

A mix of ginger phenols exhibits anti-adipogenic and lipolytic effects in mature adipocytes derived from 3T3-L1 cells

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Abstract. The prevalence of obesity has increased rapidly worldwide. Obesity is characterized by excessive adipose tissue in the body, which is related to hyperplasia and hypertrophy in adipocytes. Ginger (*Zingiber officinale Roscoe*) is a medicinal plant that possesses an anti-obesogenic effect mostly attributed to gingerols, the most abundant bioactive compounds in ginger. The anti-adipogenic and lipolytic effects of these phenols have been shown when investigated individually. Therefore, the present study aimed to evaluate the lipolytic and anti-adipogenic activity of a mix of the main ginger phenols 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 8-shogaol and 10-shogaol on the 3T3-L1 cell line. A total of four study groups were designed: Negative control (3T3-L1 preadipocytes); positive control (mature 3T3-L1 adipocytes); phenols-pre (3T3-L1 cells stimulated with the phenols mix during adipogenic differentiation); and phenols-post (mature 3T3-L1 adipocytes stimulated with the phenols mix). MTT viability cell assay and Oil Red O staining were performed. Glycerol concentration supernatants were determined using the VITROS 350 Chemistry System. Expression of mRNA was measured using qPCR. The treatment with a 2 µg/ml ginger phenol dose reduced the lipid content by 45.52±7.8 and 35.95±0.76% in the phenols-pre and -post group, respectively, compared with that in the positive control group. The phenols-post group presented a higher glycerol concentration in the supernatant compared with that in the positive control and the phenols-pre groups. The mRNA expression levels of

CCAAT/enhancer-binding protein alpha, peroxisome proliferator activated receptor-γ, fatty acid-binding protein 4 and fatty acid synthase were higher in the phenols-pre and lower in the phenols-post groups, compared with those in the positive control group. To the best of our knowledge, the current study demonstrated for the first time the anti-adipogenic and lipolytic effects of a mix of the main bioactive compounds found in ginger, and it also established the basis to use this mix of phenols in *in vivo* studies and clinical trials.

Introduction

The prevalence of obesity has increased rapidly. According to the World Health Organization (WHO), obesity rates have increased 3-fold in adults and 5-fold in children and adolescents from 1975 to 2016 (1). Physiologically, obesity is the result of an excessive increase of adipose tissue in the body, which is a process related to hyperplasia and hypertrophy in adipocytes (2). The differentiation from preadipocytes into adipocytes to store lipid droplets is called adipogenesis, a process regulated by the activation and expression of different genes that participate in both the early and late differentiation stages, such as peroxisome proliferator-activated receptor-γ (PPAR-γ), CAAT enhancer binding protein α (C/EBPα), acetyl-coenzyme A carboxylase (ACACA), fatty acid synthase (FASN) and fatty acid binding protein 4 (FABP4) (3,4). Fatty tissue and adipocytes are a research focus for treatment of metabolic diseases (5).

Different treatment options were proposed for the control and reduction of adiposity. The first-line intervention includes diet modification, limiting the consumption of total fats and sugars and increasing the consumption of fruits, vegetables, legumes, whole grains and nuts, and increased physical activity (1). However, some patients need to complement this intervention with pharmacological therapy. Despite the benefits of the anti-obesity drugs, such as orlistat, lorcaserin, liraglutide, or phentermine-topiramate, some important barriers exist to widespread application, including high financial burden and a wide number of reported side effects like nausea, vomiting, constipation, hypoglycemia, diarrhea, headache, and abdominal pain (2). For these reasons, some

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bioactive food compounds have attracted attention in the treatment of obesity, such as capsaicin from chili or caffeine and ephedrine from guarana due to their properties that activate vasodilator and endorphin-releasing nerve signals, or thermal properties, which they increase energy and reduce weight, respectively (6-9). Therefore, it is necessary to continue the search and validation of active compounds that could be used in the treatment of obesity.

Ginger (*Zingiber officinale Roscoe*) is a medicinal plant with various beneficial effects, including anti-inflammatory, antioxidant, anti-nausea, antiemetic, lipid-lowering, and recently, the anti-obesogenic effect (10-12). Ginger contains a variety of phenolic compounds, with the most notable being gingerols, shogaols and paradols (13). Gingerols constitute the majority of phenolic compounds in fresh ginger and include 6-, along with 4-, 5-, 8-, 10- and 12-gingerols (14). Gingerols are thermally labile due to the presence of a β -hydroxy keto group and they are converted under high temperature to the corresponding shogaols (15). Gingerols (23-25%) and shogaols (18-25%) are the main bioactive constituents of ginger (14) and potential mediators of its major therapeutic effects (16).

Some studies demonstrated the anti-adipogenic and lipolytic effects of these phenols individually. The ability of 6-gingerol to inhibit adipocyte hypertrophy and hyperplasia both *in vitro* and *in vivo* was clearly demonstrated (17-20). Suk *et al.* (10,11) showed that treatment with 40 μ M of either 6-shogaol, 8- or 10-gingerol decrease the content of lipids in 3T3-L1 adipocytes, which is a murine embryonic preadipocyte cell line widely used for adipogenesis research. Even 6-shogaol was used in clinical trials where mean body weight, BMI and body fat level were lower in the group that received capsules of steamed ginger ethanolic extract rich in 6-shogaol than in the placebo group (21). However, to the best of our knowledge, there are no studies that evaluated the anti-obesogenic effect of the 6-, 8- and 10-gingerol plus 6-, 8- and 10-shogaol on adipose tissue. Therefore, the current study aimed to evaluate the lipolytic and anti-adipogenic activity of a mix of the main ginger phenols in the 3T3-L1 cell line.

