Immunohistochemical Identification And Quantification Of Brown Adipocytes In Human Adipose Tissue.

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ABSTRACT

Background: Obesity is a worldwide challenge and is closely connected to many metabolic diseases. Two types of adipose tissue, white adipose tissue (WAT) and brown adipose tissue (BAT) have been identified. White fat cells store chemical energy, brown adipocytes defend against hypothermia, obesity and diabetes.

Objective: To localize and quantify brown adipocytes in human subcutaneous (S) and visceral (V) adipose tissue by histology and immunohistochemistry.

Type of the study: A cross -sectional study.

Methods: Adipose tissue was obtained from histopathology specimens taken from ten patients, of different age, sex and body mass index (BMI), undergoing surgery for different pathologies. Immunohistochemistry for detection of UCP (Uncoupling Protein) in S and V adipose tissue depots was performed, and percentage of the positive pixels for UCP color intensity was measured and statistical analysis performed.data was expressed as mean ± standard deviation (SD) and analyzed using student t-test to compare values

Results: Brown adipocytes with typicalmultivesicular appearance were detected as clusters of cells among white fat in both S and V adipose tissue. The percentage of the positive pixels for UCP color intensity of brown adipocytes was significantly ($P < 0.001$ and $P < 0.05$) higher in S than in V adipose tissue, significantly higher levels ($P < 0.05$) when BMI was 30 or more, and non-significant higher levels ($P > 0.05$) in females than males.

Conclusion: Brown a dipocytes are more abundant in S than V adipose tissue, and have a positive correlation to BMI.

Keywords: human brown adipocytes, immunohistochemistry, UCP.

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Obesity is a worldwide challenge and not unique to anyone country. Furthermore, obesity is closely connectedto many metabolic diseases. Essentially, obesity and overweightare caused by the energy imbalance between thecalories consumed and calories expended.

Adipose tissue,which iscomposed mostly of adipocytes, is a major endocrineorgan and plays a key role in energy homeostasis.Two types of adipose tissue, white adipose tissue (WAT)and brown adipose tissue (BAT), have been identified (1)

While white fat cells are specialized to store chemical energy, brown adipocytes defend mammals against hypothermia, obesity and diabetes. Brown fat utilizes a high mitochondrial content and high mitochondrial uncoupling protein one (UCP1) to uncouple respiration and dissipate chemical energy as heat. Rodents and other small mammals have copious brown fat deposits, but larger mammals often lose prominent brown fat depots after infancy. (2, 3, 4, 5, 6)

The physiological significance of adult human brown fat has not yet been fullyexplored. It has been known for many years that some white adipose tissues contain cells that can express high levels of UCP1 and take on a multilocular appearance upon prolonged stimulation by cold or pathways that elevate intracellular cyclic AMP. (7, 8)

In humans and other large mammalian species, BAT was traditionally thought to be restricted to the neonatal and early childhood periods (9, 10). However, positron emission tomography (PET)scanning technology was recently adopted for detecting metabolically active sites for oncology diagnosis; this application is based on the uptake of radio labeled non-metabolizable glucose derivatives. The results obtained from a scanning experiment using PET to analyze BAT clearly demonstrated that active BAT is present in adult humans at discrete anatomical sites, especially in the upper trunk, such as cervical, supraclavicular, paravertebral, pericardial, and to some extent, mediastinal and mesenteric areas. (2, 5, 11, 12)

Recently, a new type of brown-like adipocyte was discovered that shows distinct gene expression patterns from those of white or brown adipocytes. These novelbrown-like cells that reside within WAT, especially inguinal WAT, were termed beige/brite adipocytes. (13)

Using Heaton's definitionof BAT as fat containing multilocular adipocytes stainedby hematoxylin-eosin on

light microscopy (14)BAT was designated as being visceral (V) or subcutaneous (S), subdividingeach category into separate depots accordingto their contiguous organ or tissue. (11, 15) In healthy lean male and female adults, age40 ± 9 years, mean weight of cold-activated human BATcalculated by PET-CT was 34 g (range 9-90) (4). Based on the calculation of 63 g cervical BAT reported in a differentstudy by Virtanen et al. (12) it was estimated thatactivated BAT thermogenesis contributed 4.5% to wholebodyenergy expenditure, which was considered to be significant (16) and commensurate with the goal of exploitingBAT to burn excess calories stored in WAT for weight lossin obesity and type 2 diabetes (15, 17). The 5% figure citedabove could be an underestimate by a factor of two to threein some subjects because of their greater BAT mass.

More extensive analysis was necessary in human BAT studies, thus we performed this work to localize and quantify brown adipocytes in human adipose tissue by histology and immunohistochemistry.

