Moderated-fat Diet Supplemented with Green Tea Reduces oxLDL Levels and Fat Mass in Obese Women

Elizabeth Gordillo-Bastidas¹, Irinea Yañez-Sanchez², Arturo Panduro³ and Erika Martínez-Lopez*¹

¹Department of Molecular Biology in Medicine, Hospital Civil de Guadalajara “Fray Antonio Alcalde”, Department of Molecular Biology and Genomics, Health Sciences University Center, University of Guadalajara, Guadalajara, Jalisco, Mexico
²Department of Medical Clinics, Health Sciences University Center, University of Guadalajara, Guadalajara, Jalisco, Mexico
³Department of Natural and Exact Sciences, Institute of Nano sciences and Nanotechnology, University Center of Valles, University of Guadalajara, Ameca, Jalisco, Mexico

Abstract

Background: Obesity is a chronic degenerative disease, considered as cardiovascular risk factor, and characterized by systemic inflammation and high levels of oxidized LDL (oxLDL). Clinical studies suggest that drink green tea could improve these complications. The aim of this study was analyze the effect of a moderate-fat diet supplemented with green tea on oxLDL, fat mass and Tumoral necrosis factor α (TNFα) in obese women.

Methods: Obese women, without other chronic-degenerative disease, were divided into control group (n=32) and intervention group (n=32), and were instructed to consume a moderate-fat diet and intervention group was instructed to supplement the diet with green tea. Anthropometric and biochemical measurements were measured, and oxLDL and TNFα levels were determined by ELISA. All parameters were realized at baseline and at 1st, 2nd and 3rd months post-intervention. TNFα mRNA expression was determined by real-time RT-PCR.

Results: The intervention group showed a significant reduction of oxLDL versus the control group in the 2nd month (p<0.05). At the end of the follow-up period, intervention group had a higher body weight loss percentage (84.2 ± 15.1%) in relation to fat mass (p < 0.05), and this group had less increment in serum TNFs than the control group. The intervention group demonstrated other metabolic improvements like a reduction in triglycerides (-39.0 ± 16.6mg/dL), while high density lipoprotein increased (4.2 ± 0.9mg/dL) versus control group (p<0.05).

Conclusions: Our results demonstrate that consuming a moderate-fat diet supplemented with green tea reduce oxLDL, fat mass, and modulate TNFα expression. This therapy could aid reduces the cardiovascular risk in obese patients.

Keywords: Obesity; Epigallocatechin 3 gallate; Dietary intervention; Oxidized LDL; TNFα

Introduction

Currently, obesity is a global health problem linked with the onset and progression of chronic diseases. It has also been associated with high levels of reactive oxygen species (ROS) and tumor necrosis factor α (TNFα). This condition evokes inflammation due to the oxidation of low-density lipoprotein (LDL) particles, which in turn activate macrophages to secrete TNFα while changing into an inactive foam cell phenotype. Subsequently, foam cells and oxidized LDL (oxLDL) are accumulated on the endothelium, inducing thrombosis and atherosclerosis. Thus, by this mechanism, oxidative stress has been considered a risk factor for cardiovascular disease in patients who have high levels of oxLDL [1].

Green tea (Camellia sinensis) is a widely consumed beverage in many cultures and it has been attributed beneficial effects against degenerative diseases and weight control [2-4]. In vivo studies have shown that rodents fed with a meal containing green tea lost fat mass and reduced food consumption, weight, insulin, glucose, total cholesterol and triglycerides [5,6]. Clinical trials have showed that a consumption of 302-525 mg (equivalent to 4-6 cups of tea) of epigallocatechin-3-gallate, the main catechin of green tea, is safe. This reduces triglycerides [7,8] and elevates high-density lipoprotein (HDL) and adiponectin levels. The Rotterdam study showed an inverse association between severe aortic atherosclerosis and ≥ 500 mL/green tea/day, in a 3-year follow-up [9]. In a further study, the same group of patients was followed for 5.6 years observing that the relative risk for infarction was lower in patients that drank ≥375 mL/green tea/day than in patients that did not drink green tea [10].

On the other hand, there is evidence that the consumption of green tea may have beneficial effects on metabolic and anthropometric profile. The aim of the present study was to analyze the effect of a moderate-fat diet supplemented with green tea on oxLDL, fat mass and TNFα in obese women.

Materials and Methods

Study population

We assessed 114 women with the following eligibility criteria: obese women older than 18 years, in good health, as determined by a medical history questionnaire, and normal clinical laboratory tests.