Materials and methods

3T3-L1 cell culture and differentiation. Mouse 3T3-L1 preadipocytes were obtained from the Immunology Laboratory of the University Center of Health Sciences, University of Guadalajara, Mexico. Cells were cultured in DMEM (cat. no. SIG-D6429; Merck KGaA) supplemented with 10% Bovine Calf Serum (cat. no. 12389812; HyClone; Cytiva) and 1% antibiotics (100 U/ml penicillin and 100 μ g/ml streptomycin; cat. no. 15140122; Thermo Fisher Scientific, Inc.). (maintenance medium) at 37°C and 5% CO₂ in a cell incubator (cat. no. SCO6AD; Sheldon Manufacturing, Inc.). The cells were seeded in Petri dishes at a density of 1x10⁵ cells/dish and the maintenance medium was replaced every 2-3 days until the cells reached 100% confluence. At 1 day post-confluence (day 0), medium was substituted with DMEM/F-12 (cat. no. 21041025; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (cat. no. 16000044; Thermo Fisher Scientific, Inc.), 1% antibiotics and an adipogenic cocktail including 500 μ M 3-isobutyl-1-methylxanthine (IBMX), 1 μ M dexamethasone, 1.5 μ g/ml insulin and 1 μ M rosiglitazone (3T3-L1

Differentiation Kit; cat. no. SIG-DIF001-1KT; Merck KGaA) for 3 days, according to the manufacturer's instructions. On day 3, the medium was replaced with DMEM/F-12 supplemented (cat. no. 21041025; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (cat. no. 16000044; Thermo Fisher Scientific, Inc.), 1% antibiotics and an adipogenic cocktail including 500 μ M IBMX, 1 μ M dexamethasone, 1.5 μ g/ml insulin and 1 μ M rosiglitazone (3T3-L1 Differentiation Kit; cat. no. SIG-DIF001-1KT; Merck KGaA) for 3 days. On day 3, the medium was replaced with DMEM supplemented with 10% FBS, 1% antibiotics and 5 μ g/ml insulin, and the cells were cultured for 5 days. The total duration of adipogenesis was 8 days from the induction of differentiation with adipogenic cocktail and the cells were incubated at 37°C and 5% CO₂.

Mix of the main ginger phenols. A solution from ginger gingerols and shogaols mix was acquired from Merck KGaA (cat. no. SIG-G-027-1ML). The solution contained the main phenols of ginger (6-, 8- and 10-gingerol and 6-, 8- and 10-shogaol) at a standard concentration of 500 μ g/ml for every component (Fig. 1).

3T3-L1 cell treatment. A dose-response curve was generated using 1, 2, 3, 4 and 5 μ g/ml phenol-mix during the adipogenesis process (8 days) and in mature 3T3-L1 adipocytes (48 h) to determine the dose to be used in subsequent experiments. A total of four study groups were formed: i) Negative control (3T3-L1 preadipocytes); ii) positive control (mature 3T3-L1 adipocytes); iii) phenols-pre; and iv) phenols-post groups. In the phenols-pre group, confluent preadipocytes were incubated with phenol-mix until mature adipocytes were formed (day 8). In the phenols-post group, after 8 days of differentiation, cells were treated for a period of 48 h with phenol-mix. After the treatment with the phenol-mix, supernatants were collected in tubes and stored at -80°C; the 3T3-L1 adipocytes were cryopreserved for 1 month. No prior treatment was performed on the samples at the time of its use in the experimental analysis.

MTT assay. 3T3-L1 preadipocytes were seeded at 5x10³ cells/well in 96-well plates (cat. no. 701001; Nest Scientific USA Inc.). Cells were differentiated as aforementioned in the presence or absence of the mix of main ginger phenols. After 8 (phenols-pre group) and 10 days (phenols-post group), 1 mg/ml of MTT (cat. no. M6494; Thermo Fisher Scientific, Inc.) solution was added and cells were incubated for 1 h at 37°C. The dark blue formazan crystals were dissolved in an extraction buffer containing 20% SDS and 50% dimethylformamide. Absorbance at 570 nm was measured using a microplate reader (Multiskan™ GO; cat. no. 51119300; Thermo Fisher Scientific, Inc.).

Oil Red O staining. 3T3-L1 preadipocytes were differentiated as aforementioned in the presence or absence of the mix of main ginger phenols. Fully differentiated cells were fixed with 10% (v/v) formaldehyde solution for 60 min at room temperature and washed 3 times with distilled water. Lipid droplets in mature adipocytes were then stained with Oil red O (cat. no. O0625; Merck KGaA) solution for 15 min and washed 3 times with distilled water. The stained Oil Red O was then dissolved in 100% isopropanol (cat. no. IBI5730; IBI

