RESEARCH PAPER

Phloridzin Inhibits Dipeptidyl Peptidase-IV Activity and Enhances Glucose Uptake in L6 Myoblasts and 3T3-L1 Preadipocytes: An In Vitro Study

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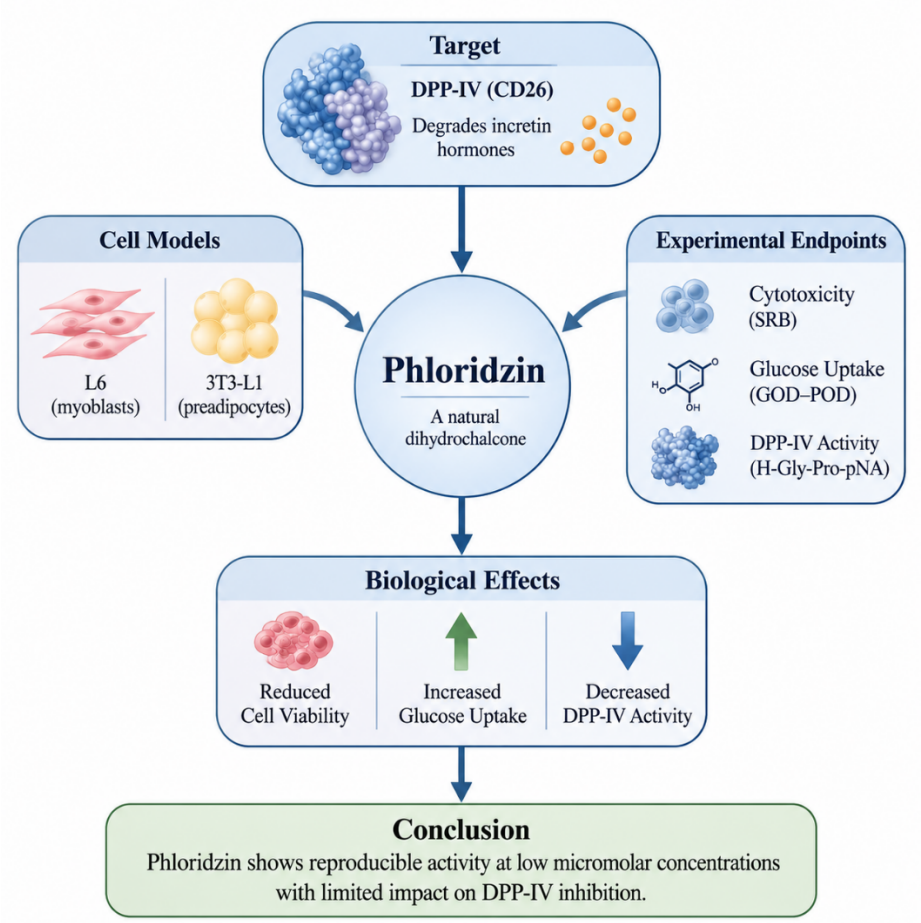
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Abstract

Dipeptidyl peptidase-IV (DPP-IV, CD26) shortens the half-life of the incretin hormones, and blocking it is an established route to glycaemic control in type 2 diabetes mellitus. Dietary polyphenols are widely screened for this activity, yet their reported potency in cell-based systems is rarely interpreted alongside the concentrations at which they begin to compromise the cells themselves. We examined phloridzin, the principal dihydrochalcone of apple, in rat L6 myoblasts and mouse 3T3-L1 preadipocytes, using sitagliptin as the reference inhibitor. Cytotoxicity was measured by sulforhodamine B staining after 72 h, glucose uptake by the glucose oxidase and peroxidase method after a 30 min exposure, and DPP-IV activity in cell lysates after 24 h treatment using H-Gly-Pro-*p*-nitroanilide as substrate. Phloridzin was cytotoxic with half-maximal growth-inhibitory concentrations of 2.42 µM in L6 and 2.19 µM in 3T3-L1 cells, the L6 concentration response being linear over 1 to 3 µM ($R^2 = 0.925$). Glucose uptake rose steeply with concentration in both lines, from 18.60% of the insulin response at 1 µM to 94.26% at 3 µM in L6 cells and from 19.82% to 92.34% in 3T3-L1 cells. DPP-IV activity fell in a concentration-dependent manner across 0.5 to 2.5 µM, reaching 70.41 ± 0.74% in L6 and 74.71 ± 1.75% in 3T3-L1 cells at 2.5 µM, against 72.28 ± 2.45% and 78.16 ± 1.41% for sitagliptin; phloridzin was the weaker of the two at every concentration in both lines. The glucose uptake result, obtained under acute exposure, is unlikely to be confounded by loss of viable cells and is consistent with published reports of phloridzin acting on glucose transport in these cell types. The DPP-IV result is more difficult to read, because the concentrations producing half-maximal loss of enzyme activity coincide with those producing half-maximal growth inhibition, and a lysate-based assay cannot separate inhibition of the enzyme from a reduction in the number of cells supplying it. Phloridzin therefore shows reproducible activity at low micromolar concentrations in two metabolically distinct cell types, while its effect on DPP-IV, measured in this format, is not established as inhibition of the enzyme.

