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Protective effect of the hydroalcoholic extract from *Lampaya medicinalis* Phil. *(Verbenaceae)* on palmitic acid- impaired insulin signaling in 3T3-L1 adipocytes

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ABSTRACT

Background: Obesity is strongly associated with insulin resistance (IR). IR at the molecular level may be defined as a diminished activation of insulin signaling-related molecules (IRS-1/Akt/AS160) as well as reduced glucose uptake. Subject with obesity have elevated plasma levels of saturated fatty acids, such as palmitic acid (PA), which triggers insulin signaling disruption *in vivo* and *in vitro*. Infusions of *Lampaya medicinalis* Phil. *(Verbenaceae)* are used in folk medicine of Northern Chile to counteract inflammatory diseases. Hydroethanolic extracts of lampaya (HEL) contain considerable amounts of flavonoids that may explain the biological activity of the plant. The aim of this study was to assess whether HEL exposure protects against PA-disrupted insulin signaling and glucose uptake in adipocytes.
Methods: Cytotoxicity of a range of HEL concentrations (0.01–10 μg/mL) was evaluated in 3T3-L1 adipocytes. Cells were exposed or not to 0.1 μg/mL of HEL before adding 0.65 mM PA or vehicle and incubated with 100 nM insulin (or vehicle) for 15 min. Phosphorylation of Tyr-IRS-1, Ser-Akt, Thr-AS160 was evaluated by Western blot. Glucose uptake was assessed using the 2-NBDG analogue.
Results: HEL was not cytotoxic at any concentration assessed. PA-induced reduction in insulin-stimulated phosphorylation of IRS-1, Akt and AS160 and glucose uptake were abolished by co-treatment with HEL.
Conclusion: These findings give new insights about the effect of HEL ameliorating PA- impaired IRS-1/Akt/AS160 pathway and glucose uptake in adipocytes. More studies should focus on lampaya, since might represent a preventive approach in individuals whose circulating PA levels contribute to IR.

Introduction

Obesity has reached epidemic proportions worldwide and is associated with several pathologies such as dyslipidemia, insulin resistance (IR), type 2 diabetes mellitus (T2DM), cardiovascular diseases and cancer [1]. A hallmark of obesity pathophysiology at the cellular level is defective insulin signaling involving defective activation of the insulin receptor (INSRβ)/IRS-1/Akt/AS160 pathway, in insulin-responsive cells such as adipocytes. This impairment leads, among others, to a lower insulin-induced glucose uptake [2].

Saturated fatty acids (SFAs) such as palmitic acid (PA) are elevated in plasma of subjects with obesity, and this condition is aggravated considering that western diets are usually abundant in PA [3,4]. SFAs are strongly associated with the occurrence of obesity-related metabolic complications such as IR and chronic inflammation [5]. *In vitro* evidence indicates that PA exposure leads to a decrease in IRS-1, Akt and AS-160 phosphorylation as well as insulin-induced glucose uptake in 3T3-L1 adipocytes [6]. In this

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context, the implementation of new therapeutic approaches aimed to improve insulin sensitivity, glycemic control, as well as T2DM complications is encouraged. In this regard medicinal plants have received much attention due to their traditional use in the alleviation and treatment of diseases [7].

Lampaya medicinalis Phil. *(Verbenaceae)*, also known as "lampaya" is a small bush growing in the "Puna atacameña" in the Northern region of Chile [8,9]. Oral reports from herbal medical practitioners indicate that the infusion of the plant is given for treating colds, stomach pain, urinary bladder discomforts, as an antitussive and against rheumatism, arthritis and joint pain [10]. Hydroethanolic extracts of lampaya (HEL) contain large amounts of flavonoids, while a minor fraction is composed of other compounds such as phenolic acids, p-hydroxyacetophenones derivatives, naphthalenic and iridoid glycosides (Supplementary Table 1) [11]. These results are consistent with the antioxidant activity of the extract as well as with its anti-inflammatory potential *in vivo* and *in vitro*, as previously reported [8,9,11].

Low grade inflammation, particularly in adipose tissue, is essential in the pathogenesis of insulin resistance [12]. Interestingly, the exposure to HEL *in vitro* ameliorates PA-induced proinflammatory response in human macrophages [11], whose infiltration in adipose tissue is key in obesity pathophysiology. However, it is not clear whether HEL is also able to preserve insulin signaling pathway in adipocytes, the main cells in adipose tissue, whose insulin response is key for safely managing excess fat in obesity. Therefore, in the present study, we investigated the effect of HEL on PA-disrupted insulin signaling and insulin-induced glucose uptake in 3T3-L1 adipocytes.

