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ORIGINAL ARTICLE

Occurrence of periadrenal brown adipose tissue in adult Slovak population

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Abstract

OBJECTIVES: The brown adipose tissue is distributed only in specific locations of the mammalian body. Evidence has been presented that the brown fat occurs in human foetuses and new-borns. At later stages of life, most brown fat cells disappear and only persist in the perirenal and periaortic adipose tissue. However, detailed data on the occurrence of brown adipose tissue in adult humans differ among existing studies.
DESIGN: The aim of this study was to determine the prevalence of brown adipose tissue in adult Slovak population.
SUBJECTS AND METHODS: The samples of periadrenal adipose tissue were taken from 379 consecutive autopsies of subjects aged between 26 and 94 years, fixed in formalin, processed by routine methods and stained with hematoxylin-eosin. The sections were examined by light microscopy.
RESULTS AND CONCLUSIONS: Brown adipose tissue cells were found in 240 (63%) cases. The prevalence of brown adipose tissue decreases below the age of 50 and later slightly increases. No significant differences in the prevalence of brown adipose tissue between men and women were found. With the exception of Germany, this study is currently the first extensive morphological autopsy study of brown adipose tissue prevalence in adults in Central and Eastern Europe.

Abbreviations:

BAT	- brown adipose tissue
PET-CT	- positron-emission tomography – computed tomography
MRI	- magnetic resonance imaging

INTRODUCTION

Multilocular brown adipose tissue (BAT), first described by Welsch in 1670 (as reported in Rasmussen, 1923), is a highly specialized tissue distributed (in contrast to the white adipose tissue) only in a restricted range of locations of a mammalian body (Figure 1). In the body of

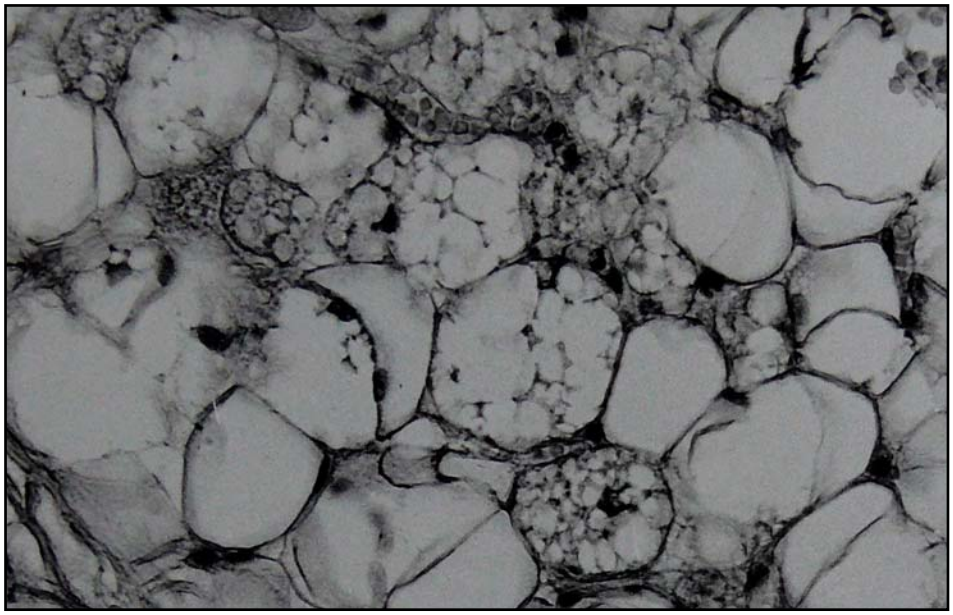

Fig. 1. Brown adipose tissue from periadrenal region of a 76-year-old woman (H&E, 200x).