Graphical Abstract:



Keywords: Phloridzin; dipeptidyl peptidase-IV; sitagliptin; glucose uptake; L6 myoblasts; 3T3-L1 preadipocytes; sulforhodamine B assay

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1. Introduction

Type 2 diabetes mellitus develops when insulin secretion can no longer keep pace with insulin demand. Among the therapeutic strategies developed over the past two decades, few have been as widely adopted as interference with the incretin axis. Glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide are secreted by the intestine after a meal and amplify glucose-stimulated insulin release, but both are cleaved within minutes by dipeptidyl peptidase-IV (DPP-IV, also known as CD26), a serine exopeptidase that removes N-terminal dipeptides from substrates bearing proline or alanine in the penultimate position. Preventing that cleavage extends incretin signalling and lowers blood glucose without forcing insulin release, which is the pharmacological logic of the gliptin class [1].

The structural basis of this chemistry is well understood. The catalytic triad lies at the bottom of a large internal cavity that substrates reach either through a side opening or through the channel of an eight-bladed propeller domain. An S1 pocket accounts for the preference for proline and alanine, while the neighbouring S2 subsite carries a pair of glutamate residues that grip the free N-terminus of a substrate or of an inhibitor bearing a basic amine [2]. Sitagliptin, the first agent of the class to be approved, exploits exactly this arrangement and inhibits the human enzyme with a half-maximal inhibitory concentration of 18 nM [3,4]. Potency, however, was never the only requirement. DPP-IV sits in a family that also contains DPP-8 and DPP-9, and inhibitors that hit those two enzymes caused alopecia, thrombocytopenia, multi-organ histopathology and mortality in rats and gastrointestinal toxicity in dogs. Selectivity within the family consequently became a baseline expectation for anything developed against this target [5].

The enzyme is not confined to the gut and pancreas. Its expression shifts in inflammation, obesity, cancer and diabetes, it is shed from the cell surface and circulates as an adipokine, and its substrate list extends well beyond the incretins [1]. Skeletal muscle and adipose tissue both express it, which makes cell lines derived from those tissues reasonable places to look for activity by a candidate compound, even though neither reproduces the intestinal context in which incretin cleavage matters most.

Dietary polyphenols have been examined extensively as a source of DPP-IV inhibitors. Phloridzin, chemically phloretin 2'-O-β-D-glucopyranoside, is the principal dihydrochalcone of apple bark, core and peel and is distributed throughout the genus *Malus* [6]. Its structure (Figure 1) joins a glucose unit to a dihydrochalcone carrying four phenolic hydroxyl groups. The compound occupies an unusual position in the history of diabetes pharmacology: it is the molecule from which sodium-glucose cotransporter inhibition was first described, and it retains documented anti-inflammatory, antioxidant, antimicrobial and antidiabetic activity [6]. More directly relevant here, phloridzin has been shown to raise glucose uptake in differentiated 3T3-L1 adipocytes and in C2C12 muscle cells, with increased phosphorylation of AMP-activated protein kinase and Akt, and to inhibit protein-tyrosine phosphatase MEG2 with a half-maximal inhibitory concentration of 32 ± 1.06 μM [7]. Phenolic compounds more broadly have been reported to inhibit DPP-IV in enzymatic and cell-based formats [8], and one study that combined extract screening with computational prediction identified phlorizin, a synonym of phloridzin, as binding the enzyme with low predicted binding energy [9].

What this literature less often addresses is the concentration at which an apparent cellular enzyme inhibition stops being separable from a loss of viable cells. An assay that measures DPP-IV activity in the lysate of treated cells reports the total enzyme recovered from the well. If the test compound also reduces cell number, substrate turnover falls for a reason that has nothing to do with the active site, and the calculation converts that fall into an apparent percentage inhibition. Measuring cytotoxicity in the same cells, over the same concentrations, is therefore not an optional addition to such a screen; it determines whether the primary result can be interpreted at all.

