

## Research Article

### Effect of Curcumin on the Plasticity of Inguinal Adipose Tissue in Experimental Model of Hypothyroidism in Rat: Histological Study

Bosy Ahmed Abdelaziz, Walaa Baher\*, Manal Shaaban Hafez, Faika Hassan El Ebiary

Department of Histology and Cell Biology, Faculty of Medicine, Ain Shams University, Egypt.

\*Corresponding author: Walaa Baher, Department of Histology & Cell Biology, Faculty of Medicine, Ain Shams University, Cairo, Egypt. Tel: +201002050563; Email: walaa\_baher@med.asu.edu.eg

**Citation:** Abdelaziz BA, Baher W, Hafez MS, El Ebiary FH (2019) Effect of Curcumin on the Plasticity of Inguinal Adipose Tissue in Experimental Model of Hypothyroidism in Rat: Histological Study. Cytol Histol Rep 2: 112. DOI: 10.29011/2688-6421.1000012

**Received Date:** 16 September 2019; **Accepted Date:** 25 September 2019; **Published Date:** 30 September, 2019

#### Abstract

**Background and objectives:** Obesity is closely related to hypothyroidism. Mild thyroid dysfunction is linked to significant changes in body weight. Curcumin is regarded as a preventive agent against obesity.

This study aimed to explore the effect of curcumin on the structure of inguinal adipose tissue in Propylthiouracil (PTU) induced hypothyroid rat model.

**Materials and methods:** Thirty-six male Wistar rats were divided into three groups: Group I (Control, n=18): equally subdivided into: Subgroup Ia (Euthyroid), Subgroup Ib (Euthyroid- corn oil): received 1ml of corn oil and sacrificed after 2 and 6 weeks. Subgroup Ic (Euthyroid- curcumin): received 1ml of corn oil for 2 weeks followed by 1ml of curcumin solution for another 6 weeks. Group II (PTU- hypothyroid, n=12): equally subdivided into: Subgroup IIa (PTU): received daily 5 mg PTU in corn oil for 2 weeks and Subgroup IIb (Recovery): received daily 5 mg PTU in corn oil for 2 weeks then left for recovery for 6 weeks. Group III (hypothyroid- curcumin): received 5 ml of PTU for 2 weeks followed by 1ml of Curcumin for 6 weeks. At sacrifice, the inguinal adipose tissue was dissected and processed for different histological, ultrastructure, immunohistochemically and morphometric analyses.

**Results:** Areas of brown like adipocytes were detected among white adipocytes in Groups Ic, IIB and III. corrugated adipocytes' cell membrane was evident in subgroup IIa. Densely stained collagen appeared around the cell clusters in subgroup IIB. Findings were further confirmed by statistical analysis of the morphometric results.

**Conclusion:** Curcumin resulted in the appearance of brown like adipocytes in between WAT in the inguinal region.

**Keywords:** Beige cell; BAT; Curcumin; PTU; WAT

#### Introduction

Obesity and hypothyroidism are two common clinical conditions that have been linked together closely. Obesity is an accelerating worldwide health crisis associated with co-morbidities that include diabetes, hyperlipidemia, and hypertension. Moreover, clinical evidence suggested that mild thyroid dysfunction even in the form of subclinical hypothyroidism is linked to significant changes in body weight and represents a risk factor for overweight and obesity [1]. Propylthiouracil (PTU) is a thionamide anti-thyroid drug, which is the mainstay of pharmacologic treatment of Graves' disease. It acts primarily by inhibiting thyroid hormone synthesis through interference with the organic binding of iodide

into thyroglobulin, as well as through inhibiting the peripheral conversion of  $T_4$  to  $T_3$  [2]. Hypothyroidism induce oxidative stress in different tissues [3]. On the other hand, thyroxine administration was found to increase the oxidative stress, therefore supplementation of exogenous antioxidant may be a preferable therapeutic agent against hypothyroid-induced oxidative stress [4]. Curcumin is a naturally occurring curcuminoid of turmeric, which is a member of the ginger family [5]. It has an antioxidant, anti-inflammatory, anti-microbial, and anti-carcinogenic activities as well as, hypoglycemic and anti-rheumatic properties. Moreover, curcumin has been shown in various animal models and human studies to be extremely safe even at very high doses [6]. The objective of this study was to detect the effect of curcumin on the structure of inguinal adipose tissue of the hypothyroid rat model induced by PTU.

## Materials and Methods

### Drugs

**Propylthiouracil:** Thyrocil<sup>®</sup>, was purchased from Amoun Pharmaceutical Co, Egypt as 50 mg tablets. The drug was given to the experimental animal at a daily dose of 5mg per rat [7].

**Curcumin:** Curcumin crystalline (C.I.No.75300), was purchased from El -Goumhoria Co for trading medicines, chemicals and medical appliances, Egypt. It appeared as yellowish powder in 100 gm package. It was given at a daily dose 100 mg/kg [8].

### Animal groups

The study included thirty-six, 7 weeks old Wistar male rats - weighting 70- 80 gms- purchased and housed in MASRI Center, Faculty of medicine, Ain Shams University. All animal procedures were performed in accordance with the general guidelines for the care and use of laboratory animals and approved by the animal ethical committee at Faculty of Medicine, Ain Shams University. The animals were housed in groups of six in galvanized iron cages with mesh wire covers at room temperature and were allowed free access to water and food ad libitum. The animals were exposed to 12 hours of artificial light and 12 hours of darkness per day.

The animals were divided into three main groups:

**Group I:** (n=18) subdivided into three equal subgroups:

Subgroup Ia (Euthyroid): (n=6), half of the rats were concurrently sacrificed with other experimental groups after 2 weeks and the other half, after further 6 weeks) to obtain inguinal adipose tissue specimens from euthyroid rats.

Subgroup Ib (Euthyroid- corn oil): (n=6) animals were given 1ml of corn oil by gastric tube. Then, three rats were sacrificed concurrently with experimental groups after 2 weeks and the other three rats after further 6 weeks.

Subgroup Ic (Euthyroid- curcumin): (n=6) rats, animals were given 1ml of corn oil for 2 weeks through gastric tube. Then, after 24 hours of the last dose of corn oil, 1ml of curcumin solution (100 mg of curcumin dissolved in 10 ml of corn oil) was given through gastric tube, for another 6 weeks. Then, sacrificed 24 hours after the last dose of curcumin solution (i.e. eight weeks from the beginning of experiment).

**Group II (PTU- hypothyroid):** (n=12), divided into two equal subgroups:

Subgroup IIa (PTU): (n= 6), each rat was given PTU dissolved in corn oil in dose of 5 mg per day. The dose was prepared such that, each 50 mg tablet of thyrocil<sup>®</sup> was dissolved in 10 ml of corn oil. Such preparation ensured that each 1 ml of the solution contains 5 mg of the drug (5 mg/ml). Each rat received 1ml of solution for 2 weeks through gastric tube, then sacrificed 24 hours after the last dose.

