Chronic Green Tea Consumption Decreases Body Mass, Induces Aromatase Expression, and Changes Proliferation and Apoptosis in Adult Male Rat Adipose Tissue

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Abstract

Green tea (GT) and its components have been shown to possess antiobesity properties and the corresponding mechanisms of action are being investigated, given the epidemic proportions of obesity incidence. In the current work, we used 12-mo-old male Wistar rats to test the effect of 6 mo of treatment with GT as the sole drinking beverage (52.8 ± 6.4 mL/d) on adipose tissue (AT). AT aromatase expression was determined by Western blotting, plasma concentrations of 17β-estradiol and testosterone were determined by RIA, and adipocyte size determined by measuring diameter in tissue sections. Proliferation and apoptosis were also assessed by Ki67 immunostaining and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling, respectively. Evaluations were made in subcutaneous (sc) AT and visceral (v) AT. Body weight increased over time in both groups (P < 0.001), but the increase was more pronounced in controls (P < 0.001) and food and fluid intake did not influence that effect. At the end of the experiment, aromatase expression increased in the AT (318.5 ± 60.6% of control in scAT, P < 0.05, and 285.5 ± 82.9% of control in vAT, P < 0.01). AT of GT-treated rats had a higher percentage of proliferating cells (204.1 ± 3.2% of control in vAT, P < 0.001) and smaller adipocytes (78.3 ± 3.2% of control in vAT, P < 0.05). GT also increased the number of apoptotic cells in vAT (320.4 ± 21.9% of control; P < 0.001). These results suggest new mechanisms for GT on body weight and highlight its potential benefit to prevent or treat obesity and the metabolic syndrome.

Introduction

Obesity has become a major health problem as a growing proportion of the population worldwide is overweight and very likely to become obese (1). One of the major concerns related to the increasing obesity incidence is its association with other pathological signs, including hypertension, dyslipidemia, insulin resistance, and glucose intolerance. The combination of these features constitutes the so-called metabolic syndrome that dramatically increases the risk to develop cardiovascular disease or diabetes (2). Furthermore, visceral (v) fat accumulation is the main feature of this syndrome, whereas subcutaneous (sc) fat is a minor contributor to obesity complications (3). Obesity is generally associated with systemic inflammation, with increased production of cytokines from inflammatory cells, such as macrophages, and adipocytes (4,5). It has been shown that cytokine and hormone release profiles of adipose tissue (AT) from various locations are distinct, although the reasons for these differences are not fully clarified (6).

Estrogens can be produced in the AT from aromatization of circulating androgens by the action of aromatase (7) and its expression is higher in scAT in comparison to vAT (8). Evidence from estrogen deficiency models shows that estrogen signaling contributes to weight regulation and its absence is related to v obesity, insulin resistance, and glucose intolerance along with other features of the metabolic syndrome (9,10). In men, aromatase mutations that give rise to lack of enzyme function are related with undetectable estrogen concentration and normal or high testosterone and gonadotropins associated with excess weight or obesity. Body fat accumulation in the v region and alterations of lipid metabolism are present as well (11). In women, aromatase deficiency also results in developmental and

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3 Abbreviations used: AT, adipose tissue; DAPI, 4',6-diamidino-2'-phenylindole dihydrochloride; EC, (–)-epicatechin; EGCG, (–)-epigallocatechin-3-gallate; ER, estrogen receptor; GT, green tea; sc, subcutaneous; v, visceral.

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metabolic alterations. Surprisingly, these disturbances are ameliorated by estrogen treatment (12). Furthermore, alterations in AT distribution and inflammatory status are common after estrogen production ceases in menopause and may also be reversed by hormone replacement treatment (13). vAT has a relative estrogen insufficiency due to its lower expression of aromatase compared with scAT (7). Although estrogens influence body fat accumulation by altering appetite and energy expenditure, direct AT effects are possible, including lipoprotein lipase inhibition and hormone-sensitive lipase stimulation, as well as preadipocyte proliferation and differentiation alterations (14,15). This hormone is thus likely to induce its antiobesity effects locally, as its tissue concentration may be 10 times higher than circulating concentrations (16) and because AT accounts for most of the estrogen production in males and postmenopausal females (10).

