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Effect of *Lemon Verbena* Polyphenol on Glycerol Channel Aquaporin 7 Expression in 3T3-L1 Adipocytes

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ABSTRACT

Objective: Polyphenols are of great interest in obesity prevention approaches. The aquaglyceroporin 7 (*AQP7*) channel is involved in the transport of glycerol across cell membranes in adipose tissue. This study aimed to explore how *lemon verbena* (LV) polyphenols affect the expression of the glycerol channels *AQP7* and *perilipin 1* (*PLIN1*) in 3T3-L1 hypertrophic adipocytes.

Materials and Methods: Hypertrophic adipocyte cells (H) were treated with LV at two different doses of 200 µg/mL (H-LV200) and 400 µg/mL (H-LV400). In addition, 0.1 µM β3-AR agonist (CL316243) and 0.1 µM β3-AR antagonist (L7483337) were applied to the cells at both doses. *AQP7* and *PLIN1* gene expressions were determined by real time-polymerase chain reaction (RT-PCR), and glycerol levels were determined by enzyme-linked immunosorbent assay (ELISA).

Results: Hypertrophic adipocytes showed increased *AQP7* and *PLIN1* gene expression and glycerol content compared with the control group. H-LV200 and CL316243 treatment together increased *AQP7* gene expression, whereas H-LV400 and L7483337 treatment together decreased *AQP7* gene expression.

Conclusion: The data indicated that both doses of LV inhibited glycerol production by suppressing *AQP7* and *PLIN1* gene expression. Approaches to regulate *AQP7* gene expression in adipose tissue using plant-derived polyphonic compounds are considered a healthy and innovative approach to combat and manage metabolic diseases, including obesity.

Keywords: Polyphenol, adipocyte, aquaglyceroporin, glycerol, obesity

INTRODUCTION

Obesity is a global public health problem that leads to chronic diseases, such as diabetes, cardiovascular disease, and cancer (1). In obesity, adipose tissue homeostasis is disturbed, leading to the proliferation and enlargement of adipocytes (2). Currently, bioactive compounds derived from natural foods are attracting considerable interest as potential strategies to combat obesity. Among these compounds, polyphenols, which are abundant in fruits, vegetables, cereals, and beverages, stand out. In fact, specific plant-derived polyphenols are increasingly being considered viable alternatives to obesity (3-5). Numerous polyphenols play an important role in the regulation of essential cellular processes, including growth, differentiation, energy balance, and metabolic homeostasis. These compounds contribute to metabolic health by modulating molecular signalling pathways (6). *Lemon verbena* (LV) is a notable example of these polyphenols. One of its major constituents, verbascoside, a phenylpropanoid, plays a crucial role in imparting the plant's antioxidant and anti-inflammatory properties. Polyphenolic extracts from LV have been shown

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to suppress intracellular lipid accumulation, oxidative stress due to high glucose levels, and inflammation in adipose tissue by acting on various metabolic pathways. Research has potential applications in the prevention and treatment of metabolic diseases (7, 8).

LV extract has been reported to reduce high-glucose-induced metabolic stress in hypertrophic adipocytes and hyperlipidaemia mice through 5'-adenosine monophosphate-activating protein kinase (AMPK)-dependent mechanisms (11). LV has been shown to exert this effect by activating adiponectin through a transcriptional peroxisome proliferation activating receptor gamma (PPAR-γ)-dependent mechanism that correlates with AMPK activation. Through this pathway, LV oxidation increases fatty acid oxidation, suppresses lipogenesis in obesity, and alleviates obesity-related disorders (9).

Aquaporins (AQP) are channel proteins that enable the passage of water and small molecules. In mammals, 13 forms have been identified and classified into subgroups. Recently, the effects of AQPs on adipose tissue homeostasis have been clarified, providing a new perspective in this field. Research indicates that AQPs could serve as targets for drug development aimed at treating diseases related to adipose tissue function and/or dysfunction (10-14). The function of *AQP7*, an aquaglyceroporin, a subgroup of AQPs, primarily stems from its role in enabling glycerol transport across cell membranes (15). Therefore, it plays an important role in regulating glycerol transport from adipose tissue to maintain lipid and energy balance. Under lipogenic conditions of increased insulin stimulation, such as feeding, *AQP7* co-localises with the protein *perilipin 1* (*PLIN1*) at the lipid droplet interface, and the movement of AQP7 to the plasma membrane is inhibited by *PLIN1*. Under lipolytic conditions, such as fasting and exercise, when the energy demand increases, triacylglycerol (TAG) in adipose tissue is hydrolysed, releasing free fatty acids (FFA) and glycerol. Protein kinase A (PKA), which is activated by signalling under these metabolic conditions, weakens the association between *AQP7* and *PLIN1*. The inhibitory effect of *PLIN1* on *AQP7* is removed. *AQP7* translocates to the plasma membrane to release glycerol into the circulation (16). Glycerol is an important organic molecule in carbohydrate and lipid metabolism because of its role in determining plasma glucose levels (17-19). No studies have been identified in the existing literature that investigate the impact of LV plant extract on the expression of the glycerol channel *AQP7* and associated *PLIN1* in 3T3-L1 cells. There are only a few studies on the effects of plant-derived polyphenol applications on *AQP7* and *PLIN1* gene expression (19, 20). Among these studies, no studies have investigated the effects of LV therapy. In our study, we sought to explore the effects of LV polyphenols on *AQP7* and *PLIN1* expression in mature and hypertrophic adipocytes.

MATERIALS AND METHODS

Chemicals and Reagents

In order to investigate into the impact of LV polyphenols on lipid and glucose metabolism, LV polyphenolic extract (27% verbascoside, dry weight) was kindly provided by Dr. María Herranz-López (University Miguel Hernandez, Elche, Spain) (9). The LV aqueous extract contained iridoid glycosides (% dry weight w/w; 2.5%), phenylpropanoid (w/w; 33.5%), and flavonoids (w/w; 4.8%). The total w/w of these identified compounds was 40.8%. Verbascoside comprises 27% phenylpropanoids. LVs were freshly prepared before use and filtered after thawing in cell culture medium. LV polyphenol doses of 200 µg/mL (mature) and 400 µg/mL (hypertrophic adipocytes) were selected and applied to the cells as previously described (9, 21). Under conditions that promote fat breakdown, sympathetic nerves become active, resulting in an increase in catecholamine levels and stimulation of adrenergic receptors in adipose tissue. The β3-AR receptor agonist CL316243 (CL; Cat. No:138908-40-4, Cayman Chemical, Michigan, USA) was used to stimulate fat breakdown in adipose tissue, while the β3-AR antagonist L7483337 (L; Cat. No: 244192-94-7, Tocris Bioscience-Bristol, UK) was used to obstruct its stimulation.

Cell Culture

The preadipocyte cell line 3T3-L1 (American Type Culture Collection, Manassas, VA, USA, CL173™) were cultured in low-glucose (1 g/L) DMEM supplemented with 10% calf serum, 100 g/mL streptomycin, and 100 U/mL penicillin, known as complete medium. The cells were cultured in flasks, with the complete medium being replaced every 2-3 days, and incubated at 37°C in a humidified atmosphere (5% CO_2, 95% air).

Differentiation of the 3T3-L1 Cell Line

Differentiation of 3T3-L1 preadipocyte was performed according to standard protocols (22). Initially, preadipocyte were seeded in 6-well plates using complete medium and cultured until they reached confluence. Next, differentiation was induced by incubating with differentiation medium I (DMI), which contains high-glucose DMEM (4.5 g/L) supplemented with 10% FBS and adipogenic reagents such as 10 µg/mL insulin, 1 µM DXMT, and 0.5 mM IBMX. This DMI was left for 48 h and then changed to differentiation medium II (DMII), which contains insulin and 10% FBS in high-glucose DMEM (4.5 g/L). The DMII was changed every 2-3 days for 10-12 days, obtaining mature adipocytes. After obtaining mature adipocytes, The cells underwent 24-h treatment with LV (200 µg/mL, 400 µg/mL), L (0,1 µM), CL (0.1 µM), LV (200 µg/mL)+L(0.1 µM), LV (400 µg/mL)+L (0.1 µM), LV (200 µg/mL)+CL (0.1 µM), and LV (400 µg/mL)+CL 0.1 µM). Before cell treatment, all extracts were dissolved in either medium or DMSO-enhanced medium and subsequently filtered for sterilisation.