endotherms, the adipose tissue performs a variety of functions (Arbuthnott, 1989; Cannon & Nedergaard, 2004; Iatropoulos & Williams, 2004; Sethi & Vidal-Puig, 2007). BAT has a thermogenic role and is an important source of cold- and diet-induced non-shivering thermogenesis. The heat production is under autonomic control and is activated in response to an acute drop in ambient temperature or to overfeeding (Smith, 1961; 1962; Himms-Hagen, 1984; Stock, 1989; Bartness & Song, 2005). BAT masses are distributed in all hibernators, rodents, and humans in specific regions of the body, and named according to their locations: cervical, axillary, interscapular, intrathoracic (mediastinal), abdominal (including perirenal and periaortic) and inguinal (Aherne & Hull, 1966; Itoh & Kuroshima, 1967; Mrosowsky & Rawlatt, 1968; Zancanaro *et al.* 1995). Hatai (1902) and several other researchers presented evidence of BAT in human foetuses and newborns. But in humans – in contrast to hibernators – the brown fat cells gradually disappear at later stages of life. According to Heaton (1972) and Nedergaard *et al.* (2007), active brown fat cells persist longest in the abdominal brown fats including the perirenal and periaortic brown fat. The aim of the present study was to determine the occurrence of BAT in periadrenal adipose tissue in adult Slovak population.

SUBJECTS AND METHODS

Subjects and samples

Adipose tissue samples from periadrenal adipose tissue were obtained from 379 consecutive autopsies of adult persons (208 men and 171 women) aged 26 to 94 years (the lower age limit for selection of cases was set to 20 years). The composition of the study sample according to age and gender is shown in Table 1. Samples of adipose tissue were taken from the periadrenal region bilaterally. The incision was made at approx. 45 degrees with respect to aorta perpendicularly down into the tissue in the region of the largest diameter of the adrenal gland following a standard autopsy procedure (Šikl, 1953) (Figure 2). From the adipose tissue adjoining

Tab. 1. Subjects by gender and age category.

Age	Gender		Total
	male	female	
20-39	7	5	12
40-49	18	10	28
50-59	40	20	60
60-69	56	52	108
70-79	66	43	109
80-99	21	41	62
Total	208	171	379

Tab. 2. BAT positivity scale.
The following scale was used to evaluate the presence of BAT in histology samples.

Grade	Histology finding in a sample
0	1 – 10 cells
1	11 to 100 cells
2	Up to 25% BAT cells
3	Up to 50% BAT cells
4	Up to 75% BAT cells
5	Up to 100% BAT cells

BAT – brown adipose tissue

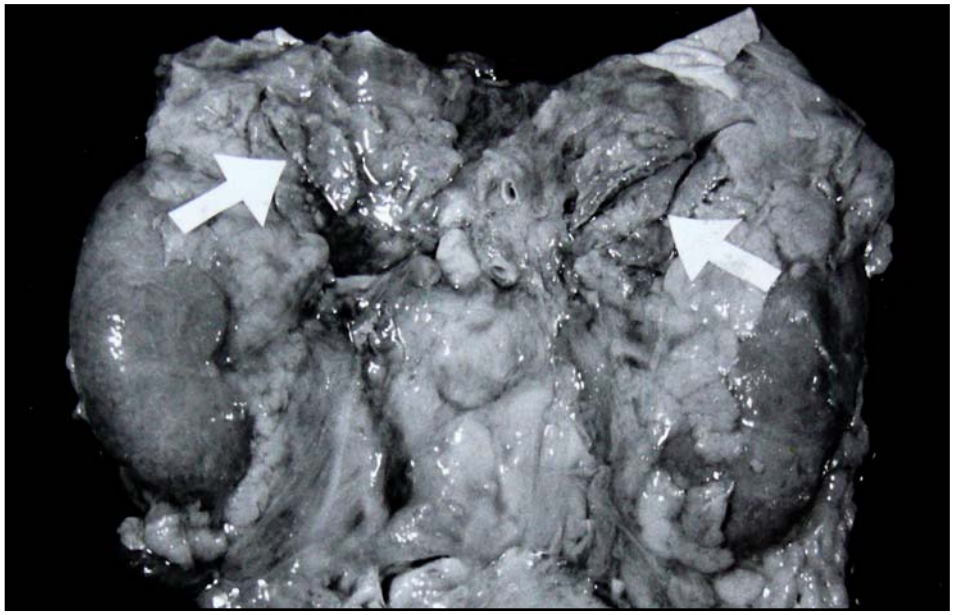

Fig. 2. Locations from where the samples of adipose tissue were taken (arrows).