The investigation of the antiobesity properties of food components is a popular field of research, because it may lead to the discovery of new naturally occurring agents to prevent or treat obesity. There are studies on green tea (GT) and GT components, especially catechins, demonstrating their effects on energy expenditure, fat oxidation, blood lipids, and preadipocyte differentiation and adipocyte apoptosis (17,18). Yet, to date, little is known about its ability to interfere with estrogen synthesis in vivo, particularly in the AT. Aromatase activity inhibition by some GT components has been demonstrated on placental microsomes (19) and choriocarcinoma-derived JAR cells (20), but in vivo long-term studies are lacking. We have also recently found that red wine, a beverage containing compounds able to inhibit aromatase activity in cellular lines, could induce an increase in aromatase expression in animal models after prolonged treatment (21). Taking this information into account, together with the known effects of locally produced estrogens, it is possible that GT components may modulate aromatase in the AT and that, as a consequence, alterations in tissue dynamics may occur. For this reason, we intended to determine the effect of chronic consumption of GT on the expression of aromatase in the AT from different anatomical locations. We also sought to determine the effect of GT intake on adipocyte size, AT apoptosis, and proliferation. To approach this subject, we treated adult male Wistar rats with GT, performed evaluations in scAT and vAT, and measured circulating 17β-estradiol and testosterone concentrations.

Materials and Methods

Rats and treatments. Ten 12-mo-old male Wistar rats weighing 918.3 ± 22.8 g were individually housed and maintained under standard temperature and light conditions (21-22°C; 12-h-light-dark cycles). Rats were randomly assigned to 2 groups: control rats with access to tap water and GT-treated rats with access to GT as the only liquid source. During the 6-mo treatment, all rats had access to standard laboratory animal food (Panlab S.L.; 15,733 kJ/kg, 156 g/kg protein, 28 g/kg fat, 48 g/kg cellulose, 49 g/kg mineral salts, 22.5 mg/kg retinol equivalents, 0.375 mg/kg cholecalciferol equivalents, and 150 mg/kg α-tocopherol equivalents). Bottles containing the beverages were protected from light to avoid oxidation of light-sensitive components and beverages were renewed every 2–3 d with water (controls) or freshly prepared GT. We monitored ad libitum consumption of both food and fluid every other day and recorded rat weight every week. To prepare GT infusion, 1 L of boiling water (100°C) was dispensed over 3 tea bags (1.3 g/bag) for 5 min. The infusion was allowed to cool to room temperature before being transferred to the bottles and supplied to the rats. The tea used during the treatments was from the same batch and the composition of the final beverage was determined after 3 randomly chosen infusion preparations. Tea composition in catechins was determined using HPLC (22) and was as follows: (-)-epigallocatechin-3-gallate (EGCG), 1.21 ± 0.256 mmol/L; (-)-epicatechin (EC), 0.84 ± 0.071 mmol/L; EC-3-gallate, 0.29 ± 0.067 mmol/L; (+)-gallocatechin-3-gallate, 0.07 ± 0.001 mmol/L. Mean caffeine content was 0.52 ± 0.022 mmol/L, measured using GC-ion trap MS, as described (23). Animal procedures were according to the European Community guidelines (86/609/EEC) and the Portuguese Act (129/92) for the use of experimental animals.

Tissue collection and preparation. At the end of treatment, fed rats were anesthetized (intraperitoneal sodium pentobarbital; 80 mg/kg body weight) and perfused with saline at 4°C. All rats were killed between 1000 and 1200. Samples from sc (inguinal) and v (mesenteric) AT were removed and frozen in liquid nitrogen for protein extraction. A fraction of each sample was immersed in 10% formalin solution for paraffin embedding and 4-μm-thick tissue sections were made for histological analysis, apoptosis determination, and immunohistochemistry.

