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Decaffeinated green tea extract rich in epigallocatechin-3-gallate prevents fatty liver disease by increased activities of mitochondrial respiratory chain complexes in diet-induced obesity mice

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Abstract

Nonalcoholic fatty liver disease has been considered the hepatic manifestation of obesity. It is unclear whether supplementation with green tea extract rich in epigallocatechin-3-gallate (EGCG) influences the activity of mitochondrial respiratory chain complexes and insulin resistance in the liver. EGCG regulated hepatic mitochondrial respiratory chain complexes and was capable of improving lipid metabolism, attenuating insulin resistance in obese mice. Mice were divided into four groups: control diet+water (CW) or EGCG (CE) and hyperlipidic diet+water (HFW) or EGCG (HFE). All animals received water and diets *ad libitum* for 16 weeks. Placebo groups received water (0.1 ml/day) and EGCG groups (0.1 ml EGCG and 50 mg/kg/day) by gavage. Cytokines concentrations were obtained by ELISA, protein expression through Western blotting and mitochondrial complex enzymatic activity by colorimetric assay of substrate degradation. HFW increased body weight gain, adiposity index, retroperitoneal and mesenteric adipose tissue relative weight, serum glucose, insulin and Homeostasis Model Assessment of Basal Insulin Resistance (HOMA-IR); glucose intolerance was observed in oral glucose tolerance test (OGTT) as well as ectopic fat liver deposition. HFE group decreased body weight gain, retroperitoneal and mesenteric adipose tissue relative weight, HOMA-IR, insulin levels and liver fat accumulation; increased complexes II-III and IV and malate dehydrogenase activities and improvement in glucose uptake in OGTT and insulin sensitivity by increased protein expression of total AKT, IRα and IRS1. We did not find alterations in inflammatory parameters analyzed. EGCG was able to prevent obesity stimulating the mitochondrial complex chain, increasing energy expenditure, particularly from the oxidation of lipid substrates, thereby contributing to the prevention of hepatic steatosis and improved insulin sensitivity.

Keywords: EGCG; NAFLD; Obesity; Mice; Respiratory chain; Insulin resistance

1. Introduction

Obesity has grown at an alarming rate over the last decades and is actually considered a public health problem. Genetic and environmental factors are implicated in the development of obesity, with emphasis on unsuitable diets [1]. It is known that the western dietary patterns, particularly the diets high in saturated fat and low in dietary fiber, contribute to the development of several chronic diseases [2–4]. Nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver illness [5]. The increased incidence of NAFLD is closely related to the increase in obesity, metabolic syndrome and type 2 diabetes mellitus in this populations [6,7].

NAFLD, caused by excessive lipid accumulation in liver, is the hepatic manifestation of insulin resistance and the metabolic syndrome [8,9]. These conditions are caused by an imbalance between triglyceride deposition and synthesis compared with oxidation and secretion by lipoproteins. This initially represents a protective mechanism against the toxicity arising from an increased flux of nonesterified fatty acids (NEFAs) to the liver [10]. The NEFAs can modify hepatic metabolism; one of the effects is to reduce hepatic insulin extraction, contributing to systemic maintenance of hyperinsulinemia [11,12].

Common in obesity is the installation of insulin resistance in visceral adipose tissue. It is well known that adipose tissue insulin resistance has increased lipolysis pathway activity in proportion to the expansion of visceral adipose tissue [13–15]. The activation of this pathway leads to the increase NEFA flows to the liver [10,16].

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In the liver, NEFAs can be oxidized or esterified to form triacylglycerols (TAG). The overload NEFAs that are not used in the oxidative metabolic pathways for production of energy during catabolism (skeletal muscle activity and prolonged fasting) are directed to *de novo* lipogenesis, responsible for generating TAG and phospholipids from long-chain fatty acids (LCFAs) and glycerol-3-phosphate [11,15,17].

In review, Aubert *et al.* report that excess LCFAs in the portal circulation are capable of activating cytochrome P450 (CYP450). These data suggest that these lipids can increase hepatic CYP450 expression in NAFLD. In fact, CYP450 induction in NAFLD could be a mechanism of adaptation to lipid overload limit since CYP450-mediated hydroxylation of fatty acids is an alternative route for mitochondrial and peroxisomal oxidation [18].

Mitochondria are the primary cellular organelles for the oxidation and metabolism of fatty acids and glucose. A reduction in mitochondrial function may contribute to the increase in lipid accumulation leading to insulin resistance [10]. These modifications may be related to the reduction in maximal activities of enzyme systems, such as carnitine palmitoyl transferase and mitochondrial respiratory chain complexes, which are responsible for the transport of LCFAs into the organelle and generation of ATP molecules, respectively [19,20]. All of these changes contribute to the exacerbation of fatty liver.

Green tea (*Camellia sinensis*) is one of the most well-known and oldest beverages, consumed in large quantities all over the world even today. The polyphenols found in green tea have many beneficial metabolic effects [21–23]. The major catechins in green tea are epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate, epigallocatechin and epicatechin, but EGCG is the most abundant catechin and the most biologically active [24,25]. Some studies suggest that EGCG has antisteatotic effects on the liver [21,26,27], as well as positive effects on lipid metabolism as described previously [22,25,28].

Currently, it is not clear whether EGCG regulates hepatic mitochondrial respiratory chain complexes able to improve the lipid metabolism and attenuates insulin resistance. Given both the importance of mitochondrial functionality and the beneficial effect of EGCG on pathologic liver conditions, the aim of this study was to demonstrate the effects of EGCG supplementation on the activity of mitochondrial respiratory chain complexes and insulin resistance in mice fed with a high-fat diet (HFD).