*Corresponding author: Erika Martínez-Lopez, Department of Molecular Biology in Medicine, Hospital Civil de Guadalajara “Fray Antonio Alcalde”, Department of Molecular Biology and Genomics, Health Sciences University Center, University of Guadalajara, Guadalajara, Jalisco, Mexico, Tel: +52(33) 36147743; E-mail: erikamitz27@yahoo.com.mx

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excluding lipids. Exclusion criteria was a history of cardiovascular, hepatic, gastrointestinal, or renal disease; alcoholism, smoking, exogenous hormone use or other medication; no supplemental vitamin or infusion drinking (tea, coffee); or treatment for weight loss 3 months before the start of the study. A total of 64 female with BMI ≥ 30 kg/m2 were included. We conducted the trial between April 2009 and December 2009 in Guadalajara, Jalisco, Mexico. All studies were conducted at the Department of Molecular Biology in Medicine, Hospital Civil de Guadalajara “Fray Antonio Alcalde”. This study was approved by the Ethical Committee for Human Research, Universidad de Guadalajara (registration number CI-10808; 028/10 and Clinicaltrials.gov, ID: NCT01628705). The procedures were in accordance with this institution’s guidelines, and written consent was obtained from each study subject.

**Study design**

This study was a randomized controlled clinical trial. Sample size was calculated based on a standard deviation observed to detect a reduction in oxLDL with a 95% confidence and 20% of power [11]. All subjects were screened by a complete medical history and signed the informed consent form. The subjects were randomly assigned to 2 different groups using a computer-generated random sequence. The follow-up period for the intervention was three months. The control group (n=32) and intervention group (n=32) were instructed to consume a moderate-fat diet and a moderate-fat diet supplemented with green tea, respectively. At the end of the study, 20 patients in the control group and 23 patients in the intervention group remained (Figure 1). During the follow-up. Anthropometric and biochemical measurements, oxLDL, and TNFa serum levels were performed at baseline and 1st, 2nd and 3rd months post-intervention. Also, TNFa mRNA expression was determined at baseline and at 3rd-month post-intervention by real-time RT-PCR. Blood pressure was measured manually on the upper arm using a sphygmomanometer [12,13].

**Moderate-fat Diet and Green Tea**

A qualified dietitian interviewed each patient at baseline. All the enrolled subjects were asked to record their daily dietary intake for three days, including a weekend day. Moderate-fat diet was composed of 25% fat (saturated fat <7%, monounsaturated 10% and polyunsaturated 10%, relative to total calories), 60% carbohydrates, and 15% protein. Each participant’s habitual caloric intake was reduced by 500 kcal per day [14-16]. Dietary adherence and adherence to drinking tea were assessed by a 3-day food record and questionnaire, both of which were measured monthly. The patients drank *sencha* green tea. Subjects of the intervention group were instructed on how to prepare the green tea infusion. Each cup was prepared using 3 g of dried green tea in 300 mL of hot water (temperature 80°C) for 4 min. It was drunk fresh and without sugar. The treatment consisted of 2 cups/day of green tea, one taken in the morning, and one at evening 30 min after meals. With a total amount of epigallocatechin-3-gallate of 498 mg/day.

**Anthropometric measurements**

Subjects came in the morning after 12 h fasting and with light clothes. Waist circumference was determined using the National Health and Nutrition Examination Survey III procedure [17]. Body weight, Body mass index (BMI), fat mass (kg) and percentage of fat mass were determined by tetra polar bio impedance analysis (In Body 3.0, Bio space CO, Korea). A qualified dietitian assessed this determination three times each interview.

Fat mass 5 percentage was calculated with this data. The percentage of body weight loss was relative to fat mass determined at baseline, 1st, 2nd and 3rd-month post-intervention.

**Blood sampling**

Fasting (12 hours) blood samples were collected in dried tubes and then were centrifuged at 3000g for 15 minutes at room temperature in order to obtain the serum. Serum samples were divided into aliquots and stored at -80°C for subsequent analysis of insulin, oxLDL, and TNFa. Further, an EDTA tube was used to quantify the TNFa mRNA expression.

**Assays**

Biochemical analysis for triglycerides, total cholesterol, very low density lipoprotein (VLDL), LDL, HDL, glucose and C reactive protein (CRP) were determined immediately using dry chemistry on
a Vitros250 analyzer (Ortho Clinical Diagnostics, Johnson & Johnson Co, Rochester, NY). Insulin was determined by Microparticle Enzyme Immunoassay (Abbott-AxSym chemistry analyzer, Abbott laboratories, Abbott Park, IL, USA). oxLDL (Biomedical Gruppe, ALPCO Immunoassays, and Glasgow, UK) and TNFα (R&D Systems Inc, Minneapolis, USA) were assessed by Enzyme-Linked Immuno Sorbent Assay (ELISA) according to the manufacturer’s instructions. Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR). HOMA-IR was calculated using the following formula: HOMA index=[fasting insulin (µU/mL) x fasting glucose (mmol/L)] × 22.52, as shown by Matthews et al. [18]. An HOMA-IR >2.5 was considered to be insulin resistance.

