Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 361 (2007) 208–213
BBRC
www.elsevier.com/locate/ybbrc

Phloretin enhances adipocyte differentiation and adiponectin expression in 3T3-L1 cells

Meryl Hassan [a,b,c,d], Claire El Yazidi [a,b,c], Jean-François Landrier [a,b,c], Denis Lairon [a,b,c], Alain Margotat [a,b,c], Marie-Josèphe Amiot [a,b,c,*]

[a] INSERM, U476 "Nutrition Humaine et Lipides", Marseille F-13385, France
[b] INRA, UMR1260, Marseille F-13385, France
[c] Univ Méditerranée Aix-Marseille 2, Faculté de Médecine, IPHM-IFR 125, Marseille F-13385, France
[d] ANDROS & Cie, Biars sur Cere F-46130, France

Received 3 July 2007
Available online 16 July 2007

Abstract

Adipocyte dysfunction is strongly associated with the development of cardiovascular risk factors and diabetes. It is accepted that the regulation of adipogenesis or adipokines expression, notably adiponectin, is able to prevent these disorders. In this report, we show that phloretin, a dietary flavonoid, enhances 3T3-L1 adipocyte differentiation as evidenced by increased triglyceride accumulation and GPDH activity. At a molecular level, mRNA expression levels of both PPARγ and C/EBPα, the master adipogenic transcription factors, are markedly increased by phloretin. Moreover, mRNA levels of PPARγ target genes such as LPL, aP2, CD36 and LXRα are up-regulated by phloretin. We also show that phloretin enhances the expression and secretion of adiponectin. Co-transfection studies suggest the induction of PPARγ transcriptional activity as a possible mechanism underlying the phloretin-mediated effects. Taken together, these results suggest that phloretin may be beneficial for reducing insulin resistance through its potency to regulate adipocyte differentiation and function.

Keywords: Phloretin; Flavonoid; 3T3-L1 Adipocytes; Adipocyte differentiation; PPARγ; C/EBPα; Adiponectin; Insulin resistance

Adipose tissue, as a metabolic and endocrine organ, plays an essential role in the regulation of energy balance [1]. Accordingly, adipocytes are emerging as a potential therapeutic target for obesity, type 2 diabetes, and cardiovascular disease [2]. Adipogenesis is a complex process accompanied by coordinated changes in morphology, hormone sensitivity, and gene expression. Members of the CCAAT/enhancer binding protein (C/EBP) transcription factor family and peroxisome proliferator-activated receptor γ (PPARγ) act in concert to regulate the adipocyte differentiation program [3]. Thiazolidinediones (TZDs), as synthetic PPARγ ligands and anti-diabetic agents, are able to stimulate adipogenesis and enhance insulin sensitivity by stimulating the transcriptional activity of PPARγ [4,5].

In addition to the central role of lipid storage, adipocytes express and secrete numerous bioactive substances called adipokines [1,6]. Among these, adiponectin regulates energy metabolism mainly by increasing insulin sensitivity. Plasma levels of adiponectin and mRNA expression in adipose tissue are decreased in obese and insulin resistant states. Furthermore, administration of adiponectin to obese or diabetic mice reverses insulin resistance by decreasing plasma triglycerides and improving glucose tolerance [7]. Adiponectin also displays anti-inflammatory and anti-atherogenic properties [7].

* Corresponding author. Address: UMR 476 INSERM-1260 INRA-Université Aix-Marseille 2, Faculté de Médecine, 27, Boulevard Jean Moulin, 13385 Marseille Cedex 5, France. Fax: +33 491 78 2101.
E-mail address: marie-jo.amiot-carlin@medecine.univ-mrs.fr (M.-J. Amiot).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.07.021

More recently, much attention has been focused on flavonoids, a group of phenolic compounds that have been linked to a reduced risk of major chronic diseases [8]. Phloretin belongs to the chalcone class of flavonoids and is present as glucosides in apples [9], and strawberries [10]. Phloretin displays antioxidant properties as many flavonoids [9], and is known to be a competitive inhibitor of sodium D-glucose cotransporter 1 [11]. Recently, biological actions of flavonoids have been attributed to their ability to modulate signalling pathways [12]. In this line, phloretin has been reported to prevent cytokine-induced expression of endothelial adhesion molecules and to reduce activation of human platelets [13]. The present study was designed to investigate whether phloretin can exert modulatory effects on 3T3-L1 adipocyte differentiation process and adiponectin expression.