The present study was designed with that constraint in mind. We asked whether phloridzin lowers DPP-IV activity in L6 myoblasts and 3T3-L1 preadipocytes relative to sitagliptin, whether it alters glucose uptake in the same cells, and over what concentration range these effects can be distinguished from loss of viability. Both lines are established models for assessing plant-derived compounds against muscle and adipocyte metabolism [10,11]. The work was not intended to demonstrate advantage over an approved drug, to establish a mechanism, or to support any therapeutic claim.

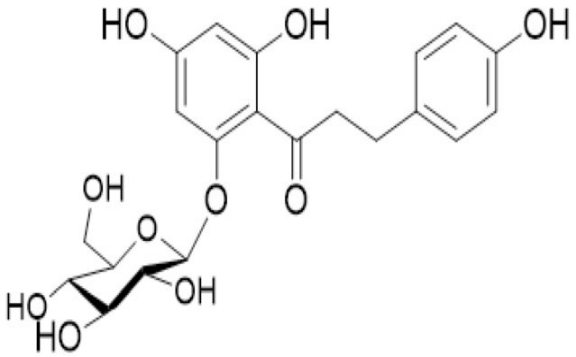


Figure 1. Structure of phloridzin (phloretin 2'-O-β-D-glucopyranoside), $C_{21}H_{24}O_{10}$, relative molecular mass 436.41, PubChem CID 6072.

2. Materials and methods

2.1 Chemicals and reagents

Phloridzin and sitagliptin were used as the test compound and reference inhibitor respectively. Dulbecco's Modified Eagle Medium, inactivated fetal bovine serum, penicillin, streptomycin, amphotericin B, trypsin, EDTA, trichloroacetic acid, sulforhodamine B, Tris base, acetic acid, dimethyl sulfoxide, RIPA lysis buffer, Krebs-Ringer phosphate buffer, bovine serum albumin, insulin, H-Gly-Pro-*p*-nitroanilide and the glucose oxidase and peroxidase reagent were of cell culture or analytical grade.

2.2 Cell lines and culture conditions

L6 and 3T3-L1 cells were obtained from the National Centre for Cell Science, Pune, India. Their characteristics are summarized in Table 1.

Table 1. Characteristics of the cell lines used in this study.

Property	**L6**	**3T3-L1**
Morphology	Fibroblastoid	Fibroblastoid
Tissue of origin	Skeletal muscle	Whole embryo
Species	Rat	Mouse (Swiss)
Developmental stage	Embryonic	Embryonic
Ploidy	Not stated	Aneuploid

Distinguishing features	Forms myotubes	Contact inhibited, readily transformed

Stock cultures were grown in Dulbecco's Modified Eagle Medium containing 10% inactivated fetal bovine serum, penicillin 100 IU/mL, streptomycin 100 µg/mL and amphotericin B 5 µg/mL, in a humidified atmosphere of 5% CO_2 at 37 °C, and were carried to confluence before use. Monolayers were dissociated with 0.2% trypsin and 0.02% EDTA in phosphate-buffered saline. Stock cultures were maintained in 25 cm^2 flasks and cytotoxicity experiments were performed in 96-well microtitre plates. Both lines were used in the undifferentiated state. L6 cells were not induced to form myotubes, and 3T3-L1 cells were not taken through an adipogenic differentiation protocol.

2.3 Cytotoxicity by sulforhodamine B assay

Cells in exponential growth were washed, trypsinised and resuspended in complete medium, seeded at 10,000 cells per well in 96-well plates and left for 24 h to form a partial monolayer. Phloridzin was then added across a range of concentrations in quadruplicate, with control wells receiving maintenance medium alone. Plates were held at 37 °C in 5% CO_2 for 72 h, during which treated and control cells were examined by inverted microscopy at intervals for morphological change.

At the end of the exposure, 50 µL of 50% trichloroacetic acid was layered gently over the medium to give a final concentration of 10%, and plates were kept at 4 °C for 1 h. Plates were then flicked, washed five times with tap water to clear medium, compound and serum, and air dried. Fixed cells were stained with sulforhodamine B for 30 min, excess dye was removed by four rapid washes in 1% acetic acid, and the plates were air dried again. Bound dye was dissolved in 100 µL of 10 mM Tris base with vigorous shaking for 5 min, and absorbance was read at 540 nm on a microplate reader. Sulforhodamine B binds the basic amino acid residues of protein fixed by trichloroacetic acid and gives a signal proportional to total cellular protein over a wide range of cell density, which makes it a stable and inexpensive index of cell number [12-14].