2. Materials and methods

2.1. Animal treatment

All animals were performed according to protocols approved by the Experimental Research Committee of Universidade Federal de São Paulo (CEUA no. 975418). A total of 54 male Swiss mice at 30 days old were used. They were maintained in collective cages in a room with controlled temperature (25±2°C), humidity (60±5%) and lighting (12-h light/dark cycle) and received water and diet *ad libitum*. After 1 week of acclimatization, the mice were divided evenly into four groups: control diet+water (CW), control diet+EGCG (CE), high-fat diet+water (HFW) and high-fat diet+EGCG (HFE). The animals were fed with control diet (AIN-93) [29] or high-fat diet (AIN-93 adapted) during the next 16 weeks (Table 1). The placebo groups received water (0.1 ml of water by gavage/day) and treatment EGCG (0.1 ml EGCG and 50 mg/kg/day). The animals of placebo groups were weighed once a week, the weight of the EGCG groups was measured every day during the experimental period. We considered as an adiposity index ($\sum$WAT) the sum of three adipose depots (mesenteric, epididymal and retroperitoneal) and the relative weight (tissue weight/final weight×100), and delta (Δ) was calculated (initial weight−final weight). At the end of the experimental period, the mice were euthanized after 12 h of fasting, and blood and liver were collected. All tissues were weighed and stored at −80°C.

2.2. Serum parameters

The content of insulin (Millipore, Inc.) and glucose were analyzed by colorimetric method using commercial kits (Labtest). The Homeostasis Model Assessment of Basal Insulin Resistance (HOMA-IR) method of Turner *et al.* [30] was calculated as the product of fasting insulin (nanograms per milliliter) and fasting glucose (milligrams per deciliter) divided by the constant 22.5 or [HOMA-IR (mmol/L)=(fasting insulin×fasting glucose)/22.5].

Table 1
Composition of experimental diets, CD and HFD (AIN-93 modified) [29], growth (G) and maintenance (M)

Ingredients	CD (G/M)	HFD (G/M)
Corn starch (%)	62. 95/72.07	40. 92/40.87
Casein (%)	20. 0/14.0	13. 95/14.0
Soybean oil (%)	7 0.0/4.0	7.0/4.0
Lard (%)	–	28. 08/31.2
Cellulose (%)	5	5
Mixture of vitamins (%)	1.0	1.0
Mixture of mineral (%)	3.5	3.5
L-Cystine (%)	0. 3/0.18	0.18/0.18
Choline bitartrate (%)	0.25	0.25
Hydroquinone (g/kg)	0.014/0.008	0.014/0.008
Energy (kcal/kg)	3948/3.802	5.352/5.362

2.3. Oral glucose tolerance test (OGTT)

Test was performed after 15 weeks of treatment. After 8 h of fasting, blood was collected from the tail vein to assess basal glucose concentration. Then, a glucose (Merck) solution (2 g/kg) was administrated by gavage. Blood samples were collected after 15, 30, 45, 60, 90 and 120 min to measure glucose concentration using a glucose analyzer (AccuCheck Roche).

2.4. Histological analysis

Sections from right lobe liver were collected immediately after euthanasia, fixed in paraformaldehyde (4%) for 16 h and washed with 70% ethanol overnight. The samples were processed, paraffin embedded and mounted on glass slides. The histological sections of 5 μM were stained using hematoxylin and eosin for analysis of the presence of hepatic steatosis.

2.5. Folch analysis for hepatic TAG content

To quantify ectopic fat accumulation in the liver, the extraction of the total lipids of the sampled tissues was performed with a solution of chloroform:methanol:water (2:1:0.5). According to the method of Folch *et al.* [31], the samples were homogenized at 25,000 rpm for 45 s and centrifuged for 5 min at 655,1g. After centrifugation, the organic layer was removed by pipette technique and doubled the total lipid extract evaporated under nitrogen atmosphere. Samples of lipids extract were analyzed for their content of TAG by colorimetric method, using (Labtest) commercial kit.

2.6. Western blot analysis

Hepatic tissue were homogenized in lyses buffer containing 100 mM Tris–HCl (pH 7.5), 1% Triton X-100, 10% sodium dodecylsulfate (SDS), 10 mM EDTA, 100 mM sodium fluoride, 10 mM sodium pyrophosphate, 10 mM sodium orthovanadate, 2.0 mM phenylmethylsulfonyl fluoride and 0.1 mg/ml aprotinin. The homogenate was centrifuged at 14,000 rpm for 45 min at 4°C, and the supernatant was collected. The total protein concentration was measured with Bradford Reagent. Proteins in the lysates were electrophoretically separated in 10% SDS polyacrylamide gel and transferred to nitrocellulose membrane. We used precast gels in 4–15% Mini-PROTEAN TGX Precast Gels of Bio-Rad Laboratories, Inc., to antibodies of IRS1 and IR.

The membranes were blocked in 1% bovine serum albumin overnight at room temperature and then incubated overnight with the following primary antibodies: TLR4, IRS1 (insulin receptor substrate 1) and IRα (insulin receptor α); TNFR-1 (sc-7895), IL-6R (sc-660) and IL-10Rα (sc-984) were purchased from Santa Cruz Biotechnology, Inc.(Santa Cruz, CA, USA). The β-tubulin was obtained from Cell Signaling Technology (Beverly, MA, USA). The membranes were next incubated with horseradish-peroxidase-conjugated secondary antibodies during 1 h at room temperature. The bands were visualized with enhanced chemiluminescence scanned at UVITec (Cambridge) after adding the ECL reagent (GE Healthcare Bio-Sciences, AB, UK), and the intensities of the bands were quantified in ImageJ software (ImageJ, National Institute of Health, Bethesda, MD, USA).