Materials and methods

Plant material and preparation of the Hydroethanolic Extract of Lampaya *(HEL)*

Leaves and aerial parts of *Lampaya medicinalis* Phil. were collected near Socaire in Northern Chile (23° 36́40 s S; 67° 50́33 s W, 3230 m above sea level). The botanical material was identified by academic personal and voucher specimens are kept at the Herbarium of Universidad de Concepción, Chile.

The air-dried leaves of *Lampaya medicinalis* Phil. (1.2 kg) were mechanically grounded to obtain a fine powder that was deposited into a cotton bag and exhaustively extracted with a mixture of EtOH:H_2O (1:1, 10 L) during one week at room temperature. The EtOH:H_2O extract was filtered and evaporated under reduced pressure to eliminate the ethanol. The aqueous extract was freeze-dried with a Labconco 4.5 FreeZone lyophilizer obtaining a viscous dark green mass (HEL). The yield of the lyophilized solution was 12.5% (w/w). The dried extract was sealed in a bottle and stored at 4 °C until use. Just prior to use, the extract was dissolved in dimethyl sulfoxide (DMSO, Sigma–Aldrich, USA) to prepare a 10 mg/mL stock solution. Further dilutions were prepared from the stock in culture media.

Culture and differentiation of 3T3-L1 preadipocytes

Mouse 3T3-L1 preadipocytes were kindly donated by Dr Rodrigo Moore, Universidad de Talca, Chile. Preadipocytes were cultured in high-glucose Dulbecco modified Eagle's medium (DMEM) (Sigma–Aldrich, USA) supplemented with 10% fetal bovine serum (FBS, Biological Industries, USA) and antibiotics (penicillin-streptomycin) at 37 °C in a controlled atmosphere incubator (5% CO_2). At two days postconfluence, preadipocytes were incubated in DMEM with 10% FBS and supplemented with 0.5 mM 1-methyl-3-isobutyl-xanthine (Calbiochem®, Germany), 1 μM dexamethasone (Sigma–Aldrich, USA) and 10 μg/mL insulin (Insuman®, Sanofi-Aventis, France) for 2 days, followed by insulin alone in DMEM 10% FBS for another 2 days. Thereafter, the cells were cultured in DMEM with 10% FBS for three days and differentiated adipocytes were obtained at day 8. In order to confirm fully mature adipocyte phenotype, cells were fixed with 4% formaldehyde and stained with the lipophilic dye Oil Red O (Sigma–Aldrich, USA).

Cell viability and treatments

In order to determine the concentration of HEL to use in this study, the viability of adipocytes exposed to different concentration of HEL was assessed. After differentiation (day 8), adipocytes were treated with 0, 0.01, 0.1, 1 and 10 μg/mL of HEL for 18 h, then Trypan blue exclusion test was performed by counting viable cells (clear cytoplasm) using Neubauer chamber, according to the protocol described by Strober [13].

To select proper concentrations of PA that reduce insulin-stimulated phosphorylation of Akt by at least 50%, adipocytes were incubated with concentrations ranging from 0.1 to 0.8 mM PA for 16 h, which are conditions widely used to impair insulin response in cultures of 3T3-L1 adipocytes [14–16]. Such treatments with PA have been reported do not show cytotoxic effects on adipocytes [17]. In addition, adequate morphology and phenotype of adipocytes as well as full attachment of the cell monolayer to the surface were observed in 3T3-L1 adipocytes upon PA exposure. PA was prepared complexed with fatty acid-free Bovine Serum Albumin (FFA-BSA, Sigma–Aldrich, USA), according the protocol from Cousin et al. [18].

Differentiated 3T3-L1 adipocytes were incubated with PA or vehicle for 16 h in the presence or not of HEL (2-h preincubation) and thereafter stimulated with insulin or vehicle. Thereby, experimental conditions were: control (untreated cells), 0.65 mM PA, 0.1 μg/mL of HEL and 0.1 μg/mL of HEL (2 h before) +0.65 mM PA for 16 h, in insulin-stimulated or basal conditions.