Hypertrophic Adipocyte Model

Once the differentiation to mature adipocytes has occurred, the hypertrophic state of adipocytes can be obtained to simulate obesity-induced adipose tissue via prolonged incubation under high-glucose conditions. To obtain hypertrophic adipocytes from mature adipocytes, the cells were maintained with DMII for at least an additional 7 days. Nearly 20 days

after incubation, a hypertrophic adipocyte model has been observed. It is a well-established model of insulin resistance in adipocytes exposed to metabolic stress (23). After obtaining hypertrophic adipocytes, the cells were treated for 48 h with LV (200 µg/mL, 400 µg/mL), L (0.1 µM), CL (0.1 µM), LV (200 µg/mL)+L (0.1 µM), LV (400 µg/mL)+L (0.1 µM), LV (200 µg/mL)+L (0.1 µM), and LV (400 µg/mL)+CL (0.1 µM). Each extract was dissolved in medium or DMSO-supplemented medium, sterilised by filtration, and then administered to the cells. The planned study groups are as follows;

1. Mature adipocytes without any treatment (control (K))
2. Untreated hypertrophic adipocyte (hypertrophic (H))
3. Hypertrophic adipocytes treated with LV 200 µg/mL alone (H-LV200)
4. Hypertrophic adipocytes treated with LV 400 µg/mL alone (H-LV400)
5. Hypertrophic adipocytes treated with CL alone (H-CL)
6. Hypertrophic adipocytes treated with L alone (H-L)
7. Hypertrophic adipocytes treated with a combination of 200 µg/mL LV and CL (H-LV200+CL)
8. Hypertrophic adipocytes treated with a combination of 400 µg/mL LV and CL (H-LV400+CL)
9. Hypertrophic adipocytes treated with 200 µg/mL combination of LV and L (H-LV200+L)
10. Hypertrophic adipocytes treated with 400 µg/mL combination of LV and L (H-LV400+L)

Cell Viability

To assess the percentage of viable 3T3-L1 cells, approximately 2×10^4 cells per well were seeded in a 96-well plate and treated with the indicated agents. Different incubation protocols were applied to cells, followed by incubation for 24 and 48 h. Cell viability was assessed using the WST-8 Cell Proliferation Kit-8 (Elabscience), following the manufacturer's instructions for proliferation assays. WST-8 reagent was added to each well for 4 h. Upon completion of the assay, the absorbance for each sample was recorded at 450 nm using a microplate reader (BioTec-USA) (24).

Oil-Red-O Staining

To test the suitability of the cell culture model created in our experiments was done in 2 stages. A staining kit (Oil-red-O staining kit, K580-24, Biovision, USA) was used. Cells were seeded in 6-well plates at a density of 100,000 cells/well, as previously described. Following the steps described in the adipocyte differentiation protocol, Oil red O staining was performed at different stages of the cells, and intracellular lipid accumulation was evaluated.