Methods: Adipose tissue was obtained from histopathology specimens taken from patients undergoing surgery for trauma to chest or abdomen in Al Kindy Teaching Hospital in Baghdad. These patients were ten in number and of different age, sex and body mass index (BMI). Study protocol was approved by scientific and ethical committee in Alkindy College of Medicine.

Immunohistochemistry: for detection of UCP in adipose tissue, 4µm paraffin sections were mounted on Fisher brand positively charged slides (18). Actual assessment of immunohistochemistry was performed by image analysis of tissue sections using Aperio image scope v11.1.2.760 software program and positive pixel count algorithms were used to quantify the amount of a specific color in a slide image. This algorithm has a set of default input parameters when first selected. These inputs were pre- configured for brown color quantification in the three intensity ranges (weak, positive, and strong).

Immunohistochemistry staining in adipose tissue sections were quantified as the percentage of the positive pixels for UCP color intensity. The data was expressed as mean ± standard deviation (SD) and analyzed using student t-test to compare values.

Differences between groups were considered highly significant at ($P < 0.001$), significant at ($P< 0.05$) or non-significant at ($P > 0.05$).

Results : Brown adipocytes were detected as clusters of cells among white fat cells (Figure 2). They were found in both subcutaneous and visceral adipose tissue that was collected from different body regions (Figure a, Figure b). At a higher magnification, the brown adipocytes showed the typical appearance of multi vesicle - filled cytoplasm (Figure 3). The percentage of the positive pixels for UCP color intensity of brown adipocytes detected by immunohistochemistry staining in adipose tissue sections showed that there was significant higher levels in subcutaneous than in visceral adipose tissue regions (Table 1). The percentage of positive pixels for brown adipocytes in adipose tissue with respect to BMI showed significantly higher levels ($P < 0.05$) when BMI was 30 or more (Table 2). Concerning gender, there was a non-significant higher levelin the percentage of positive pixels for brown adipocytes in females than males ($P > 0.05$) (Table-3).

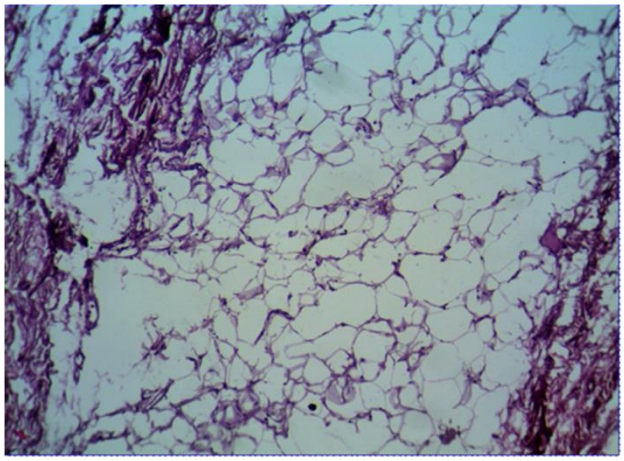

Figure 1- Subcutaneous adipose tissue. Hematoxylin and Eosin, 100x.

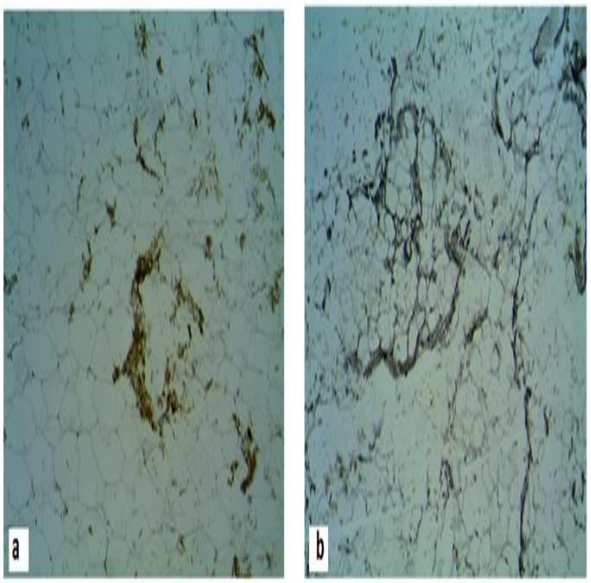

Figure 2- Immunohistochemistry for UCP identification in brown adipocytes. a. subcutaneous adipose tissue b. visceral adipose tissue. Staining by peroxidase/DAB (brown).100x.