RNA extraction and real-time PCR

Leukocytes were separated by density gradient (LymphoprepTM, Axis-Shield, and Oslo, Norway) and total RNA was obtained with the TRIZOLmethod [19]. cDNA synthesis was performed with MMLV enzyme (Invitrogen TM) according to previous reports [20]. Expression of TNFα mRNA (PN 432705ST, Applied Bio systems) was determined by real-time RT-PCR (ABI Prism 7300 System, Applied Bio systems) using master mix (Applied Bio systems), AmpliTaq Gold® polymerase and the 18s rRNA as constitutive gene (PN 4369510, Applied Bio systems). The expression levels of TNFα gene in each sample were calculated by the comparative Ct method (2^–ΔCt formula), after normalizing with the Ct value of the 18s rRNA housekeeping gene. Each experiment was performed in triplicate in samples at baseline and at 3rd-month post-intervention.

Statistical analysis

Data is presented as mean ± standard deviation (SE). Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) (v19, Chicago, IL) and a p value<0.05 was considered statistically significant. The non-parametric Friedman and Wilcoxon post hoc tests were used when intragroup means were compared. Non-parametric Mann-Whitney test was used when means were compared between groups. We calculated the delta value of each measurement. Delta (∆) is the result of the subtraction of the baseline data from the 3rd-month data (3rd month - baseline). mRNA expression levels are presented as relative quantification units (RQU) using the 2^–ΔΔCt method [21,22].

Results

Anthropometric, biochemical characteristics and nutritional intake at baseline

Sixty-four patients were enrolled in this study, and only 43 participants completed the follow-up period for three months. The mean age was 36.8 ± 1.3 years and the mean BMI was 36.3 ± 0.9 kg/m². The average weight loss at the end of the intervention was of 4.1 ± 0.38 kg (4.4%).

Participant characteristics by group of intervention at baseline are shown in Table 1. There were no significant differences in demographics or clinical, anthropometric characteristics. Only total cholesterol and LDL levels were higher in the control group compared to the intervention group. The average nutritional intake of patients at baseline in both groups was calculated. In the control group, caloric intake was 2008 ± 104.3 kcal/day and diet composition was 36.8 ± 1.4% fat (10.5 ± 0.5% saturated fat, 12.4 ± 0.8% monounsaturated fat and 4.4 ± 0.3% polyunsaturated fat), 46.9 ± 1.5% carbohydrates and 17.6 ± 0.6% protein. In the intervention group, caloric intake was 2364 ± 122.9 kcal/day and diet composition was 32.8 ± 1.4% fat (8.7 ± 0.7% saturated fat, 10.9 ± 0.7% monounsaturated fat and 4.5 ± 0.3% polyunsaturated fat), 50.5 ± 1.4% carbohydrates and 17.1 ± 0.8% protein. Saturated fat ingestion was higher in the control group (p<0.05), and caloric intake was higher in the intervention group (p<0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>37.2 ± 2.1</td>
<td>35.5 ± 1.4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>91.6 ± 3.4</td>
<td>94.1 ± 5.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>36.5 ± 0.8</td>
<td>36.9 ± 1.4</td>
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<tr>
<td>Fat mass (kg)</td>
<td>40.5 ± 1.7</td>
<td>40.9 ± 3.0</td>
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<tr>
<td>Fat mass (%)</td>
<td>44.4 ± 1.0</td>
<td>43.1 ± 1.0</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>112 ± 2.2</td>
<td>109.8 ± 3.5</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>195.7 ± 8.9</td>
<td>173. ± 6.3</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>38.5 ± 1.7</td>
<td>36.8 ± 1.4</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>125.2 ± 7.3</td>
<td>102.1 ± 4.9</td>
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<tr>
<td>Glucose (mg/dL)</td>
<td>94.9 ± 3.0</td>
<td>93.5 ± 2.8</td>
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<tr>
<td>Insulin (µU/mL)</td>
<td>20.6 ± 2.2</td>
<td>17.8 ± 1.9</td>
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<tr>
<td>HOMA-IR</td>
<td>4.9 ± 0.6</td>
<td>4.2 ± 0.5</td>
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<tr>
<td>CRP (mg/L)</td>
<td>8.6 ± 1.6</td>
<td>7.9 ± 1.1</td>
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<tr>
<td>oxLDL (mg/dL)</td>
<td>3.2 ± 0.4</td>
<td>3.3 ± 0.6</td>
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<td>Systolic blood pressure (mmHg)</td>
<td>117.0 ± 5.2</td>
<td>110.7 ± 2.5</td>
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<tr>
<td>Total cholesterol (mg/dL)</td>
<td>195.7 ± 8.9</td>
<td>181.3 ± 6.3</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.0 ± 4.1</td>
<td>71.9 ± 1.9</td>
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<tr>
<td>TNFα mRNA (RQU)</td>
<td>1.0 ± 2.8</td>
<td>0.6 ± 2.0</td>
</tr>
<tr>
<td>TNFα protein (pg/mL)</td>
<td>25.8 ± 12.5</td>
<td>14.3 ± 3.5</td>
</tr>
</tbody>
</table>