Subgroup IIb (Recovery): (n= 6), each rat was given PTU dissolved in corn oil in dose of 5 mg per day for two weeks through gastric tube, then left for spontaneous recovery for another 6 weeks, before sacrifice.

**Group III (hypothyroid- curcumin):** (n= 6) rats, each rat was given PTU as the previous group for 2 weeks followed by daily doses of 1ml of curcumin solution for another 6 weeks. Then, sacrificed 24 hours after the last dose of curcumin (i.e. eight weeks from the beginning of experiment).

### Histological studies

At the end of the experiment, all animals were sacrificed, the inguinal adipose tissue was dissected out, washed and divided into 2 parts. One part was fixed in 10% formol saline, then; dehydrated, cleared, embedded in paraffin and cut into serial sections of 5  $\mu$ m thickness. Sections were stained with different histological stains (H&E and Masson's trichrome) [9], and immune-histochemical avidine-biotin peroxidase technique for detection of Caspase-3) [10]. The other part was fixed in 2.5% formol buffered glutaraldehyde solution overnight, post fixed for 2 h in 2% osmium tetroxide and processed to epoxy resin blocks. Semithin sections of 1  $\mu$ m thick were stained with toluidine blue. Ultrathin sections of 50-60 nm were prepared and stained with uranyl acetate and lead citrate [11].

### Serological tests

Serum levels of TSH, free T<sub>3</sub>, and T<sub>4</sub> were measured in all animal groups at the time of sacrifice. Blood samples were collected in the mornings (9:00-10:30 am) after overnight deprivation of food. The blood was collected from the orbital sinus of rats under ether anesthesia. The measured values were expressed as mean  $\pm$  SD and subjected to statistical analysis.

### Morphometric measurements

The following parameters were measured in randomly chosen five fields/section, in five sections for every rat in each subgroup, at a constant magnification for each parameter.

- The perimeter (circumference)of the adipocytes in H&E stained sections using 20X objective lens. Morphometric measurements were done manually by circumscribing well defined fat cells in the microscopic field.
- The area percentage of collagen fibers in adipose tissue in Masson's trichrome stained sections using 20X objective lens.
- The area field percentage of positive reaction to caspase-3 in immunohistochemically stained sections using 20X objective lens.

All measurements were performed using Leica microscope DM 2500 connected to a camera (Leica DFC 295) and Leica Q win V3

image analysis software (Leica microsystems, Germany) at image analysis unit, Histology and Cell Biology Department, Faculty of Medicine, Ain Shams University.

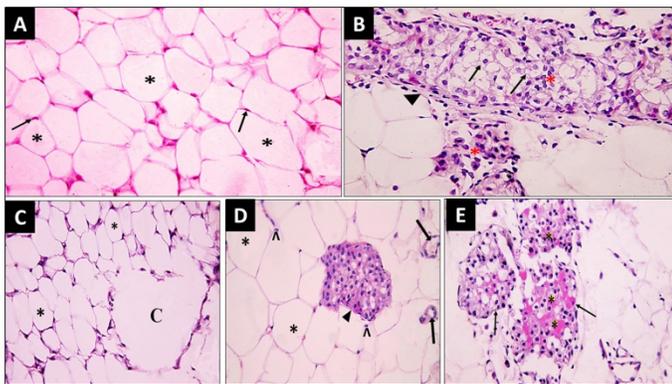
### Statistical analysis

All values of the morphometric results were expressed as mean  $\pm$  Standard Deviation (SD). Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS), software program, version 20 (IBM Corporation, Armonk, New York, USA). Statistical difference among groups was determined using two-way Analysis of Variance (ANOVA) followed by post hoc Least Significance Difference (LSD) for comparison between more than two groups. P values  $< 0.05$  were considered statistically.

### Results

Examination of adipose tissue sections by light and electron microscopes, revealed almost similar results in both subgroups Ia (euthyroid) and Ib (euthyroid - corn oil). Both groups are referred as control group in the results and discussion sections.

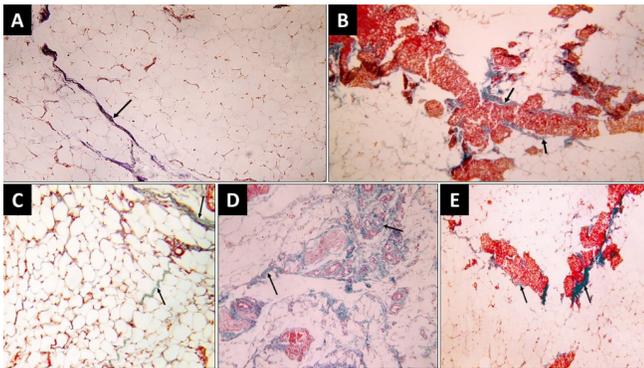
#### Light microscopic results



**Figure 1:** A photomicrograph of inguinal adipose tissue sections showing; (A): Control group: variable sized polygonal unilocular adipocytes having clear cell boundaries (\*). The cytoplasm contains single large unstained vacuoles with compressed flat nuclei at the periphery of cells giving them "signet ring appearance" ( $\uparrow$ ). (B): Euthyroid-curcumin group: A large Cluster of cells surrounded by connective tissue septa ( $\blacktriangle$ ). These cells have many small vacuoles in the cytoplasm and rounded nuclei ( $\uparrow$ ). Many nuclei of connective tissue cells are noticed in-between them (\*). (C): PTU group: group of unilocular adipocytes which are variable sizes have corrugated cell membranes (\*). Notice coalescence of adjacent adipocytes (C). (D): Hypothyroid-recovery group: group of cells ( $\blacktriangle$ ) have acidophilic cytoplasm and rounded nuclei. Their cytoplasm contain many small vacuoles variable in size. The surrounding adipocytes are unilocular fat cells having single large unstained cytoplasmic vacuole and compressed flat nucleus at the periphery of cells (\*). Notice nearby blood capillaries (thick arrow) and nuclei of connective tissue cells ( $\wedge$ ). (E): Hypothyroid-curcumin: multiple scattered small clusters of vacuolated cells ( $\uparrow$ ) as well as acidophilic cells (\*). (H&E X400).