Western blot

Total cell extracts were obtained sonicating 3T3-L1 cells at 4 °C in a buffer containing 150 mM NaCl, 50 mM Tris base, 1% NP40, 100 mM NaF, 10 mM Sodium Pyrophosphate, 2 mM Ortovanadate, pH 8.0, supplemented with Complete® protease inhibitor cocktail (Roche, Mannheim, Germany) plus PhosSTOP (Roche, Mannheim, Germany). Protein concentration of the lysate was determined by a method based on bicinchoninic acid (Pierce, Rockford, IL). Fifty micrograms of protein were heat-denatured in SDS-PAGE loading buffer (240 mM Tris–HCl, pH 6.8, 8% SDS, and 40% glycerol, 20% 2-mercaptoethanol). Proteins were electrophoresed on 10% polyacrylamide gels and electrotransfered to nitrocellulose 0,22 μm (Amersham™ Protran®, Munich, Germany) membranes using a buffer containing 24 mM Tris, 194 mM glycine and 20% methanol. The immunoreaction was achieved by incubation of the membranes, previously blocked with a 5% BSA solution in Tris-buffered saline (TBS) with 0.05% Tween 20 (Sigma–Aldrich), with antibodies against phospho-IRS1 (Tyr612) (Invitrogen, USA), IRS-1, phospho-Akt (Ser473), Akt, phospho-AS160 (Thr642), anti-phospho-NF-κB p65 (S536), anti-NF-κB p65 (Cell Signaling Technology, Danvers, MA). β-actin (Santa Cruz Biotechnology) was used as internal loading control. Detection of immune complexes was performed by incubation with peroxidase-conjugated secondary antibodies, followed by incubation with the substrates of the enzyme using the gel documentation system LI-COR C-DiGit Blot Scanner (Lincoln, NE). The images were digitalized, and the band densities were quantified with ImageJ software (NIH, Bethesda).

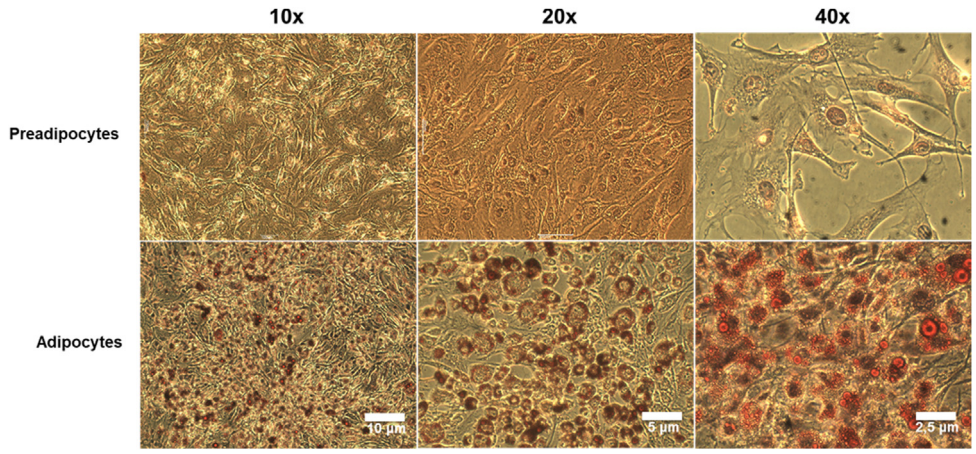

Fig. 1. Oil Red O staining in 3T3-L1 preadipocytes and adipocytes. 3T3-L1 cells were differentiated for 8 days, as described in Material and Methods. Triglyceride accumulation is visualized by red staining of lipid droplets. Total magnification: 100×, 200× and 400×. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Glucose uptake

Glucose uptake assay was performed according to the protocol described by Yamamoto et al. [19] with some modifications. Equal amounts of 3T3-L1 preadipocytes were seeded into 6-well plates and at two days postconfluence cells were differentiated according the protocol previously described. On day 8, adipocytes were incubated with PA or vehicle for 16 h in the presence or not of HEL as described before. Once the experiments were completed, the cells were rinsed and serum starved for 2 h in DMEM (Sigma–Aldrich, USA). Media was removed and cells washed twice with Kreb's Ringer Buffer without glucose (KRB w/o glu) (145 mM NaCl, 5 mM KCl, 2.6 mM CaCl2, 1 mM MgCl2, 10 mM HEPES, pH 7.4). Adipocytes were stimulated with 100 nM insulin in KRB w/o glu for 30 min at 37 °C in a 5% CO_2 atmosphere. After cell wash, the fluorescent analogue of glucose, 2-NBDG (300 μM) was added and cells were further incubated for 15 min. The 2-NBDG uptake was stopped by removing the incubation medium and cells were washed twice with KRB w/o glu. Lysis buffer (150 mM NaCl, 50 mM Tris base, pH 8.0, and 1% NP40) was added to the wells and cells were collected and transferred in a black, clear-bottom 96-well culture plate. Fluorescence was measured at 465/540 nm (wave length excitation/emission) using a fluorimeter (Synergy 2 fluorimeter, BioTek Instruments).