NA: Not applicable; UD: Undetermined. Data are presented as mean ± SE and percentage. Non-parametric U Mann-Whitney test was used. *p<0.05. They compared with baseline value.
Changes in anthropometrics, biochemistry, and nutritional Intake during follow-up

During the follow-up period, the control group improved anthropometric and biochemical parameters, such as body weight, BMI, fat mass (kg), waist circumference, glucose, insulin determinations and HOMA-IR (p<0.05). Furthermore, the TNFα protein showed an upward trend (25.8 ± 12.5 to 64.4 ± 29.1 pg/mL, NS) (Table 2).

In the intervention group, major changes in anthropometric and biochemical parameters were the decrease in body weight, BMI, fat mass kg, fat mass percentage, waist 8 circumference, and more body weight loss percentage, triglycerides, glucose, insulin, HOMA-IR, CRP, oxLDL, and an increase in HDL levels. TNFα serum levels were increased (14.3 ± 3.5 to 25.4 ± 5.7 pg/mL, p<0.05) (Table 3). As for the effect on nutritional status, BMI was determined in both groups every month throughout the study. An important beneficial improvement was observed at the 3rd month in both groups (p<0.05). However in the intervention group, the improvement was better that the control group at the same time in body weight loss, triglycerides, HDL (84.2%, -39.0 mg/dL, 4.2 mg/dL, respectively; p<0.05).

Comparison between moderate-fat diet alone and moderate-fat diet supplemented with green tea

In order to compare the effect of both treatments, the delta (Δ) for each parameter was calculated as shown in Table 4. By this perspective, at the end of the follow-up period, the parameters showed a significant improvement in the intervention group vs. the control.
group were in body weight loss percentage, triglycerides and HDL (p<0.05). With respect to the TNFα mRNA expression, it decreased 0.4 times more in the intervention group than in the control group, but this was not significant. The serum levels of this cytokine tended to increase in both groups. However, was higher in the control group than in the intervention group but not statistical significant (NS).

To know whether the observed effects were due to the treatment (green tea) or the moderate-fat diet, we assessed the adherence to diet in both groups. The adherence percentages were similar in both groups at the 1st and 2nd month, however at the 3rd month the adherence was lower in the intervention group compared to the control group (66.4 ± 4.8% vs. 78.6 ± 2.9%, respectively; p<0.05) (Tables 2 and 3). In addition, The adherence percentage to drinking tea in the intervention group assessed by intake questionnaire diminished significantly at the 3rd month (p<0.05) (Table 3).

**Effect green tea/moderate-fat diet on oxLDL levels and fat mass**

At baseline both groups showed similar oxLDL levels. However, in the follow-up period the oxLDL levels had changed significantly at the 1st and 2nd months in the intervention group compared with the control group (p<0.05) (Figure 2A). However, at the end of the study the intervention group maintained the lowest oxLDL levels at 3rd month versus baseline (2.7 ± 0.5 vs. 3.3 ± 0.6 mg/dL), respectively (Table 3). This result was only observed in the intervention, but not in the control group (4.1 ± 0.8 vs. 3.2 ± 0.4 mg/dL) (Table 2). Body weight loss percentage relative to fat mass was assessed. The control group lost 40 to 50%, while in the intervention group it was between 81 to 85%. These differences were statistically significant (p<0.05) (Figure 2B).

**Discussion**

This study demonstrates that moderate-fat diet supplemented with green tea reduces fat mass, weight, triglycerides, oxLDL levels and TNFα serum levels in obese women (Tables 2 and 3). Baseline data of both groups were similar in demographics, anthropometric and biochemical parameters and TNFα mRNA expression; except in total cholesterol, and LDL levels (p<0.05). This could be because the patients maintained a saturated fat uptake that was higher than in the intervention group (10.5% vs. 8.7%, respectively). Freedman et al. has reported that this dyslipidemia is induced by diet [23].