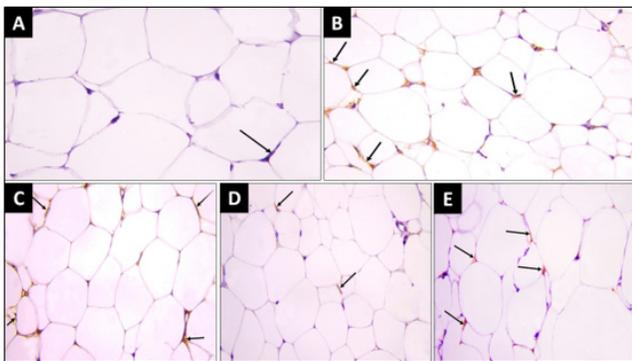
Examination of H&E stained sections of inguinal adipose tissue of rats of the control group revealed that it was formed of white adipose tissue. It contained polygonal adipocytes of variable sizes with well-defined cell boundaries. The adipocytes were of the unilocular type, each cell contained a single large unstained vacuole occupying most of the cytoplasm and sparing a thin rim of peripheral cytoplasm. The nucleus compressed peripherally against the cell membrane giving signet ring appearance. In-between the adipocytes, there were few nuclei of connective tissue cells and blood capillaries (Figure 1A). In Subgroup Ic (euthyroid-curcumin), inguinal adipose tissue was formed mainly of unilocular type with characteristics signet ring appearance. In addition to large cluster of cells which had multiple small vacuoles and rounded vesicular nuclei in the cytoplasm. This cluster was surrounded with connective tissue. Many nuclei of the connective tissue cells in-between were also noticed (Figure 1B). In subgroup IIa (PTU group) most of the inguinal adipose tissue was formed of unilocular adipocytes in addition to formation of large spaces of fusion of adjacent adipocytes. Inguinal adipose tissue was formed of polygonal fat cells of variable size and distinct cell boundaries. Each cell had a unilocular large unstained vacuole with compressed flat nucleus at the periphery of cell giving signet ring appearance. The cell membranes of most adipocytes were corrugated (Figure 1C). In subgroup IIb (hypothyroid-recovery group) group of cells with acidophilic vacuolated cytoplasm and rounded nuclei. The surrounding adipocytes are unilocular fat cells having single large unstained cytoplasmic vacuole and compressed flat nucleus at the periphery of cells (Figure 1D). In Group III (hypothyroid-curcumin) multiple scattered small clusters of vacuolated cells were present. These cells in clusters showed multiple small vacuoles and rounded nuclei. In between these cells, polygonal cells with acidophilic cytoplasm and rounded nuclei were detected (Figure 1E).

In Masson's trichrome stained sections, control group showed fine collagen fibers forming thin connective tissue septa that separated groups of adipocytes into adipose tissue lobules (Figure 2A). In subgroup Ic (euthyroid-curcumin), scattered collagen fibers were seen surrounding the cluster of cells (Figure 2B). In subgroup IIa (PTU group), fine collagen bundles appeared, separating groups of adipocytes into adipose tissue lobules (Figure 2C). In subgroup IIb (hypothyroid-recovery group), dense collagen bundles appeared separating groups of adipocytes into lobules (Figure 2D). In Group III (hypothyroid-curcumin), scattered collagen fibers surrounding groups of cells in between unilocular adipocytes (Figure 2E).



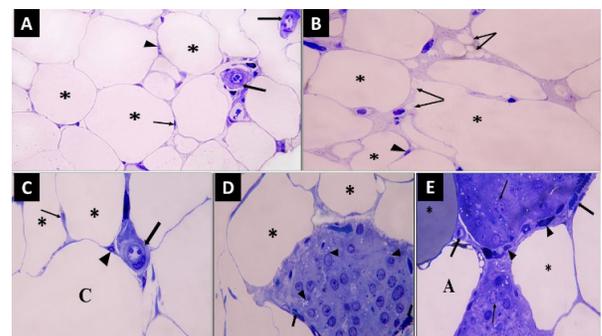
**Figure 2:** A photomicrograph of inguinal adipose tissue sections showing; (A): Control group: thin bundles of collagen fibers (↑) separating groups of adipocytes into adipose tissue lobules. (B): Euthyroid - curcumin: scattered collagen fibers surrounding cells cluster (↑) are noticed. (C): PTU group: fine collagen bundles separating groups of adipocytes into adipose tissue lobules (↑). (D): Hypothyroid-recovery group: thick septa of collagen fibers especially around blood vessels separating groups of adipocytes into lobules (↑). (E): Hypothyroid-curcumin: scattered collagen fibers surrounding clusters of cells (↑). (Masson Trichrome stain X 100).

Immune histochemical staining for detection of caspase-3 in adipose tissue sections showed that in control group, there was negative caspase 3 reaction in the adipocytes (Figure 3A). In subgroup Ic (euthyroid - curcumin) positive caspase 3 reactions in most adipocytes was detected (Figure 3B). In subgroup IIa (PTU group), positive caspase 3 reaction in most adipocytes was found (Figure 3C). In subgroup IIb (hypothyroid-recovery), revealed positive. caspase-3 reaction inn few adipocytes (Figure 3D). On the other hand, Group III (hypothyroid curcumin) showed positive caspase-3 reaction in most adipocytes (Figure 3E)



**Figure 3:** A photomicrograph of inguinal adipose tissue sections showing; (A): Control group: negative caspase-3 reaction in the adipocytes (↑). (B): Euthyroid - curcumin group: many caspase-3 positive reaction in the peripheral cytoplasm of most adipocytes (↑). (C): PTU group: scattered caspase-3 positive reaction in the peripheral cytoplasm of adipocytes (↑). (D): Hypothyroid-recovery group: scattered caspase-3 positive reaction in few adipocytes (↑). (E): Hypothyroid-curcumin group: caspase-3 positive reaction in peripheral cytoplasm of many adipocytes (↑). (Caspase-3 immune histochemical stain x400).

Semi thin section of inguinal adipose tissue of control group, showed that adipocytes were of the unilocular type having thin rim of cytoplasm and flat peripheral nuclei. Small blood vessels and some nuclei of connective tissue cells were also detected in between (Figure 4A). In subgroup Ic (euthyroid - curcumin) there were other cells in-between the unilocular adipocytes. These cells were irregular in shape and had variable sized vacuoles relative to large cytoplasmic area (Figure 4B). In subgroup IIa (PTU group), most adipocytes were of the unilocular type with apparent large size. Each cell contained a large single unstained vacuole with compressed flat nuclei at their periphery. In between the cells, connective tissue cells and blood vessels were detected. Large space contained unstained vacuole was also noticed (Figure 4C). In subgroup IIb (hypothyroid-recovery), one or more clusters of cells in between unilocular adipocytes were detected. The cells forming these clusters had rounded vesicular nuclei and contain small vacuoles. This group of the cells were surrounded with blood vessels and some connective tissue cells. The surrounding unilocular adipocytes contained large single unstained cytoplasmic vacuoles (Figure 4D). Likewise, in Group III (hypothyroid curcumin) clusters of small cells, containing few small vacuoles and rounded nuclei were detected in between unilocular adipose tissue. These clusters were surrounded by blood capillaries and connective tissue cells. Unilocular adipocytes around these clusters appeared formed of large single unstained cytoplasmic vacuole with compressed flat nuclei at their periphery and thin rim of cytoplasm. Occasionally, adipocytes that contained a large cytoplasmic vacuole in the center and multiple small vacuoles at their peripheral cytoplasm were detected (Figure 4E).