2.7. Cytokine concentrations in liver

The hepatic tissue was homogenized and centrifuged at 14,000 rpm for 45 min at 4°C. The supernatant was saved and used in the commercial kits of ELISA (R&D Systems) to measure the concentrations of TNF-α, IL-6 and IL-10 following the recommendations of the manufacturer.

2.8. Enzymatic activity

2.8.1. Mitochondrial respiratory chain enzymes

The liver were homogenized (1:10, w/v) in SETH buffer (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base and 50 IU/ml heparin, pH 7.4). The homogenates were centrifuged at 800g for 10 min and the supernatants were used to determine the activities of the

mitochondrial respiratory chain enzymes (complexes I, II, II-III and IV). On the day of the assays, the samples were frozen and thawed thrice in hypotonic assay buffer to fully expose the enzymes to substrates and achieve maximal activities. NADH dehydrogenase (complex I) was evaluated according to the method described by Cassina and Radi [32] by the rate of NADH-dependent ferricyanide reduction at l=420 nm. The activities of succinate-2,6-dichloroindophenol (DCIP)-oxidoreductase (complex II) and succinate-cytochrome c oxidoreductase (complex II-III) were determined by the method described by Fischer *et al.* [33]. Complex II activity was measured by the decrease in absorbance due to the reduction of 2,6-DCIP at l=600 nm. Complex II-III activity was measured by cytochrome c reduction from succinate at l=550 nm. The activity of cytochrome c oxidase (complex IV) was assayed according to the method described by Rustin *et al.* [34], measured by the decrease in absorbance due to the oxidation of previously reduced cytochrome c at l=550 nm. The activities of the mitochondrial respiratory chain complexes were calculated as nanomoles per minute per milligram of protein.

2.8.2. Succinate dehydrogenase (SD) activity

SD activity was determined according to the method of Fischer *et al.* [33], by following the decrease in absorbance, due to the reduction of 2,6-DCIP at 600 nm, with 700 nm as a reference wavelength [ε=19.1 mM^{-1} cm to 140 mM potassium phosphate (pH 7.4), 16 mM succinate and 8 μM 2,6-DCIP], and was preincubated with 40–80 μg homogenate protein at 30°C, for 20 min. Subsequently, 4 mM sodium azide, 7 μM rotenone and 40 μM 2,6-DCIP were added, and the reaction was initiated by the addition of 1 mM phenazinemethasulfate (PMS) and monitored for 5 min, in the presence of PMS.

2.8.3. Malate dehydrogenase activity

Aliquots (20 mg protein) were transferred into a medium containing 10 mM rotenone, 0.2% Triton X-100, 0.15 mM NADH and 100 mM potassium phosphate buffer, pH 7.4, at 37°C. The reaction was started by the addition of 0.33 mM oxaloacetate. Absorbance was monitored as described above.

2.8.4. Creatine kinase (CK)

CK activity was measured in liver homogenates pretreated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60 mM Tris–HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM $MgSO_4$ and approximately 0.4–1.2 mg protein in a final volume of 100 ml. After 15 min of preincubation at 37°C, the reaction was started by the addition of 0.3 mmol of adenosine diphosphate plus 0.08 mmol of reduced glutathione. The reaction was stopped after 10 min by the addition of 1 mmol of *p*-(hydroxymercuri)benzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes [35]. The color was developed by the addition of 100 ml of 2% *a*-naphthol and 100 ml of 0.05% diacetyl in a final volume of 1 ml and analyzed spectrophotometrically after 20 min at 540 nm. Results were expressed as nanomoles per minute per milligram protein.

3. Statistical analyses

Data were submitted to the quality tests Shapiro–Wilk (normality), Levenne (homogeneity) and/or Mauchly (sphericity). If necessary, data were standardized to Z score. We used corrected values F (Greenhouse-Geisser) in case of nonspherical. The descriptive analysis was performed using the mean±standard error. To verify the interactions between groups, we used *two-way* analysis of variance followed by *post hoc* test of Bonferroni. The level of significance was P less than or equal to 5%. For statistical analyses, we used the software SPSS version 17.0.

4. Results

4.1. Effect of EGCG treatment and body weight and tissues

The final weight and the delta (Δ) weight were significantly higher in the group fed HFW compared with both the CW (P=.013; P=.008) group and the group with HFE (P=.011; P=.001). Less mass gain in the CE group was shown on delta (Δ) weight (P=.021) when compared to the CW group (Table 2).

The relative weights of liver and epididymal adipose deposits did not differ among the groups. However, the relative weights of mesenteric and retroperitoneal adipose tissues were increased in the group fed HFW when compared with the CW (P=.012; P=.034) and the HFE (P=.046; P=.007) groups. In the ∑WAT, we observed increased fat mass in HFW when compared to CW (P=.01) and HFE (P=.007); the supplementation of EGCG significantly suppressed a greater gain of mass in these adipose depots during the experimental period (Table 2).