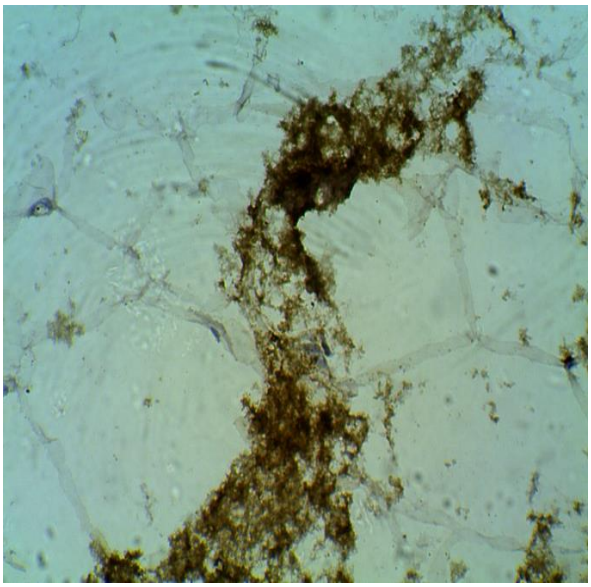

Figure 3- Immunohistochemistry for UCP identification in brown adipocytes (sucutanous) showing multilocular appearance. Staining by peroxidase/DAB (brown).400x.

	S Brown adipocytes Mean ± SD	V Brown adipocytes Mean ± SD	P values
s1 vs v1	31.97 ± 3.05	21.25 ± 2.84	0.004
s2 vs v2	25.77 ± 2.80	17.84 ± 2.49	0.008
s3 vs v3	25.64 ± 9.17	24.5 ± 14.1	0.896
s4 vs v4	20.86 ± 8.12	5.330 ± 0.618	0.032
s5 vs v5	36.62 ± 7.95	43.70 ± 5.17	0.196
s6 vs v6	24.73 ± 1.06	5.8315 ± 0.0721	0.000
s7 vs v7	44.6 ± 15.7	11.91 ± 6.20	0.030
s8 vs v8	58.52 ± 9.03	11.91 ± 6.20	0.000
s9 vs v9	33.70 ± 2.26	37.1 ± 36.7	0.864

Table-1: Percentage of positive pixels for brown adipocytes in subcutaneous (S) versus visceral (V) adipose tissue. (No. 1,2,3..etc. referred to the no. of patient)

(P < 0.001): highly significant
(P < 0.05): significant
(P > 0.05): non- significant

BMI <30	BMI ≥30	
Brown adipocytes Mean ± SD	Brown adipocytes Mean ± SD	P value
20.50 ± 9.05	29.6 ± 18.4	0.005

Table-2: Percentage of positive pixels for brown adipocytes in adipose tissue with respect to BMI.
(P < 0.05): significant

Male	Female	
Brown adipocytes Mean ± SD	Brown adipocytes Mean ± SD	P value
22.7 ± 14.8	28.8 ± 17.6	0.124

Table-3: Percentage of positive pixels for brown adipocytes in adipose tissue with respect to gender.
(P > 0.05): non- significant

Discussion : The ability of brown fat to suppress obesity through increased energy expenditure has caused an explosion of interest in the development and function of brown adipocytes (17). With the use of different techniques, many studies have been performed to identify and localize brown adipose tissue (2, 5, 11 and 12), and brown adipocytes within white adipose tissue (13), however non has aimed to quantify the number and distribution of brown adipocytes among white fat cells using immunohistochemical technique in human adipose tissue depots. At present all known adipose-specific genes are expressed in both white and brown adipocytes (adipocyte lipid binding protein, adipsin, etc.) or in brown adipocytes only (UCP), with none being expressed exclusively in white adipocytes (20, 21, 22 and 23). Thus the demonstration of brown color intensity using immunohistochemistry for UCP would indicate brown adipocytes.

Our finding that there is significant difference in number of brown adipocytes, reflected by the percent of positive pixels of brown color, in subcutaneous and visceral adipose tissues, being more in the subcutaneous region may be due to the presence of a greater source of precursor cells for brown adipocytes in the subcutaneous region which are myf-5, muscle-like cellular lineage (24); or from transdifferentiation from mature white adipocytes into beige (brite) adipocytes (25) or may be due to the presence of distinct genetic loci that control the amounts of UCP1-positive cells in the white and classical brown fat depots (24, 26, 27, 28 and 29) and that these loci are present more in the subcutaneous adipose tissue depots (30). We found a positive relation between the quantity of brown adipocytes and BMI. This result is in contrast to what has been found by others who found a negative correlation between body fat and brown adipose tissue (31, 32). However, all these studies were correlating brown adipose tissue and different functional parameters as glucose metabolism (31), exposure to cold, age, sex and lipid profile (32). Our results showed non- significant higher levels of brown adipocytes in females than males. This finding was similar to what has been found in one study (32), where there was a significant sexual difference (5.5% in the females vs 1.3% in the males; P<0.000).

Conclusion

Brown and browning Adipose tissue are more abundant in subcutaneous than visceral adipose tissue and there is positive correlation between brown adipocytes and body mass index.

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