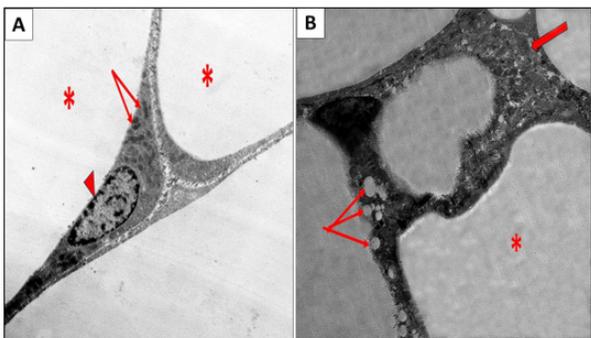


**Figure 4:** A photomicrograph of inguinal adipose tissue semi-sections showing; (A): Control group: variable sizes polygonal fat cells (\*). The cells have single large unstained vacuoles in the cytoplasm and flat peripheral nuclei (↑). Some nuclei of connective tissue cells (▲) and blood vessels (thick arrow) are noticed in-between the cells. (B): Euthyroid – curcumin group: variable sizes polygonal fat cells (\*). Most adipocytes contain a single large unstained vacuole in the cytoplasm with compressed flat nucleus at their periphery (↑). Notice the multiple vacuoles at the periphery of some cells (↑). (C): PTU group: large sized adipocytes (\*). Each cell contains a single large unstained vacuole with compressed flat nuclei at their periphery (↑). In between the cells, there

are nuclei of connective tissue cells (▲) and blood vessels (thick arrow). Notice the presence of large space of fusion of adjacent adipocytes (C). (D): Hypothyroid-recovery group: a cluster of cells in between unilocular adipocytes. The cells (▲) have central rounded vesicular nuclei and contain very small vacuoles. The cluster of cells is surrounding by blood vessels (thick arrow). The surrounding unilocular adipocytes contain large single unstained cytoplasmic vacuoles with compressed flat nuclei at their periphery (\*). (E): Hypothyroid-curcumin group: clusters of cells in between unilocular adipocytes (\*). These cells are smaller in size, contain few small vacuoles and rounded vesicular nuclei (↑). These cells are surrounded by blood capillaries (thick arrow) and connective tissue cells (▲). Notice the presence of an adipocyte (A) that contains a large unstained cytoplasmic vacuole in the center and multiple small vacuoles at their peripheral cytoplasm. (Toluidine blue stain X1000).

### Electron microscope results

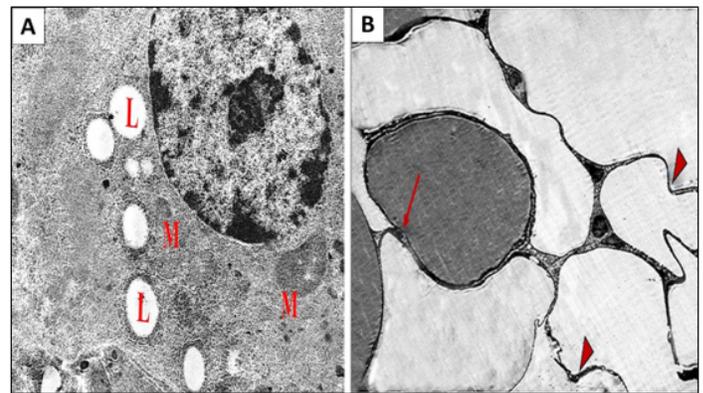
Examination of ultrathin sections inguinal adipose tissue showed that in control group, it was formed of unilocular adipocytes containing single large fat globules occupying most of the cells. The cytoplasm appeared as peripheral thin rim around the fat globule. The nuclei were compressed peripherally against the cell membrane. The cytoplasm of the adipocytes showed few small elongated or filamentous mitochondria (Figure 5A). In subgroup Ic (euthyroid - curcumin) adipocytes appeared containing large single fat globules occupying most of their cytoplasm leaving thin peripheral rim of the cytoplasm. Irregularly shaped cells in between, contained fat globules in their cytoplasm, whereas, their peripheral cytoplasm contained multiple small fat globules and multiple mitochondria (Figure 5B). Moreover, there were cells characterized by granular cytoplasm, few variable sized lipid droplets, multiple mitochondria. Their nuclei are rounded and had aggregated peripheral heterochromatin (Figure 6A).



**Figure 5:** An electron micrograph of an ultrathin section of the inguinal adipose showing; (A): control group: unilocular adipocytes

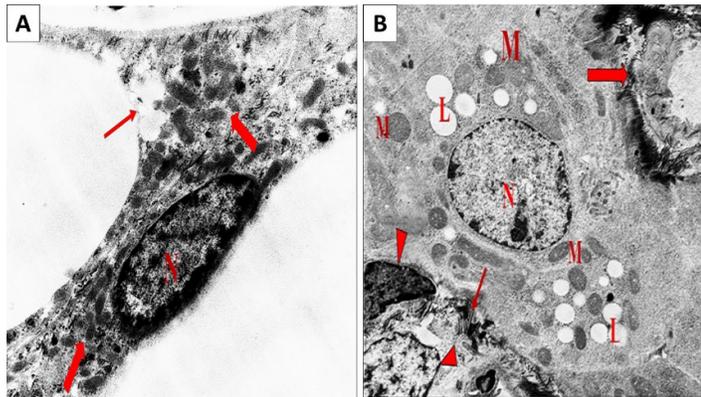
containing single large fat globules (\*) occupying most of the cell. The nucleus is compressed peripherally against cell membrane (▲). The cytoplasm appears as peripheral thin rim containing multiple small filamentous mitochondria (↑). (TEM x 8000). (B): Euthyroid - curcumin group: unilocular adipocytes containing a large single fat globule occupying most of their cytoplasm leaving thin peripheral rim of the cytoplasm (\*). Other adipocyte contains a large fat globule in the cytoplasm, whereas, their peripheral cytoplasm contains multiple small fat droplets (↑) and numerous small mitochondria (thick arrow). (TEM x5000).

On the other hand, adipose tissue of subgroup IIa (PTU group) showed unilocular adipocytes of variable sizes. The cells had corrugated cell membranes and thin rim of cytoplasm containing compressed nuclei (Figure 6B).



