The possible effect of green tea (catechin) on perinephric fat adipocytes of adult male albino rat after induction of obesity: a histological study
Mona A. Mohamed, M. Zaki, Omayma K. Helal and Mohamed M. Yousef

Department of Histology and Cell Biology, Faculty of Medicine, Benha University, Benha, Egypt
Correspondence to Omayma K. Helal, Department of Histology and Cell Biology, Faculty of Medicine, Benha University, Benha, Egypt
Tel: + 127169605; e-mail: dromima_helal@yahoo.com

Introduction
Green tea is one of the most widely consumed beverages in the world, and its antioxidant properties have been widely explored. Its active ingredients (polyphenols) are believed to be responsible for most of green tea’s roles in promoting good health. Obesity and its associated metabolic disorders are an increasingly prevalent condition in different societies.

Aim of the study
The aim of this research is to study the effect of diet-induced obesity on the histological structure of adipocytes and to evaluate the possible protective role of green tea.

Materials and methods
Forty adult male rats were divided into three groups. Group I rats (control group, n = 10) were given a balanced diet for 6 weeks. Group II rats (n = 10) were given a high-energy fatty diet for 6 weeks and served as the affected group. Group III rats (green tea group, n = 20) were divided into two subgroups. Subgroup IIIa rats (low-dose group, n = 10) were given a high-energy fatty diet for 6 weeks and a low dose of green tea extract (325 mg/kg/day) by an oral tube for the last 4 weeks. Subgroup IIIb rats (high-dose group, n = 10) were given a high-energy fatty diet for 6 weeks and a high dose of green tea extract (500 mg/kg/day) by an oral tube for the last 4 weeks.

After 6 weeks, the animals were weighed, killed, and specimens from perinephric fat were prepared for light microscopic (sudan III and osmic acid stains) and electron microscopic (transmission and scanning electron microscopic) studies. The mean area of unilocular fat cells (micrometer square) was measured and statistically studied.

Results
There was a significant increase in body weight and in marked adipocyte morphological and cytological changes (size of adipocytes, saturated fatty acids within fat cells, and increased mitochondrial content) in groups II and IIIa compared with the control group. Such effects were ameliorated by concomitant administration of high-dose green tea extract in group IIIb.

Conclusion
It could be concluded that high-dose green tea extract is effective in lowering the increased body weight due to a high-energy fatty diet. Hence, it is advised to consider a high dose of green tea extract effective against diet-induced obesity through its effect on size and structure of adipocyte.

Keywords:
adipocytes, green tea, obesity

Introduction
Green tea, a product of the dried leaves of Camellia sinensis, is the most widely consumed beverage in the world with no known serious side effects [1].

Green tea polyphenols have shown significant antioxidant, anticarcinogenic, anti-inflammatory, thermogenic, and antimicrobial properties in numerous human, animal, and in-vitro studies. The main catechins in green tea are epicatechin, epicatechin-3-gallate, epigallocatechin (EGC), and epigallocatechin-3-gallate [2]. Furthermore, its catechins affect lipid metabolism by different pathways and prevent the appearance of atherosclerotic plaque. Green tea extract (GTE) intake decreases the absorption of triglycerides and cholesterol [3]. Green tea catechins decrease plasma total cholesterol and blood triglycerides levels [4]. Green tea ingestion also decreases low-density lipoprotein cholesterol and increases high-density lipoprotein cholesterol [5]. GTE intake decreased serum glucose levels, suggesting that catechins interact with glucose metabolism [6].
Adipose tissue represents a large amount of adult tissues. The presence of preadipocytes throughout life was shown using primary culture technology from cells derived from adipose tissue [7].

Adipose tissue is classified into two types according to whether it is composed of unilocular or multilocular adipocytes. Other differences between the two types of adipose tissue are color, vascularity, and metabolic activity [8]. White adipose tissue is the predominant type in adult humans. Brown adipose tissue presents many features of an adipose tissue. However, it has specific morphology, innervation, vasculation, body location, as well as a unique physiological role in regulatable thermogenesis [9].