Materials and methods

Reagents. Dulbecco's modified Eagle's medium (DMEM) was purchased from Invitrogen (Carlsbad, CA), fetal bovine serum (FBS) was obtained from PAA laboratories (Pasching, Austria) and charcoal-stripped FBS was from Biowest (Nuaille, France). Phloretin, isobutylmethylxanthine, dexamethasone, and insulin were purchased from Sigma–Aldrich (St. Louis, MO). TRIzol reagent, random primers and Moloney murine leukemia virus reverse transcriptase were obtained from Invitrogen (Carlsbad, CA). SYBR Green reaction buffer was purchased from Eurogentec (Angers, France). JetPEI reagent was purchased from Polyplus Transfection (Illkirch, France). Unless otherwise specified, all other reagents were purchased from Sigma.

Cell culture and stimulation. 3T3-L1 preadipocytes (ATCC, Manassas, VA) were seeded in 6-cm diameter dishes at a density of 15×10^4 cells/well. Cells were grown in phenol red-free DMEM supplemented with 10% charcoal-stripped FBS at 37 °C in a 5% CO_2 humidified atmosphere. To induce differentiation, 2-day postconfluent 3T3-L1 preadipocytes (day 0) were stimulated for 48 h with 0.5 mM isobutylmethylxanthine, 0.25 μM dexamethasone, and 1 μg/ml insulin in phenol red-free DMEM supplemented with 10% charcoal-stripped FBS. Then preadipocytes were maintained in and refed every 2 days with phenol red-free DMEM supplemented with 10% charcoal-stripped FBS and 1 μg/ml insulin. To examine the effect of phloretin on adipocyte differentiation, 2-day postconfluent 3T3-L1 preadipocytes received 50 μM phloretin every 2 days until the end of the experiment at day 12. Phloretin was reconstituted as 50 mM stock solutions in DMSO and stored at −20 °C. The data are the mean of three independent experiments each performed in triplicate.

Oil Red O staining. 3T3-L1 Adipocytes were washed with PBS and fixed with 10% formalin for 30 min. After two washes with distilled water, cells were stained for at least 1 h at room temperature in freshly diluted Oil Red O solution (six parts Oil Red O stock solution and four parts H_2O; Oil Red O stock solution is 0.5% Oil Red O in isopropanol).

Triglyceride assay. 3T3-L1 Adipocytes were washed with PBS and harvested into 25 mM Tris buffer (pH 7.5) containing 1 mM EDTA, sonicated to homogenize the cell suspension, and assayed for total triglyceride with the GPO-Trinder kit (Sigma, St. Louis, MO). An aliquot of the homogenate was used to determine protein content with a protein assay kit (Pierce, Rockford, IL).

Glycerol-3-phosphate dehydrogenase (GPDH) activity. 3T3-L1 Adipocytes were washed twice with PBS and harvested into 25 mM Tris buffer (pH 7.5) containing 1 mM EDTA and 1 mM DTT. Cells were disrupted by sonication and then centrifuged at 12,000*g* for 20 min at 4 °C. The supernatants were assayed for GPDH activity according to the Wise and Green method [14].

RNA preparation and real-time quantitative RT-PCR. Total cellular RNA was extracted from 3T3-L1 cells at various times after adipogenic induction using TRIzol reagent according to the manufacturer's instructions. The cDNA was synthesized from 1 μg of total RNA in 20 μl using random primers and Moloney murine leukemia virus reverse transcriptase. Real time quantitative RT-PCR analyses for the genes described in Table 1 were performed using the Mx3005P Real-time PCR System (Stratagene, La Jolla, CA). Reactions were performed in a 25 μl volume containing 12.5 μl of 2× SYBR Green reaction buffer, 5 μl of cDNA (corresponding to 50 ng of reverse transcribed total RNA) and 300 nM of each primer. After an initial incubation for 2 min at 50 °C, the cDNA was denatured at 95 °C for 10 min followed by 40 cycles of PCR (95 °C, 15 s, 60 °C, 60 s). For each condition, expression was quantified in duplicate, and 18S mRNA was used as the endogenous control in the comparative cycle threshold (C_T) method [15].