**Figure 6:** An electron micrograph of an ultrathin section of the inguinal adipose showing; (A): Euthyroid - curcumin group: cell with a single euchromatic nucleus. It has fine granular cytoplasm and some small lipid droplets that are not surrounded by membrane (L) and. Multiple mitochondria (M) are of variable sizes and shapes. (TEM x 15000). (B): PTU group: unilocular adipocytes of variable sizes. The cells have corrugated cell membranes (▲) and thin rim of cytoplasm containing compressed nuclei (↑). (TEM x 1500).

In subgroup IIb (hypothyroid-recovery), some adipocytes appeared with multiple small lipid droplets and numerous mitochondria at their peripheral cytoplasm. Other adipocytes showed peripheral compressed nuclei surrounded by numerous mitochondria (Figure 7A). In group III (hypothyroid curcumin), group of polygonal cells having euchromatic nuclei were detected. Some of them contained multiple small lipid droplets and numerous mitochondria. In between, many blood capillaries lined with flat endothelial cells (Figure 7B).



**Figure 7:** An electron micrograph of an ultrathin section of the inguinal adipose showing; (A): Hypothyroid-recovery group: two adipocytes of unilocular type with many small mitochondria (thick arrow) and some small lipid droplets (↑) in the cytoplasm. Notice the flat nucleus with peripheral heterochromatin (N). (TEM x 12000). (B): Hypothyroid-curcumin group: irregular shaped cell having central euchromatic nucleus (N). The cytoplasm is finely granular with multiple small lipid droplets (L) and abundant variable size mitochondria (M). Notice nearby blood vessels (thick arrow), nuclei of connective tissue cells (▲), and collagen fibers (↑). (TEM x 8000).

**Serological tests’ results (Table-1)**

Statistical analysis of the serological values of T<sub>3</sub> and T<sub>4</sub>, the levels of T<sub>3</sub> and T<sub>4</sub> were significantly increased (P<0.05) in subgroup Ic (euthyroid-curcumin group), subgroup Iib (recovery group) and group III (hypothyroid-curcumin group) in comparison to the control group. On the other hand, the lowest values for both were measured in subgroup Iia (PTU group). These values showed significant decrease (P<0.05) in comparison to all other groups.

Moreover, in group III (hypothyroid-curcumin) the levels of T<sub>3</sub> and T<sub>4</sub> were significantly increased (P<0.05) in comparison to subgroup Ic (euthyroid-curcumin group) and subgroup Iib (recovery group). Also, in subgroup Iib (recovery group) the levels of T<sub>3</sub> and T<sub>4</sub> were significantly increased (P<0.05) in comparison to subgroup Ic (euthyroid-curcumin group).

Regarding the serological values of TSH showed significant increase (P<0.05) in subgroup Iia (PTU group) in comparison to subgroup Ic (euthyroid-curcumin group), subgroup Iib (PTU recovery group) but with no significant difference to group Ia (euthyroid-control group). Also, the level of TSH in subgroup Ia (control group) showed significant increase (P<0.05) in comparison to subgroup Ic (euthyroid-curcumin group), subgroup Iib (PTU group) and group III (hypothyroid-curcumin group).

Groups / Test	Subgroup Ia (euthyroid-control)	Subgroup Ic (euthyroid-curcumin)	Subgroup Iia (PTU)	Subgroup Iib (recovery)	Group III (hypothyroid-curcumin)
TSH	0.043 ± 0.0015 (♦♣)	0.0098±0.00130 (**)	0.0272±0.00148 (♦)	0.0096±0.00270 (**)	0.0214±0.02718 (•)
T3	1.778 ± 0.01304 (*)	2.3320 ± 0.00837 (**)	0.1376 ± 0.20259 (•)	3.1820 ± 0.00837 (**♦♣)	4.5260±0.01140 (**♦)
T4	0.5620±0.00837 (*)	0.9320±0.00837 (**)	0.0860 ± 0.0114 (•)	1.5240± 0.0114 (**♦♣)	2.4620±0.00837 (**♦)

(•) Significant change compared to control group (subgroup Ia). (\*) Significant change compared with PTU group (subgroup Iia), (□) Significant change compared with recovery group (subgroup Iib), (♦) Significant change with euthyroid-curcumin group (subgroup Ic), (♣) significant change with hypothyroid-curcumin group (group III)

**Table 1:** Showing mean values ± SD of TSH, T<sub>3</sub> and T<sub>4</sub> in different groups.

**Morphometric and statistical results (Table-2)**

**The mean perimeter (circumference) of fat cells:** Statistical analysis of fat cells’ diameter of different groups showed that, subgroup Ic (euthyroid-curcumin group) was having the smallest fat cells among all groups. This value showed significant decrease (P< 0.05) compared to subgroup Ia (euthyroid-control group), Iia (PTU group), Iib (recovery group) and group III (hypothyroid-curcumin group).

Moreover, group III (hypothyroid-curcumin) showed significant decrease (p< 0.05) in the perimeter of fat cells compared to subgroup Iia (PTU group). Also there was non-significant change (P> 0.05) between subgroup Ia (euthyroid control group) and subgroup Iia (PTU group).

**The mean area percentage of collagen (mean ± SD):** Statistical analysis of the morphometric results of the area percentage of collagen in different groups, revealed that subgroup IIb (recovery group) recorded the highest values. These values showed significant increase (P<0.05) in comparison to subgroup Ia (euthyroid-control), Ic (euthyroid-curcumin), IIa (PTU group) and group III (hypothyroid-curcumin).

Likewise, group III showed significant increase (P<0.05) of area percentage of collagen fibers compared to subgroup Ia (euthyroid-control group), Ic (euthyroid-curcumin group) and IIa (PTU group).

On the other hand, statistical results of subgroups Ia (euthyroid-control group), subgroup Ic (euthyroid-curcumin) and

IIa (PTU group) showed non-significant change (P>0.05)

**The mean area percentage of caspase-3 positive reaction (mean ± SD):** Morphometric measurements and statistical analysis results revealed highest area percentage for caspase-3 positive reaction in subgroup Ic (euthyroid-curcumin). This value showed significant increase (p<0.05) compared to subgroup Ia (euthyroid control), subgroup IIa (PTU group) and IIb (recovery group).

Group III (hypothyroid-curcumin) showed significant increase (p<0.05) in area percentage of caspase-3 positive reaction compared to subgroup IIa (hypothyroid group) and subgroup IIb (hypothyroid-recovery group) and non-significant to subgroup Ic (euthyroid-curcumin).

	perimeter (circumference) of fat cells	Area percentage of collagen/HPF	Area percentage of Caspase-3 positive reaction /HPF
Subgroup Ia	238.2 ± 30.2 (♦)	0.5 ± 0.26	0.44± 0.16
Subgroup Ic	199.5± 25.1	0.62 ± 0.16	2.12 ± 0.27 (▲*♣)
Subgroup IIa	262.6 ± 24.4 (♦♦)	0.67 ± 0.26	0.94 ± 0.22 (*♦)
Subgroup IIb	245.5 ± 25.5 (♦)	2.48 ± 0.33 (*♦▲♦)	0.71± 0.16 (♦♦)
Subgroup III	225.6 ± 29.9 (♦)	1.42 ± 0.21 (*♦▲)	1.88 ± 0.19 (▲*♣)

(♦): Significant difference compared to euthyroid curcumin (subgroup Ic), (♦♦): Significant difference compared to hypothyroid curcumin (group 0000000000000000), (\*): Significant difference compared to euthyroid control group (subgroup Ia), (▲): Significant difference compared to PTU group (subgroup IIa), (♣): Significant difference compared to recovery group (subgroup IIb).