4.2. Effect of EGCG treatment on serum parameters and OGTT

The analyses of serum glucose and insulin levels are shown in Table 3. Serum glucose and insulin levels (P=.021 and .001, respectively) and HOMA-IR (P=.004) showed higher levels in the HFW group when compared to the CW group. Insulin and HOMA-IR showed significantly higher levels in the HFW group versus HFE (P=.005 and .015, respectively). The OGTT showed similar results with other insulin resistance biomarkers. The absolute values at baseline and at 30 min (P=.020 and .019, respectively) were significantly higher in the high-fat group when compared to the CW group; the 30-min point also showed differences from the HFE (P=.031). When calculating area under the curve (AUC), these differences were reaffirmed between the HFW and CW groups (P=.001) and the HFE group (P=.003) (Fig. 1).

4.3. Effects of EGCG on ectopic fat accumulation in the liver

EGCG supplementation (Fig. 2) was able to reduce the accumulation of ectopic fat in the hepatic tissue of the animals fed with high-fat diet (P=.007).

4.4. Effect of EGCG treatment in inflammatory profile

The concentration of cytokines [TNF-α, IL-6 and IL-10 (Table 4)] and protein expression of TLR4, TNFR-1, IL-6R and IL-10Rα were not different among the groups (Fig. 3D–G).

4.5. EGCG and modulation of protein expression of insulin pathway

Protein expression analysis demonstrated that treatment with EGCG improved protein levels of total AKT (P=.045) and IRα (P=.013) and revealed a significant increase in the HFE group in comparison with the HFW group. Supplementation with EGCG enhanced the protein expression of IRS1 (P=.046) in the CE group when compared to the CW group (Fig. 3A–C).

4.6. EGCG in the glycolytic and oxidative metabolism enzymes in liver

Assessing the activity of the enzymes in the mitochondrial complex in order to observe oxidative metabolism, we observed the increase in the activities of complex I (P=.001) and complex IV (P=.04) in the HFE group versus the HFW group. Complex II did not show change among the experimental groups; however, complexes II-III and IV were increased in the HFW group when compared to CW [P=.003 and .015, respectively (Fig. 4A–D)]. In the glycolytic metabolic pathway,

Table 2
Body weight, absolute and relative tissue weight

Parameters	CW	CE	HFW	HFE
Initial weight (g)	25.9±1.6	23.2±0.7	23.5±1.8	26.1±1.4
Final weight (g)	48.3±2.4	40.2±1.7	57.3±2.7 **	45.5±2.4 ***
Delta weight (g)	23.7± 2.71	17.3±1.3 *	33.8±2.2 **	19.3±2.1 ***
Epididymal (%)	4.14±0.51	4.07±0.42	5.33±0.72	4.23±0.91
Retroperitoneal (%)	1.14±0.10	1.09±0.07	1.44±0.12 **	0.98±0.05 ***
Mesenteric (%)	1.85±0.30	1.65±0.22	3.04±0.30 **	2.02±0.52 ***
∑WAT (g)	3.75±0.60	2.76±0.33	5.56±0.30 **	3.32±0.56 ***
Liver (g)	2.1±0.16	1.57±0.08 *	2.33±0.26	1.8±0.15
Liver (%)	4.13±0.09	3.93±0.19	4.01±0.19	3.96±0.30

* P<.05, control diet and EGCG (CE) group versus control diet and water (CW) group (n=14).
** P<.05, high-fat diet and water (HFW) group versus CW group (n=13–14).
*** P<.05, high-fat diet and EGCG (HFE) group versus HFW group (n=13).

Table 3
Serum concentrations of fasting glucose and fasting insulin and homoeostasis model assessment (HOMA-IR) in different experimental groups

Parameters	CW	CE	HFW	HFE
Glucose (mg/dl)	135.6±5.18	128.5±4.42	168.7±16.6 **	149.1±7.1
Insulin (ng/ml)	1.5±0.18	0.82±0.13	3.4±0.64 **	1.52±0.21 ***
HOMA-IR	9.21±1.25	4.65±0.77	15.78±2.32 **	9.91±1.2 ***

** $P<.05$, high-fat diet and water (HFW) group versus CW group ($n=13$–14).
*** $P<.05$, high-fat diet and EGCG (HFE) group versus HFW group ($n=13$).

we found an improvement in the activity of malate dehydrogenase ($P=.001$) in HFW when compared to the CW group; nevertheless, we did not find differences in the activities of CK and SD (Fig. 5A–C).

5. Discussion

The major findings of the present study involve the capacity of EGCG to prevent fatty liver in mice fed with HFD. Our data demonstrated that EGCG treatment produced improvements in metabolic profiles and insulin resistance and increased activities of mitochondrial respiratory chain complexes in liver obese mice.

Several studies in humans [36–39] and rodents [8,16,19,40,41] presented strong evidence that HFD, particularly rich in saturated fatty acids, promoted the development of three pathological features associated with the metabolic syndrome: dyslipidemia, insulin resistance/glucose intolerance and increased visceral adiposity [42]. Our experimental model of diet to induce obesity proved to be effective in simulating obesity with treatment of 16 weeks. This finding was clear when we observe value of delta (Δ) and the adiposity index of mice fed the high-fat diet for 16 weeks (Table 2). Serum parameters also corroborated the effectiveness of the diet in inducing obesity and metabolic syndrome, with higher insulin and fasting blood glucose levels (Table 3). The decaffeinated green tea extract rich in EGCG played an obesity protective role and caused a reduction in weight gain and fat deposits (Table 2). It also caused an improvement in measured serum parameters and insulin sensitivity in the groups supplemented with EGCG (Table 3).