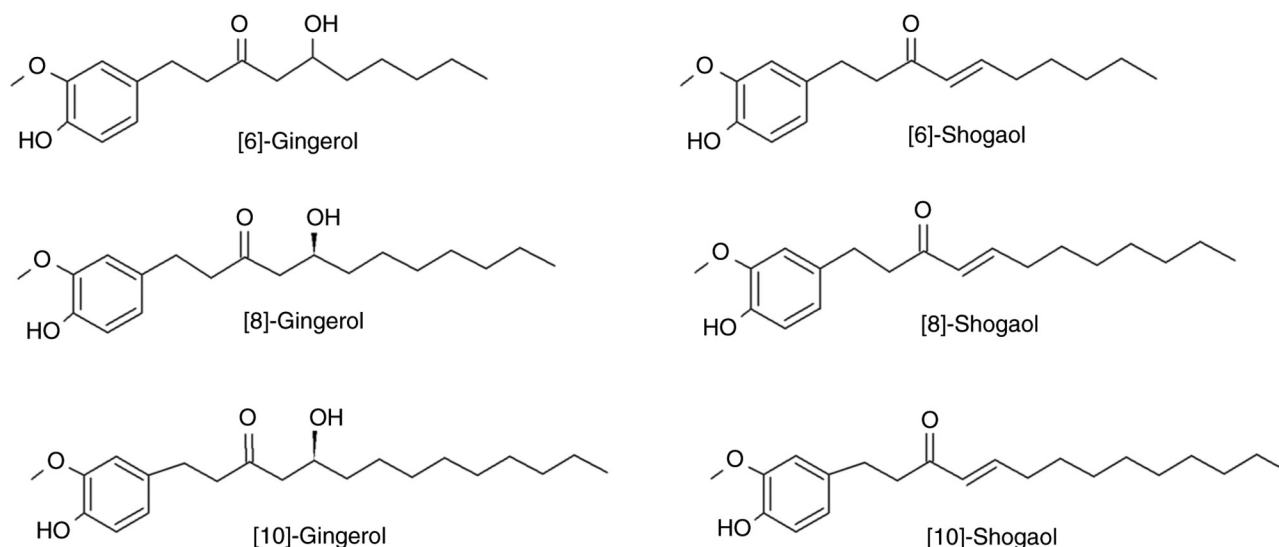


Figure 1. Molecular structure of ginger phenols contained in the mix 6-, 8-, 10-gingerol and 6-, 8- and 10- shogaol.

Scientific) and the absorbance was measured at 515 nm using a microplate reader (Multiskan GO; cat. no. 51119300; Thermo Fisher Scientific, Inc.).

Determination of glycerol release. Glycerol quantification in supernatants was performed using the VITROS 350 Chemistry System (QuidelOrtho Corporation) with TRIG slides (cat. no. OCD-MS-1336544; QuidelOrtho Corporation), according to the manufacturer's instructions.

mRNA expression quantification using reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from the study groups with the RNeasy Mini Kit (cat. no. 74104; Qiagen, Inc.) following the manufacturer's instructions. Total RNA (1 μg) was reverse transcribed into cDNA using the M-MLV Reverse Transcriptase (cat. no. 28025013; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The mRNA expression was quantified using qPCR (LightCycler 96 thermocycler; Roche Diagnostics) using the OneTaq[®] Hot Start Master Mix (cat. no. N01-M0484L; New England Biolabs, Ltd.) and the following TaqMan[®] Real-Time PCR Assays for Rn18s (cat. no. Mm03928990_g1), PPAR- γ (cat. no. Mm00440940_m1), C/EBP α (cat. no. Mm00514283_s1), ACACA (cat. no. Mm01304257_m1), FASN (cat. no. Mm00662319_m1) and FABP4 (cat. no. Mm00445878_m1), all from Thermo Fisher Scientific, Inc. The following thermocycling conditions for qPCR were used: Initial preincubation at 95°C for 300 sec; 30 cycles of denaturation at 95°C for 20 sec and amplification at 60°C for 60 sec; and 1 cycle of extension at 68°C for 300 sec. The relative gene expression was quantified using the $2^{-\Delta\Delta C_t}$ method (19) and normalized against the expression level of 18S as an internal reference gene. Each analysis and quantification were independently repeated 3 times.

Statistical analysis. Experimental analyses were performed in triplicate. Data are expressed as mean \pm SD. Differences among groups were evaluated using one-way ANOVA followed by Tukey's post hoc test. Data were analyzed using

SPSS (version 25; IBM Corp.). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Cell viability and lipid content dose-response curves with phenol-mix. A dose-response curve with phenol-mix was performed in the 3T3-L1 cell line to establish the treatment dose. Subsequently, cell viability was evaluated with the MTT assay and lipid content with Oil Red O staining. Treatment of immature 3T3-L1 cells with $\geq 3 \mu\text{g/ml}$ phenol-mix during the differentiation process significantly decreased the percentage of living cells ($P < 0.01$; Fig. 2A); these concentrations were excluded from subsequent experiments. However, treatment with the phenols mix did not significantly decrease the cell viability of mature 3T3-L1 adipocytes (Fig. 2B). Regarding the lipid content, the 3T3-L1 cells treated with phenol-mix during the adipogenesis process showed a decreased percentage of lipids compared with that in the positive control group at every concentration ($P < 0.001$). Particularly, the $2 \mu\text{g/ml}$ dose lead to the greatest reduction in lipid content compared to the positive control group ($P < 0.001$; Fig. 3A). Similarly, the mature 3T3-L1 adipocytes treated with 2, 4 (both $P < 0.01$) and $5 \mu\text{g/ml}$ ($P < 0.001$) phenol-mix presented a significantly lower percentage of lipids than the positive control group (Fig. 3B).

