Histological study of adipose tissue and kidney in rat model of diet induced obesity and the possible protective role of green tea

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Presented by

Mohamed Ahmed Abdou Hegazi
Assistant Lecturer Of Histology
Faculty Of Medicine-Ain Shams University

Under Supervision Of

Prof. Dr. Samy Hosny Hamed
Professor Of Histology
Faculty Of Medicine-Ain Shams University

Prof. Dr. Maher Mohamed Hussein Emara
Professor Of Histology
Faculty Of Medicine-Ain Shams University

Prof. Dr. Suzi Sobhy Atallah
Professor Of Histology
Faculty Of Medicine-Ain Shams University

Prof. Dr. Suzi Hanafy Ibrahim Mahmoud
Professor Of Histology
Faculty Of Medicine-Ain Shams University

Faculty Of Medicine
Ain Shams University

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Introduction

The adipose tissue represents a large amount of adult tissues. For long time, it was considered as a poorly active overgrown and undesirable tissue even if its usefulness was demonstrated in reconstructive surgery. It was studied for its main involvement in energy metabolism and disorders as diabetes and obesity. More recently, its endocrine functions emerged and appeared to play a key role in many physiological situations such as inflammation and immunity. The presence of preadipocytes throughout life was demonstrated using primary culture technology from cells derived from adipose tissue. **(Castiela et al., 2005)**

Guebre et al. (2005) found that adipose tissue releases inflammatory cytokines, they also reported that obesity is associated with elevated C-reactive protein level in general population.

Moreover, adipose tissue functions as endocrine organ secreting hormones e.g. ***Leptin.*** This small peptide hormone (Leptin) is mainly but not exclusively produced by adipose tissue ***(Hall et al., 2003).***

Hausman et al. (2003) found that feeding of high fat diet in rodents leads to age related increase in body fat, fat cell size and number as well as an increased circulating level of leptin.

The world wide prevalence of obesity and its associated metabolic and cardiovascular disorders has risen dramatically in the past two decades **(Hall et al., 2003)**.

Meanwhile **Aneja et al. (2004)** reported that excess weight gain is a major risk factor for essential hypertension and for end stage renal diseases. He also recorded proteinurea and focal glomerulosclerosis on biopsy in patients with chronic obesity.

In view of this point **Smith (2004)** reported that chronic obesity caused marked structural changes in the kidneys that led to loss of function with increase in blood pressure.

Guebre et al. (2005) declared that in obese rats with severe proteinurea, ***Leptin*** was found to be the trigger for an increase in the expression of extracellular matrix of both glomerular epithelium and mesangeal cells.

Moreover **Kume et al. (2007)** high-fat diet leads to an altered balance between renal lipogenesis and lipolysis, subsequent renal accumulation of lipid, and renal injury. They suggested that modulation of lipid metabolism could serve as a new therapeutic target to prevent chronic kidney disease in patients with obesity.

On the other hand **Choo (2003)** Reported that green tea extract exerts potent body fat suppressive effect by two mechanisms, either by reduction in digestibility of fat or rather increase in thermo genesis in brown fat.

Rah et al. (2007) found that green tea polyphenoles can reduce renal injury by preventing the oxidative stress**.**

Furthermore **Ribaldo et al. (2009**) suggested that the consumption of green tea may ameliorate nephropathy in diabetic hypertensive patients.

Aim of the Work:

The aim of this work is to study the histological effect of diet induced obesity on the structure of adipose tissue and the reflective effect on renal tissue, and to evaluate the possible protective role of green tea on both.

Adipose tissue:

White fat and brown fat constitute two different subtypes of adipose tissue . white fat comprises 10% to 20% of the body weight in normal adult males. It constitutes a relatively large diffuse organ which is very active metabolically. It is primarily engaged in the uptake , synthesis, storage, and mobilization of lipid.

(Cormack, 1987).

It is now prevailing view that there are two processes for adipose tissue formation: primary fat formation, which occurs in early fetal life resulting in formation of small aggregates of brown fat. Secondary fat formation, which occurs later in fetal life and the early postnatal life resulting in the widely disseminated deposits of unilocular fat found in the adult human. It is also well settled that the function of unilocular fat cell is affected by a variety of hormones e.g. insulin, epinephrine, and nor epinephrine. **(Fawcett, 1994)**.

Gartner (2001) stated that adipocytes differentiate from special precursor cell of mesenchymal origin, called preadipocytes.

Adipose tissue has long been considered to be metabolically

passive. The recent scientific advances have altered our

understanding of the fat cell function. The fat cell was found to be a transducer of energy supply, modulating glucose homeostasis and hypothalamic function **(Diamond and Eichler, 2002).**

Moreover, adipose tissue functions as endocrine organ secreting hormones and cytokines e.g. ***Leptin and adiponectin***. This small peptide hormone (Leptin) is mainly but not exclusively produced by adipose tissue ***(Wolf et al., 2002*)**.

There is noticeable difference in the map of fat distribution amongst different species. As rodents unlike human have minimal subcutaneous fat depot. **(Tholpady et al., 2003)**

Hausman et al. (2003) found that feeding of high fat diet in rodents led to age related increase in body fat, fat cell size and number as well as an increased circulating level of leptin.

Bains et al. (2004) found that late-onset obesity in male rat resulted in gradual accumulation of large amounts of fat in visceral (perirenal and epididymal) but not peripheral fat depots.

Casteilla et al. (2005) demonstrated evidences that supported a link between adipose tissue and immunocompetent cells. This link was evident in obesity, where excess adiposity and

impaired immune function had been described in both humans and

genetically obese rodents. They added that numerous factors involved in inflammatory response were secreted by both preadipocytes and macrophages. They showed that proliferating preadipocytes in cell lines and primary cultures, developed phagocytic activity toward microorganisms. This was demonstrated by phagocytosis assays and confocal microscopy. This function disappeared when preadipocytes stopped proliferation and differentiated into adipocytes.

Gross et al. (2005) stated that in mature adipocytes, triglyceride was stored within lipid droplets. Triglycerides were coated with the protein perilipin, which regulated lipolysis by controlling lipase access to the droplet in a hormone-regulatable fashion. Adipocyte-differentiation related protein (ADRP) was another widely expressed lipid droplet-binding protein that was co-expressed with perilipin in differentiating fat cells. It preserved lipid droplet morphology and structure.

The objective of the study done by **Hong et al. (2005)** was to understand the onset of obesity. They stated that the increase in fat mass associated with obesity resulted from recruitment and differentiation of adipocyte progenitor cells. The precise origin of these cells was unknown, although accumulating evidence

suggested that circulating stem cells could differentiate into cells of

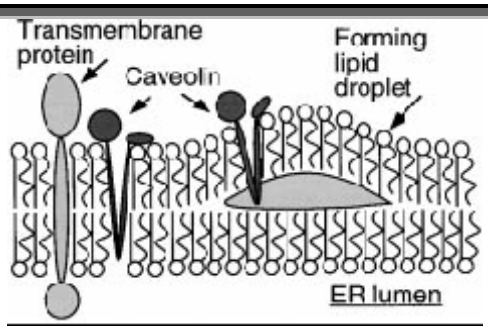
mesenchymal lineage. They added that it was unclear whether a progenitor adipocyte population existed in circulation. Fibroblast might represent a common progenitor cell for several mesenchymal lineages. They demonstrated that these circulating progenitors become adipocytes when cultured under adipogenic conditions, with intracellular lipids accumulation and up-regulation of proteins specific for adipocyte differentiation, including leptin.

Ost et al. (2005) stated that synthesis of triacylglycerol (TG) from exogenous fatty acids was a principal metabolic function of adipocytes. The level of fatty acids had to be tightly controlled in the adipocyte, as they could act as detergents that rapidly dissolved the plasma membrane, causing cell lysis if allowed to accumulate. Fatty acids therefore should be efficiently converted to TG and stored in the central lipid droplet. They reported that in intact primary adipocytes exogenous oleic acid was taken up and directly converted to TG in the plasma membrane, in a novel subclass of caveolae that specifically contained the protein perilipin. Electron microscopy revealed the presence of caveolin and perilipin in caveolae in the plasma membrane, and fluorescence microscopy demonstrated 2 classes of caveola. The first class contained perilipin at the plasma membrane. A second caveolae fraction was isolated, which lacked perilipin and the triacylglycerol synthesizing enzymes.

Both caveolae fractions contained caveolin-1 and the insulin

receptor. Their findings demonstrated that specific subclasses of caveolae carried out specific functions in cell metabolism. In particular, triacylglycerol was synthesized at the site of fatty acid entry in one of these caveolae classes.

Ostermeyer et al. (2006) discribed Caveolin-1 as a normally localized protein in plasma membrane caveolae and the Golgi apparatus in mammalian cells. They stated that caveolin-1 has a role in lipid droplet formation. They suggested that neutral lipids accumulate in the hydrophobic core of the phospholipid bilayer, forming a bulge that eventually buds from the ER membrane to form a free droplet. Accumulation of neutral lipids in the bilayer core would initially force opposite leaflets of the bilayer apart, increasing bilayer thickness. Such a thickened bilayer would not be able to accommodate transmembrane proteins that have hydrophilic domains on both sides of the membrane, and these proteins would be excluded from the forming droplets. Caveolins, by contrast, lacked luminal hydrophilic domains, and could diffuse freely between the ER membrane and the monolayer surrounding the enlarging droplet. Thus, caveolins would not need to dissociate from the ER membrane.



(figure I) **(Ostermeyer et al., 2006)**

Listenberger et al. (2007) studied with much detail the formation and turnover of lipid droplet inside fat cells, they stated that newly synthesized neutral lipids accumulate inside the endoplasmic reticulum membrane, forming a disk that eventually buds into the cytoplasm surrounded by an endoplasmic reticulum derived phospholipid monolayer. Conversely, lipid droplet turnover occurs via the hydrolysis of stored neutral lipids by cytosolic lipases. They focused on the regulation of lipolysis and tried to describe its sequence:

In response to hormone stimulation, protein **kinase A** phosphorylated two key substrates: hormone-sensitive lipase (HSL) and perilipins. Phosphorylation of HSL stimulated both its activity and its association with lipid droplets, in a manner that depended on perilipins.

They added that Perilipins regulate TG. hydrolysis in two ways. First, they greatly enhanced TG. turnover under lipolytic conditions by recruiting HSL to lipid droplets and/or by activating it on the lipid droplet surface. Second, perilipins inhibited TG. turnover under basal conditions, at least in part by reducing lipid droplet-associated lipase activity.

Adipogenesis:

An accurate chronology of the earliest steps in adipocyte differentiation has not been elucidated. The expression of lipoprotein lipase (LPL) mRNA has often been cited as an early sign of adipocyte differentiation **(Macdougald and Lane, 1995).** It is also secreted by mature adipocytes and plays a central role in controlling lipid accumulation **(Goldberg, 1996**).

Although the developmental origin of adipocytes is not known, several studies have suggested that the adipocyte lineage derives from an embryonic **pluripotent** stem cell precursor with the capacity to differentiate into the Mesenchymal cells. The mesenchymal stem cells (**multipotent** stem cells) would proliferate and undergo change in gene expression with either repression or activation of genes (four sets of genes: stromagenic, osteogenic, chondrogenic, and adipogenic. They could give rise to the different populations of the cells (stromal, endothelial, osteoblasts or adipocytes **(Tong and Hotamisligil 2001).**

Once multipotent MSCs become committed to the adipoblast lineage (cells committed to fill with lipid and become adipocytes) adipogenesis is initiated and started with a phase of growth of

adipoblasts. Following confluence of these adipoblasts, the cells enter into a cell cycle arrest (contact inhibition), they re-enter the

cell cycle and pass through a limited number of cell divisions, start multiple lipid droplets formation (**preadipocyte**) and finally differentiate into fully mature adipocytes. **(Koutnikova and Auwerx, 2001).**

Nakae et al. (2003) reported that, MSCs give rise to adipocytes in response to adipogenic hormones. An outstanding question in adipocyte biology is how hormonal cues are relayed to the nucleus to activate the transcriptional program that promotes adipogenesis. They suggested that fork head transcription factor (Foxol) plays an important role in the integration of hormone-activated signaling pathways with the complex transcriptional cascade that promotes adipogenesis.

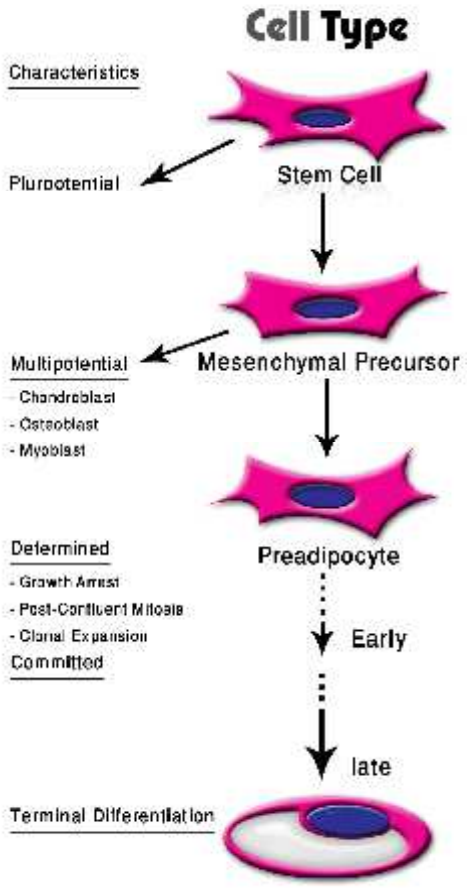


Figure II : Schematic diagram of stages of adipogenesis, a pluripotent stem cell gives rise to a mesenchymal precursor cell with the potential to

differentiate along lineages of myoblast, chondroblast, osteoblast and adipocyte. Under appropriate environmental factors and gene expression the preadipocyte can differentiate into mature adipocyte. Selected

(Gregoire et al., 1998).

Obesity:

Obesity, developing in the adult life, is commonly due to accumulation of excess lipid in normal number of unilocular adipose cells (hypertrophic obesity). The fat cells in such individuals may be four times their normal size. In severe obesity, there may also be a greater number of cells (hypercellular obesity).

(Fawcett, 1994).

However it should be noticed that the body fat distribution has a remarkable effect on the metabolic state. **Wajchenberg (2000)** found that the visceral fat mass contributed significantly to the free fatty acids levels in the systemic circulation. They added that subjects with visceral abdominal obesity are more insulin resistant than those with peripheral obesity

Altomontea et al. (2003) attributed this to the more rapid mobilization of fatty acids from visceral fat cells because of the higher lipolytic activity in visceral adipocytes, in both nonobese and