Adiponectin quantification. The adiponectin concentration in cell culture medium was measured by an enzyme-linked immunosorbent assay (mouse/rat adiponectin ELISA kit, Otsuka Pharmaceutical, Tokyo, Japan). The media of the cells were centrifuged 5 min at 1000*g* and the supernatants were diluted 100-fold. The adiponectin levels in cell culture supernatants were determined according to the method recommended by the manufacturer. A reference curve was obtained in the range of adiponectin standard from 0.25 to 8 ng/ml.

Plasmids. The L-FABP 275^{wt} and L-FABP 275^{del} constructs [16], derived from the −4000/+22 rat L-FABP promoter (kindly provided by Dr. J.I. Gordon, St. Louis, MO), were subcloned into pGL3-basic vector. In L-FABP 275^{del} construct, the functional PPAR-responsive element (PPRE) was deleted [16]. The Adipo 908^{wt} construct [17], was provided by Dr. I. Shimomura (Osaka, Japan). The mutation of the PPRE (Adipo 908^{mut}) was generated by site-directed mutagenesis (QuickChange™ site-directed mutagenesis kit, Stratagene, La Jolla, CA) using the following oligonucleotides: 5′-CCTGCTGTGGTTT**TGATATCTGCCCC**ATCTTC TGTTGCTGTTG-3′ and the reverse one (PPRE in bold, point mutations are underlined). All constructs were sequenced.

Transfection and reporter assays. COS-1 cells were seeded in 24-well plates at a density of 2.7×10^4 cells/well. Cells were grown in DMEM supplemented with 10% FBS at 37 °C in a 5% CO_2 humidified atmosphere. Co-transfection mixes contained 300 ng of L-FABP 275^{wt}, L-FABP 275^{del},

Table 1
Sequences of primers used for real-time PCR

Gene name	Accession No.	Forward primer	Reverse primer
18S	X00686	CGCCGCTAGAGGTGAAATTCT	CATTCTTGGCAAATGCTTTCG
PPARγ	NM_011146	CAAGAATACCAAAGTGCGATCAA	GAGCTGGGTCTTTTCAGAATAATAAG
C/EBPα	NM_007678	AGCAACGAGTACCGGGTACG	TGTTTGGCTTTATCTCGGCTC
C/EBPβ	NM_009883	GCAAGAGCCGCGACAAG	GGCTCGGGCAGCTGCTT
C/EBPδ	NM_007679	TTCCAACCCCTTCCCTGAT	CTGGAGGGTTTGTGTTTTCTGT
LPL	NM_008509	GGCCAGATTCATCAACTGGAT	GCTCCAAGGCTGTACCCTAAG
aP2	NM_024406	AGTGAAAACTTCGATGATTACATGAA	GCCTGCCACTTTCCTTGTG
CD36	NM_007643	TTGTACCTATACTGTGGCTAAATGAGA	CTTGTGTTTTGAACATTTCTGCTT
LXRα	NM_013839	AGGAGTGTCGACTTCGCAAA	CTCTTCTTGCCGCTTCAGTTT
Adiponectin	NM_009605	TCCTGGAGAGAAGGGAGAGAAAG	TCAGCTCCTGTCATTCCAACAT

Adipo 908^{wt} or Adipo 908^{mut} reporter plasmids, 150 ng of the mouse PPARγ expression vector (pSG5-PPARγ) or empty pSG5 and 50 ng of β-galactosidase expression vector (pSV-βgal). Cells were transfected overnight using JetPEI reagent at a ratio of 1 μL JetPEI per 0.5 μg DNA. After this, the medium was changed to DMEM supplemented with 10% FBS and 50 μM phloretin, 10 μM troglitazone or the vehicle DMSO. After an additional 24 h-incubation, cells were lysed for luciferase activity using a Luciferase Assay System (Promega, Madison, WI), which was normalized to β-galactosidase activity. The transfection experiments were performed in triplicate and repeated at least two times independently.

Statistical analysis. The data are expressed as means ± SD. Significant differences between the control and the treated group, set at $P < 0.05$, were determined by Student's *t*-test using Statview software (Abacus Concepts, Cary, NC).

Results

Phloretin enhances 3T3-L1 adipocyte differentiation

Two-day postconfluent 3T3-L1 preadipocytes (day 0) were treated with phloretin at 50 μM every 2 days for 12 days. No significant effect has been observed with phloretin concentrations under 50 μM (1, 2, 10, and 20 μM; data not shown). When preadipocytes differentiated into adipocytes, morphological alterations were observed due to the accumulation of lipid droplets in the cytoplasm. As evidenced by Oil Red O staining, phloretin significantly increased lipid accumulation compared with control cells (Fig. 1A). To further characterize the effects of phloretin on differentiation, cellular triglyceride content and GPDH enzyme activity were measured. By the time of full differentiation at day 12, cells treated with phloretin accumulated 1.8 times more triglycerides ($P < 0.01$) compared with the controls (Fig. 1B). Consistent with the observed increase in triglyceride accumulation, GPDH activity was enhanced by 1.7-fold ($P < 0.001$) in cells treated with phloretin (Fig. 1C).