Treatment with the $2 \mu\text{g/ml}$ dose both during adipogenesis and in mature 3T3-L1 adipocytes showed the greatest reduction of lipid content ($P < 0.001$ and $P < 0.01$, respectively) without affecting cell viability ($P = 0.812$). For this reason, the $2 \mu\text{g/ml}$ treatment dose was chosen for the following experiments.

Effects of the phenol-mix on cell viability and adipocyte differentiation. After determining the optimal treatment dose ($2 \mu\text{g/ml}$), the negative control, positive control, phenols-pre and phenols-post study groups were formed to analyze the differences in cell viability and adipocyte differentiation with or without treatment with phenol-mix. The percentage of viable cells was not affected by the treatment with $2 \mu\text{g/ml}$

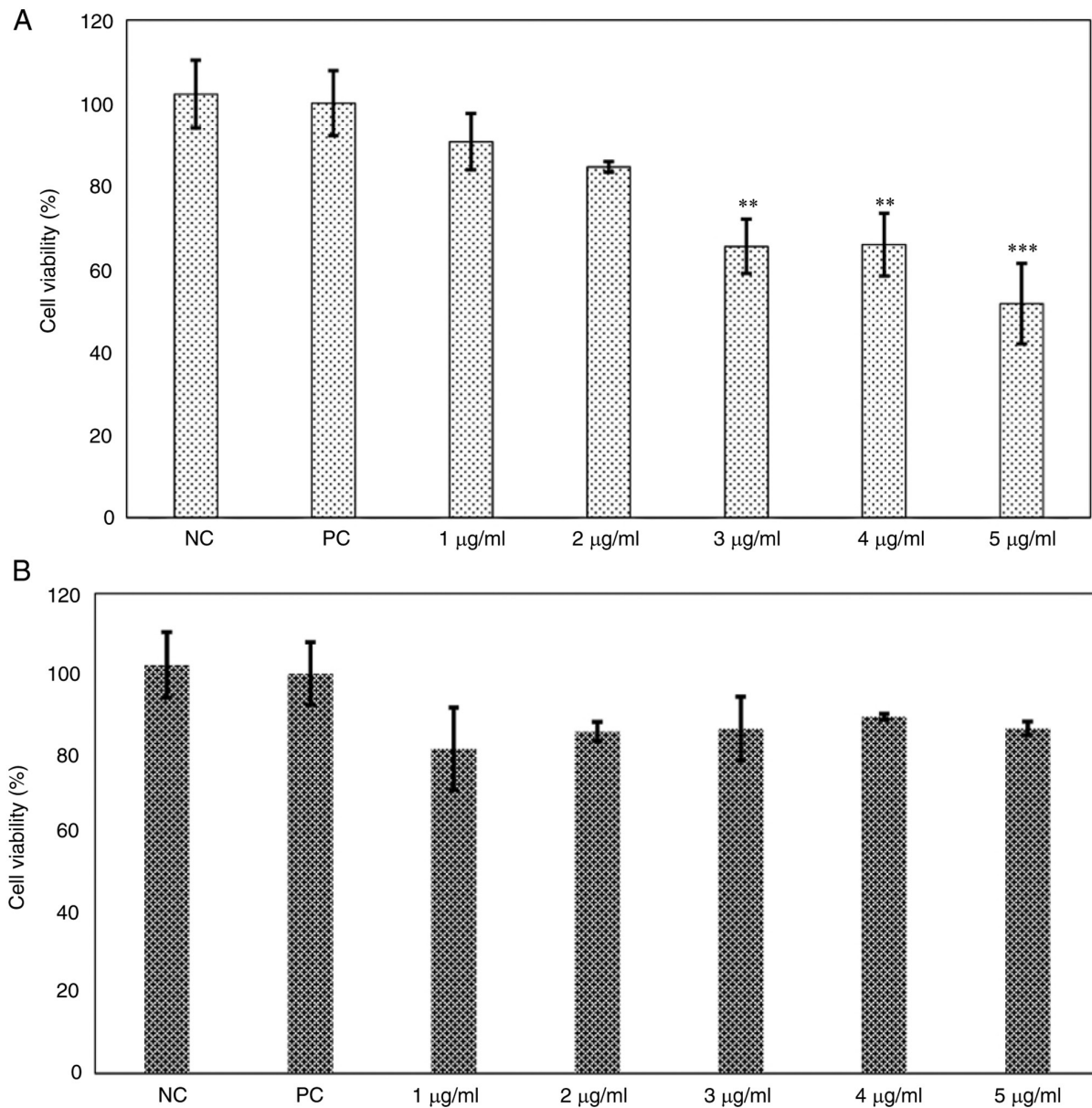


Figure 2. Cell viability dose-response curve of phenol-mix. (A) 3T3-L1 cells were treated with the phenol-mix at 1, 2, 3, 4 and 5 µg/ml during adipogenic differentiation and cell viability was determined using MTT assay. (B) Mature 3T3-L1 adipocytes were treated with the phenol-mix at 1,2,3,4 and 5 µg/ml for 48 h and cell viability was measured using MTT assay. Each bar represents the mean \pm standard deviation of three experiments. ** $P < 0.01$ and *** $P < 0.001$ vs. positive control. NC, negative control (3T3-L1 cells); PC, positive control (mature 3T3-L1 adipocytes); phenols-pre, 3T3-L1 cells stimulated with the phenols mix during adipogenic differentiation; phenols-post, mature 3T3-L1 adipocytes stimulated with the phenols mix.

in both phenols-pre ($P=0.275$) and phenols-post ($P=0.710$) groups compared with that in the positive control group (Fig. 4A). Notably, the treatment with 2 µg/ml induced a reduction of the lipid content by 45.52 ± 7.8 and $35.95 \pm 0.76\%$ in the phenols-pre and -post groups, respectively, compared with that in the positive control group ($P < 0.001$; Fig. 4B). Oil red O staining revealed lipid content differences between groups. As expected, negative control 3T3-L1 cells showed no staining, while high staining was observed in the positive control (PC; mature 3T3-L1 adipocytes) which is indicative that adipogenic differentiation has occurred successfully. Finally, in the groups treated with the phenol-mix during and after adipogenesis, a qualitative decrease of the dye is observed in comparison with PC, which is an indirect indicator of a lower amount of stored lipids (Fig. 4C).

Effects of phenol-mix on glycerol release from mature 3T3-L1 adipocytes. The glycerol content in the supernatant was measured as a marker of lipolysis in the four study groups. Results showed that the phenols-pre group released a lower glycerol amount than that of the positive control group ($P < 0.001$). Conversely, the phenols-post group presented a higher glycerol concentration in the supernatant compared with that in the positive control ($P < 0.001$) and was numerically increased compared with that in the phenols-pre group (Fig. 5).

Effects of phenol-mix on proadipogenic and lipogenic genes in 3T3-L1 adipocytes. Finally, the expression of genes related to the regulation of adipogenesis and lipogenesis in the study groups was analyzed. The phenols-pre group showed higher expression at the mRNA level of C/EBP α , PPAR- γ , FABP4,