Total RNA Isolation and Gene Expression

Mature cells treated with LV, CL, and L for 24 h, along with hypertrophic adipocytes treated for 48 h, were collected by centrifugation at 1,000 g for 10 min. The cell pellets were lysed using 100 µL of cold lysis buffer. Following a 10-min incubation on ice, the lysates were centrifuged at 14,000 g for 1 min to separate the supernatants. After centrifugation, the supernatants were decanted into fresh microcentrifuge tubes. Mature and hypertrophic cells were harvested using Trizol™ (Cat.No:15596018, Ambion, Invitrogen, California, USA). To determine *AQP7* and *PLIN1* gene expression in LV polyphenol-treated cells, cells were removed from the flask and centrifuged at 1500 rpm for 5 min. Following removal of the medium, total RNA was extracted from the pellets using Trizol™ (Cat.No:15596018, Ambion, Invitrogen, California, USA) reagent, according to the manufacturer's instructions. The quality and quantity of the isolated RNAs were assessed using a Nanodrop ND-2000c (Thermo Scientific-USA). Subsequently, mRNAs were converted into cDNA using a cDNA Reverse Transcription kit (A.B.T.™ Laboratory Industry, Ankara, Turkiye). cDNA-converted samples were stored at -20°C until used in real time-polymerase chain reaction (RT-PCR) analyses. The effects of LV administration to 3T3-L1 hypertrophic adipocyte cells on *AQP7* (F: 5'-TATGGTGCGAGAGTTTCTGG -3', R: 5'-GCCTAGTGCACAATTGGTGA -3') and *PLIN1* (F: 5'-ACAGAGAATATGCCGCCAA-3', R: 5'-GGCTGACTCCTTGTCTGGTG -3') gene expressions were analyzed by RT-PCR. For normalisation of the data obtained because of RT-PCR, glyceraldehyde-3-phosphate-dehidrogenase (GAPDH) (F: 5'-CCTGCCAAGTATGACATCAA -3', R: 5'- AGCCCAGGATGC CCTTTAGT-3') reference gene was used for normalization. We obtained the analysis results from three replicates and normalized the data by calculating the $2^{-\Delta\Delta CT}$ value.

Measurement by ELISA

Glycerol levels in cell medium collected and stored at -80°C were measured using an enzyme-linked-immunosorbent assay (ELISA) kit (BioVision Cat. No: K630-100, CA, USA) according to the manufacturer's instructions (Bio Tek Instruments, Inc, USA).

Statistical Analyses

Results are presented as mean ± standard deviation. The normality of data distribution was assessed using the Shapiro-Wilk test. For comparisons between groups, the Kruskal-Wallis Test and the Mann–Whitney U test were used. A value of $p<0.05$ was considered statistically significant. In this study, the SPSS 20.0 package was used for the statistical analysis of the data.

RESULTS

Association Between *AQP7*/*PLIN1* Gene Expression and Glycerol Concentration in Hypertrophic Adipocytes

A comparison was made between *AQP7* and *PLIN1* gene expression and glycerol concentrations in hypertrophic and non-hypertrophic (mature) control adipocytes. The results indicated that *AQP7* and *PLIN1* gene expression was elevated in hypertrophic adipocytes relative to the control group (Figure 1,

2 and 3). Although the increase in *AQP7* gene expression was not statistically significant (p=0.704), the increase in *PLIN1* gene expression was statistically significant (p<0.001). The glycerol concentration in the medium surrounding hypertrophic adipocytes was significantly elevated compared with that in the medium surrounding mature control group adipocytes (K; 11.77 ± 0.40 ng/mL vs H; 43.79 ± 0.59 ng/mL, p=0.014; Figure 4). Therefore, we inferred that *AQP7*, which is modulated by *PLIN1* in hypertrophic adipocytes, acts as a mediator of glycerol extrusion.

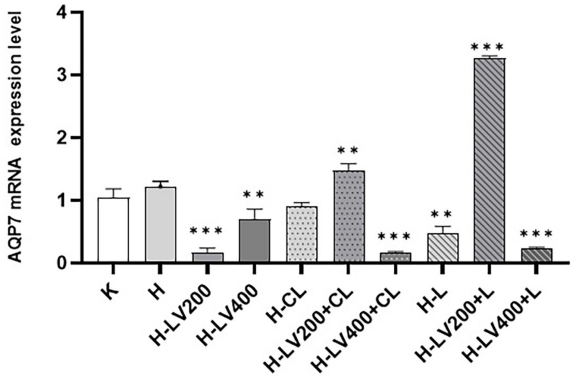

Figure 1. Changes in *AQP7* gene expression on application of 200 and 400 ng/mL doses of LV, the β3-AR receptor agonist-CL and the β3-AR receptor antagonist-L.
K, control; H, hypertrophic adipocyte; LV, *lemon verbena*; CL, CL316243 (β3-AR receptor agonist); L, L7483337 (β3-AR antagonist); **p<0.001; ***p<0.0001.

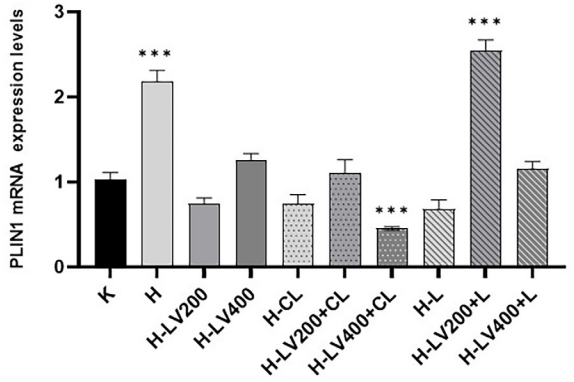

Figure 2. Changes in *PLIN1* gene expression on application of 200 and 400 ng/mL doses of LV, the β3-AR receptor agonist-CL and the β3-AR receptor antagonist-L.
K, control; H, hypertrophic adipocyte; LV, *lemon verbena*; CL, CL316243 (β3-AR receptor agonist); L, L7483337 (β3-AR antagonist); **p<0.0001.

Application of LV at Different Concentrations

When LV polyphenol was administered to hypertrophic adipocytes (H) at doses of 200 and 400 µg/mL, it resulted in the inhibition of *AQP7* gene expression compared with hypertrophic adipocytes (H vs H-LV200 p<0.0001 and H vs H-LV400 p=0.005, respectively; Figure 1). Although gene expression of *PLIN1* was significantly decreased at a dose of 200 µg/mL, there was no statistically significant difference at both doses (H vs H-LV200 p=0.279 and H vs H-LV400 p=0.611, respectively; Figure 2). The glycerol content in the medium of LV-treated cells was decreased (H; 43.79 ± 0.59 ng/mL vs H-LV200; 32.05 ± 1.59 ng/mL, p=0.009; H; 43.79 ± 0.59 ng/mL vs H-LV400; 36.83 ± 0.73 ng/mL, p=0.009; Figure 4). These findings indicate that both LV doses diminished glycerol efflux by suppressing *AQP7* and *PLIN1* gene expression.

Administration of the β3-AR Receptor Agonist CL

The β3-AR receptor agonist CL, which is known to induce anti-obesity effects, did not affect the gene expression of *AQP7* and *PLIN1* when applied alone to hypertrophic cells. This was when compared with the non-hypertrophic mature adipocyte control (K), and the results were non-significant (For *AQP7*, K vs H-CL p=0.846 and for *PLIN1*, K vs H-CL p=0.272, respectively) (Figure 1 and 2). However, the amount of glycerol in the medium of CL-treated hypertrophic adipocytes was increased compared with non-hypertrophic mature adipocytes and hypertrophic control adipocytes. The increase observed in the hypertrophic control group failed to reach statistical significance (K: 11.77 ± 0.40 ng/mL vs H-CL: 62.81 ± 3.63 ng/mL, p<0.0001; H: 43.79 ± 0.59 ng/mL vs H-CL: 62.81 ± 3.63 ng/mL, p=0.098; Figure 4).

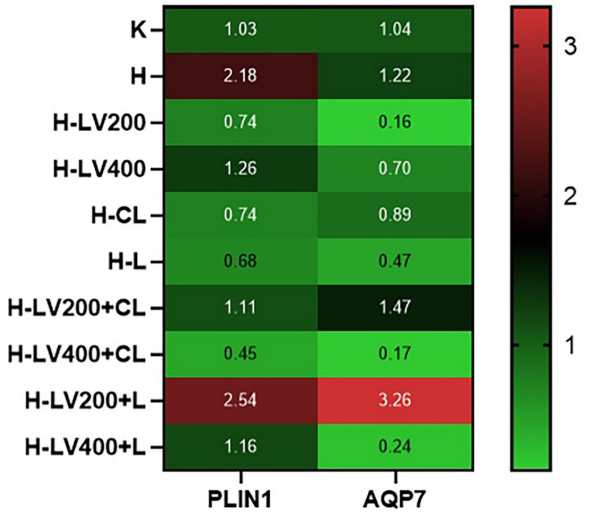

Figure 3. Whole cell *AQP7* and *PLIN1* gene expression levels in the groups are shown together.
K, control; H, hypertrophic adipocyte; LV, *lemon verbena*; CL, CL316243 (β3-AR receptor agonist); L, L7483337 (β3-AR antagonist); **p<0.001; ***p<0.0001.

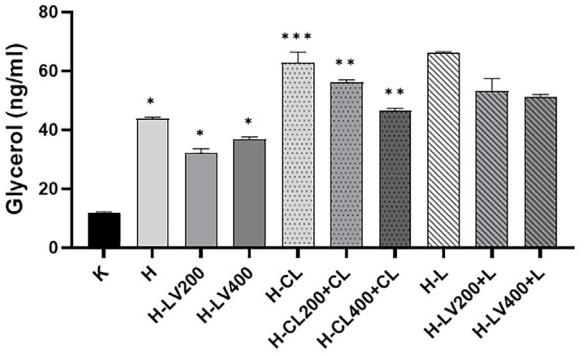

Figure 4. Change of glycerol content in the adipocytes medium when 200 and 400 ng/ml doses of LV, the β3-AR receptor agonist-CL and the β3-AR receptor antagonist-L. K, control; H, hypertrophic adipocyte; LV, *lemon verbena*; CL, CL316243 (β3-AR receptor agonist); L, L7483337 (β3-AR antagonist); *p<0.05; **p<0.001; ***p<0.0001.

When a 200 µg/mL dosage of LV and CL was applied to hypertrophic adipocytes, an increase in *AQP7* gene expression was observed (p=0.005), but no changes in *PLIN1* gene expression were noted (p=0.999).

An analysis of glycerol content showed that both non-hypertrophic mature adipocytes and hypertrophic adipocytes exhibited increased glycerol content compared with the controls. However, although there was an increase in glycerol content in hypertrophic adipocytes compared with the control, it did not reveal any statistical significance (K; 11.77 ± 0.40 ng/mL vs H-LV200+CL; 56.20 ± 0.67 ng/mL, p<0.0001, H; 43.79 ± 0.59 ng/mL vs H-LV200 + CL; 56.20 ± 0.67 ng/mL, p=0.226). When 400 µg/mL dose of LV and CL was applied to hypertrophic adipocytes, *AQP7* and *PLIN1* gene expressions were significantly decreased compared with the non-hypertrophic mature adipocyte control (p<0.0001, p<0.0001, respectively) (Figure 1 and 2). When compared in terms of glycerol concentration released in the media of the cells, glycerol was found to be increased in the LV-treated groups (K; 11.77 ± 0.40 ng/mL vs H-LV400 + CL; 46.35 ± 0.45 ng/mL p=0.005). When hypertrophic adipocytes were compared with the control group, the glycerol content was similar (H; 43.79 ± 0.59 ng/mL vs H-LV400 + CL; 46.35 ± 0.45 ng/mL p=0.729; Figure 4). When CL was co-administered with 200 µg/mL dose of LV, the amount of glycerol increased due to an increase in *AQP7* gene expression. This indicates that CL has different effects on *AQP7* gene expression and glycerol output depending on the LV application dose.

β3-AR Receptor Antagonist L and LV Application at Different Concentrations

AQP7 and *PLIN1* gene expression levels were assessed following the administration of the β3-AR antagonist, L, alone and in combination with two different concentrations of LV. L treatment decreased the gene expression of both *AQP7* and *PLIN1* in hypertrophic adipocytes compared with non-hypertrophic mature adipocytes. Notably, a statistically significant decrease in *AQP7* expression was observed (p<0.0001) and *PLIN1* gene expression was not significant (p<0.079). Treatment with L and LV at a concentration of 200 µg/mL resulted in a noteworthy increase in the gene expression of *AQP7* and *PLIN1* (p<0.0001 vs p<0.0001), in comparison to treatment with L alone. Conversely, treatment with L and LV at a concentration of 400 µg/mL led to a significant decrease in *AQP7* gene expression (p<0.0001; Figure 1). The experimental conditions did not affect *PLIN1* gene expression (p=0.991; Figure 2). When comparing glycerol content, it was found that the administration of L along with LV doses of 200 µg/mL and 400 µg/mL led to a significant increase in comparison with the non-hypertrophic mature adipocyte control (K; 11.77 ± 0.40 ng/mL vs H-LV200+L; 53.13 ± 4.27, p=0.001, K; 11.77 ± 0.40 ng/mL vs H-LV400+L; 51.08 ± 0.99, p=0.002; Figure 4). These results indicate that the application of the β3-AR antagonist L at a concentration of 0.1 µM, did not exert the expected inhibitory effect. The gene expressions of *AQP7* and *PLIN1* in all cell groups are shown in Figure 3.

DISCUSSION

In vitro models, particularly preadipocyte cell lines such as 3T3-L1 cells, are widely used to study the processes of adipocyte proliferation, differentiation, adipokine secretion, and gene/protein expression. Under high-glucose conditions, the 3T3-L1 cell line undergoes hypertrophy and becomes insulin-resistant. These cells have been extensively utilised to gain a deeper understanding of the fundamental cellular processes relating to obesity and associated disorders (3, 25). *In vitro* experiments using the 3T3-L1 model have demonstrated that phytochemicals, including polyphenols, induce adipose tissue browning by reducing adipogenesis/lipogenesis or enhancing lipolysis/SYA oxidation, thereby producing an anti-obesity effect. This study was designed to assess the potential effects of LV polyphenol on the expression of the *AQP7* and *PLIN1* genes in response to molecules that simulate lipogenesis/lipolysis. Despite the initial expectations, both doses of LV decreased *AQP7* and *PLIN1* gene expression and reduced glycerol output. However, administration of a low dose of LV in combination with CL, which mimics β3-AR activation, led to an increase in *AQP7* and *PLIN1* gene expressions, as well as glycerol levels in the cell medium. These findings suggest that the expected lipolytic effects of LV are mediated through β3-AR activation.

Polyphenols, renowned for their pharmacological properties, are among the most extensively researched natural compounds. A typical diet contains over 500 different polyphenols. Although extracts simplify this variety into a complex mixture, the relationship between this complexity and human health remains unclear. Several recent studies have suggested that polyphenols have a significant effect on obesity, a condition that is becoming increasingly common. Adipocyte hypertrophy impairs cellular functions, elevates

reactive oxygen species (ROS) production, and contributes to inflammation, thereby worsening obesity-related metabolic disorders (26). Polyphenols found in LVs have been shown to inhibit several damaging effects commonly associated with obesity (9). LV polyphenols reduce lipid accumulation in adipocytes, resulting in cellular ROS production (8). These findings were validated in an animal model of diet-induced obesity (9). The LV polyphenol doses used in our study were determined based on the quantities described in the research carried out by M. Herranz-López et al. (9).

Accumulating evidence indicates that *AQP7*, a glycerol channel, significantly influences glycerol availability in tissues where it is expressed. A deficiency of *AQP7* in adipose tissue is linked to increased triglyceride (TG) accumulation and the onset of obesity (15). In addition, *AQP7* overexpression contributes to insulin resistance in hypertrophic adipocytes (19, 27, 28). In our study, the increased expression of *AQP7* in hypertrophied 3T3-L1 cells was consistent with the literature. While targeting *AQP7* in anti-obesity treatment is being considered, several studies, including those on other tissues in which *AQP7* is expressed, are needed.

In this study, the effects of LV polyphenol, which plays a role in the regulation of lipolysis in 3T3-L1 hypertrophic cells, on *AQP7, PLIN1,* and glycerol levels were evaluated. The regulatory mechanism in humans and rodents is unknown. In addition, the results would have benefited from the western blot method. Further studies are necessary to explain interspecies regulation.

PLIN1 serves as a protective barrier against lipid droplets in adipocytes, shielding them from the effects of natural lipases, including HSL. In addition, it inhibits the movement of *AQP7* to the plasma membrane in the presence of feeding. *PLIN1* gene expression is known to be high in obese human and animal models (29). As reported in the literature, *PLIN1* expression was increased in the hypertrophied model.

β3-AR is acknowledged as a validated therapeutic target for obesity and associated metabolic disorders (30, 31). CL, a selective systemic β3-AR agonist, has been demonstrated to stimulate UCP-1 expression in adipose tissue and exert anti-obesity effects (32, 33). This study is the first to investigate the effects of CL, a β3-AR agonist, and LV polyphenol, a β3-AR antagonist, on the gene expressions of *AQP7* and *PLIN1*, as well as glycerol levels and release, in hypertrophic adipocyte cells and high glucose-induced insulin-resistant hypertrophic adipocytes. CL, which mimics the β3-AR receptor agonist, yielded different dose-dependent results when administered with LV polyphenol. Coadministration of CL with a low dose of LV polyphenol was found to be more effective in the formation of lipolytic conditions. *AQP7* gene expression is positively regulated by fasting and PPARγ agonists, (21, 36), whereas factors such as nutrition, increased circulating insulin levels, dexamethasone administration, and tumour necrosis factor-α (TNF-α) suppress *AQP7* gene expression (35). Administration of L, which mimics the activity of β3-AR antagonists, reduced the expression of *the AQP7* and *PLIN1* genes in hypertrophic adipocyte cells. Furthermore, coadministration of LV polyphenol at a dose of 400 µg/mL suppressed *AQP7* gene expression. However, it led to an unexpected increase in the glycerol content of the cell medium, and the cause of this phenomenon remains unclear. This situation requires further investigation for better clarification. The regulation of *AQP7* gene expression and glycerol levels in adipose tissue could be a key determinant of fat storage and overall glucose regulation in the body (11, 14, 18).

Few studies have investigated polyphenol interactions with *AQP7* and *PLIN1*. Apple polyphenols, a distinct group of polyphenolic compounds, have been demonstrated to inhibit adipose tissue formation in Wistar rats (36). and to decrease triglyceride absorption by inhibiting pancreatic lipase activity in both mice and humans (37). Furthermore, a study on diet-induced obesity in Wistar rats demonstrated that supplementation with apple polyphenols prevented an increase in adipose tissue by inhibiting adipose tissue hypertrophy (20). Apple polyphenol consumption resulted in higher *AQP7* expression in epididymal adipose tissue, potentially clarifying the rise in glycerol release observed through *ex vivo* lipolysis testing as well as the decrease in adipose tissue volume observed in apple polyphenol-fed rats (20). Similarly, the low-dose LV used in this study and β3-AR activation together increased *AQP7* gene expression and glycerol content. These results indicate that the beneficial effects of LV therapy are triggered by the activation of β3-AR (with effects such as fasting, cold exposure, and exercise). The Dietary polyphenols are potential therapeutic agents for the management of obesity (38).

Additional studies are needed to pinpoint the specific metabolites responsible for the anti-obesity properties of LV extract, thereby aiding the development of polyphenolic blends for potential therapeutic applications in obesity management. Further analysis of the identified metabolites could provide insights into their potential health benefits in humans.

CONCLUSION

Although the mechanisms of action of polyphenols remain unclear, their beneficial effects are not only mediated by their antioxidant properties. *AQP7* modulation in adipose tissue is a potential innovative therapeutic approach. The identification of *AQP7* inhibitors or polyphenols, which are largely found in plant-derived foods, represents a healthy and innovative approach to the prevention and treatment of metabolic diseases, including obesity. Further studies are required to explain the mechanisms of action more clearly.

Ethics Committee Approval: Ethical approval is not applicable as cell line is used.

Peer-review: Externally peer-reviewed.

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