**Table 2:** Showing mean values ± SD of perimeter of fat cells, area percentage of collagen/HPF and area percentage of Caspase-3 positive reaction/HPF in different groups.

## Discussion

The aim of the present study was to evaluate the effect of curcumin on the structure of the inguinal adipose tissue of rat in an experimental hypothyroid model induced by propylthiouracil. In the current work, examination of H&E stained and semithin sections of the inguinal adipose tissue of the rats of the euthyroid group and euthyroid-corn oil subgroup were almost similar and showed the structural characteristics of the white adipose tissue which consisted of variable sized unilocular adipocytes, polygonal in shape with well-defined cell boundaries. Each cell contained a single large unstained vacuole occupying most of the cytoplasm and sparing a thin rim of peripheral cytoplasm. The nucleus is compressed peripherally against the cell membrane giving a signet ring appearance. In-between the adipocytes, there were nuclei of connective tissue cells and blood capillaries. These in accordance with [12,13]. Moreover, ultrastructural sections of the inguinal adipose tissue of the rats revealed unilocular adipocytes with single

large fat droplet and small filamentous mitochondria. Similar results were described by [13]. However, in hypothyroid group, the inguinal adipose tissue was mainly formed of unilocular adipocytes comparable to that of subgroups Ia and Ib (control group). However, the cell membranes of most adipocytes were corrugated with fusion of multiple adjacent adipocytes. Moreover, there was an apparent increase in the size of the unilocular adipocytes. Ultrathin sections of the inguinal adipose tissue confirmed the previous findings. Statistical analysis of the adipocyte circumference in different groups revealed a significant increase in the mean circumference of adipocytes in comparison to subgroup Ic and Group III. Jena et al, 2012 [3] reported that induction of hypothyroidism in adult rats by PTU resulted in augmentation of lipid peroxidation, which was considered as an index of oxidative stress. Therefore, the corrugation of cell membranes could be attributed to the PTU induced oxidative stress that led to peroxidation of lipid content of the cell membranes of the adipocytes.

Weiner et al, 2016 [14] reported that the adipocytes of the hypothyroid mice were larger in size with increased lipid accumulation in White Adipose Tissue (WAT) when compared with hyper- and euthyroid mice. Similarly, Santini et al, 2014 [15] reported the increase in fat accumulation within WAT in case of hypothyroidism. Hence, the significant increase of the size of the unilocular adipocytes of WAT of the rats of subgroup IIa might be attributed to the increase of the lipid accumulation within their cytoplasm. Wu et al, 2012 [16] stated that the adipose tissue of the mouse is subdivided into two main types, WAT and Brown Adipose Tissue (BAT). A new type of brown like adipocytes was identified known as beige cells. These newly identified cells were considered as thermogenic fat cells as brown adipocytes. Both brown adipocytes and beige cells played an important role in regulation of energy homeostasis in mammals. In the present study, examination of H&E stained sections of the inguinal adipose tissue of the rats of euthyroid-curcumin subgroup revealed the appearance of large areas of cells' clusters among the white adipocytes. These clusters were surrounded by connective tissue separating them from the surrounding white adipocytes. The cells within the cluster had multiple cytoplasmic vacuoles and small central rounded nuclei. In between these cells, few branched cells having deep acidophilic cytoplasm were detected. Moreover, semithin sections revealed the appearance of multiple small vacuoles at the periphery of some cells.

Orava et al, 2011 [17] and Cedikova et al, 2016 [18] stated that the beige cells had an intermediate cell morphology as they appeared as unilocular adipocytes similar to WAT under basal conditions, while under certain stimuli as cold exposure or chronic treatment of beta3 adrenergic agonists, they appeared as brown adipocytes with multilocular lipid droplets with expression of brown fat specific genes like UCP1 and PGC<sub>1</sub>  $\alpha$ . Moreover, classical brown adipocytes were reported to be located in certain areas in rodents and infants, such as the interscapular regions and around the kidney (perirenal BAT), while, beige cells are an inducible form of thermogenic adipocytes that sporadically reside within WAT depots [19]. Therefore, the cells which had multiple cytoplasmic vacuoles and small central rounded nuclei that were detected in between the unilocular adipocytes of the WAT of the rats of euthyroid-curcumin subgroup are most probably clusters of beige cells (brown like cells), and the deeply stained acidophilic cells that appeared among them are hypothesized to be their precursors. Regarding the origin of the beige cells, several studies suggested the de novo generation of the beige cells. Beige cells that appear in WAT, were not derived from myogenic lineage (Myf5 positive) that gave rise to brown adipocytes. Rather, they were derived from endothelial and perivascular cells in WAT [20-22]. However, other studies suggested another source of the origin of beige cells. These studies proposed the trans differentiation of white adipocytes into beige cells rather than de novo generation [23-25]. Many studies reported the trans-differentiation of white adipocytes into brown-

like adipocytes under several physiological and pathological conditions. Exercise was suggested to induce browning of WAT through releasing of irisin and meteorin-like hormone. They were identified as exercise-induced myokines induced in muscle through the PGC<sub>1</sub>  $\alpha$  pathway leading to browning of WAT [26,27]. Also, browning of subcutaneous WAT in burn patients was documented and was attributed to elevated circulating levels of catecholamines; epinephrine and norepinephrine [28]. Recently, Ikeda et al, 2018 [19] performed an in vivo lineage-tracing study in transgenic mice, using transient and permanent fluorescent cell labelling. Their results proved that inguinal WAT had beige cells after period of cold exposure. After rewarming, these beige cells re-acquired white adipocytes characters, then the same cells regained their multilocular appearance and their specific genes after a second period of cold. These results supposed the trans differentiation theory. It was reported that brown adipocytes varied from beige cells in that, brown adipocytes express high levels of Ucp<sub>1</sub> and other thermogenic genes under basal (unstimulated) conditions. In contrast, beige cells express these genes only in response to activators such as agonists of the  $\beta$ -adrenergic receptor or peroxisome proliferator-activated receptor [21,22]. On the other hand, it was reported that isolated beige cells under culture directly acquired a white fat phenotype by having unilocular lipid droplets and loss of UCP<sub>1</sub> expression within 10 days following withdrawal of  $\beta_3$ -AR agonist. In contrast, under the same culture conditions, classical brown adipocytes preserved their multi-locular lipid morphology for up to 10 days [29]. In the present study, examination of H&E stained sections of the inguinal adipose tissue of rats of hypothyroid-recovery and hypothyroid-curcumin revealed multiple small clusters of cells among the white adipocytes. However, these clusters were smaller in size than that detected in the euthyroid curcumin group. Moreover, semithin stained sections confirmed the appearance of clusters of cells in between unilocular adipocytes containing few small cytoplasmic vacuoles and rounded vesicular nuclei Wang et al, 2015 [12] studied the effect of different doses of curcumin on the white adipose tissue of mice. They reported that the brown like cells that were detected in the WAT inguinal adipose tissue of mice were beige cells. Moreover, several studies reported that hyperthyroidism induced browning of WAT of mice with appearance of beige cells clusters and a significant increase in adrenoreceptors expression in WAT in addition to increase the markers for adipose tissue browning such as UCP<sub>1</sub> [14,30,31].

Curcumin effect on inguinal WAT was suggested to be through stimulating norepinephrine release from activated macrophage in adipose tissue and/or at sympathetic terminals in WAT and/or from the adrenal medulla. The released norepinephrine resulted in either immediate activation of existing beige adipocytes and /or differentiation of beige adipocytes from their precursors [32,33]. Therefore, the appearance of beige cells within inguinal WAT of rats of euthyroid-curcumin could be attributed to the stimulating effect of curcumin on the norepinephrine release. In hypothyroid-

recovery group, the appearance of beige cells might be attributed to the elevated levels of  $T_3$  and  $T_4$  upon PTU discontinuation. However, in hypothyroid-curcumin, the appearance of beige cells within inguinal WAT might be attributed to dual effect of both elevated level of  $T_3$  and  $T_4$  (after discontinuation of PTU) and the effect of curcumin administration. This is supported by statistical results that revealed a significant increase of level of  $T_3$  and  $T_4$  in hypothyroid-recovery subgroup and hypothyroid-curcumin compared to other groups. In the current study, examination of Masson's trichrome stained sections of the inguinal adipose tissue of rats showed scattered collagen fibers around the cells cluster in euthyroid-curcumin group and hypothyroid-curcumin group. However, in hypothyroid-recovery subgroup, collagen fibers appeared as dense thick septa in between adipocytes. These results were confirmed by statistical analysis that revealed significant increase in area percentage of collagen in hypothyroid-recovery compared to all groups.

Zhang et al, 2011 [34] mentioned that curcumin had anti fibrotic effect in mice through its an anti-proliferative activity on fibroblasts and interference with transforming growth factor  $\beta_1$  (TGF-  $\beta_1$ ) mediated signaling pathways. Moreover, Guo et al, 2018 [35] reported that curcumin had anti fibrotic effect in heart tissue of diabetic rats through suppression of the deposition of type I and type III collagens as well as reducing the production of TGF- $\beta_1$ . Therefore, the significant decrease in collagen in the groups in which curcumin was administrated (euthyroid-curcumin and hypothyroid curcumin) could be explained by the anti-fibrotic effect of curcumin on collagen formation. In the present study, immune histochemical staining sections of inguinal adipose tissue of rats for caspase-3 of different groups, showed a significant increase in area percentage of the immunostaining reaction in most of the adipocytes in groups in which curcumin was administrated (euthyroid-curcumin and hypothyroid curcumin) in comparison to other groups. Caspases are well known crucial mediators of apoptosis. Caspase-3 is one of most frequently activated death protease that causes cleavage of key cellular proteins responsible for apoptosis as poly (ADP) ribose polymerase (PARP). PARP is thought to have a multifunctional role in apoptosis, DNA repair, as well as in the maintenance of chromosomal stability. Activation of caspase-3 led to the cleavage of PARP with subsequent its inactivation and failure of DNA repair cycle [36]. The apoptotic effect of curcumin was explained through the activation of caspase-3 and PARP cleavage pathways. Also, curcumin stimulated the release of cytochrome c from mitochondria into cytoplasm with up regulation of Bax/Bcl-2 ratio [36,37]. Bcl-2 is antiapoptotic protein involved in cell survival and inhibiting cell apoptosis, while Bax is a proapoptotic protein [36]. Therefore, the increase of area percentage of immune histochemical staining in euthyroid-curcumin and hypothyroid-curcumin as compared to other groups could be attributed to apoptotic effect of curcumin on adipocytes.

## Conclusion

Administration of curcumin either in euthyroid or hypothyroid rats resulted in appearance of clusters of brown like adipocytes in between the white adipocytes of the inguinal adipose tissue.

## Conflict of Interest

The authors have no financial conflict of interest.

## References

1. Sanyal D, Raychaudhuri M (2016). Hypothyroidism and obesity: An intriguing link. *Indian J Endocr Metab* 20: 554-557.
2. Emiliano A, Governale L, Parks M, Cooper D (2010) Shifts in propylthiouracil and methimazole prescribing practices: Antithyroid Drug use in the United States from 1991 to 2008. *J Clin Endocrinol Metab* 95: 2227-2233.
3. Jena S, Chainy GBN, Dandapat J (2012): Expression of antioxidant genes in renal cortex of PTU-induced hypothyroid rats effect of vitamin E and curcumin. *Mol Biol Rep* 39: 1193-1203.
4. Mogulkoc R, Baltaci AK, Aydin L, Oztekin E, Sivrikaya A (2005) The effect of thyroxine administration on lipid peroxidation in different tissues of rats with hypothyroidism. *Acta Physiol Hung* 92:39-46.
5. Shishodia S, Sethi G, Aggarwal B (2005) Curcumin: getting back to the roots. *Ann N Y Acad Sci* 1056: 206-217.
6. Anand P, Thomas S, Kunumakkara A, Sundaram C, Harikumar KB, et al. (2008) Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochem pharmacol* 76: 1590-1611.
7. Yi X, Yamamoto K, Shu L et al. (1997) Effects of Propylthiouracil (PTU) Administration on the Synthesis and Secretion of Thyroglobulin in the Rat Thyroid Gland: A Quantitative Immunoelectron Microscopic Study Using Immunogold Technique. *Endocr Pathol* 8: 315-325.
8. Papiez MA, Kaja M, Gebarowska A (2008) Age-dependent different action of curcumin in thyroid gland. *Folia Histochem Cytobiol* 6: 205-211.
9. Bancroft J D and Layton D (2013) Connective tissue and other mesenchymal tissues with their stains. In: *Bancroft's theory and practice of histological techniques*. Survana S K, Layton C and Bancroft J D (Eds.) 7<sup>th</sup> edition, Elsevier 165: 166.
10. Sanderson T, Wild G, Cull AM, Marston J, Zardin G (2013) Immunohistochemical and immunofluorescent techniques. In: *Bancroft's theory and practice of histological techniques*. Survana S K, Layton C and Bancroft J D (Eds.) 7<sup>th</sup> edition, Elsevier 337-394.
11. Bancroft JD, Layton C, Suvarna SK (2013) *Bancroft's Theory and Practice of Histological Techniques*. 7th ed. Elsevier Churchill Livingstone: London, United Kingdom 386-535.
12. Wang S, Wang X, Ye Z, Xu C, Zhang M, et al. (2015) Curcumin Promotes Browning of White Adipose Tissue in a Norepinephrine-Dependent Way. *Biochem Biophys Res Commun* 466: 247-253.
13. Lone J, Choi J H, Kim S W, Yun J W (2016) Curcumin Induces Brown Fat-Like Phenotype in 3T3-L1 and Primary White Adipocytes. *J Nutr Biochem* 27: 193-202.