Endocrine functions of adipose tissue emerged and appeared to play a key role in many physiological situations such as inflammation and immunity [7]. It releases inflammatory cytokines. Obesity is associated with elevated C-reactive protein level in general population [10]. Adipose tissue acts as an endocrine organ secreting hormones, for example, leptin. This small peptide hormone (leptin) is mainly, but not exclusively, produced by adipose tissue [11].

For a long time it was considered as a poorly active, overgrown, and undesirable tissue, even after its usefulness was shown in reconstructive surgery. It was studied for its main involvement in energy metabolism and disorders such as diabetes and obesity [7]. Feeding of high-fat diet in rodents leads to an increase in body fat, fat cell size, number, as well as an increased circulating level of leptin [12]. The worldwide prevalence of obesity and its associated metabolic and cardiovascular disorders has risen dramatically in the last two decades [13]. In obesity, large adipocytes strongly express adipocytokine genes such as leptin and adipin, which lead to increase the content of saturated fatty acid [14]. Excess weight gain is a major risk factor for essential hypertension and cardiovascular disease [15].

The aim of this research is to study the effect of diet-induced obesity on the histological structure of adipocytes and to evaluate the possible protective role of green tea.

Materials and methods
This study was performed on 40 adult male albino rats weighing 180–210 gm. They were obtained from the Animal House of Cairo University Faculty of Medicine (Giza, Egypt) and acclimatized to the laboratory condition.

Two types of diet were used in this study: balanced diet [16] and high-energy fatty diet [17]. They were prepared in the Animal House of the Cairo University. Diets were freshly prepared every week and then stored at 4°C.

GTE was obtained from the Technomade Group (Nasr City, Cairo, Egypt) as a 350-gm powdered extract. It was freshly dissolved in distilled water before administration by gastric tube in two doses: low (325 mg/kg/day) [5] and high (500 mg/kg/day) [6].

Rats were divided into three groups as follows: group I rats (control group, n = 10) were given a balanced diet for 6 weeks. Group II rats (n = 10) were given a high-energy fatty diet for 6 weeks and served as the affected group. Group III rats (green tea group, n = 20) were divided into two subgroups. In subgroup IIIa (low-dose group, n = 10), each animal was given a high-energy fatty diet for 6 weeks and a low dose of GTE by an oral tube for the last 4 weeks (2 weeks from the start of experiment). In subgroup IIIb (high-dose group, n = 10), each animal was given a high-energy fatty diet for 6 weeks and a high dose of GTE by an oral tube for the last 4 weeks.

After 6 weeks of the experiment, the animals were weighed and killed under thiopental sodium anesthesia. The perinephric fat was dissected out of the animals under study. Specimens were processed through the frozen section technique for light microscopic examination and stained with Sudan III (with hematoxylin counterstain) and osmic acid [18]. For electron microscopic preparation, perinephric fat samples were transferred into buffered formol gluteraldehyde (3%) and buffered osmic acid (1%) and processed. Ultrathin sections were prepared for transmission electron microscopy [19] along with sections (fixed as soon as possible in 1.5% gluteraldehyde in phosphate buffer saline) for scanning electron microscopy [20], and examined by a Jeol transmission and scanning electron microscope in Tanta EM unit.

Statistics
The body weight of all groups was measured. The mean area of unilocular fat cells (micrometer square) was measured from five different fields of five serial sections using an Olympus soft imaging system and an analysis life science program.

All data were expressed as mean ± standard error of the mean. Differences between groups were compared by Student’s t-test, with a P value of less than 0.05 selected as the level of statistical significance. The statistical analysis was carried out using Microsoft Excel 2003.

Results
Statistical results
Mean weight of animals (grams)
The mean weight of animals of group I (control group) was 189.76 ± 7.81 g. There was a significant increase in body weight of animals of the affected group (received high-fat diet) and group IIIa (received high-fat diet and green tea at a dose of 325 mg/kg/day) when compared with the control group. There was no significant change in the body weight of animals in group IIIb (received high-fat diet and green tea at a dose of 500 mg/kg/day) compared with the control group (Table 1 and Histogram 1).