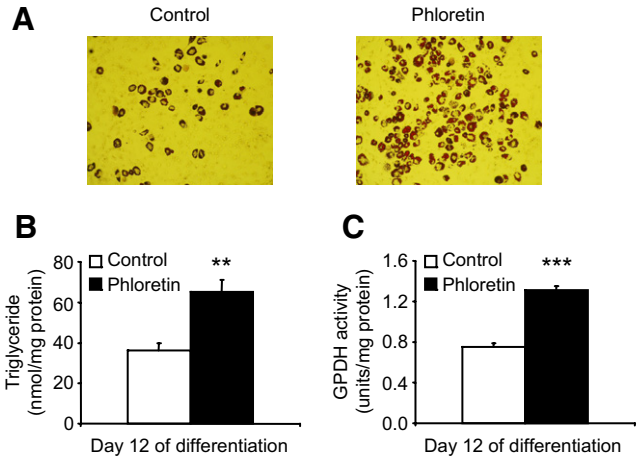

Fig. 1. Effects of phloretin on adipocyte differentiation of 3T3-L1 cells. Two-day postconfluent 3T3-L1 preadipocytes (day 0) were treated with 50 μM phloretin every 2 days for 12 days. Cells treated with 0.1% DMSO were used as controls. The assays were performed on fully differentiated adipocytes (day 12). (A) Intracellular lipid was stained with Oil Red O. (B) Triglyceride content was measured using the GPO-Trinder method. (C) GPDH activity was measured with the Wise and Green method. The bars represent mean values ± SD. $^{**}P < 0.01$; $^{***}P < 0.001$.

Phloretin up-regulates the expression of adipogenic transcription factors PPARγ and C/EBPα, and PPARγ target genes

The PPARγ and C/EBPα mRNA levels were significantly increased by 1.9- to 2.3-fold from day 4 to day 12 during adipocyte differentiation in cells treated with phloretin compared with control cells (Fig. 2A). We also determined C/EBPβ and δ mRNA levels, the upstream regulators of both PPARγ and C/EBPα. As expected, C/EBPβ and δ were highly expressed at day 1 after induction and were down-regulated during terminal differentiation. Phloretin was without effect on their expression pattern (Fig. 2A). Since PPARγ mRNA level was increased by phloretin, we hypothesized that expression of PPARγ target genes may also be up-regulated. Indeed, under phloretin treatment, the mRNA levels of LPL, aP2, CD36, and LXRα were significantly increased by 1.5- to 2.8-fold during adipocyte differentiation (Fig. 2B).

Phloretin increases transcriptional activity of PPARγ

We used a short version of rat L-FABP promoter containing almost only the functional PPRE as a natural model for PPARγ transcriptional activity assessment. COS-1 cells were co-transfected with PPARγ expression vector (pSG5-PPARγ) or pSG5 alone and with L-FABP 275^{wt} or L-FABP 275^{del} reporter plasmids. As shown in Fig. 3, the TZD troglitazone used at 10 μM induced luciferase activity by 2.1-fold ($P < 0.01$) in cells co-transfected with PPARγ expression vector and L-FABP 275^{wt} reporter plasmid. In same conditions, phloretin at 50 μM induced luciferase activity by 1.6-fold ($P < 0.05$). No gene reporter activation was observed in the absence of PPARγ or in the presence of deleted PPRE either under troglitazone or phloretin stimulation.