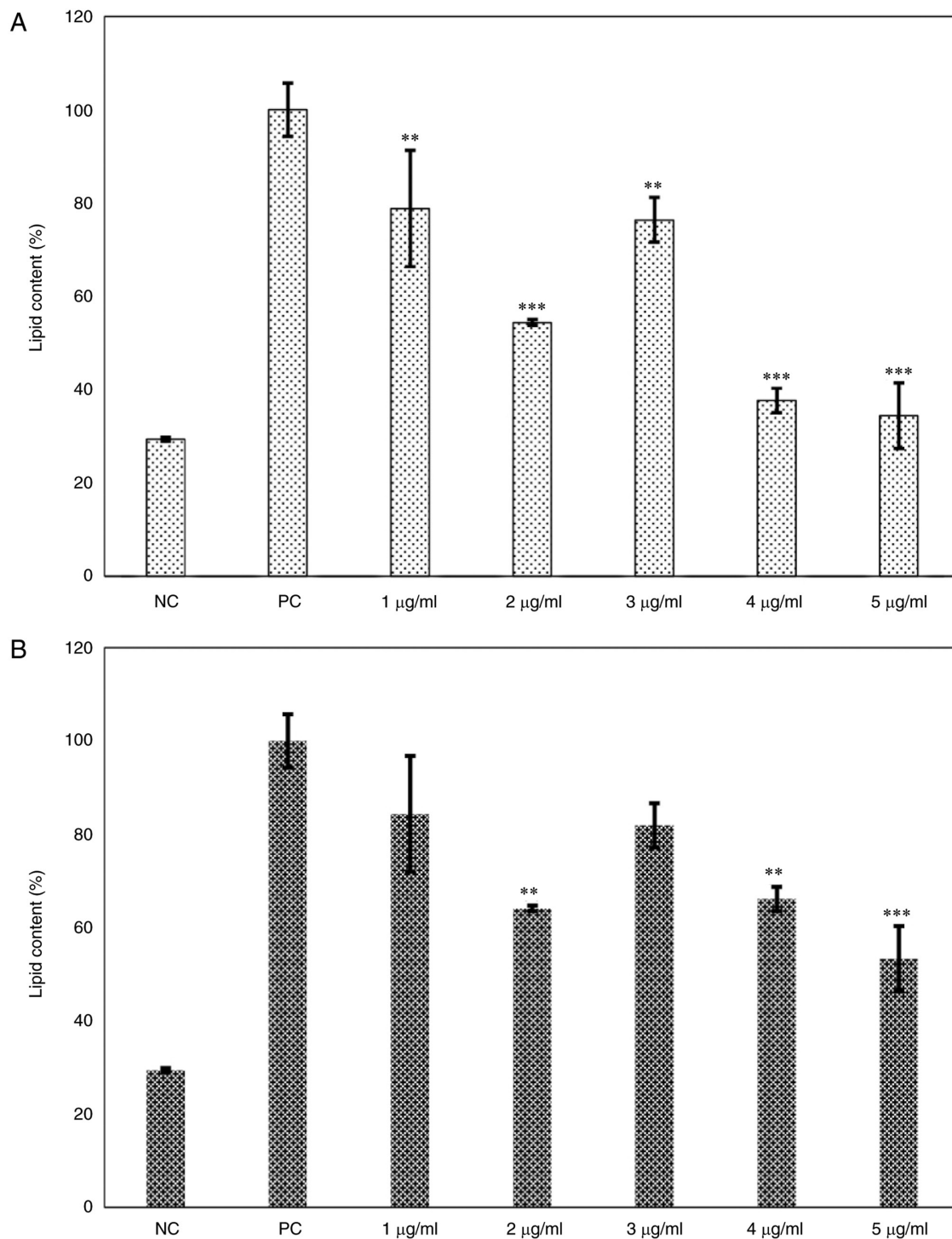


Figure 3. Dose-response curve of the main ginger phenols mix effects on lipid content. (A) 3T3-L1 cells were treated with the mix of the main ginger phenols at various concentrations (1,2,3,4 and 5 µg/ml) during adipogenic differentiation and lipid content was measured. (B) Mature 3T3-L1 adipocytes were treated with the mix of the main ginger phenols at various concentrations (1,2,3,4 and 5 µg/ml) for 48 h and lipid content was measured. Each bar represents the mean \pm standard deviation of three experiments. ** $P < 0.01$ and *** $P < 0.001$ vs. positive control. NC, negative control (3T3-L1 cells); PC, positive control (mature 3T3-L1 adipocytes); phenols-pre, 3T3-L1 cells stimulated with the phenols mix during adipogenic differentiation; phenols-post, mature 3T3-L1 adipocytes stimulated with the phenols mix.

ACACA and FASN than that of the positive control group ($P < 0.05$; Fig. 6A-E). By contrast, the phenols-post group presented a significantly lower expression of C/EBP α , FABP4 and FASN compared with that in the positive control group ($P < 0.001$; Fig. 6A, C and E). The expression of PPAR- γ and ACACA was not significantly different compared with that in the positive control (Fig. 6D).

Discussion

This study demonstrated that the six main phenols contained in ginger, i.e. 6-, 8-, 10-gingerol and 6-, 8- and 10-shogaol, could decrease the lipid content in both 3T3-L1 cells undergoing adipogenic differentiation and in mature adipocytes derived from this cell line.

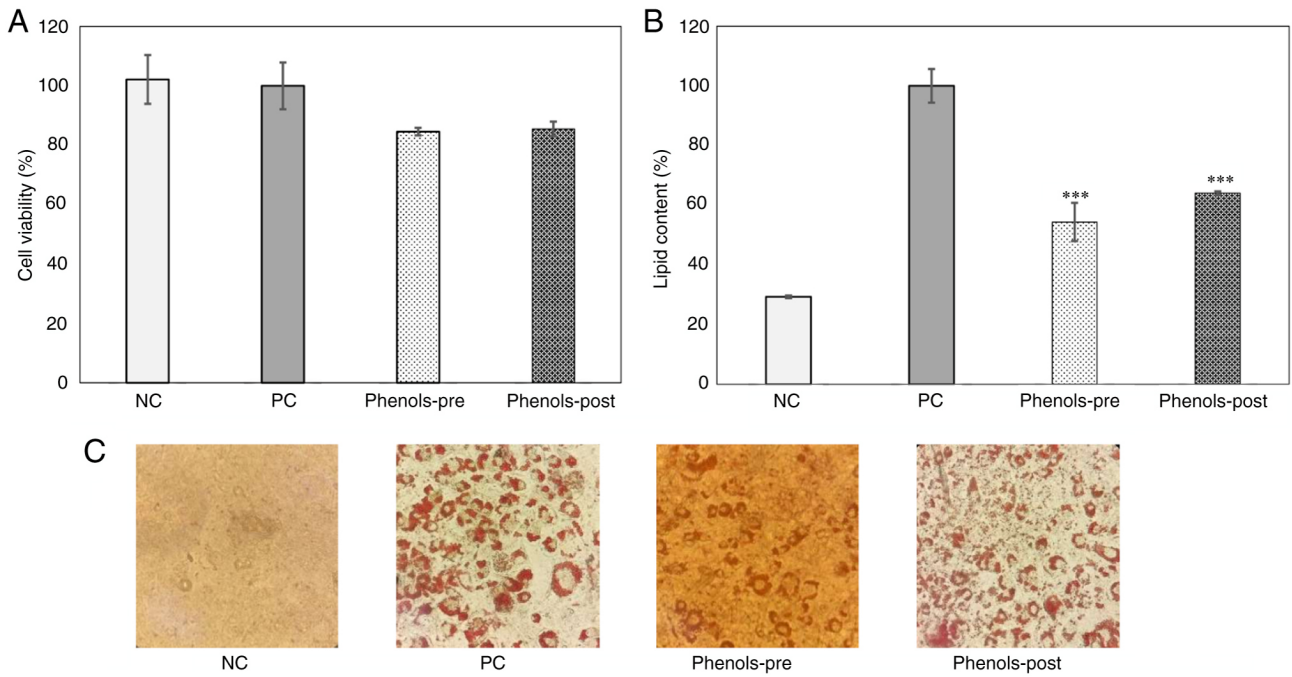


Figure 4. Effects of phenol-mix on cell viability and lipid content. (A) The study groups were treated with 2 $\mu\text{g/ml}$ phenol-mix and (A) Cell viability (MTT assay) and (B) Lipid content were measured. (C) Representative images of the lipid content in the four groups after Oil Red O staining (magnification, x40). Each bar represents the mean \pm standard deviation of three experiments. *** $P < 0.001$ vs. positive control. NC, negative control (3T3-L1 cells); PC, positive control (mature 3T3-L1 adipocytes); phenols-pre, 3T3-L1 cells stimulated with the phenols mix during adipogenic differentiation; phenols-post, mature 3T3-L1 adipocytes stimulated with the phenols mix.

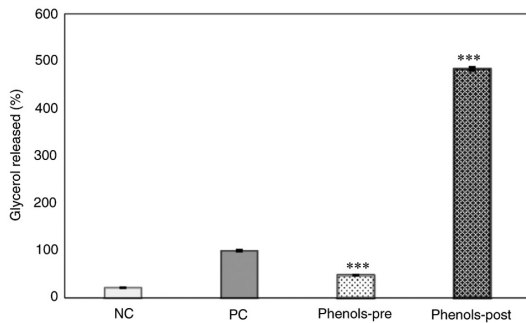


Figure 5. Effects of the main ginger phenols mix on glycerol-release activity. 3T3-L1 cells were treated with 2 $\mu\text{g/ml}$ of the mix of the main ginger phenols during adipogenic differentiation, while mature 3T3-L1 adipocytes were treated with the same dose for 48 h. Glycerol determination in supernatant was performed using VITROS 350 Chemistry System following the manufacturer's instructions. Each bar represents the mean \pm standard deviation of three experiments. *** $P < 0.001$ vs. positive control. NC, negative control (3T3-L1 cells); PC, positive control (mature 3T3-L1 adipocytes); phenols-pre, 3T3-L1 cells stimulated with the phenols mix during adipogenic differentiation; phenols-post, mature 3T3-L1 adipocytes stimulated with the phenols mix.

Several studies evaluated the ability of plant-derived extracts to reduce the lipid content in the 3T3-L1 cell line (8,23-26). These phenols were evaluated individually in different experimental models that support their antitumor (27,28), anti-inflammatory (29,30) and anti-obesogenic capacities (31). Nonetheless, to the best of our knowledge, there were no previous studies that tested these six phenols together. Notably, the anti-adipogenic effect of different gingerols were widely studied in both *in vitro* and *in vivo* models (11,19,20,32-34). The coordination of the stages of adipocyte differentiation was

shown to be regulated by the sequential activation of transcriptional factors, including PPAR- γ and various members of the C/EBP family of transcriptional factors (35). Therefore, the decrease in lipid content in *in vitro* model could be attributed to the suppression of the transcriptional factors that regulate adipogenesis, i.e. PPAR- γ and C/EBP α (34,36). However, although the results of the present study in the phenols-pre group did not reflect the trend observed in other previous studies, a reduction in the concentration of these pro-adipogenic markers at the protein level could not be rejected since studies found discrepancies between the transcriptional and translational levels of C/EBP α (37,38). Some studies showed that other natural bioactive compounds could inhibit the PPAR- γ translocation to the nucleus (23,39,40). Hence, more studies would be needed to clarify the molecular mechanism by which gingerols and shogaols decrease the lipid content during differentiation of 3T3-L1 cells to adipocyte.

Recently, Cheng *et al* (20) conducted a study using 6-gingerol as an intervention in a C57BL/6J high-fat-diet-induced obese mice that resembled the phenols-post group of the present study. The authors reported decreases in the expression of PPAR- γ , C/EBP α and FABP4 from epididymal white adipose tissue both at the transcriptional and translational levels, consistent with the results of the current study for the phenols-post group. PPAR γ and C/EBP α were known to form a positive feedback loop with each other and to promote the expression of genes that encode proteins related to the adipogenic phenotype (3), including FABP/aP2 (FABP4 gene), which is induced during adipocyte differentiation and expressed in adipocytes (41). Therefore, the significant reduction of these adipogenic markers in the phenol-post group of the present study indicated that the suppression of

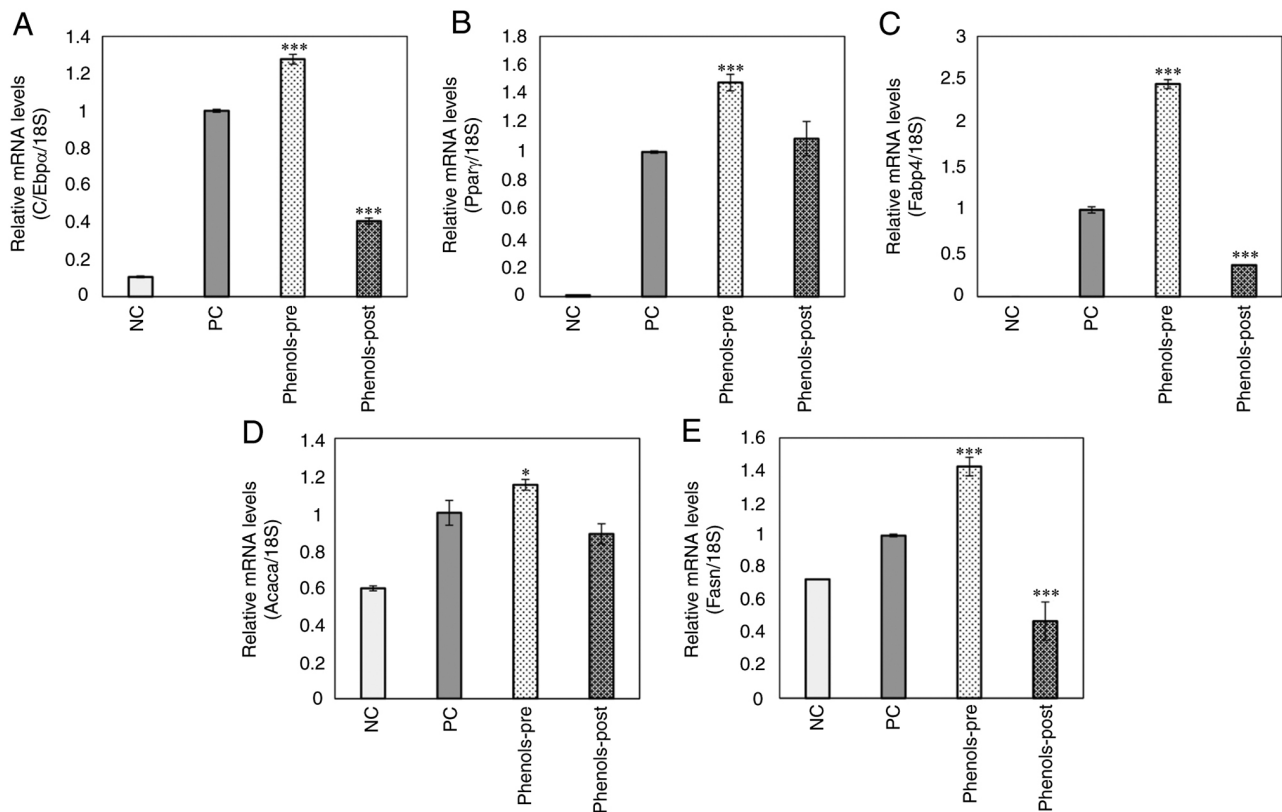


Figure 6. Effects of the main ginger phenols mix on pro-adipogenic and -lipogenic genes expression. (A) CCAAT enhancer-binding protein α , (B) peroxisome proliferator-activated receptor- γ , (C) fatty acid binding protein 4, (D) acetyl-coenzyme A carboxylase and (E) fatty acid synthase mRNA levels in 3T3-L1 mature adipocytes were examined using reverse transcription-quantitative PCR. Each bar represents the mean \pm standard deviation of three experiments. * $P < 0.05$ and *** $P < 0.001$ vs positive control. ACACA, acetyl-coenzyme A carboxylase; C/EBP α , CCAAT enhancer-binding protein α ; FABP4, fatty acid binding protein 4; FASN, fatty acid synthase; PPAR- γ , peroxisome proliferator-activated receptor- γ ; NC, negative control (3T3-L1 cells); PC, positive control (mature 3T3-L1 adipocytes); phenols-pre, 3T3-L1 cells stimulated with the phenols mix during adipogenic differentiation; phenols-post, mature 3T3-L1 adipocytes stimulated with the phenols mix.

adipogenesis could be a key factor for the anti-obesogenic effect of this group. Noteworthy, the regulation of the lipid metabolism by these transcriptional factors could be involved in this process.

The decrease of lipids in mature 3T3-L1 adipocytes may also be related to the suppression of lipogenesis and lipid accumulation (31). Some ginger compounds, specifically 6-shogaol and 6-gingerol, may serve as PPAR- δ agonists with subsequent effects on energy homeostasis, such as a reduction of lipid deposition in skeletal muscle and adipose tissue (42). Moreover, the mechanisms that mediate the suppression of lipogenesis are intrinsically linked to the expression levels of lipogenic enzymes such as acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS) (43,44). ACC catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, the rate-limiting step in fatty acid synthesis; in turn, FAS is involved in energy homeostasis by converting energy into lipid storage (45). These enzymes are expressed in adipose tissue at high levels and their expression and activity are acutely and chronically regulated through transcriptional control and post-translational modifications are associated with nutritional status (fasting and feeding) and substrate availability (46). Results from the present study revealed significantly decreased expression of FASN in the phenol-post group, suggesting that the observed decrease in lipid accumulation in mature adipocytes achieved by these ginger phenols could be related to the inhibition of the

expression of these lipogenic enzymes. Future *in vitro* studies should evaluate the protein expression and activity of FAS and ACC, as well as how these are regulated by the main bioactive compounds in ginger.

Finally, the current study also showed lipolytic activity of the mix of phenols derived from ginger on mature 3T3-L1 adipocytes. The lipolytic effect was previously reported for 6-shogaol (11). However, it should be noted that considerably lower doses were used in the present study than those reported by previous studies with individual phenols. For instance, a greater pro-lipolytic effect was observed in the present study than that achieved by Suk *et al* (10,11) who only used 6-shogaol (40 μ M), an observation that also occurred with the anti-obesogenic effect found in the present study. Therefore, the rest of the phenols contained in the mix would be responsible for enhancing this effect. Further studies are required to elucidate the individual lipolysis-promoting capacities of the rest of the phenols in the mix.

In conclusion, the present study shows for the first time the anti-adipogenic and lipolytic effects of a mix of the main bioactive compounds found in ginger, i.e. 6-, 8-, 10-gingerol and 6-, 8- and 10-shogaol, during the differentiation of 3T3-L1 cells to adipocytes as well as on mature adipocytes. Future approaches will need to test the *in vivo* effects of this mix of ginger phenols to provide more compressive data that would validate its use in clinical protocols, as perhaps all or some

of them could be useful for the treatment and prevention of obesity.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

JRV and EML contributed to the experimental design. GGO, MPO and SCRR performed the experiments. WCP and EML performed the validation of data and statistical analysis. GGO, MPO and JRV wrote the manuscript. MPO and JRV confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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