Growth inhibition was calculated as:

% Growth inhibition = 100 − (mean OD of test group / mean OD of control) × 100

The half-maximal growth-inhibitory concentration was obtained from the resulting concentration response.

2.4 Glucose uptake assay

Cells were seeded in 24-well plates and incubated for 48 h at 37 °C in 5% CO_2. Once a semi-confluent monolayer had formed, the medium was exchanged for serum-free Dulbecco's Modified Eagle Medium containing 0.2% bovine serum albumin and the cells were left for a further 18 h. That medium was then discarded, the monolayer was washed once with Krebs-Ringer phosphate buffer, and cells were treated with phloridzin at 1.0, 1.5, 2.0, 2.5 and 3.0 µM, or with insulin as the reference, in the presence of glucose. After 30 min at 37 °C the cells were lysed by three cycles of freezing and thawing and the lysate was collected. Glucose uptake was taken as the difference between the initial and final glucose content of the incubation medium, measured by the glucose oxidase and peroxidase method [11], and is expressed as a percentage of the uptake produced by insulin in the same experiment.

2.5 DPP-IV activity assay

Phloridzin was dissolved in dimethyl sulfoxide and diluted in culture medium to 0.5, 1.0, 1.5, 2.0 and 2.5 µM. Sitagliptin was prepared at matching concentrations as the reference inhibitor. The final concentration of dimethyl sulfoxide did not exceed 0.1% (v/v). Three groups were studied in each cell line: untreated controls, phloridzin at the five concentrations, and sitagliptin at the same five concentrations.

L6 and 3T3-L1 cells were grown in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum and 1% antibiotics at 37 °C in 5% CO_2 and used at 70 to 80% confluence. After 24 h of treatment the monolayers were washed with ice-cold phosphate-buffered saline, lysed in RIPA buffer on ice for 30 min and centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was taken as the source of enzyme.

The chromogenic reaction was run in triplicate. Each well received assay buffer and enzyme followed by inhibitor or vehicle, with a pre-incubation of 1 to 2 h. The reaction was started with 50 µL of H-Gly-Pro-*p*-nitroanilide, incubated for 30 to 60 min at 37 °C, and the *p*-nitroaniline released was read at 405 nm. Chromogenic assays of this design are well established for screening plant-derived DPP-IV inhibitors [15-17]. Reduction in enzyme activity was calculated as:

% Reduction in DPP-IV activity = [(A_control − A_treated) / A_control] × 100

2.6 Statistical Analysis

Values are reported as mean ± standard deviation of triplicate determinations for the DPP-IV assay. Cytotoxicity experiments were run in quadruplicate. Comparisons between phloridzin and sitagliptin, and between the two cell lines, are presented descriptively; no inferential statistical test was applied, and no claim of statistical significance is made anywhere in this report. The linear regression shown in Figure 2 was fitted by least squares to the L6 concentration response.

3. Results

3.1 Cytotoxicity

Both cell lines reached maximum proliferation under the culture conditions used, and cells in exponential growth were selected for the cytotoxicity work. In L6 cells, cytotoxicity rose with concentration across 1 to 3 µM, from 17.19% at 1 µM to 66.29% at 3 µM (Table 2, Figure 2). The linear trendline fitted to these points is $f(x) = 24.098x - 8.71$ with $R^2 = 0.9247$; solving that expression for 50% gives 2.44 µM. The point at 2.5 µM (45.06%) falls marginally below the point at 2.0 µM (46.12%), so the response flattens briefly in the middle of the range before rising again.

Half-maximal growth inhibition was reached at 2.42 µM in L6 cells and at 2.19 µM in 3T3-L1 cells. The L6 value agrees closely with the 2.44 µM predicted by the regression in Figure 2, which supports the internal consistency of that dataset.

Table 2. Cytotoxicity of phloridzin in L6 cells after 72 h exposure, measured by sulforhodamine B assay, together with the half-maximal growth-inhibitory concentrations determined in both cell lines.

Concentration (µM)	Cytotoxicity in L6 cells (%)
1.0	17.19
1.5	22.77
2.0	46.12
2.5	45.06
3.0	66.29
IC_{50}, L6	2.42 µM
IC_{50}, 3T3-L1	2.19 µM

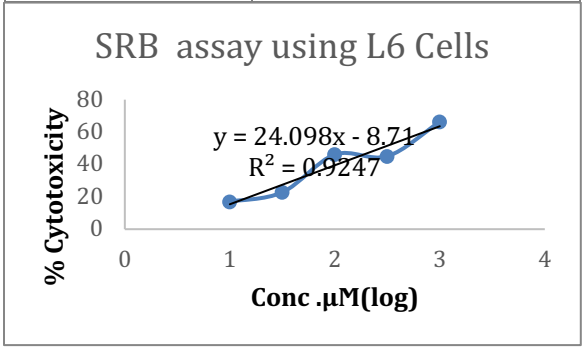
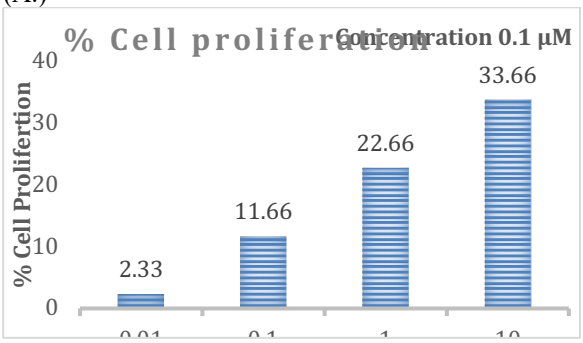


Figure 2. Cytotoxicity of phloridzin in L6 cells after 72 h exposure, measured by sulforhodamine B assay. The straight line is the linear trendline fitted to the five points, with its equation and coefficient of determination displayed on the chart.

A separate preliminary experiment, performed over the wider range 0.01 to 10 µM, is shown in Figure 3. Values labelled as cell proliferation increased with concentration in both lines, from 2.33% at 0.01 µM to 33.66% at 10 µM in L6 cells and from 6.00% to 35.66% over the same interval in 3T3-L1 cells (Figure 3). These values were recorded over a different concentration range from the data in Figure 2 and do not extend the concentration response of Figure 2; they are reported here for completeness and are not used in the interpretation that follows.

(A.)



(B.)

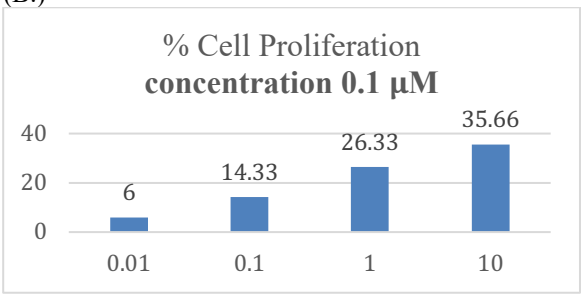


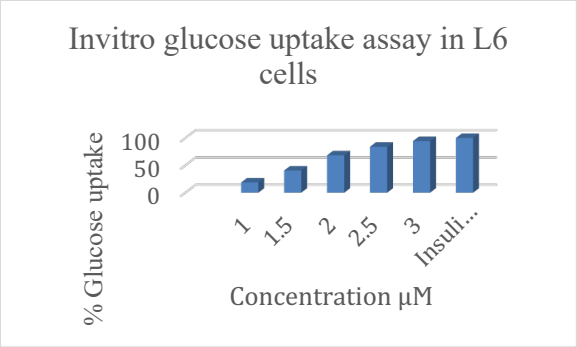
Figure 3. Preliminary range-finding data recorded over 0.01 to 10 µM phloridzin, (A.) L6 cells, (B.) 3T3-L1 cells. Values above each bar are percentages.

3.2 Glucose uptake

Phloridzin increased glucose uptake in both cell lines, and the increase tracked concentration closely (Figure 4). In L6 cells, uptake rose from 18.60% of the insulin response at 1.0 µM to 40.46% at 1.5 µM, 68.25% at 2.0 µM, 83.89% at 2.5 µM and 94.26% at 3.0 µM. The pattern in 3T3-L1 cells was almost identical: 19.82%, 37.56%, 69.87%, 81.06% and 92.34% across the same concentrations. At the highest concentration tested, the uptake produced by phloridzin came within 6 to 8 percentage points of that produced by insulin in both lines.

The steepest part of the response lay between 1.5 and 2.0 µM in both cell lines, where uptake gained roughly 28 to 32 percentage points for a 0.5 µM increment. Above 2.5 µM the curves flatten as they approach the insulin reference.

(A.)



(B.)