facies dorsalis of the adrenal gland, a 3–4 mm thick excision was extracted. The excision size was subsequently adjusted to 20 by 17 mm. A narrow strip (up to 2 mm wide) of adrenal cortex tissue was left at the edge of the excision. The resulting dimension of the adipose tissue sample was 20 by 15 mm (Figure 3). Samples were fixed in 10% neutral formalin, processed by standard methods, embedded in paraffin blocks and cut. Sections were stained with hematoxylin-eosin. The slides were examined for the presence of BAT by light microscopy. The amount of BAT in each sample was evaluated semiquantitatively by a method similar to that described by Medeiros *et al.* (1985) using the criteria listed in Table 2. A part of the results presented below is based on reduced data about the presence of BAT (i.e., present / not present): a *BAT positive sample* is a sample with grade higher than 0.

Statistical analysis

The collected data allow us to study the dependence of an ordinal response variable (BAT finding, 0 to 5, cf. Table 2) on two factors – age and gender. To simplify the analysis, patients were divided into age categories based on the decade of their age. The extreme age categories were combined (20–39 and 80 to 99) to partially compensate for the low number of cases in these categories (cf. Table 1).

The effects of age and gender on occurrence of BAT were analysed using three statistical techniques:

a. Independence ($\chi 2$) tests on contingency tables (Zar, 1999). Resampling-based p-values are reported for tables with small counts for some factor combinations.

b. (Binomial) logistic regression to fit a linear model to observed log-odds for reduced (positive/negative) response (Agresti, 2002).

c. Ordinal logistic regression to fit a linear model to cumulative log-odds for the complete ordinal response (Agresti, 2002).

The calculations were carried out using the Microsoft Excel spreadsheet and the R statistical environment (R Development Team, 2009).

RESULTS

The study data are summarized in Table 3. The table lists percentages of specific BAT findings by age category and gender, together with the total number of cases for individual age group/gender combinations. The data of

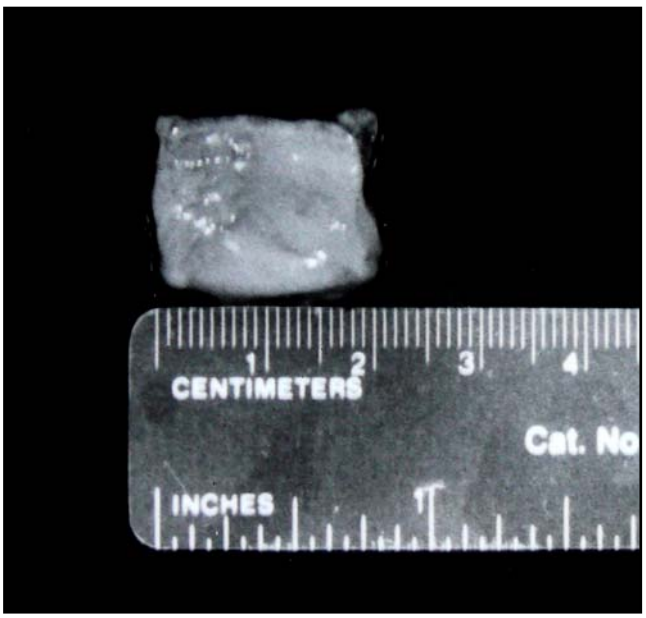

Fig. 3. Size of adapted samples for processing for microscopic examination.

Tab. 3. BAT findings by age and gender.
For BAT finding codes, cf. Table 2. N denotes the number of subjects in the respective category.