Plasma 17β-estradiol and testosterone measurement. Before perfusion, blood was drawn from the left ventricle into heparinized tubes and plasma fractions were frozen at −80°C until analysis. 17β-Estradiol and testosterone were measured with RIA using antisera against 17β-estradiol and 125I-17β-estradiol (Coat-a-Count TKE2, Diagnostic Products) or antisera against testosterone and 125I-testosterone (Testo-RIA-CT, Biosource), respectively. The commercial kits used to measure 17β-estradiol and testosterone were specific for these hormones and had very low reactivity with estrone and androstenedione. The method for measuring 17β-estradiol had a sensitivity of 29 pmol/L; the detection limit was 174 nmol/L for the test measuring testosterone.

Measurement of adipocyte size. Hematoxylin-eosin–stained tissue sections were observed and photographed under specimen identity occultation. We estimated adipocyte mean diameter using NIS Elements BR software (Nikon). Fifty to 100 adipocytes from 5 randomly selected different fields were measured for each sample.

Apoptosis assay. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling was used according to the manufacturer (Roche) in paraffin-embedded tissue sections. Cell nuclei were counterstained with 4’,6-diamidine-2-fluorescein dihydrochloride (DAPI; Roche). Slides were visualized under a fluorescence microscope (Nikon 50i) at 200× magnification. Apoptosis was determined as the percentage of positive over total counted cells. We counted 200 nuclei from different section fields for each sample.

Determination of proliferation. Proliferation was measured through immunohistochemical labeling of proliferation-characteristic nuclear protein Ki67. This cellular marker is expressed in all active phases of the cell cycle (G1, S, G2, and mitosis) and is absent in quiescent (G0) cells (24). Anti-Ki67 primary and fluorescein isothiocyanate-conjugated secondary antibodies (1:50 and 1:200 in 4% bovine serum albumin, respectively; Santa Cruz Biotechnology) were used. Total nuclei were counterstained with DAPI and visualized at 200× magnification. Nuclei stained with anti-Ki67 antibody were counted in 5 randomly selected section fields. Total DAPI-stained nuclei were counted for the same optical fields. Proliferation is given as a percentage of proliferating cells over total cells. We counted 400–600 nuclei in scAT and 200–300 in vAT from different section fields for each sample.

Western blotting. AT samples (0.8–1 g) were incubated at 4°C for 2 h in 1.5 mL of detergent protein extraction buffer (50 mmol/L Tris-HCl, pH 7.4, 1% Triton X-100, 0.2% sodium deoxycholate, 0.2% sodium dodecylsulfate, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride) containing 25 μL protease inhibitor cocktail (Sigma) as described (25). Each sample was incubated at 4°C with agitation for 2 h in the buffer and subsequently homogenized in a Teflon-glass homogenizer and centrifuged at 10,000 × g for 10 min. The infranatant protein solution was collected, quantified by bicinchoninic acid protein assay (Pierce), and stored at −80°C. Before SDS-PAGE separation, proteins were dissolved 1:1 in loading buffer (Bio-Rad Laboratories)
containing 2% mercaptoethanol, denatured for 90 s at 95°C, and 20 μg of protein was loaded per well. Anti-aromatase polyclonal antibody (1:200, Santa Cruz Biotechnology) and horseradish peroxidase-conjugated polyclonal antibody (1:1000) were used followed by chemiluminescent detection. β-Actin primary antibody (1:1000, Lab Vision) and secondary antibody hybridizations were conducted using the same procedure. Band intensity was determined using Gel Pro Analyzer (Media Cybernetics) and aromatase was normalized to β-actin expression (expressed in arbitrary units). Determinations were made in membranes containing both control and GT-treated rat proteins in 2 separate experiments.

Statistical analyses. Results are expressed as means ± SEM. Mixed effects model with random intercept was used to assess the progression of body weight with time (26 repeated measures of the body weight) adjusted for the liquid and food intake. This model allows the incorporation of both fixed (population-specific) and random (subject-specific) variables and assumes that the vector of repeated measures on each subject follows a linear regression model. Using the random intercept, it is tested if variability in subject-specific slopes can be ascribed to treatment. The differences between 2 groups were assessed by unpaired Student’s t-test or Mann Whitney U-test when variance of measurements was significantly different. We used Fisher’s exact test to determine the difference between groups in 17β-estradiol measurements, comparing the number of detectable and undetectable measurements in controls and treated animals. Association between outcome variables was determined with the data pooled from the 2 groups using Pearson or Spearman correlations for data with normal or nonnormal distributions, respectively, and AT measurements were compared within the same tissue. The normality of the distribution for each outcome variable was tested using Shapiro’s test. Statistical analyses were performed using Graph Pad Prism software (Graph Pad Software version 3.0) and SPSS (SPSS 12.0 for Windows). Differences were considered significant when $P < 0.05$.