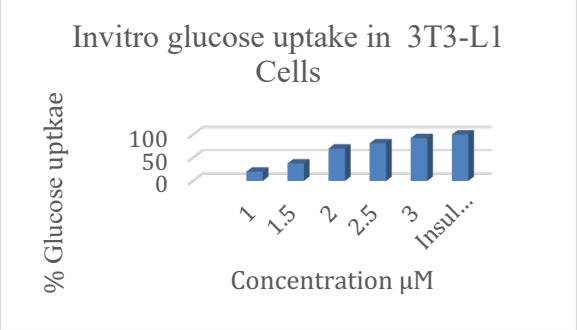


Figure 4. Glucose uptake by phloridzin after 30 min exposure, measured by the glucose oxidase and peroxidase method and expressed as a percentage of the uptake produced by insulin in the same experiment. (A.) L6 cells, (B.) 3T3-L1 cells. The right-hand category in each chart is the insulin reference.

3.3 DPP-IV activity

Phloridzin lowered DPP-IV activity in a concentration-dependent manner in both cell lines (Table 3, Figure 5). In L6 cells the reduction climbed from 11.61 ± 0.99% at 0.5 µM to 70.41 ± 0.74% at 2.5 µM, against 20.22 ± 1.12% rising to 72.28 ± 2.45% for sitagliptin. In 3T3-L1 cells phloridzin gave 12.26 ± 2.51% at 0.5 µM and 74.71 ± 1.75% at 2.5 µM, against 18.00 ± 2.13% and 78.16 ± 1.41% for sitagliptin. At every concentration tested, in both lines, phloridzin produced the smaller reduction.

Table 3. Reduction in DPP-IV activity produced by phloridzin and sitagliptin in L6 and 3T3-L1 cells. Values are mean ± SD of triplicate determinations.

Concentration (µM)	**L6, phloridzin (%)**	**L6, sitagliptin (%)**	**3T3-L1, phloridzin (%)**	**3T3-L1, sitagliptin (%)**
0.5	11.61 ± 0.99	20.22 ± 1.12	12.26 ± 2.51	18.00 ± 2.13
1.0	28.09 ± 1.12	35.95 ± 1.29	33.33 ± 0.66	38.69 ± 2.68
1.5	37.07 ± 3.37	48.68 ± 1.98	44.44 ± 1.91	50.57 ± 1.32
2.0	45.69 ± 1.35	66.66 ± 1.63	56.38 ± 2.02	64.36 ± 2.43
2.5	70.41 ± 0.74	72.28 ± 2.45	74.71 ± 1.75	78.16 ± 1.41

The gap between the two compounds did not narrow steadily. In L6 cells phloridzin trailed sitagliptin by 8.6 percentage points at 0.5 µM, by 21.0 points at 2.0 µM, and by only 1.9 points at 2.5 µM. The same shape appeared in 3T3-L1 cells across a narrower span, with 5.7 points at 0.5 µM, a maximum of 8.0 points at 2.0 µM and 3.5 points at 2.5 µM. The convergence at the top of the range reflects how steeply the phloridzin response rises over the final interval: between 2.0 and 2.5 µM phloridzin gained 24.7 percentage points in L6 cells while sitagliptin gained 5.6, and in 3T3-L1 cells the corresponding gains were 18.3 and 13.8 points. Figure 6 shows the phloridzin response in the two cell lines side by side together with the size of the shortfall at each concentration.

Reading directly from Table 3, phloridzin crosses 50% reduction between 2.0 and 2.5 µM in L6 cells and between 1.5 and 2.0 µM in 3T3-L1 cells. No half-maximal inhibitory concentration is reported for this assay, because a five-point response that stops close to its steepest segment does not support a reliable fit.

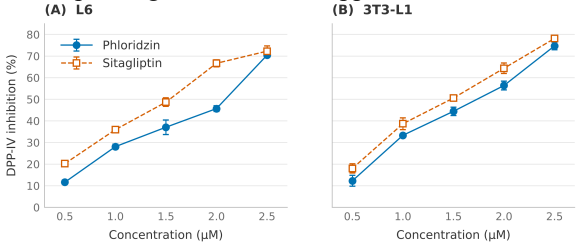


Figure 5. Reduction in DPP-IV activity produced by phloridzin and sitagliptin in (A) L6 and (B) 3T3-L1 cells after 24 h treatment. Points are mean ± SD of triplicate determinations and are joined by straight segments; no curve has been fitted.