There are extensive descriptions in the literature about the role of low-grade inflammation in the development and establishment of obesity [13]. It is important to remember that obesity is a complex and multifactorial disease, and there are several factors that appear to influence its biomarkers such as gender [43], age [44], type of fatty acid offered [45] and duration of therapy [46]. Interestingly, our data showed that high-fat feeding during the 16-week period did not alter the content of proinflammatory or antiinflammatory cytokines IL-10, IL-6 or TNF-α and respective receptors, nor did it alter the TLR4 protein expression levels in the liver (Table 4 and Fig. 3). Some studies have reported dissociation between insulin resistance and inflammation in the liver. In agreement with our results, Turner *et al.* [47] demonstrated that insulin resistance and steatosis was clearly higher in rats after a few days of a high-fat diet, but even after 16 weeks of high-fat diet, alterations in inflammation in the liver were not detected. Carillon *et al.* confirmed that insulin resistance and obesity inflammation were disassociated in the liver [48]. They proposed that the inflammatory imbalance in obesity may not always be evident, particularly in the liver, whereas the tissue may exert a strict regulatory role, which could occur after long periods of exposure to a high-fat diet.

There is evidence that green tea catechins such as EGCG have potential as antiobesity tool to its comorbidities. The functions of green tea catechins operate through several different mechanisms, and these mechanisms interact to change the energy balance, the formation of reactive oxygen species and the activities of obesity-related cells [49]. Focusing on energy balance, EGCG has been shown to be such an effective weapon against obesity by acting on two different levels that together provide a reduction in weight gain. The EGCG on the one hand, in numerous studies, has shown to be a potent activator of AMPK pathway that is renowned for increase energy expenditure and activates lipid oxidation pathways [50]. On the other hand, AMPK also acts on the lipogenesis routes, reducing the activity of enzymes that activate these pathways [51]. Therefore, EGCG is able to increase energy expenditure and at the same time reduce the pathways that make lipid storage in adipose tissue. These two factors together make the effect of EGCG be so evident in obesity.

It has been suggested that ectopic accumulation of liver triglycerides is an important mediator of hepatic insulin resistance. Some groups [6,52] have defended the hypothesis that lipid accumulation in the liver leads to hepatic insulin resistance with evidence of a dose–response relationship between the content of liver lipids and insulin action. This demonstrated that the prevention of hepatic fat accumulation nullified the development of insulin resistance in the liver. In this study, we observed that their results were similar to ours. The TAG content was reduced under EGCG supplementation, as can be clearly seen in the representative images from histological sections (Fig. 2). The OGTT and HOMA-IR indicated change in insulin responsiveness altered by HFD; in contrast, supplementation with EGCG improved insulin sensitivity and glucose uptake in the HFE group when compared to the HFW group (Fig. 1). This effect might be observed due to an increase in the expression of some proteins in the insulin signaling pathway, such as AKT, IRα and its coactivator substrate IRS1 in total fractions (Fig. 3). This improvement was observed even in mice fed high-fat diets supplemented with EGCG.

The liver plays a crucial role, particularly in lipid metabolism, and is a key organ for the maintenance of systemic homeostasis glucose. Evidence linking mitochondrial dysfunction, insulin resistance and

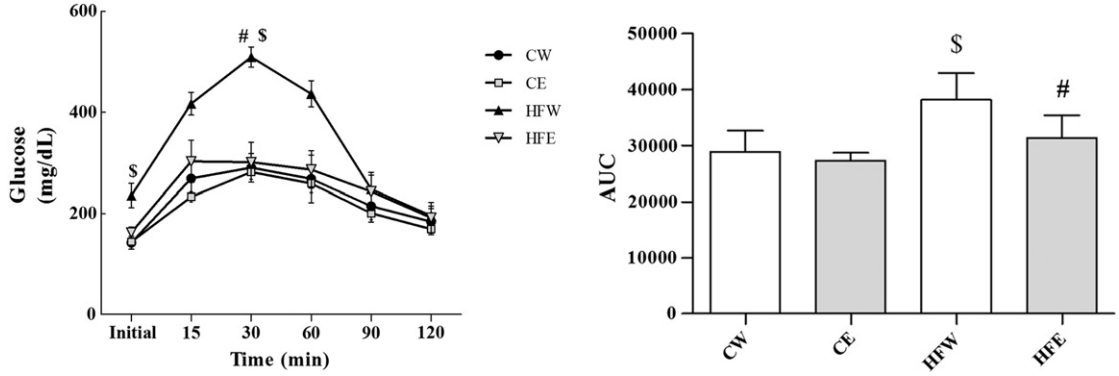

Fig. 1. OGTT and AUC. Glycemia in initial and in 15, 30, 60, 90 and 120 min after gavage of 2 g/kg body weight of glucose. Data are expressed in mean±S.E.M. $^{\$}P<.05$, high-fat diet and water (HFW) group versus CW group ($n=4$–5). $^{\#}P<.05$, high-fat diet and EGCG (HFE) group versus HFW group ($n=4$–5).