Results

Body weight and food and fluid ingestion. Body weight did not differ between groups at the beginning of the experiment (Table 1) and increased gradually throughout treatment (Fig. 1). Food and fluid intakes also did not differ between the 2 groups (Table 1). However, body weights increased during each week of the study ($P < 0.001$) and the increase in GT-treated rats ($2.83 ± 0.20 \text{ g} \times \text{wk}^{-1}$) was less than in control rats ($5.26 ± 0.25 \text{ g} \times \text{wk}^{-1}$; $P < 0.001$). Food and fluid intakes did not affect the changes in body weight (Table 2). After 6 mo of treatment, the weight gain of GT rats ($64.8 ± 16.0 \text{ g}$) was $47.5\%$ that of controls ($136.4 ± 25.4 \text{ g}$; $P < 0.05$).

Plasma 17β-estradiol and testosterone concentrations. The plasma concentration of 17β-estradiol was undetectable in control rats, whereas that of GT-treated rats was $39.2 ± 10.4 \text{ pmol/L}$ ($P < 0.01$). The plasma testosterone concentration was lower in GT-treated rats ($790 ± 177 \text{ pmol/L}$) than in controls ($2720 ± 769 \text{ pmol/L}$; $P < 0.01$).

Aromatase expression. Relative aromatase expression in homogenates from scAT was $213\%$ higher than that of vAT ($P < 0.05$) in control rats (Fig. 2). Interestingly, GT treatment induced a marked increase in aromatase expression in both AT locations to $318.5 ± 60.6\%$ of control in scAT ($P < 0.05$) and $285.5 ± 82.9\%$ of control in vAT ($P < 0.01$).

Adipocyte size. In control rats, the diameters of scAT ($123.7 ± 2.3 \mu m$) and vAT adipocytes ($113.3 ± 5.0 \mu m$) did not differ. Chronic GT consumption resulted in a reduction in adipocyte mean diameter compared with controls, both in scAT ($96.9 ± 2.1 \mu m$; $P < 0.05$) and in vAT ($99.6 ± 3.7 \mu m$; $P < 0.01$) (Fig. 3).

AT apoptosis and proliferation. Apoptotic cell number in vAT was greater in GT-treated rats ($24.1 ± 1.6\%$) than in controls ($7.5 ± 1.4\%$) ($P < 0.01$) and tended to be greater in scAT ($P = 0.08$). The number of apoptotic cells in the AT of control rats did not differ between depots. Moreover, GT treatment increased the number of proliferating cells both in scAT and vAT ($P < 0.01$). The percentage of proliferating cells also differed between the locations, as the sc depot had more proliferating cells ($P < 0.05$). GT treatment raised proliferation in vAT to levels similar to those in control scAT, with most Ki67 immunostaining appearing in the stromal fraction of the tissue (Fig. 4).

![FIGURE 1](https://academic.oup.com/jn/article-abstract/138/11/2156/4670057) Body weight throughout the 6 mo of treatment in control (C) and GT-treated rats. Results are given in mean and dotted lines represent 1 SEM, $n = 5$.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Mixed model for the variation of body weight with time, adjusted for liquid and food intake (random-intercept), in GT and control rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
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</tr>
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</tr>
<tr>
<td>Time, wk</td>
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</tr>
<tr>
<td>GT, g</td>
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<tr>
<td>Control, g</td>
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</tr>
<tr>
<td>GT x time, wk</td>
<td>0$^1$</td>
</tr>
<tr>
<td>C x time, wk</td>
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</tr>
<tr>
<td>Liquid intake, mL</td>
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</tr>
<tr>
<td>Food intake, mL</td>
<td>0.2</td>
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</tbody>
</table>