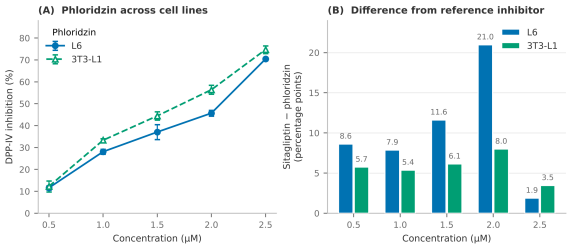


Figure 6. (A) Reduction in DPP-IV activity by phloridzin compared between L6 and 3T3-L1 cells; points are mean ± SD of triplicate determinations. (B) Difference between sitagliptin and phloridzin at each concentration, in percentage points, in both cell lines.

3.4 Relationship between the active and cytotoxic concentration ranges

The three endpoints were measured over overlapping concentrations but under very different exposure times, and this governs how each can be read.

Glucose uptake was measured after 30 min. Over so short an exposure, the 72 h growth inhibition described in Section 3.1 cannot have developed, so the values in Figure 4 are not attributable to a change in cell number. DPP-IV activity was measured after 24 h at 0.5 to 2.5 µM. The half-maximal growth-inhibitory concentrations, 2.42 µM in L6 and 2.19 µM in 3T3-L1 cells, lie inside that range, and the highest concentration tested exceeds both. The concentrations at which phloridzin crossed 50% reduction in DPP-IV activity, between 1.5 and 2.5 µM depending on cell line, therefore coincide with the concentrations producing half-maximal growth inhibition over the longer exposure. How far growth inhibition had progressed by 24 h was not measured.

4. Discussion

4.1 Principal findings

Phloridzin dose-dependently increased glucose uptake towards insulin-treated levels and reduced cellular DPP-IV activity, although less effectively than sitagliptin. It also showed cytotoxicity, with 72-h IC_{50} values of approximately 2.2–2.4 µM, indicating that these effects occur within a closely related concentration range.

Both cell lines exhibited similar glucose uptake and DPP-IV responses despite their different lineages and metabolic profiles, suggesting a shared underlying mechanism rather than a cell-type-specific effect.

4.2 Glucose uptake

The glucose uptake result is the most securely interpretable of the three, for a simple reason: it was obtained after 30 min of exposure, far too short for the growth inhibition seen at 72 h to contribute. The effect is also sizeable. At 3 µM, phloridzin delivered 94.26% of the insulin response in L6 cells and 92.34% in 3T3-L1 cells.

A reader familiar with this compound may find that direction counter-intuitive. Phloridzin is the classical inhibitor of sodium-glucose cotransport and was the starting point for the development of the gliflozin class [6]. The resolution is that sodium-glucose cotransporters are not the principal route of glucose entry into skeletal muscle cells or adipocytes; those tissues rely on the facilitative GLUT transporters, and GLUT4 in particular. Published work supports the direction observed here. Phloridzin has been reported to stimulate glucose uptake in differentiated 3T3-L1 adipocytes and in C2C12 muscle cells, together with increased phosphorylation of AMP-activated protein kinase and Akt, and to act as an inhibitor of protein-tyrosine phosphatase MEG2, a phosphatase whose inhibition promotes insulin signalling [7]. The present data are consistent with that account, although they do not test it: no transporter, kinase or phosphatase was measured here.

Two differences from the published work should be kept in view. That study used differentiated adipocytes and myotubes, whereas both lines here were undifferentiated, and the differentiated and undifferentiated states differ in glucose transporter expression. The reported MEG2 inhibitory concentration of 32 µM is also an order of magnitude above the concentrations used here, so if MEG2 inhibition contributes to the present result it is unlikely to be the whole of it.

4.3 DPP-IV activity

The reduction in DPP-IV activity is harder to interpret, and the cytotoxicity data are the reason. Sulforhodamine B quantifies total cellular protein, so an agent with a half-maximal growth-inhibitory concentration of 2.19 to 2.42 µM is removing a substantial fraction of the cell population at concentrations that were also used in the enzyme assay. That assay reads activity in the lysate of treated cells. Unless the lysate is normalised to total protein, fewer cells yield less enzyme, less substrate turnover and a lower absorbance at 405 nm, and the calculation in Section 2.5 converts that lower absorbance directly into an apparent reduction in activity. The two effects are arithmetically identical in such a design.

Several considerations temper this. The exposures differ, 72 h for the cytotoxicity measurement against 24 h for the enzyme assay, so growth inhibition at the time of lysis would be expected to be considerably less than the 72 h figure. The concentration responses in Figure 5 are smooth and orderly in both lines, which fits a graded pharmacological effect better than progressive cell loss. Sitagliptin, which is not cytotoxic at these concentrations, generated a comparable profile, indicating that the assay is responding to something other than cell number alone. None of this, however, replaces the measurement that would settle the question. The defensible conclusion is that phloridzin produces a concentration-dependent

reduction in recoverable DPP-IV activity, and that the present design does not establish this as inhibition of the enzyme.