Age	Gender	BAT finding					N
		0	1	2	3	4	
20-39	male	0%	14%	0%	71%	14%	7
	female	20%	20%	20%	20%	20%	5
	all	8%	17%	8%	50%	17%	12
40-49	male	56%	6%	17%	11%	11%	18
	female	30%	20%	20%	20%	10%	10
	all	46%	11%	18%	14%	11%	28
50-59	male	38%	13%	20%	30%	0%	40
	female	45%	20%	15%	20%	0%	20
	all	40%	15%	18%	27%	0%	60
60-69	male	45%	13%	18%	18%	7%	56
	female	35%	15%	15%	33%	2%	52
	all	40%	14%	17%	25%	5%	108
70-79	male	38%	12%	30%	14%	6%	66
	female	35%	16%	21%	28%	0%	43
	all	37%	14%	27%	19%	4%	109
80-99	male	33%	14%	29%	24%	0%	21
	female	27%	5%	29%	29%	10%	41
	all	29%	8%	29%	27%	6%	62
Total	all	37%	13%	22%	24%	5%	379

BAT – brown adipose tissue

Table 3 are visualized in the three bar charts of Figure 4. Figure 5 shows a subset of the dataset – frequencies of positive BAT findings (that is, BAT findings different from 0) for individual age group/gender combinations. This plot also shows standard errors of frequency estimates. Overall, BAT was found in 240 of the total 379 cases, which gives a frequency estimate of (63.3±2.5) %. The frequency of positive findings is slightly higher in women than in men, (66.7±3.3) % vs. (60.6±3.4) %. The difference is well within the error bars of the common mean frequency, and is statistically insignificant (p=0.22 for independence test on the *BAT finding* × *gender* contingency table). Similarly, the difference between age categories is insignificant by independence test on the *BAT finding* × *age category* contingency table (p=0.13). However, independence test on the full contingency table *(BAT finding* × *age category* × *gender)* is significant ($\chi 2$=71.58 with 49 d.f. gives p=0.02). As seen in Figures 4 and 5, the most remarkable differences between men and women is in the two youngest age categories. Statistically, these are marginally significant (p=0.052 for age × gender interaction coefficient in the logistic and ordinal logistic fit to subset of subjects with age <50 years). Figures 4 and 5 indicate that the occurrence of BAT decreases between 20–49 years of age; and then slightly increases at higher ages. Statistically, our data only marginally confirm the slight increase, p=0.058 from logistic regression on the subset of subjects with age over 40. The decrease of BAT between the first and second age categories is statistically significant in men (p=0.02 by independence test on the corresponding contingency table, with resampling-based P, and similarly by logistic regression), but not in women (p=0.83). The decrease is also significant for genders combined (p=0.03). Figures 4 A, B indicate, that the decrease of BAT occurrence at ages between 20 and 50 is steeper in men than in women: it seems that occurrence of BAT reaches its minimum in men in the 5th decade, and in women in the 6th decade. However, this observation is hard to prove statistically with current data. Evidently, there is insufficient statistical power in the youngest age categories (there are only 40 cases – 25 men and 15 women – in the first and second age categories), where we see the most remarkable features in the data. This is an unavoidable drawback of an autopsy study, which has the power strongly concentrated at the ages close to the median lifespan. We have used ordinal logistic regression to get a complete description of the data in Figure 4. The proportional cumulative odds model (Agresti, 2002) fits the data well (p=0.33), but otherwise just confirms the observations already obtained by other methods (no overall difference between men and

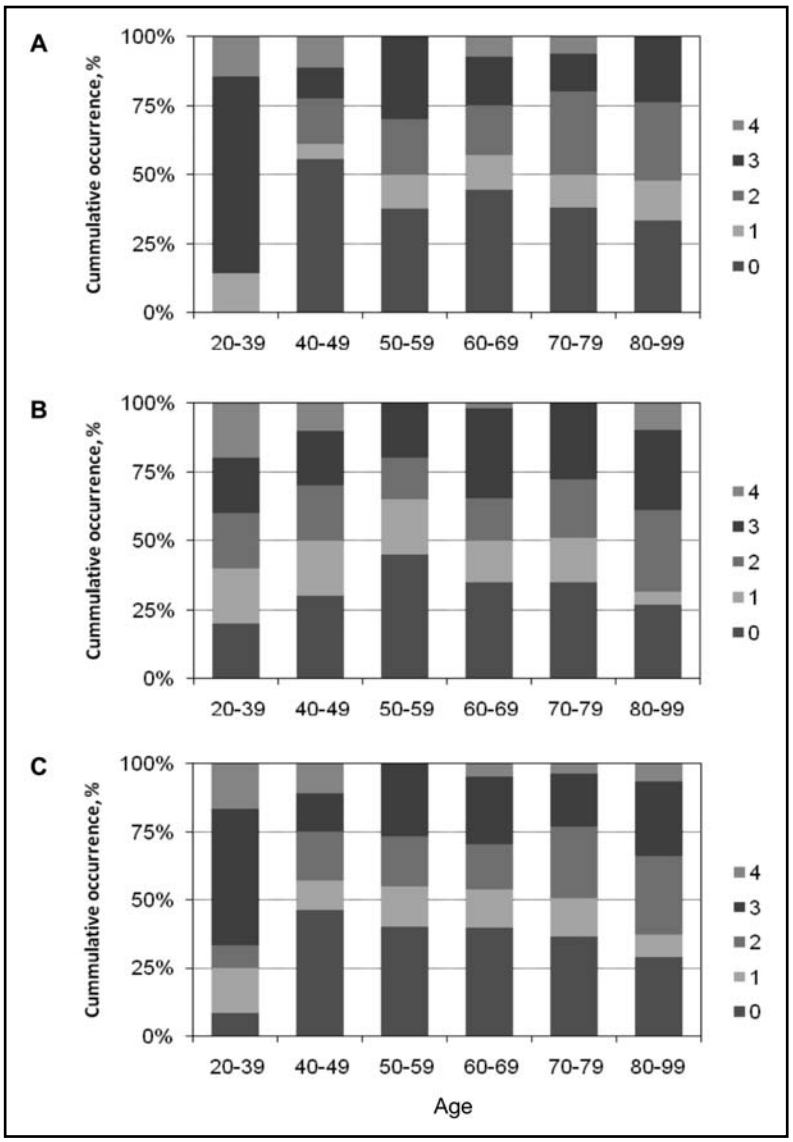

Fig. 4. BAT findings by age and gender. The bar graphs show distribution of individual BAT findings (indicated by shades of grey, see legends on the right) for individual age categories in men (A), women (B), and in total (C).

women and a significant difference between the first and second age category in men), so the results are not shown here, as they do not offer new insights into the study results.

DISCUSSION AND CONCLUSIONS

The presence and role of BAT in humans has been studied for decades. As regards BAT, human new-borns do not differ from new-borns of other mammals. The brown adipose tissue was found in perinatal period in all mammals (Aherne & Hull, 1966; Merklin, 1974; Cannon & Johanson, 1980; Zancanaro *et al.* 1995; Cannon & Nedergaard, 2004; Brucknerova & Ujhazy, 2016). BAT is widely present in human body during the first years of life (Hatai, 1902; Aherne & Hull, 1966; Itoh & Kuroshima, 1967; Heaton, 1972). It has been suggested that this is related to the immature thermoregulation system in this age (Heaton, 1972). With increasing age, the amount of BAT in the body decreases. This study was intended to find out whether adults keep the ability to develop BAT. It is reasonable to expect that the respective genetic material is retained at older age. Some authors try, similar to what we do, to morphologically determine BAT in healthy humans. According to such studies, BAT can be found in autopsy of adults of various ages. Heaton (1972) reports an increase in BAT starting at the 6th decade; this may be related to the decrease in basal metabolism that occurs at this age (Wright & Samson, 1965). Heaton (1972) also reports a decrease in BAT starting in the 8th decade. This may be related to the high risk of hypothermia at this stage of life. Several studies reported ethnical differences in BAT occurrence: BAT cells were detected in 75% of Japanese (Tanuma *et al.* 1975), in 100% of Irish (Heaton, 1972), in 35% of Ger-