The mean surface area of unilocular fat cells
The mean surface area of unilocular fat cells showed a significant increase in the affected group (group II) and
group IIIa compared with the control group. There was no significant increase in the mean surface area in group IIIb compared with the control group (Table 2 and Histogram 2).

**Light microscope study**

**Sudan III-stained sections**

In the control group, these sections showed small unilocular adipocytes containing single small fat droplets with eccentric flattened nuclei (Fig. 1). In group II (affected group), they showed closely packed and large unilocular adipocytes (Fig. 2). In group IIIa (low-dose green tea group), most adipocytes appeared large and nearly similar to group II (Fig. 3). In group IIIb (high-dose green tea group), most adipocytes appeared small and nearly similar to the control group (Fig. 4).

**Osmic acid-stained sections**

In the control group, a large amount of small black adipocytes containing unsaturated fat were detected (Fig. 5). In group II (affected group), large and closely packed white adipocytes containing saturated fat and a few scattered small black adipocytes, which contain unsaturated fat, were seen (Fig. 6). In group IIIa (low-dose green tea group), most adipocytes appeared large, white, and contained saturated fat separated by C.T septa (Fig. 7). In group IIIb (high-dose green tea group), most adipocytes containing unsaturated fat appeared small and black (Fig. 8).

**Electron microscope study**

**Scanning electron microscopy**

In the control group, electron micrograph examination showed that the adipose tissue consisted of lobules of adipocytes of different sizes. They were separated by a meshwork of collagenous fibers (Fig. 9). The surfaces of the adipocytes were observed to be smooth (Fig. 10). In group II (affected group), the adipocytes were apparently larger and more globular compared with those of the control group (Fig. 11). Adipocytes also showed deep grooves over their surface called caveolae (Fig. 12). In group IIIa (low-dose green tea group), the adipocyte appeared larger and globular, nearly similar to group II (Fig. 13). In group IIIb (high-dose green tea group), the adipocytes were of different sizes and nearly similar to the control group, separated by connective tissue fibers (Fig. 14).

**Transmission electron microscopy**

In the control group, electron micrograph examination showed unilocular adipocytes separated by extracellular spaces. Each cell was formed of a large fat globule and a thin rim of cytoplasm (Fig. 15). There were a few mitochondria within the thin rim of cytoplasm surrounding the unilocular adipocytes (Fig. 16). Group II (affected group) showed the large number and size of mitochondria within the thin rim of cytoplasm surrounding the unilocular adipocyte (Fig. 17). There was infiltration of extracellular space (between large mature adipocytes) with macrophages (Fig. 18). Group IIIa (low-dose green tea group) showed many large mitochondria within the cytoplasm of adipocyte (Fig. 19). Group IIIb (high-dose green tea group) showed a few mitochondria within the cytoplasm of the adipocyte (Fig. 20).

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**Table 1. Showing the mean weight of animals (grams) of different groups compared with control group**

<table>
<thead>
<tr>
<th>Group</th>
<th>n=10</th>
<th>Group 2</th>
<th>n=10</th>
<th>Group 3a</th>
<th>n=10</th>
<th>Group 3b</th>
<th>n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>189.76</td>
<td>237.87</td>
<td>223.93</td>
<td>192.83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD ±</td>
<td>7.81</td>
<td>16.09</td>
<td>11.97</td>
<td>6.67</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.0006</td>
<td>0.002</td>
<td>0.068</td>
<td>0.068</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>S</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

NS, nonsignificant; S, significant; SD, standard deviation.

**Histogram 1.**

Showing the mean weight of animals (grams) of different groups compared with control group.