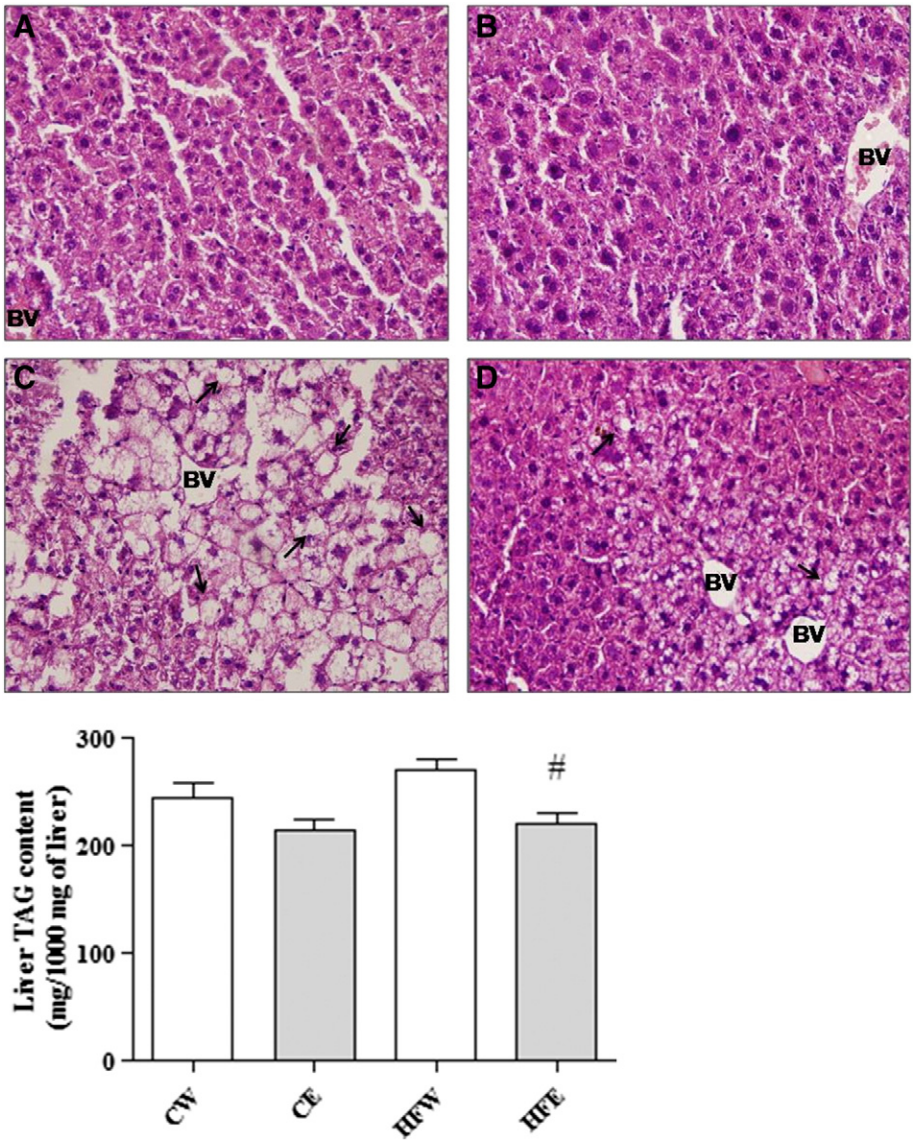

Fig. 2. Histological analysis: hematoxylin and eosin staining with original magnification ×200. (A) Control diet and water, (B) control diet and EGCG, (C) high-fat diet and saline and (D) high-fat diet and EGCG, as well as liver TAG content among the groups. $^{\#}P<.05$, high-fat diet and EGCG (HFE) group versus HFW group ($n=7$–10). Hepatic TAG content per 1000 mg of liver tissue.

glucose tolerance installation has been suggested [20,53]. The mitochondria have great adaptive potential and can easily adapt to the specific environment and energy demands of the tissue [54]. Holmström postulated that these adaptations in mitochondrial function were a consequence of transcriptional regulation observed in mRNA expression profiles and mitochondrial proteins; they could have been due to excess energy substrate supply and disorders in blood glucose and energy homeostasis of the whole body in obesity and diabetes [20].

Human studies have shown similar results, indicating that the presence of peripheral insulin resistance is associated with reduced gene expression involved in mitochondrial oxidative phosphorylation [55]. In rodents, high-fat diets led to obesity, insulin resistance and hepatic steatosis with concomitant reductions in respiratory capacity and increased oxidative stress in liver mitochondria [56–58]. In our study, we provided evidence that supplementation with EGCG improved mitochondrial respiratory capacity in livers of high-fat-induced obese mice due to an increase in mitochondrial complex IV (Fig. 4).

Complex I is the first step of the respiratory mitochondrial chain and the most common site for mitochondrial abnormalities. Dysfunction in this complex inhibits the mitochondrial electron flow from the Fe-S complex I to the ubiquinone centers and therefore may block the entire process of oxidative phosphorylation. EGCG had the effect of enhancing the activity of this complex and consequently enhancing the electron transport chain [40,55,59,60]. Complex II is the second entry point of reducing equivalents into the mitochondrial respiratory chain via FADH, and it is the only complex that pumps protons across the inner mitochondrial membrane. Complex III represents a confluence point for reducing equivalents from various dehydrogenases; it can catalyze the transfer of electrons from hydroxyquinones. This finding

Table 4
Liver concentrations of cytokines in different experimental groups

Parameters	CW	CE	HFW	HFE
TNF-α (pg/mg protein)	5.29±0.70	6.36±0.77	5.79±0.27	5.74±1.22
IL-10 (pg/mg protein)	49.50±2.37	63.05±6.26	58.34±4.81	46.47±3.14
IL-6 (pg/mg protein)	97.7±7.05	121.93±12.09	92.86±7.41	96.65±8.5

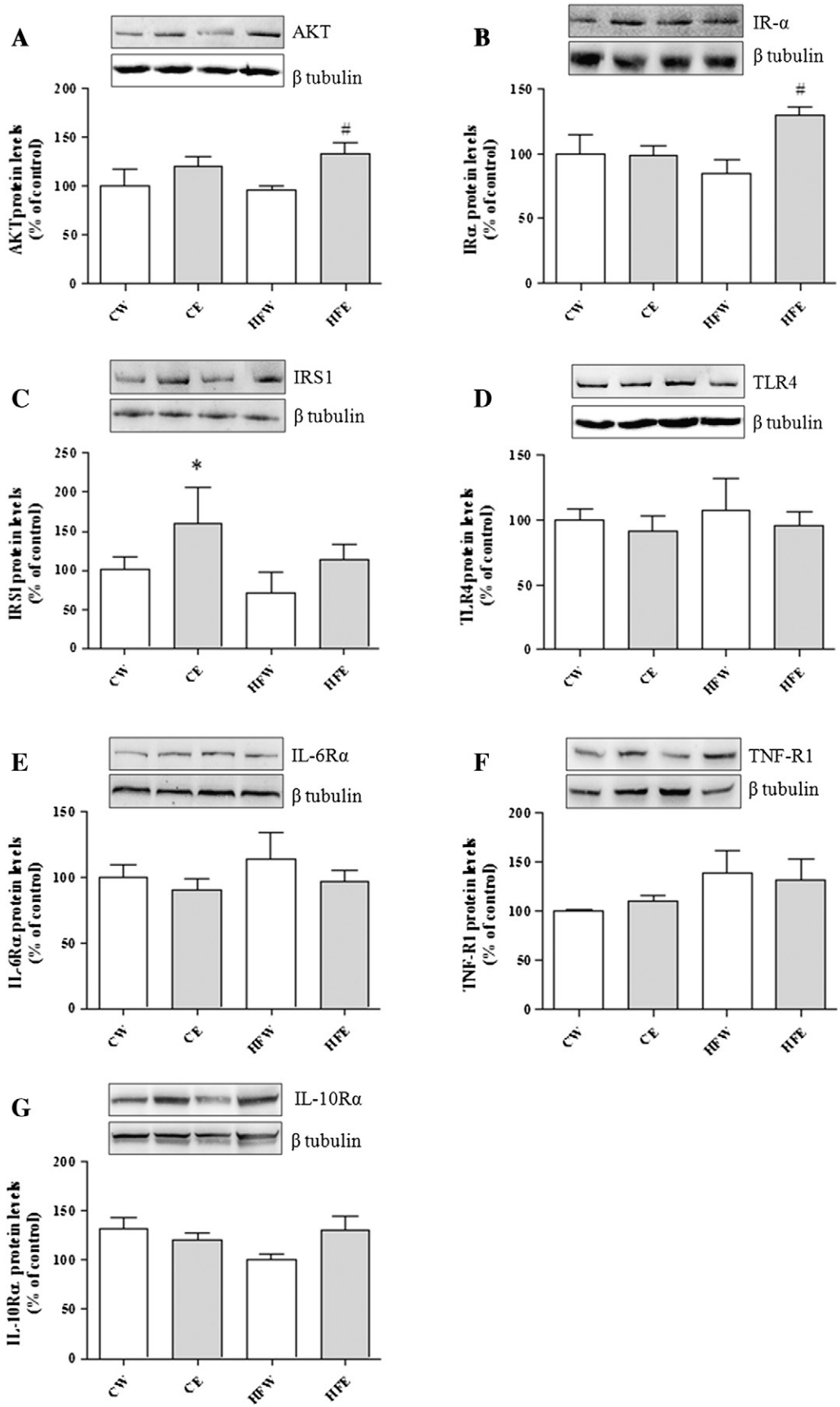

Fig. 3. Western blotting analysis of protein expression in the liver on different experimental groups. (A) AKT, (B) IRα, (C) IRS1, (D) TLR4, (E) IL-6Rα, (F) TNFR-1 and (G) IL-10Rα. $^{*}P<.05$, control diet and EGCG (CE) group versus control diet and water (CW) group ($n=4–7$). $^{\#}P<.05$, high-fat diet and EGCG (HFE) group versus HFW group ($n=4–7$).