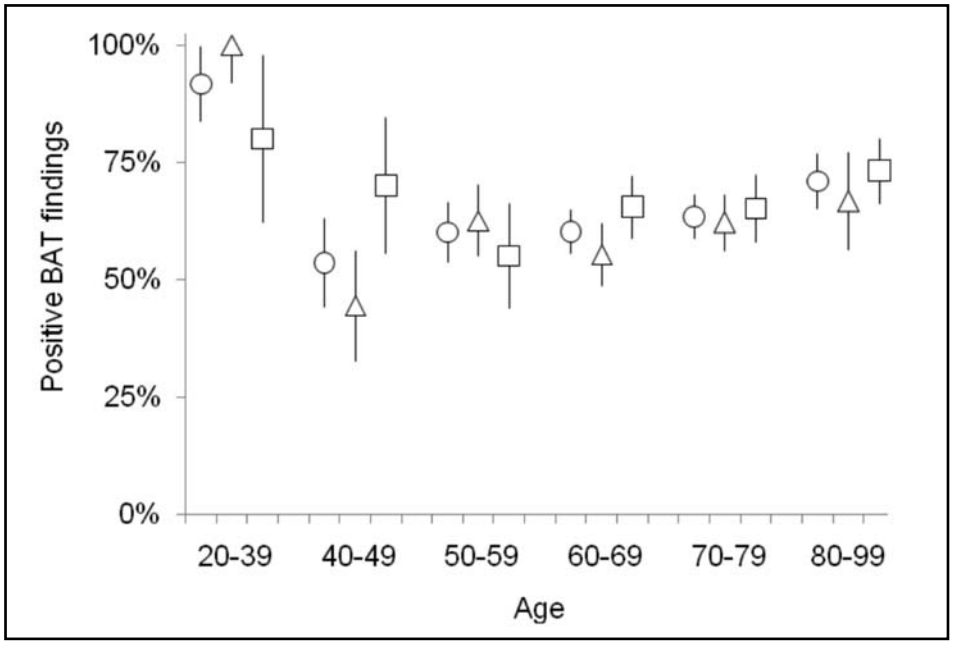

Fig. 4. Positive findings of BAT (i.e., all except „0") in subjects according to gender and age. Circles – both genders, triangles – men, squares – women. Error bars indicate standard deviations.

mans (Feyrter, 1973), in 10% of Finns (Hassi, 1977), in 51% of Californians (Medeiros *et al.* 1985) and in 24% of Brazilians (Santos *et al.* 1992). In our study, we found BAT in 63.3 % of autopsies. We observed a decrease in BAT occurrence at the youngest age categories (20 to 49 years). The amount of BAT reached its minimum in the 5th decade in men and in the 6th decade in women. Afterwards, the BAT levels slightly increase. The results of our study suffer from the fact that it was the autopsy study, so we had little cases in the most interesting age groups. A new era of BAT research started in recent years with the development of radiology methods, especially the positron-emission tomography – computed tomography (PET-CT), able to visualize BAT *in vivo* (Hadi *et al.* 2007; Goetze *et al.* 2008; Seale & Lazar, 2009; Virtanen, 2009). PET-CT imaging revealed active BAT in adult humans in the supraclavicular area, the *retroperitoneum*, and along the aorta, with the main depot in the supraclavicular area (Saito *et al.* 2009). In these studies, BAT was discovered in 33% of adults by Japanese authors (Saito *et al.* 2009), in 96% cases by Dutch authors (Lichtenbelt *et al.* 2009), and in 5.4% of cases (3.1% of men and 7.5% in women) by Israeli authors (Cypess *et al.* 2009). Lee *et al.* (2010; 2011) suggest the prevalence of BAT of 64% in the adult Australian population. Gifford *et al.* (2016) used parallel examination with PET-CT and MRI methods for the detection of BAT in adults. It has been shown that MRI is an appropriate method to investigate the presence of BAT in living people, even in cases where the brown adipose tissue is inactive, i. e., also in cases where a PET-CT examination is negative. In addition, MRI examination does not damage the organism with ionizing radiation.

With the exception of Germany, this study is currently the first extensive morphological autopsy study of brown adipose tissue prevalence in adults in Central and Eastern Europe. The use of PET-CT and/or MRI in combination with morphological (biopsy or autopsy) examinations opens, after many years, a new perspective for more extensive comparative clinical-morphological studies.

DECLARATIONS OF INTEREST

None.

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