Our results show that intervention group had a significant body weight loss percentage (p<0.05). This result may be associated with the green tea intake because previous reports indicate that some components of green tea, such as epigallocatechin-3-gallate, inhibit adipocyte differentiation through to inhibit CCAAT/enhancer binding protein α [24]. Accordingly, adipocytes would decrease its ability to store fat and promote weight loss. Other important data was that triglycerides and oxLDL levels decrease, and HDL increase even though the adherence percentage was lower than in the control group. These results can be explained due to evidence showing a direct relationship between the levels of oxLDL and obesity.

Reports previous demonstrate that oxLDL contributes to triglycerides production by induction of lipoprotein lipase expression and fatty acids accumulation in adipocytes [22]. Therefore, body weight loss percentage and decrease of oxLDL, both could be related to green tea consumption. This observed effect has been associated with reperfusion in the expression of arachidonate-5-lipoxygenase which catalyzes the oxidation of LDL, as well as an overproduction of superoxide dismutase 3 that prevents oxidation of LDL [1]. Paul Holvoet et al. reported the utility of oxLDL as a good marker of cardiovascular risk. In their study, they tested the use of the Global Risk Assessment Scoring (GRAS) to determine cardiovascular risk. The GRAS score plus oxLDL had an increase in sensitivity, specificity and an odds ratio of 5.2 to cardiovascular disease. Also, oxLDL levels correlated with arterial intima thickness and total cholesterol and LDL were not good markers of cardiovascular risk [25]. Cote et al. showed the correlation between oxLDL and in situ expression of TNFα in the arterial intima, as well as the correlation between oxLDL and waist circumference in patients with cardiovascular disease [26]. Therefore, we used oxLDL serum levels as cardiovascular risk marker.

In this study we demonstrate, at the end of the treatment (3rd month), that the intervention group showed an improvement in body weight loss percentage, triglycerides and HDL compared to the control group. However, we found that LDL levels increased and the adherence percentage to diet was lower than in the control group (p<0.05). This result could be because LDL levels and other lipids could be influenced by diet, specifically by high saturated fat intake. However, these results may not affect the results detected on the oxLDL levels. Since, high levels of the enzyme arachidonate-5-lipoxygenase and ROS, or low levels of superoxide dismutase 3, that could be modulated by diet [23,25]. Inami et al. showed that catechin (major component in green tea) decreased the plasma oxLDL concentration with significant change in plasma LDL concentration [11].

Interestingly, HDL levels increase in the intervention group vs. the control group. Other studies show that during active weight loss, HDL levels decrease, and when the patient maintains the weight loss HDL levels increase by each kg of lost weight [27]. It is reported that HDL and oxLDL levels are antagonists. HDL has associated enzymes called paraoxonase and platelet activating factor acetyl hydrolase. These enzymes inhibit the oxidation of LDL. On the other hand, if the oxLDL levels increase, the associated HDL enzymes decrease [28]. In this way, we demonstrate that a moderate-fat diet supplemented with green tea may contribute to increased HDL levels, even if the patient has an active weight loss. A similar effect has been reported about TNFα expression; because during an active weight loss, TNFα levels do not decrease [29,30] or its levels could increase [31]. However, TNFα levels decreased in obese patients that had a lost and maintained weight [32]. In our study, TNFα protein showed a tendency to increase in both groups, and TNFα mRNA expression was lower in the intervention group. This could be explained because TNFα is a cytokine that stimulates lipolysis. In this way, a diet supplemented with green tea contributes to decreasing fat mass and modulates TNFα expression, in order to avoid an exacerbated expression of this cytokine.

Our results demonstrate that adherence percentage to diet and to drinking green tea are important because we observed a significant reduction in oxLDL levels in the intervention group at the 1st and 2nd month vs. the control group. Although the statistical significance was lost at the 3rd month, the observed effects are maintained. This could be attributed to green tea intake. It might also explain the lower rate of cardiovascular disease observed in cultures where green tea consumption is prevalent. However, further studies are necessary in order to establish new strategies for individualized intervention applying the principles of nutritional genomic.

We concluded that a moderate-fat diet supplemented with green tea and a moderate-fat diet supplemented with green tea reduce oxLDL levels and fat mass in obese women.
teas showed a reduction of oxLDL and fat mass and modulated TNFα expression in obese women. The oxLDL improvement could represent a good marker of cardiovascular risk.

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Conflict of Interests, Source of Funding

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