**Table 2. Showing the mean surface area of unilocular fat cells (micrometer square) in different groups compared with control group**

<table>
<thead>
<tr>
<th>Group</th>
<th>n=10</th>
<th>Group 2</th>
<th>n=10</th>
<th>Group 3a</th>
<th>n=10</th>
<th>Group 3b</th>
<th>n=10</th>
</tr>
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<tr>
<td>Mean</td>
<td>50 409.7</td>
<td>72 972.3</td>
<td>61 012.9</td>
<td>52 402.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD ±</td>
<td>3891.6</td>
<td>7806.4</td>
<td>8011.2</td>
<td>2932.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
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<td>0.003</td>
<td>0.071</td>
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<tr>
<td>Significance</td>
<td>S</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS, nonsignificant; S, significant; SD, standard deviation.

**Histogram 2.**

Showing the mean area of unilocular fat cells (micrometer square) in different groups compared with control group.
A photomicrograph of a section of perinephric fat of adult male albino rat of group I (control group) showing small unilocular adipocytes (A). Each cell containing a single fat droplet with eccentric flattened nuclei (arrow).

Sudan III stain, × 100.

A photomicrograph of a section of perinephric fat of adult male albino rat of group II (affected group) showing large and closely packed unilocular adipocytes (A).

Sudan III stain, × 100.

A photomicrograph of a section of perinephric fat of adult male albino rat of group IIIa (low-dose green tea group) showing large unilocular adipocytes (A) separated by connective tissue septa (arrow).

Sudan III stain, × 100.

A photomicrograph of a section of perinephric fat of adult male albino rat of group IIIb (high-dose green tea group) showing small unilocular adipocytes (A).

Sudan III stain, × 100.

A photomicrograph of a section of perinephric fat of adult male albino rat of group I (control group) showing many small darkly stained adipocytes that contain unsaturated (US) fat and a few large unstained adipocytes that contain saturated (S) fat.

Osmic acid stain, × 100.

A photomicrograph of a section of perinephric fat of adult male albino rat of group II (affected group) showing many large unstained adipocytes containing saturated (S) fat and a few small darkly stained adipocytes containing unsaturated (US) fat.

Osmic acid stain, × 100.

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Figure 7. A photomicrograph of a section of perinephric fat of adult male albino rat of group IIIa (low-dose green tea group) showing many large unstained adipocytes containing saturated (S) fat separated by C.T septum (arrow) and a few small darkly stained adipocytes containing unsaturated (US) fat.

Osmic acid stain, × 100.

Figure 9. Scanning electron micrograph of a section of perinephric fat of adult male albino rat of group I (control group) showing lobules of small adipocytes (A) packed in a meshwork of connective (C) tissue fibers.

× 350.

Figure 10. A higher magnification of the previous field showing fat cell with smooth surface (arrow).

× 1000.

Figure 11. Scanning electron micrograph of a section of perinephric fat of adult male albino rat of group II (affected group) showing large and globular adipocytes (A).

× 350.

Figure 12. A higher magnification of the previous field showing adipocyte with deep grooves of the cell surface (arrows).

× 1000.
Figure 13.

Scanning electron micrograph of a section of perinephric fat of adult male albino rat of group IIIa (low-dose green tea group) showing large and globular adipocytes (A) packed in a meshwork of connective (C) tissue fibers.  × 350.

Figure 14.

Scanning electron micrograph of a section in perinephric fat obtained from male albino rat of group IIIb (high-dose green tea group) showing small adipocytes (A) separated by connective (C) tissue fibers.  × 350.

Figure 15.

Transmission electron micrograph of a section of perinephric fat of adult male albino rat of group I (control group) showing portions of three adjacent adipocytes separated by extracellular (EC) space. Each cell is formed of a large fat (F) globule, a thin rim of cytoplasm (black arrow), and a flat peripheral nucleus (N).  × 1000.

Figure 16.

Transmission electron micrograph of a section of perinephric fat of adult male albino rat of group I (control group) showing part of an adipocyte with a fat (F) globule and a thin rim of cytoplasm (black arrow) containing a few mitochondria (M) and a flat peripheral nucleus (N).  × 4000.

Figure 17.

Transmission electron micrograph of a section of perinephric fat of adult male albino rat of group II (affected group) showing adipocyte with a large fat (F) globule and a thin rim of cytoplasm containing many mitochondria (M). Extracellular (EC) space is also seen.  × 4000.

Figure 18.

Transmission electron micrograph of a section of perinephric fat of adult male albino rat of group II (affected group) showing extracellular (EC) space between two adipocytes containing macrophages (MA). Each adipocyte is formed of a large fat (F) globule and a thin rim of cytoplasm (black arrow).  × 3000.
Adipocytes are the primary site for lipid storage and mobilization. High-dietary fat intake would implicate a remarkable effect on adipose tissue in different aspects, either in morphology or composition of adipocytes [21]. Obesity is one of the major risk factors for atherosclerosis and coronary heart disease. The lowering of body weight is beneficial for reducing the risk of developing cardiovascular disease and other health problems [22].

Green tea has thermogenic properties and promotes fat oxidation. Its extract may play a role in the control of body composition by sympathetic activation of thermogenesis, fat oxidation, or both. Stimulation of thermogenesis and fat oxidation by the GTE was not accompanied by an increase in heart rate. In this respect, the GTE is distinct from sympathomimetic drugs, whose use as antiobesity thermogenic agents is limited by their adverse cardiovascular effects, and hence, are particularly inappropriate for obese individuals with hypertension and other cardiovascular complications [23].

In this study, the samples were taken from perinephric fat and this was in agreement with other researchers [24] who found that obesity in male rat resulted in accumulation of large amounts of visceral fat (perirenal and epididymal), but not peripheral fat depots. The data of this study showed a significant increase in the mean body weight of rats fed with high-energy fatty diet (affected group) for 6 weeks compared with the control group and this agreed with the scientists [25] who reported that there was an increased rate of body weight gain with respect to high-fat diet.

In the affected group (high-fat diet) of this study, there was an obvious increase in adipocytes size compared with the animals fed with a balanced diet. These results were confirmed by morphometric and statistical results and this agreed with other investigators [25] who reported that there was an increase in adipocyte size in rats, respectively, after high-fat diet feeding. There was a high degree of hyperplasia in perirenal fat compared with other fat depots in response to high-fat feeding. They attributed this finding to the fact that cells of this depot are already of near-maximal size. Reaching this maximal size could induce increased proliferation in adipocyte progenitor cells. Hence, an increase in the mean fat cell size preceded the increase in fat cell number. Other researchers [26] reported that enlarging adipocytes due to high-fat feeding might secrete growth factors, for example, insulin-like growth factor type I. These factors could induce the proliferation of preadipocytes.

In this study, staining of adipose tissue with osmic acid stain showed an increase in saturated fat content within fat cell cytoplasm in the high-fat diet group. This finding was observed in large adipocytes. This was in agreement with researchers [14] who reported that high-fat diet resulted in an increase in the content of saturated fatty acid due to large adipocytes that strongly express adipokine genes such as leptin and adipin. This is in contrast with other investigators [27] who found a negative relationship between adipocyte size and saturated fat content of adipocyte.

In this study, scanning electron microscopic examination of adipocytes from the perinephric fat depot showed an apparent increase in adipocyte size. Deep grooves and furrows were observed on the surface of most of the adipocytes in high-energy fatty diet-fed rats and this coincided with scientists [28] who characterized these large surface-connected invaginations, and termed them as ‘caves’ that sometimes reached many micrometers in diameter. These surface-connected invaginations might account for the presence of small groups of caveoleae deep within the cell interior. They emphasized that these morphological changes accompanied the process of unilocular adipocyte formation and other researchers...
In this study, low-dose green tea supplementation with high-fat diet resulted in a significant increase in body weight compared with the control group. This means that there was still an increase in body weight of rats after a high-fat diet and this agreed with investigators [30] who reported that there was no correlation between low-dose tea consumption, plasma lipid level, and body weight. According to this result, there were no changes observed in sudan III and osmic acid-stained sections and in transmission and scanning electron microscopic images compared with the affected group (high-energy fatty diet). In contrast, other investigators [37] reported that low-dose green tea supplementation reduces body weight and prevents obesity in rats.

In this study, high-dose green tea supplementation with high-fat diet (group IIIb) resulted in nonsignificant changes in body weight compared with the control group and also with the size of the adipocytes (sudan III and scanning electron microscopy), saturated fat (osmic acid), and mitochondria content (transmission electron microscopy). A similar finding was reported by investigators [31] who found that high-dose green tea supplementation reduced body weight and total cholesterol. Some researchers [32,33] reported that catechins significantly decreased body weight, and GTE helped to prevent obesity in rats. In contrast, other investigators [34] reported that the intake of a high dose of green tea polyphenols did not affect plasma lipid levels and body weight.

In addition, the morphometric studies of this study showed a nonsignificant change in adipocyte size in the high-dose green tea group compared with the control group. These findings indicated an antiobesity effect of high-dose green tea and these agreed with researchers [35] who emphasized that green tea suppresses adipocyte differentiation and intracellular lipid accumulation. They added that EGC, a major component of green tea, resulted in decreased fat cell proliferation and differentiation. Moreover, other investigators [36] found that green tea suppresses acetyl CoA carboxylase activity, a rate-limiting step in the fatty acid biosynthesis pathway. Hence, this resulted in decreased triglycerides and fatty acid accumulation within developing adipocytes. Another mechanism was suggested by investigators [37] who stated that green tea had thermogenic properties and promoted fat oxidation. This effect was mediated by increased sympathetic stimulation for adipose tissue, rather than increased mitochondria number and size. They found that green tea inhibited catechol-β-methyltransferase activity, an enzyme that degrades norepinephrine. Thus, prolonged life of norepinephrine in the sympathetic synaptic cleft resulted in prolonged stimulation of fat oxidation in adipose tissue and increased energy expenditure. Other investigators [38] confirmed the previous mechanism as they found that EGC administration was associated with an increase in the uncoupling protein-2 gene expression. Uncoupling protein-2 is a mitochondrial membrane transporter, controlling energy expenditure and thermogenesis in adipocytes. The antiobesity action of green tea was attributed to the enhancement of lipolysis within mature adipocytes.

In this study, the transmission electron microscopic image showed many large mitochondria inside adipocytes in rats fed with high-fat diet (affected group) and this is in agreement with some investigators [39] who reported that adipogenesis of white adipocytes was also accompanied by a stimulation of mitochondrial biogenesis. This was attributed to the need for a large mitochondrial mass in white fat with increased fat intake. In contrast, other investigators [40] stated that elevated fatty acid concentrations in the cytosol of adipocytes induce mitochondrial activity to remove large amount of fatty acids through mitochondrial β-oxidation. When the rate of fatty acid release into the cytosol exceeds the β-oxidation capacity, cytosolic fatty acid concentrations increase and induce mitochondrial damage, resulting in the subsequent decrease in the mitochondrial content within adipocytes.

Furthermore in this study, transmission electron microscopic images showed infiltration of adipose tissue by macrophages in rats fed with high-fat diet and this is in agreement with investigators [41] who observed that, in obesity, adipose tissue contained an increased number of resident macrophages and that, under certain circumstances, macrophages could constitute up to 40% of the cell population within an adipose tissue depot. They added that macrophages were obviously a potential source of secreted proinflammatory factors; and these correlative data have led to the concept that macrophages could directly influence adipocyte biology. Some investigators [42] proved that macrophage inflammatory activity had a causal role for insulin resistance in case of obesity. Other investigators [27] also found that adipose tissue released inflammatory cytokines. They also reported that obesity was associated with elevated C-reactive protein level in general circulation.

Conclusion
From the preceding results, it was observed that high-fat diet led to marked morphological changes in adipose tissue. Such effects were ameliorated by concomitant administration of high-dose GTE. Hence, it is advised to consider a high dose of GTE effective against diet-induced obesity. Other studies are needed to exclude the possible adverse effect of high dose of GTE on different body organs.

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