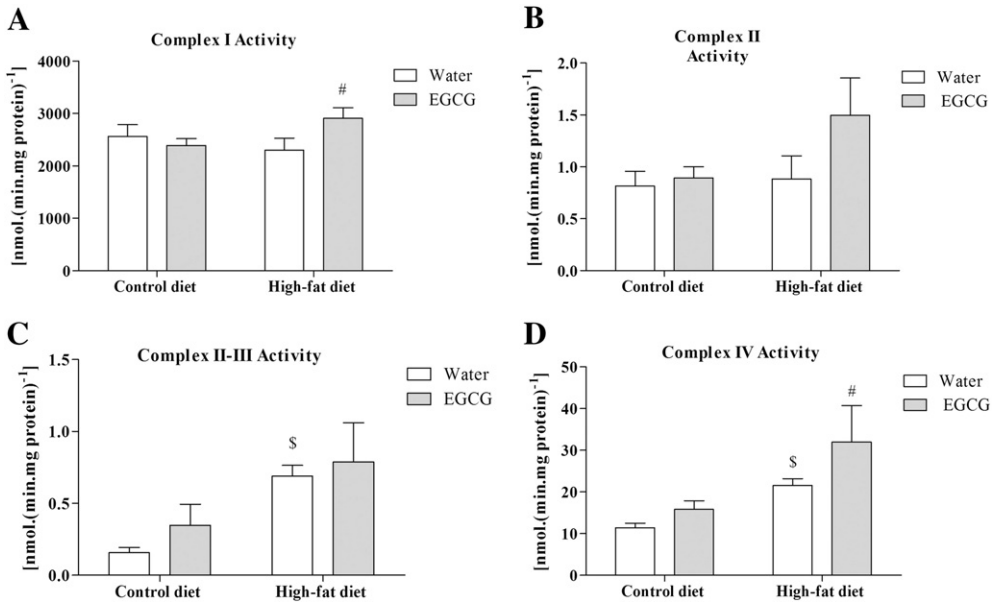

Fig. 4. Oxidative metabolism enzymes in liver. Data are express mean±S.E.M. *$P<.05$, control diet and EGCG (CE) group versus control diet and water (CW) group ($n=4–7$). $^{\$}P<.05$, high-fat diet and water (HFW) group versus CW group ($n=4–7$). $^{\#}P<.05$, high-fat diet and EGCG (HFE) group versus HFW group ($n=4–7$).

was in contrast to the striking observation of mitochondrial dysfunction in the liver from the high-fat-induced mice without catechins once there was a significant increase in activity of the II-III complexes in hyperlipidic group [19,61] (Fig. 4).

Complex IV forms part of heme-copper oxygen reductase superfamily that has the ability to catalyze the complete reduction of O_2 to water, thus promoting the translocation of protons through the mitochondrial membrane. Complex IV deficiencies can interrupt the process of oxidative phosphorylation, thus decreasing the production of energy for the cells to function properly. Other studies have shown the deleterious effects that the high-fat diet produced in this complex, by reducing the oxidative process [20,56,57,62]. In the HFE group, we found an increase in the oxidative process generated by supplementation with EGCG (Fig. 4).

Our research revealed that supplementation with EGCG potentially appeared to increase the activity of the mitochondrial complex chain and thereby increased the oxidation of lipids and prevented the rise of hepatic steatosis. Effect of EGCG appeared to be an increase in the activity of the signaling pathway associated with insulin and glucose uptake.

We concluded that supplementation with decaffeinated green tea extract, which was rich in EGCG, was able to prevent the deleterious effects associated with obesity and ingestion of a high-fat diet. This

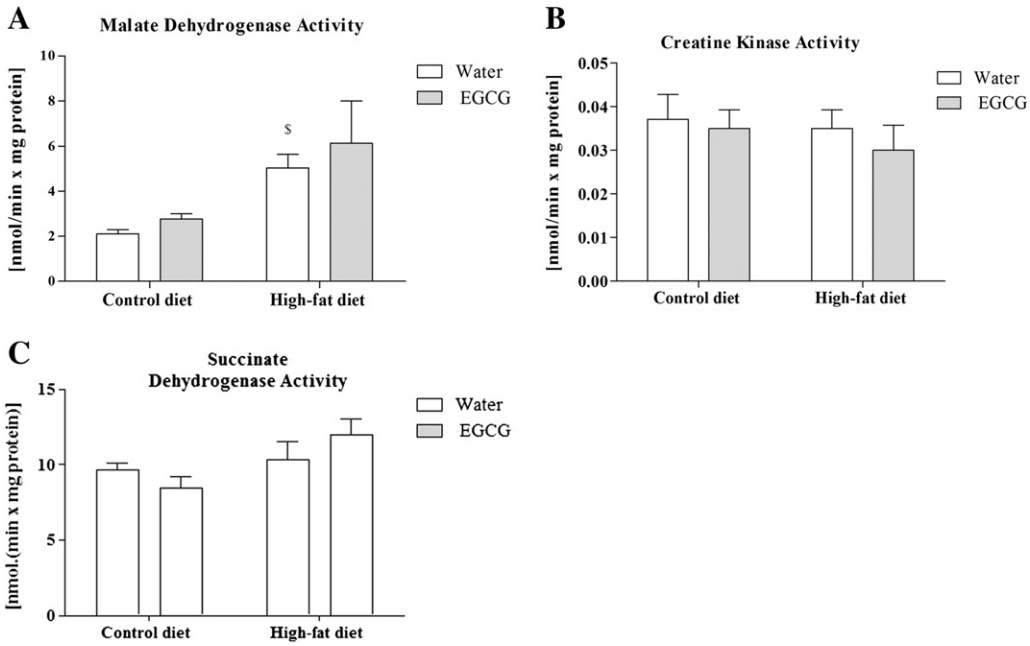

Fig. 5. Glycolytic and oxidative metabolism enzymes in liver. Data are expressed as mean±S.E.M. *$P<.05$, control diet and EGCG (CE) group versus control diet and water (CW) group ($n=4–7$). $^{\$}P<.05$, high-fat diet and water (HFW) group versus CW group ($n=4–7$). $^{\#}P<.05$, high-fat diet and EGCG (HFE) group versus HFW group ($n=4–7$).

beneficial effect may be due to the effect of stimulation of mitochondrial complex chain and increased energy expenditure, particularly from the oxidation of lipid substrates, thereby contributing to the prevention of hepatic steatosis and improved insulin sensitivity. Results from our study demonstrated that EGCG can be an important tool against obesity.

Potential Conflicts of Interest

All authors declare no conflicts of interest.

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