Phloretin increases adiponectin expression

Treatment of 3T3-L1 cells with phloretin significantly increased adiponectin mRNA level during adipocyte differentiation with a 2.3-fold increase at day 8 (Fig. 4A). Consistent with the mRNA expression, secretion of adiponectin in the medium of cells exposed to phloretin was significantly increased by 2.9- and 2.5-fold at days 8 and 12 of differentiation, respectively (Fig. 4B). In order to demonstrate the transcriptional origin of the adiponectin regulation by phloretin as well as the involvement of PPARγ and PPRE, COS-1 cells were co-transfected with two versions of human adiponectin promoter (Adipo 908^{wt} or Adipo 908^{mut}) and PPARγ expression vector. As shown in Fig. 4C, phloretin induced luciferase activity by 1.5-fold ($P < 0.05$) in cells co-transfected with Adipo 908^{wt} reporter

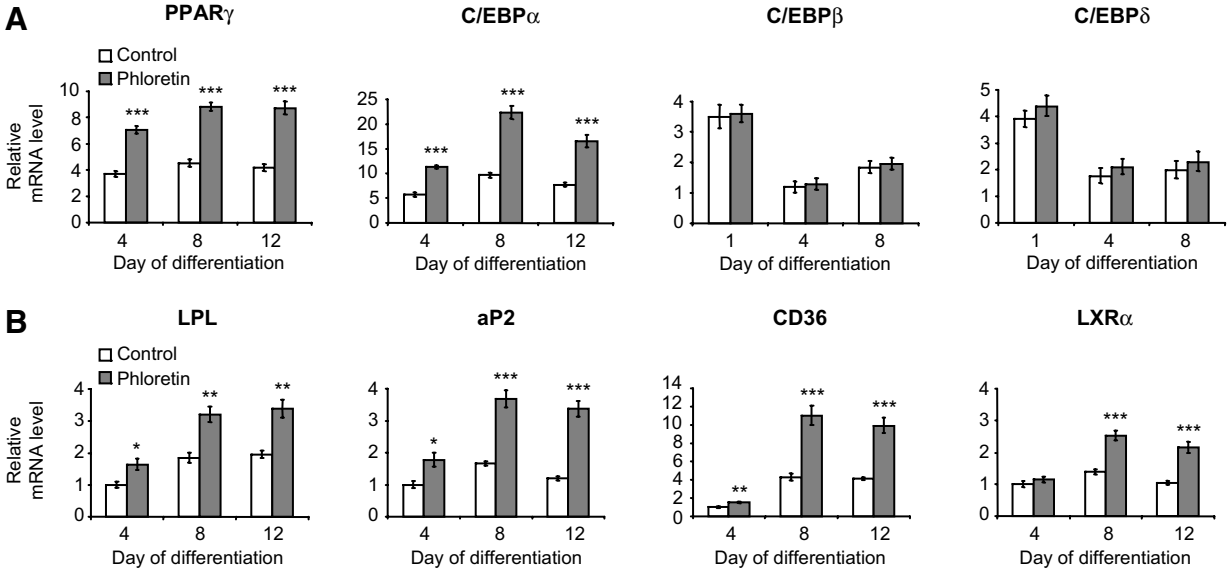

Fig. 2. Effects of phloretin on gene expression patterns during adipogenesis. Two-day postconfluent 3T3-L1 preadipocytes (day 0) were treated with 50 μM phloretin every 2 days for 12 days. Cells treated with 0.1% DMSO were used as controls. At the indicated days after inducing differentiation, total RNA was isolated and mRNA level of the indicated genes were measured by real-time quantitative RT-PCR. (A) mRNA expression of adipogenic transcription factors PPARγ, C/EBPα, C/EBPβ and C/EBPδ. Results were expressed relative to untreated cells at day 0 (not shown) after normalization to 18S rRNA. (B) mRNA expression of PPARγ target genes LPL, aP2, CD36, and LXRα. Results were expressed relative to untreated cells at day 4 after normalization to 18S rRNA. The bars represent mean values ± SD. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

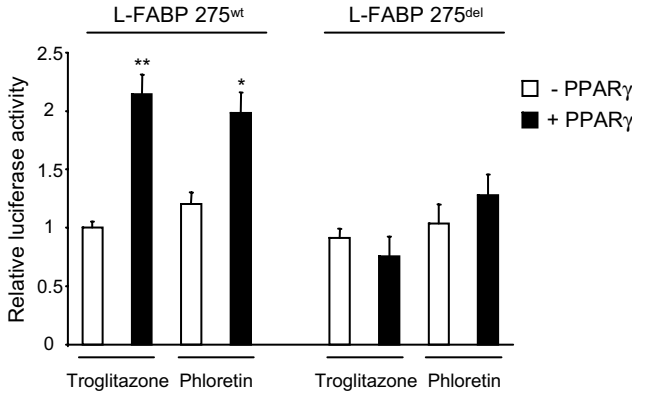

Fig. 3. Phloretin increases transcriptional activity of PPARγ. PPARγ expression vector (shaded bars) or pSG5 empty (open bars) were co-transfected with two different rat L-FABP promoter-luciferase constructs (L-FABP 275^{wt} and L-FABP 275^{del}) to COS-1 cells for 12 h. After transfection, cells were treated for a further 24 h with 50 μM phloretin or 10 μM troglitazone as positive control, and then assayed for luciferase activity which was normalized to β-galactosidase activity. Results are represented as arbitrary units of normalized luciferase activity. The bars represent mean values ± SD. $^{*}P < 0.05$; $^{**}P < 0.01$.