Statistical analysis

We used non-parametric statistics that do not have any distributional assumptions, given the low reliability of normality testing for small sample sizes such as those used in this work [20]. To compare parameters between controls and treated cells, variables were evaluated using Friedman Test with Dunn's multiple comparisons. Analysis were performed using GraphPad Prism 6 software. Data are shown in the figures as graph bars and a p-value < 0.05 was considered significant.

Results

Adipogenic differentiation in 3T3-L1 cells

Preadipocyte differentiation into adipocytes was determined by Oil Red O staining. Triglyceride accumulation was visualized by red staining of cytoplasmic lipid droplets in 3T3-L1 adipocytes (Fig. 1). Intracellular red staining was absent in 3T3-L1 preadipocytes. Mature adipocyte phenotype was confirmed after 8 days of adipogenic differentiation in 3T3-L1 cells.

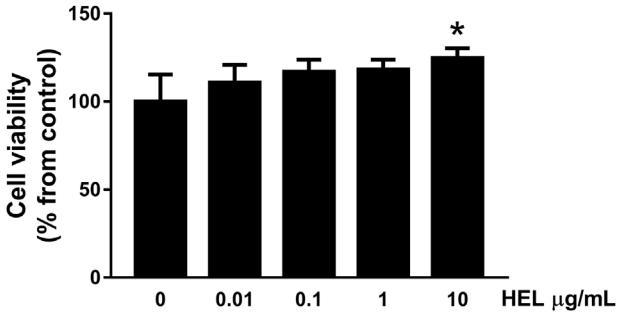

Fig. 2. Cell viability in 3T3-L1 adipocytes exposed to different concentration of HEL. Trypan blue exclusion assay was performed on 3T3-L1 cells treated with HEL at 0.01, 0.1, 1.0 and 10.0 μg/mL for 18 h, as described in Material and Methods. Data are reported as percentage of untreated cells (100%). Bars represent media ± SEM (n = 4, each independent experiment was performed in triplicate). *p < 0.05 for the difference from vehicle-treated cells, Friedman test.

HEL is not cytotoxic on 3T3-L1 adipocytes

To determine whether HEL affects 3T3-L1 cells viability, assessed as the maintenance of cell integrity, we incubated the cells with different concentrations of HEL (0.01, 0.1, 1 and 10.0 μg/mL) for 18 h; then, Trypan blue exclusion test. To our surprise, concentration of 10.0 μg/mL of HEL showed a significant increase in cell viability compared to control cells exposed to vehicle (Fig. 2). Since our aim was to assess metabolic insulin signaling downstream insulin receptor, to avoid misinterpretation of data due to a possible activation of mitogenic pathways activated when cell viability is increased, the lowest concentration of HEL (0.01 μg/mL) was chosen.

HEL restores impaired phosphorylation of IRS-1, Akt and AS160 induced by palmitic acid in 3T3-L1 adipocytes

As a first approach, in order to select a suitable working concentration of PA, insulin-stimulated phosphorylation of Akt was assessed as a marker of insulin responsiveness in 3T3-L1 adipocytes treated with different concentrations of PA (0.12, 0.24, 0.45, 0.65 mM) for 16 h. Since 0.65 mM PA reduced insulin-stimulated phospho-Akt by more than 50% (Fig. 3A, black bars), this concentration was used for further studies. Basal (non-insulin-stimulated) phosphorylation of Akt was unaffected by PA at the different concentrations assessed (Fig. 3A, white bars).

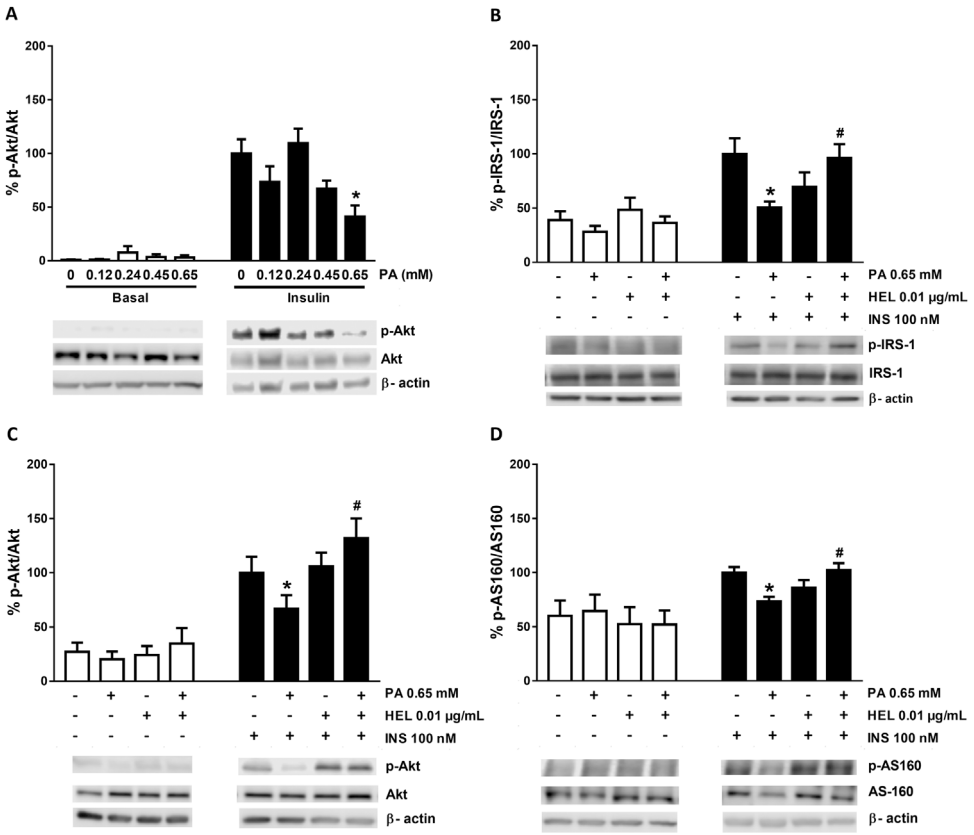

Fig. 3. HEL restores the PA-induced decrease in insulin-stimulated phosphorylation of Akt, IRS1 and AS160 in 3T3-L1 cells. (A) Phosphorylation of Akt in 3T3-L1 adipocytes treated with different concentrations of PA (0.12, 0.24, 0.45, 0.65 mM) for 16 h under basal (white bars) and insulin-stimulated (black bars) conditions. 3T3-L1 adipocytes were exposed for 16 h to 0.65 mM PA in the presence (plus 2 h pre-incubation) or not of 0.01 µg/mL HEL. (B) phospho-IRS-1/IRS-1 (C) phosho-Akt/Akt and (D) phospho-AS160/AS160 protein content was evaluated by Western blot in control (vehicle-treated), PA, HEL and HEL + PA-treated cells under basal (white bars) and insulin-stimulated (black bars) conditions, as described in Material and Methods. A representative image of Western blot is shown for each graph. Results are depicted as percentage form insulin-stimulated control cells (100%). Bars represent media ± SEM (n = 5-8). *p < 0.05 for the difference from vehicle-treated cells and # p < 0.05 for the difference from PA-treated cells, Friedman test.

To study the effect of HEL on insulin responsiveness in 3T3-L1 adipocytes treated with PA, activation of insulin signaling molecules was assessed by evaluating the phosphorylation of Tyr-IRS-1, Ser-Akt and Thr-AS160. As reported in Fig. 3B, C and D (black bars), the exposure to the fatty acid decreased insulin-stimulated IRS-1 (50%, $p < 0.05$), Akt (34%, $p < 0.05$) and AS160 (27%, $p < 0.05$) phosphorylation, as compared to untreated cells, respectively. To analyze the effect of HEL on the PA-induced signaling impairment, we evaluated the phosphorylated forms in adipocytes treated with 0.01 µg/mL HEL for 18 h (2 h prior and during the 16 h incubation with PA). The presence of HEL prevented the PA-induced decrease in IRS-1, Akt and AS160 phosphorylation (Fig. 3B–D, black bars).

HEL alone did not change the insulin-stimulated phosphorylation of IRS-1, Akt and AS160 compared to vehicle-treated cells and no differences were observed for basal phosphorylation of IRS-1, Akt and AS160 in all the experimental conditions assessed (Fig. 3B–D).

HEL counteracts impaired glucose uptake induced by palmitic acid in 3T3-L1 adipocytes

Since HEL restores the PA-induced impairment in phosphorylation of IRS-1, Akt and AS160 induced by insulin in 3T3-L1 adipocytes, and considering that glucose uptake represents one crucial endpoint of metabolic insulin signaling in these cells, we addressed whether HEL could also prevent the deleterious effects of PA on this event. As expected, glucose uptake decreased after PA treatment compared to untreated cells (46%, $p < 0.05$, Fig. 4, black bars). Interestingly, glucose uptake did not decrease in PA-treated cells when HEL was present (Fig. 4, black bars). HEL alone did not change the insulin-stimulated glucose uptake compared to vehicle-treated cells (Fig. 4, black bars). No differences were

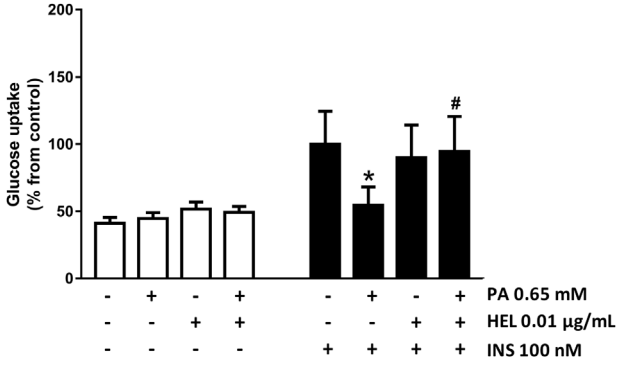

Fig. 4. HEL restores the PA-induced decrease in glucose uptake in 3T3-L1 cells. 3T3-L1 adipocytes were exposed for 16 h to 0.65 mM PA in the presence (plus 2 h pre-incubation) or not of 0.01 µg/mL HEL. Glucose uptake was evaluated using the fluorescent analog 2-NBDG in control (vehicle-treated), PA, HEL and HEL + PA-treated cells under basal (white bars) and insulin-stimulated (black bars) conditions, as described in Material and Methods. Results are depicted as percentage form insulin-stimulated control cells (100%). Bars represent media ± SEM (n = 7). *p < 0.05 for the difference from vehicle-treated cells and # p < 0.05 for the difference from PA-treated cells, Friedman test.

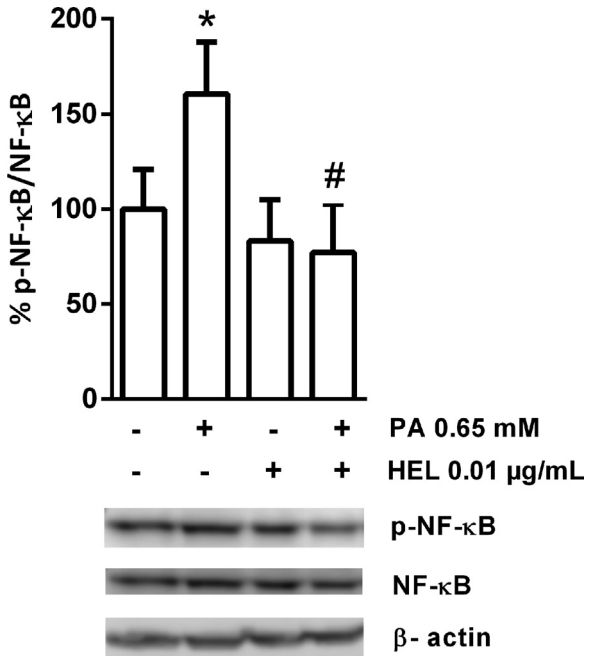

Fig. 5. HEL restores the PA-induced increase in the phosphorylation of NF-κB in 3T3-L1 cells. 3T3-L1 adipocytes were exposed for 16 h to 0.65 mM PA in the presence (plus 2 h pre-incubation) or not of 0.01 µg/mL HEL. phospho- NF-κB/NF-κB protein content was evaluated by Western blot in control (vehicle-treated), PA, HEL and HEL + PA-treated cells, as described in Material and Methods. A representative image of Western blot is shown for the graph. Results are depicted as percentage of untreated cells (100%). Bars represent media ± SEM (n = 7). *p < 0.05 for the difference from vehicle-treated cells and # p < 0.05 for the difference from PA-treated cells, Friedman test.

observed for basal (non-insulin-stimulated) glucose uptake in all the experimental conditions assessed (Fig. 4, white bars).

HEL restores impaired phosphorylation of NF-κB induced by palmitic acid in 3T3-L1 adipocytes

Since elevated PA levels activate NF-κB proinflammatory pathway [21], which is associated with impaired insulin signaling [5], and considering that HEL downregulates PA-induced NF-κB phosphorylation in macrophages, we addressed whether HEL could also prevent this activation in 3T3-L1 adipocytes. As expected, the exposure to PA increased NF-κB phosphorylation as compared to untreated cells (60%, $p < 0.05$, Fig. 5), while the presence of HEL prevented PA-induced increase in NF-κB phosphorylation. HEL alone did not change the phosphorylation of NF-κB compared to vehicle-treated cells (Fig. 5).

Discussion

The present work shows for the first time the in vitro effectiveness of *Lampaya medicinalis* in ameliorating the PA-induced disruption of metabolic insulin signaling in 3T3-L1 adipocytes. Specifically, our data suggest that the protective properties of HEL involve the modulation of the IRS-1/Akt/AS160 pathway and subsequent glucose uptake. Interestingly, HEL was not cytotoxic on 3T3-L1 cells, but the concentration of 10 µg/mL unexpectedly increased cell viability in adipocytes. The later may be associated with the action of phenolic compounds protecting DNA from oxidative damage and by enhancing the natural antioxidant system of cells [22]. However, further studies are needed to establish a causative link between phenolic composition and the observed effect of HEL on viability. Other unanticipated effects of HEL, such as genotoxicity and carcinogenicity, were not assessed since they are usually observed when plant extracts are used in cultured cells at doses one thousand times more concentrated than the working dose of HEL [23,24].

Insulin resistance is one of the main pathogenic process underlying obesity and T2DM. Therefore, seeking for new therapeutic strategies aimed at maintaining insulin sensitivity is encouraged [25]. On the other hand, high levels of FFAs in the plasma induce cellular abnormalities resulting in impaired cell function [26]. In this context, SFAs such as PA, are secreted from enlarged adipocytes in obese subjects raising their plasma levels [27]. Several studies have shown that PA plays a major role in the impairment of metabolic insulin signaling *in vitro* [15,16,28]. In the current work, we employed 3T3-L1 adipocytes exposed to PA as a validated model for metabolic dysfunction [29]. In agreement with other studies, exposure of adipocytes to PA decreased insulin-stimulated activation of IRS-1, Akt and AS160, as well as its main metabolic endpoint, glucose uptake [15,16,30]. Interestingly, we have shown that treatment with HEL prevents the effect of PA impairing insulin-induced phosphorylation of IRS-1, Akt and AS160, as well as glucose uptake in 3T3-L1 adipocytes. Therefore, this study provides *in vitro* evidence of a novel therapeutic potential of HEL counteracting PA-disrupted insulin signaling and glucose uptake in adipocytes. Such effect of HEL might be attributable, in part, to its recognized antioxidant and anti-inflammatory properties [8,9,11], however further studies should determine whether these specific properties are involved in HEL's modulatory role on insulin signaling.

Strategies for the treatment of obesity and their related comorbidities, such as insulin resistance and T2DM, include diet and exercise, as well as hypoglycemic and lipid-lowing pharmacological agents. Nonetheless, natural products have received much attention as an important source of bioactive agents useful in the treatment of pathological conditions associated with obesity. Indeed, a large number of natural products has been suggested to improve insulin signaling defects associated with T2DM *in vivo* and *in vitro* [7]. Polyphenols and flavonoids are widely present in fruits, vegetables, medicinal plants as well as other plant foods and have been shown to exert beneficial effects on health [31,32]. The phytochemical composition of extracts from *Lampaya medicinalis* has revealed the presence of considerable amounts of phenolic and flavonoid compounds [9]. Specifically, data obtained by LC-EIS-MS/MS showed flavonoids as the main components of HEL (61%), while compounds derived from phenolic acids corresponded to a minor fraction (22%) [11]. Some phenolic and flavonoid compounds present in HEL have shown potential anti- T2DM action in different models [7]. In this regard, the flavonoids quercetin and naringenin have shown to stimulate glucose transporter type 4 (GLUT4) translocation in skeletal muscle and enhance insulin receptor and GLUT4 expression in adipose tissue of diabetic rats, respectively [33,34]. On the other hand, the polyphenol epigallocatechin gallate (a derivate of epigallocatechin, present in HEL) stimulates GLUT4 translocation to the plasma membrane and glucose uptake in skeletal muscle cells and 3T3-L1 adipocytes [35,36]. Likewise, caffeic acid has shown to enhance adipocyte GLUT4 expression and glucose uptake in a mice model of obesity-induced T2DM [37]. The described experimental evidence supports a protective role of bioactive components of HEL that might explain the effect of the extract on insulin pathway and glucose uptake in murine adipocytes.

Interestingly, from this experimental approach, HEL exerts a protective effect on PA-impaired insulin signaling and glucose uptake in 3T3-L1 adipocytes undergoing an acute insulin stimulation. The later agrees with experimental data assessing the role of phytochemical compounds on insulin response in PA-exposed 3T3-L1 adipocytes [14,15,28]. In this regard, an extract consisting of 17 purified anthocyanins (all glycosides of cyanidin, peonidin, delphinidin, petunidin, and malvidin) isolated from blueberries

(*Vaccinium myrtillus* and *Ribes nigrum*) exerts positive effects on PA-disrupted insulin pathway by restoring insulin-induced Akt phosphorylation [14]. Likewise, an aspalathin-enriched green rooibos extract as well as pure aspalathin fully restored insulin responsiveness, assessed by Akt phosphorylation and glucose uptake, in PA-treated 3T3-L1 adipocytes [28]. In addition, an *Urtica dioica* extract, a medicinal plant with well-documented effects in lowering blood glucose levels, has been shown to reverse PA- reduced insulin-stimulated phosphorylation of Akt in murine adipocytes [15]. Thus, the present findings on HEL are similar to other studies on phenolic compounds or extracts from medicinal plants that have shown an ameliorative effect against agents that impair insulin response, an underlying cause for obesity-related T2DM. Natural compounds as flavonoids show senolytic properties that could account for counteracting fatty acid-increased proinflammatory marker activation as well as impaired insulin signaling [38,39]. Therefore, assessing the senolytic ability of HEL might give new insights about its role improving altered cellular function in adipocytes.

Metabolic stressors associated with obesity such as hyperlipidemia, oxidative stress and inflammation threaten the homeostatic balance in adipose tissue [40,41], leading to an increased secretion and high levels of plasma SFA, and such as PA [27]. It is well known that PA activates NF-κB signaling inducing a proinflammatory response [21]. This study shows that PA- induced upregulation of NF-κB phosphorylation was significantly inhibited when HEL was present. Such finding is similar to other studies on phytochemicals showing a reduction in the activation of NF-κB pathway [14]. More studies are needed to elucidate whether HEL modulatory action on proinflammatory marker activation could be related to its effect restoring insulin signaling in adipocytes.

It is important to point out that we decided to study HEL, and not other soluble fractions from *Lampaya medicinalis* extracts, because ethanolic extracts of plants are those that mainly comprise and concentrate molecules associated with obesity-related beneficial activities (namely anti-inflammatory and antioxidant compounds), whereas water extracts primarily contain compounds stimulating the immune system [42–44].

This study shows for the first time that HEL is able to restore insulin-stimulated activation of IRS-1/Akt/AS160 pathway and glucose uptake, as well as NF-κB phosphorylation, in 3T3-L1 adipocytes treated with PA. We suggest that the ability of HEL to restore insulin signaling and glucose uptake, as well as NF-κB activation, could be mediated by its high flavonoid content, providing a rationale to further investigate the molecular mechanisms of action of HEL against fatty acid- induced impaired insulin responsiveness. The efficacy of herbal preparations most likely rely on the synergy among several different components rather than on a single compound's activity [45]. However, future research including *in vivo* studies are mandatory to define the real effectiveness and biological activities of HEL against insulin resistance. In addition, future clinical trials assessing the bioavailability of HEL constituents as well as its efficacy and safety in humans will be required to consider the hydroalcoholic extract of lampaya as a protective/therapeutic tool.

Ethical statement

The authors of the manuscript entitled **"Protective effect of the hydroalcoholic extract from *Lampaya medicinalis* Phil. *(Verbenaceae)* on palmitic acid- impaired insulin signaling in 3T3-L1 adipocytes"** declare that they have read and have abided by the statement of ethical standards for manuscripts submitted to the ***Obesity Research & Clinical Practice***.

Declarations of interest

None.

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CRediT authorship contribution statement

Paulina Ormazabal: Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Supervision. **Karin Herrera:** Methodology, Investigation, Formal analysis, Resources. **Mariana Cifuentes:** Methodology, Formal analysis, Resources, Writing - review & editing. **Adrián Paredes:** Methodology, Investigation, Resources, Writing - original draft. **Glauco Morales:** Methodology, Investigation, Formal analysis, Resources, Writing - original draft. **Gonzalo Cruz:** Formal analysis, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.orcp.2020.11.001.

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