A second consideration is specific to the chemistry. Phloridzin is a polyphenol, and polyphenols are known to interfere with the optical readouts of DPP-IV inhibitor screens, generating signals that are not attributable to inhibition of the target [18]. A control well containing compound and substrate but no enzyme separates genuine inhibition from direct interference with the chromogenic readout, and such a control was not included here.

Taken together, these two points mean that the comparison with sitagliptin in Table 3 should be read as a comparison of assay behaviour, not of enzyme affinity. It is worth noting that berberine, the best-characterised natural DPP-IV inhibitor, inhibits the recombinant human enzyme with a half-maximal inhibitory concentration of 13.3 µM [15], roughly an order of magnitude above the concentrations used in this study. An unmodified dihydrochalcone is unlikely to be substantially more potent than berberine against this target, reinforcing the concerns raised above.

4.4 Scope of the findings

The two effects reported here are best read as separate observations about the same compound rather than as a single mechanistic sequence. An increase in glucose uptake by L6 or 3T3-L1 cells neither requires nor demonstrates DPP-IV inhibition. DPP-IV acts on incretins in the circulation, and incretin-mediated effects cannot be reproduced in a monoculture of muscle or preadipocyte cells without a pancreatic islet component.

The design carries real weight on two counts. Two independent cell lines of different tissue origin were tested in parallel against a clinical reference inhibitor across a shared concentration range, and a cytotoxicity endpoint was obtained in the same systems. That second element is what makes the boundary between the three endpoints visible at all; without it the enzyme data would have looked cleaner and meant less.

What the data can carry is correspondingly specific. All three endpoints were measured in undifferentiated cells, and L6 myoblasts and 3T3-L1 preadipocytes differ from myotubes and mature adipocytes in metabolic phenotype, glucose transporter expression included, so the findings belong to the undifferentiated state. The three experiments were also not perfectly aligned in concentration: glucose uptake and cytotoxicity were measured over 1 to 3 µM, the enzyme assay over 0.5 to 2.5 µM. That enzyme range spans only fivefold and stops close to the steepest part of the phloridzin response, which leaves the upper plateau undefined and is the reason no half-maximal inhibitory concentration is reported for it. Selectivity within the dipeptidyl peptidase family was not examined, and the toxicity associated with combined DPP-8 and DPP-9 inhibition keeps that an open question for any compound active at this target [5]. All comparisons made here are descriptive, since no inferential statistical test was applied. The work is confined to cell culture, and nothing in it speaks to bioavailability, pharmacokinetics, efficacy in an animal or clinical setting, or human safety.

5. Conclusion

Phloridzin raised glucose uptake in L6 myoblasts and 3T3-L1 preadipocytes to within 6 to 8 percentage points of the insulin response at 3 µM, and lowered the DPP-IV activity recoverable from both cell lines in a concentration-dependent manner over 0.5 to 2.5 µM, reaching 70.41 ± 0.74% and 74.71 ± 1.75% at the highest concentration tested against 72.28 ± 2.45% and 78.16 ± 1.41% for sitagliptin. It was less effective than sitagliptin at every concentration in both lines. The compound was also cytotoxic, with half-maximal growth inhibition at 2.42 µM in L6 and 2.19 µM in 3T3-L1 cells after 72 h.

The glucose uptake effect was obtained under acute exposure and is therefore not attributable to loss of viable cells; its direction and magnitude agree with published reports of phloridzin acting on glucose transport in these cell types. The effect on DPP-IV requires more caution. The concentrations producing half-maximal reduction in enzyme activity coincide with those producing half-maximal growth inhibition, and a lysate-based assay without protein normalisation cannot distinguish inhibition of the enzyme from a reduction in the number of cells supplying it. On the evidence presented, phloridzin is a compound with reproducible low-micromolar activity in two metabolically distinct cell types, and the reduction in DPP-IV activity it produces is not established as inhibition of the enzyme.

Declarations

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Author contributions. Vaishnavi Munnangi: conceptualisation, methodology, investigation. Patel Dhruv Jatinkumar: investigation and data curation. Raman Rajeshkumar: laboratory analysis. Vadivelan Ramachandran: supervision, formal analysis, writing, review and editing.

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Ethics statement. It involved no human participants and no animals, and institutional ethical approval was therefore not required.

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