plasmid. No induction was observed with Adipo 908^{mut} reporter plasmid, where the PPRE harboring mutations.

Discussion

In the present study, we demonstrate that phloretin enhances 3T3-L1 adipocyte differentiation by increasing adipogenic gene expression. The cytosolic enzyme GPDH occupies a central position in the pathway of triglyceride synthesis and is linked to the characteristic changes of adipose conversion [14]. Here we show that phloretin significantly increases both GPDH activity and triglyceride content.

At the molecular level, adipogenesis is driven by a complex transcriptional cascade involving the sequential activation of C/EBPs and PPARγ [3]. C/EBPβ and C/EBPδ are rapidly and transiently expressed after the hormonal induction of differentiation. These factors act synergistically to induce the expression of C/EBPα and PPARγ, the master adipogenic transcription factors. C/EBPα and PPARγ together promote terminal differentiation by activating the transcription of genes involved in creating and maintaining adipocyte phenotype. Loss-of-function studies have convincingly shown that PPARγ is necessary as well as sufficient to promote adipogenesis and that C/EBPα is influential by maintaining the expression of PPARγ and promoting full insulin sensitivity [3]. Our results indicate that exposing 3T3-L1 preadipocytes to phloretin during adipogenesis increases C/EBPα and PPARγ mRNA levels but does not affect the expression of C/EBPβ and C/EBPδ. Thus, these data suggest that phloretin enhances adipogenesis through the up-regulation of C/EBPα and PPARγ without affecting the expression of upstream regulators.

Activation of PPARγ induces the expression of genes controlling adipocyte fatty acid metabolism, including lipoprotein lipase (LPL), fatty acid binding protein (aP2), fatty acid translocase (CD36) and liver X receptor α (LXRα). Here we show that phloretin increases the expression of PPARγ target genes during adipogenesis. This up-regulation could be related to the increase of PPARγ mRNA level and/or to the ability of phloretin to transactivate

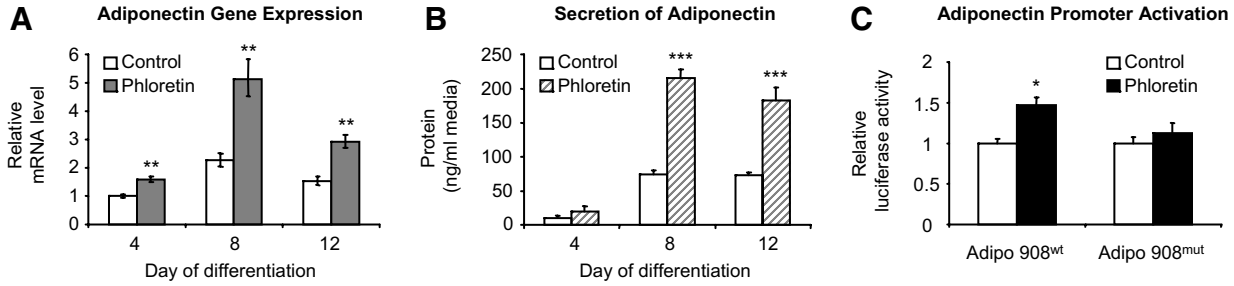

Fig. 4. Effects of phloretin on adiponectin expression and secretion. Two-day postconfluent 3T3-L1 preadipocytes (day 0) were treated with 50 μM phloretin every 2 days for 12 days. Cells treated with 0.1% DMSO were used as controls. (A) At the indicated days after inducing differentiation, total RNA was isolated and subjected to quantitative analysis of adiponectin by real-time PCR. Results were expressed relative to untreated cells at day 4 after normalization to 18S rRNA. (B) The cell culture media were sampled during adipogenesis and secreted adiponectin protein in these media were measured using an adiponectin ELISA. (C) The human adiponectin promoter-luciferase constructs (Adipo 908^{wt} or Adipo 908^{mut}) were co-transfected with PPARγ expression vector (pSG5-PPARγ) to COS-1 cells for 12 h. After transfection, cells were treated for a further 24 h with 50 μM phloretin or the vehicle DMSO as control, and then assayed for luciferase activity which was normalized to β-galactosidase activity. Results are represented as arbitrary units of normalized luciferase activity. The bars represent mean values ± SD.$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