$^1$ Reference class.
Associations between outcome variables. As expected, aromatase expression in scAT and vAT was positively correlated with 17β-estradiol and inversely correlated with testosterone plasma concentrations (Table 3). Similarly, 17β-estradiol concentration varied inversely with the plasma concentration of testosterone. Furthermore, with increasing aromatase expression, the percentage of apoptotic and proliferating cells within each corresponding AT location increased. The same relationship was observed between 17β-estradiol plasma concentration and apoptosis in vAT, as well as proliferation in scAT and vAT. Accordingly, the inverse relationship was observed for testosterone. The percentages of apoptotic cells in scAT and vAT were directly proportional to the percentages of proliferating cells within the same depot. scAT adipocyte size was positively correlated with body weight gain. In both AT locations, the 17β-estradiol plasma concentration was inversely correlated with adipocyte diameter. In vAT, aromatase expression and adipocyte size were inversely associated. The plasma testosterone concentration was positively associated with adipocyte diameter in scAT and with body weight gain. In scAT, adipocyte size was inversely correlated with the percentage of proliferating cells.

Discussion

GT is a complex beverage composed of amino acids, carbohydrates, minerals, vitamins, and xanthines such as caffeine and theophylline. It is also one of the richest food sources of polyphenols, mainly the flavan-3-ols or catechins, including EGCG (~59% of the total of catechins), (-)-epigallocatechin, EC-3-gallate, and EC (26). After oral administration of tea, catechins can be found in the plasma and widely distributed in rat tissues (26). From modulation of food intake and energy expenditure, to antiadipogenic and apoptosis-promoting activities, several relevant effects have been described for tea in the past few years; catechins, present in great amounts in GT, have been shown to be involved in most of the actions described (27–29). Caffeine, ranging from 0.51–0.86 mmol/L (26), may also account for some of the effects attributed to GT, as it increases thermogenesis and acts synergistically with other tea components such as the catechins (30). It must be stressed, however, that some studies did not support a GT effect on energetic metabolism (31,32).

As shown in the present study, prolonged GT intake by adult male Wistar rats resulted in a significantly lower weight gain compared with controls. In association with lower weight gain, increased aromatase expression was observed in both scAT and vAT of GT-treated rats. Furthermore, this increase in aromatase expression reflected upon higher plasma concentration of 17β-estradiol. We used adult rats to avoid the variations in body weight due to changes in lean mass that are associated with growth in younger rats.

Although decreased body weight gain has already been reported both in humans and animals after GT treatment (27,33,34), increased estrogen production has not yet been related to GT capacity to regulate body composition. Previous studies reported that GT catechins were able to inhibit aromatase activity in the placenta (19,20). The beverage, when placed in contact with the same cells, reduced aromatase activ-
are means were counted in different randomly selected section fields. Results quantification, Ki67-labeled nuclei, as well as total DAPI-stained nuclei, through the AT aromatase promoter 1.3 (7). cellular cAMP, a known inducer of aromatase expression difficult to predict the effects on the AT. Xanthines could also presence of distinct costimulators or corepressors, making it are not consistent between cell lines, possibly due to the receptor (ER) a with estrogen signaling through interaction with estrogen re- cipient (ER) or estrone (7). It has been proposed that the relative amount of estrogens and androgens or of their receptors (10,14) will determine the response of the AT to sex steroids. Furthermore, those ratios are thought to be closely related to the sexual dimorphism in body fat distribution and to the differential risk of men and pre- and postmenopausal women to develop metabolic diseases (41).

Estrogens have profound effects on energy metabolism as demonstrated by the amount of fat accumulation in estrogen insufficiency models (9,10), with reversion of the effect by estrogen treatment. On the other hand, estrogen therapy ameliorates the plasma lipid profile in healthy males, although no effect has been reported on body weight (42). Mice lacking ERα develop the same metabolic abnormalities as aromatase knockout mice, showing that ERα mediates the protective actions of estrogens (14). These alterations were absent in ERβ knockouts (14), although recently it has been reported that mice lacking ERβ, when fed a high-fat diet, had improved insulin sensitivity and glucose tolerance compared with wild-type mice (43).

As first described by Vague (44), the 2 main AT depots display major differences in the metabolic consequences they induce. Unfavorable cardiovascular profiles and the risk of developing type 2 diabetes are greater in individuals whose AT is located within the abdominal cavity than in those having mainly sc fat (2), although there is still no exact knowledge of the reasons for these differences. Sex steroids contribute to determine the location of fat distribution and the ability of different AT depots to produce and respond to these hormones varies tremendously (45). Confirming previous findings (8), we found the expression of aromatase in scAT was higher than that in vAT. The differential aromatase expression may contribute to the metabolic differences encountered between the accumulation of fat in the 2 locations. In fact, 2 key proteins involved in lipid deposition, lipoprotein lipase and leptin, are transcriptionally regulated by 17β-estradiol, being down- and upregulated, respectively, by this hormone (46,47). Interestingly, with GT intake, vAT expression of aromatase rose to levels comparable to those found in scAT from controls.

scAT is the main AT depot responsible for estrogen produc- tion. Because plasma 17β-estradiol was undetectable in control rats, vAT would be under little influence of this hormone due to its low endogenous capacity to produce estrogens. On the other hand, in GT-treated rats, both scAT and vAT may be influenced by estrogens and the increase in plasma concentration of 17β-estradiol may also indicate that distant tissues or organs may be affected. Food intake was not influenced, as rats from both groups ingested similar amounts of food. In fact, in models of estrogen insufficiency, increases in body fat mass are not associated with hyperphagia (48). Estrogens have, however, been shown to increase voluntary energy expenditure (48) and leptin and decrease insulin sensitivities in the hypothalamus (49).

Either induced by the elevation of 17β-estradiol production or directly by the presence of GT components, e.g. catechins or xanthines, other interesting effects were found on the AT. First,
measurement of adipocyte diameter revealed a decrease in adipocyte size in both AT depots following GT treatment. It has been pointed out that obesity and its metabolic consequences result from both increased number of adipose cells (hyperplasia) and adipocyte size (hypertrophy) (50). However, adipocyte size seems more critical for predicting morbidities related to AT excess. Inflammatory events originating in AT play a key role in obesity-associated diseases, the infiltration of macrophages in the AT being a central event (4). Cinty et al. (51) have shown that macrophages in the AT surround dead adipocytes. We have previously demonstrated that, for simple physical reasons, larger adipocytes are more prone to rupture than small ones (52), particularly when they are contained in the abdominal cavity where sudden variations of pressure frequently take place (53,54). The inflammatory events related with large adipocytes are further supported by the recent report that adiponec tin release from adipocytes is inversely correlated with adipocyte size in obese patients (50). Conversely, tumor necrosis factor-α, interleukin-6, and high sensitivity C-reactive protein circulating concentrations were positively associated with the same variable (50). Thus, by reducing adipocyte size, chronic GT treatment might have a great impact on obesity-driven inflammation. Both aromatase expression and plasma 17β-estradiol concentrations had a strong positive correlation with the percentage of proliferating cells in scAT and vAT. Thus, it is very likely that 17β-estradiol production in the AT may be responsible for the changes observed in proliferation either in cells from the adipose lineage or others. For example, endothelial, smooth muscle, and mesenchymal cells may be induced to proliferate in the presence of 17β-estradiol (56). Previous studies reported that GT catechins, particularly ECGG, have antimitogenic effects on adipose cells (29). This apparent contradiction may have arisen from the fact that the antimitogenic action was reported in vitro in 3T3-L1 preadipocytes. In that experimental setting, the effect of GT or GT catechins may not be mediated through estrogens, because these cells either lack or have residual aromatase expression. Furthermore, as mentioned previously, several other types of cells compose AT and many of them are known targets for estrogens actions. Also worth noting is the inverse correlation between plasma testosterone and the percentage of proliferating cells. This, along with the known antiadipogenic effect of testosterone (41), implicates this hormone in the reduction of AT plasticity in response to energy excess that predisposes men to metabolic disorders.

The fact that total AT or body composition was not measured is an important limitation of this study. In addition, determination of metabolic parameters and baseline and time course measurements of hormones would strengthen the conclusions. Even so, relevant alterations were observed after GT treatment, which deserve attention and further study.

In conclusion, GT intake induced profound remodeling of AT. The causal role of the GT-induced increase in aromatase plasma concentrations, in conformity with the sexual dimorphism of vAT accumulation.

AT remodeling extended also to cell proliferation. Preadipocytes and mature adipocytes are the major cell types that compose AT, but vascular endothelial and smooth muscle cells are also documented (14). Proliferation was identified mainly in the AT stromal fraction where preadipocytes and blood vessels are predominant. Although we do not know which cell types were Ki67 immunostained, proliferation of either type would reveal greater capacity for lipid trafficking between mature and maturing adipocytes and between locations of AT deposition. Both aromatase expression and plasma 17β-estradiol concentrations had a strong positive correlation with the percentage of proliferating cells in scAT and vAT. It is very likely that 17β-estradiol production in the AT may be responsible for the changes observed in proliferation either in cells from the adipose lineage or others. For example, endothelial, smooth muscle, and mesenchymal cells may be induced to proliferate in the presence of 17β-estradiol (56). Previous studies reported that GT catechins, particularly ECGG, have antimitogenic effects on adipose cells (29). This apparent contradiction may have arisen from the fact that the antimitogenic action was reported in vitro in 3T3-L1 preadipocytes. In that experimental setting, the effect of GT or GT catechins may not be mediated through estrogens, because these cells either lack or have residual aromatase expression. Furthermore, as mentioned previously, several other types of cells compose AT and many of them are known targets for estrogens actions. Also worth noting is the inverse correlation between plasma testosterone and the percentage of proliferating cells. This, along with the known antiadipogenic effect of testosterone (41), implicates this hormone in the reduction of AT plasticity in response to energy excess that predisposes men to metabolic disorders.

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**TABLE 3** Correlation coefficients between outcome variables measured in GT and control rats

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Body weight gain</th>
<th>17β-Estradiol</th>
<th>Testosterone</th>
<th>Aromatase</th>
<th>Apoptosis</th>
<th>Proliferation</th>
<th>sc/v AT, respectively$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$g$</td>
<td>pmol/L</td>
<td>arbitrary units</td>
<td></td>
<td></td>
<td>%</td>
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<tr>
<td>17β-Estradiol, pmol/L</td>
<td>$-0.61^*$</td>
<td>-0.89$^*$</td>
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<tr>
<td>Testosterone, pmol/L</td>
<td>$0.76^*$</td>
<td>-0.61$^*$</td>
<td>-0.71$^*$</td>
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<tr>
<td>scAT</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Aromatase, arbitrary units</td>
<td>$-0.50^*$</td>
<td>0.86$^*$</td>
<td>-0.74$^*$</td>
<td></td>
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<tr>
<td>Adipocyte size, μm</td>
<td>$0.71^*$</td>
<td>$0.61^*$</td>
<td></td>
<td></td>
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<td>Apoptosis, %</td>
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<td>0.42$^*$</td>
<td>-0.61$^*$</td>
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<tr>
<td>Proliferation, %</td>
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<td>$0.82^*$</td>
<td>-0.64$^*$</td>
<td></td>
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<tr>
<td>vAT</td>
<td></td>
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<td>Adipocyte size, μm</td>
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<td>0.37$^*$</td>
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<td>Apoptosis, %</td>
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<td>$0.78^*$</td>
<td>-0.76$^*$</td>
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</tbody>
</table>

$^1$ Spearman correlation. *$P$ < 0.05.

$^2$ Pearson correlation.

$^3$ AT measurements were compared within the same tissue (sc, v).
expression and circulating concentration of 17β-estradiol needs to be explored, although the strong correlations of 17β-estradiol and aromatase measurements with adipocyte size, apoptosis, and proliferation are in favor of the existence of a causal relationship. We also propose that the presence of a higher precursor adipose cell pool together with the proadipogenic effect of estrogens and attenuation of the antiadipogenic effect of testosterone would facilitate differentiation and the distribution of fat in an increased number of cells, resulting in reduced cell size. This could then contribute to the redistribution of body AT and decrease obesity-related inflammation.

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Literature Cited