14. Weiner J, Kranz M, Klötting N, Kunath A, Steinhoff K, et al. (2016) Thyroid Hormone Status Defines Brown Adipose Tissue Activity and Browning of White Adipose Tissues in Mice. *Sci Rep* 6: 38124.
15. Santini F, Marzullo P, Rotondi M, Ceccarini G, Pagano L, et al. (2014) The Crosstalk Between Thyroid Gland and Adipose Tissue: Signal Integration in Health and Disease. *Eur J Endocrinol* 171: 137-152.
16. Wu J, Boström P, Sparks L M, Ye L, Choi JH (2012) Beige Adipocytes are a Distinct Type of Thermogenic Fat Cell in Mouse and Human. *Cell* 150: 366-376.
17. Orava J, Nuutila P, Lidell ME, Oikonen V, Nojonen T, et al. (2011) Different Metabolic Responses of Human Brown Adipose Tissue to Activation by Cold and Insulin. *Cell Metab* 14: 272-279.
18. Cedikova M, Kripnerová M, Dvorakova J, Pitule P, Grundmanova M, et al. (2016) Mitochondria in White, Brown, and Beige Adipocytes. *Stem Cells Int* 2016: 6067349.
19. Ikeda K, Maretich P, Kajimura S (2018) The Common and Distinct Features of Brown and Beige Adipocytes. *Trends End Met* 29: 191-200.
20. Tran KV, Gealekman O, Frontini A, Zingaretti MC, Morroni M, et al. (2012) The Vascular Endothelium of the Adipose Tissue Gives Rise to Both White and Brown Fat Cells. *Cell Metab* 15: 222-229.
21. Harms M, Seale P (2013) Brown and Beige Fat: Development, Function and Therapeutic Potential. *Nat Med* 19: 1252-1263.
22. Kajimura S, Spiegelman M, Seale P (2015) Brown and Beige Fat: Physiological Roles beyond Heat-Generation. *Cell Metab* 22: 546-559.
23. Himms-Hagen J, Melnyk A, Zingaretti MC, Barbatelli G, Cinti S (2000) Multilocular Fat Cells in WAT of CL-316243-Treated Rats Derive Directly from White Adipocytes. *Am. J. Physiol. Cell Physiol* 279: 670-681.
24. Barbatelli G, Murano I, Madsen L, Hao Q (2010) The Emergence of Cold-Induced Brown Adipocytes in Mouse White Fat Depots is Determined Predominantly by White to Brown Adipocyte Transdifferentiation. *Am. J. Physiol. Endocrinol Metab* 298: 1244-1253.
25. Rosenwald M, Perdikari A, Rulicke T, Wolfrum C (2013) Bi-directional Interconversion of Brite and White Adipocytes. *Nat Cell Biol* 15: 659-667.
26. Bostrom P, Wu J, Jedrychowski M P, Korde A, Ye L, Lo JC, et al. (2012) A PGC,  $\alpha$  - Dependant Myokine that Drives Browning of White Fat and Thermogenesis. *Nature* 481: 463-468.
27. Rao RR, Long JZ, White JP, Svensson KJ, Lou J, et al. (2014) Meteorin-Like is a Hormone That Regulates Immune-Adipose Interactions to Increase Beige Fat Thermogenesis. *Cell* 157: 1279-1291.
28. Sidossis LS, Porter C, Saraf MK, Børsheim E, Radhakrishnan RS et al. (2015) Browning of Subcutaneous White Adipose Tissue in Humans After Severe Adrenergic Stress. *Cell Metab* 22: 219-227.
29. Altshuler-Keylin, S, Shinoda K, Hasegawa Y, Ikeda k, Hong H, et al. (2016) Beige Adipocyte Maintenance is Regulated by Autophagy-Induced Mitochondrial Clearance. *Cell Metab* 24: 402-419.
30. Lin JZ, Martagon AJ, Cimini SL, et al (2015): Pharmacological Activation of Thyroid Hormone Receptors Elicits a Functional Conversion of White to Brown Fat. *Cell Rep* 13: 1528-1537.
31. Reddy NL, Tan BK, Barber TM, Randeve HS (2014): Brown Adipose Tissue: Endocrine Determinants of Function and Therapeutic Manipulation as a Novel Treatment Strategy for Obesity. *BMC Obesity* 1:13.
32. Nguyen KD, Qiu Y, Cui X, Goh YS, Mwangi J, et al. (2011) Alternatively Activated Macrophages Produce Catecholamines to Sustain Adaptive Thermogenesis. *Nature* 480: 104-108.
33. Bartelt A, Heeren J (2014) Adipose Tissue Browning and Metabolic Health. *Nat Rev Endocrinol* 10: 24-36.
34. Zhang D, Huang C, Yang C, Liu RJ, Wang J, et al. (2011): Antifibrotic Effects of Curcumin Are Associated with Overexpression of Cathepsins K and L in Bleomycin Treated Mice and Human Fibroblasts. *Respir Res* 154: 1-12.
35. Guo S, Meng X, Yang X, Liu X, Ou-Yang, et al. (2018) Curcumin Administration Suppresses Collagen Synthesis in the Hearts of Rats with Experimental Diabetes. *Acta Pharmacologica Sinica* 39: 195-204.
36. Zhu L, Han M B, Gao Y, et al (2015): Curcumin Triggers Apoptosis via Upregulation of Bax/Bcl-2 Ratio and Caspase Activation in SW872 Human Adipocytes. *Mol Med Rep* 12: 1151-1156.
37. Ejaz A, Wu D, Kwan P, Meydani M (2009) Curcumin Inhibits Adipogenesis in 3T3-L1 Adipocytes and Angiogenesis and Obesity in C57/BL Mice. *J Nutr* 139: 919-925.