PPARγ. Therefore, we assessed this latter hypothesis by co-transfection studies with a short version of rat L-FABP promoter. Our results demonstrate that phloretin induces transcriptional activity of PPARγ. Accordingly, a recent study where a selected group of chalcone and chalcone-TZDs derivatives have been synthesized reported that a hydroxyl group in C-4' or -5' position plays a key role in determining the potency of PPARγ activation [18]. It is noteworthy that phloretin belongs to the chalcone class of flavonoids and exhibits a hydroxyl group in C-4' position. This suggests that phloretin could act as a ligand of PPARγ.

Adiponectin is exclusively expressed by mature adipocytes and is the most abundant circulating adipokine. Hypoadiponectinemia appears to play an important causal role in insulin resistance, type 2 diabetes and the metabolic syndrome [19]. Recently, marked overexpression of adiponectin in stably transduced 3T3-L1 cells has been shown to promote adipocyte differentiation through autocrine effects [20]. Interestingly, we could hypothesize that phloretin may enhance adipogenesis via increased adiponectin expression and secretion, in addition to promoting C/EBPα and PPARγ mRNA levels. Adiponectin gene transcription has been shown to be induced by PPARγ through a PPRE in its promoter [17], and by C/EBPα through an intronic enhancer [21]. Here we show that phloretin is able to stimulate PPARγ-induced transactivation of human adiponectin promoter. From a physiological point of view, an increase in adiponectin secretion would have favorable effects against insulin resistance as well as atherogenic and inflammatory processes [7,19].

A dysregulation of white adipose tissue is strongly associated with the development of cardiovascular risk factors and type 2 diabetes. The inability of enlarged adipocytes to store more fat leads to ectopic accumulation of triglycerides in muscle and liver, hepatic and muscle insulin resistance, glucose intolerance, and even overt diabetes [22]. Furthermore, not only obesity but also impaired adipogenesis is associated with insulin resistance [23]. TZDs, as activators of PPARγ, are the first line of agents that directly target the adipocyte. These anti-diabetic drugs improve insulin sensitivity by reducing peripheral insulin resistance and thus lower blood glucose levels in patients with type 2 diabetes [5]. It has been reported that TZDs promote adipocyte differentiation by increasing the number of small adipocytes more sensitive to insulin than large adipocytes [4,5]. Therefore, PPARγ activation leads to increased uptake of glucose and free fatty acids, storage of triglycerides and production of adiponectin [5]. Our *in vitro* experimental data indicate that 50 μM phloretin increases adipogenesis and adiponectin expression through PPARγ pathway similarly to the effects of TZDs on adipose tissue. Note that plasma concentrations of phloretin from phloretin-supplemented rats reach 55 to 65 μmol/l after 10 h [24]. This implies that the concentration of phloretin used in our study can be achieved in plasma after supplementation. Thus, our data suggest that phloretin as a naturally occurring flavonoid may be beneficial for reducing insulin resistance *via* its potency to regulate the adipocyte function.

In conclusion, we show that phloretin enhances 3T3-L1 adipocyte differentiation by increasing C/EBPα and PPARγ mRNA levels, and by inducing PPARγ transcriptional activity. We also provide evidence that phloretin stimulates adiponectin expression and secretion, which may contribute to promoting adipocyte differentiation and notably improving insulin sensitivity. Although the physiological relevance of these results requires further investigation, they suggest for the first time a beneficial action of phloretin in the prevention of insulin resistance and related disorders.

Acknowledgments

The present work was financially supported by ANRT (French Research Ministry) and the Andros Company.

The authors thank Christiane Malezet-Desmoulins for her technical help in co-transfection studies.

References

[1] E.D. Rosen, B.M. Spiegelman, Adipocytes as regulators of energy balance and glucose homeostasis, Nature 444 (2006) 847–853.
[2] A.R. Nawrocki, P.E. Scherer, Keynote review: the adipocyte as a drug discovery target, Drug Discov. Today 10 (2005) 1219–1230.
[3] S.R. Farmer, Transcriptional control of adipocyte formation, Cell Metab 4 (2006) 263–273.
[4] B.M. Spiegelman, PPAR-γ: Adipogenic regulator and thiazolidinedione receptor, Diabetes 47 (1998) 507–514.
[5] B. Staels, J.C. Fruchart, Therapeutic roles of peroxisome proliferator-activated receptor agonists, Diabetes 54 (2005) 2460–2470.
[6] T. Ronti, G. Lupattelli, E. Mannarino, The endocrine function of adipose tissue: an update, Clin. Endocrinol. (Oxf) 64 (2006) 355–365.
[7] T. Kadowaki, T. Yamauchi, Adiponectin and adiponectin receptors, Endocr. Rev. 26 (2005) 439–451.
[8] I.C. Arts, P.C. Hollman, Polyphenols and disease risk in epidemiologic studies, Am. J. Clin. Nutr. 81 (2005) 317S–325S.
[9] K.W. Lee, Y.J. Kim, D.O. Kim, H.J. Lee, C.Y. Lee, Major phenolics in apple and their contribution to the total antioxidant capacity, J. Agric. Food Chem. 51 (2003) 6516–6520.
[10] P. Hilt, A. Schieber, C. Yildirim, G. Arnold, I. Klaiber, J. Conrad, U. Beifuss, R. Carle, Detection of phloridzin in strawberries (Fragaria × ananassa Duch.) by HPLC-PDA-MS/MS and NMR spectroscopy, J. Agric. Food Chem. 51 (2003) 2896–2899.
[11] M.M. Raja, N.K. Tyagi, R.K. Kinne, Phlorizin recognition in a C-terminal fragment of SGLT1 studied by tryptophan scanning and affinity labeling, J. Biol. Chem. 278 (2003) 49154–49163.
[12] R.J. Williams, J.P. Spencer, C. Rice-Evans, Flavonoids: antioxidants or signalling molecules? Free Radic Biol. Med. 36 (2004) 838–849.
[13] V. Stangl, M. Lorenz, A. Ludwig, N. Grimbo, C. Guether, W. Sanad, S. Ziemer, P. Martus, G. Baumann, K. Stangl, The flavonoid phloretin suppresses stimulated expression of endothelial adhesion molecules and reduces activation of human platelets, J. Nutr. 135 (2005) 172–178.
[14] L.S. Wise, H. Green, Participation of one isozyme of cytosolic glycerophosphate dehydrogenase in the adipose conversion of 3T3 cells, J. Biol. Chem. 254 (1979) 273–275.
[15] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2-Delta Delta CT method, Methods 25 (2001) 402–408.
[16] J.F. Landrier, C. Thomas, J. Grober, H. Duez, F. Percevault, M. Souidi, C. Linard, B. Staels, P. Besnard, Statin induction of liver fatty acid-binding protein (L-FABP) gene expression is peroxisome proliferator-activated receptor-α-dependent, J. Biol. Chem. 279 (2004) 45512–45518.
[17] M. Iwaki, M. Matsuda, N. Maeda, T. Funahashi, Y. Matsuzawa, M. Makishima, I. Shimomura, Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors, Diabetes 52 (2003) 1655–1663.
[18] S.H. Jung, S.Y. Park, Y. Kim-Pak, H.K. Lee, K.S. Park, K.H. Shin, K. Ohuchi, H.K. Shin, S.R. Keum, S.S. Lim, Synthesis and PPAR-γ ligand-binding activity of the new series of 2′-hydroxychalcone and thiazolidinedione derivatives, Chem. Pharm. Bull (Tokyo) 54 (2006) 368–371.
[19] T. Kadowaki, T. Yamauchi, N. Kubota, K. Hara, K. Ueki, K. Tobe, Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome, J. Clin. Invest. 116 (2006) 1784–1792.
[20] Y. Fu, N. Luo, R.L. Klein, W.T. Garvey, Adiponectin promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation, J. Lipid Res. 46 (2005) 1369–1379.
[21] L. Qiao, P.S. Maclean, J. Schaack, D.J. Orlicky, C. Darimont, M. Pagliassotti, J.E. Friedman, J. Shao, C/EBPα regulates human adiponectin gene transcription through an intronic enhancer, Diabetes 54 (2005) 1744–1754.
[22] H. Bays, L. