 1 Correlations between the estimated weights of body parts and the weights recorded at slaughter

Estimated traits	Independent variable in the equations	Correlation coefficients
Scapular fat	Number of fat pixels in the scapular region	0.71
Perirenal fat	Number of fat pixels in the renal region	0.76
Fore part	Number of muscle pixels in the scapular region	0.78
Intermediate part	Number of muscle pixels in the renal region	0.84
Hind part	Number of muscle pixels in the pelvic region	0.98

Due to the exponential deposition of fat the amount of fat estimated more than tripled, while that of the muscle only doubled during the experimental period.

CONCLUSIONS

As a conclusion of this work it can be established that computer tomography is a useful tool for determining changes in the body composition of growing rabbits. By means of this method the same animals could be measured several times in the experimental period and the changes in their fat and muscle content could be measured with good accuracy.

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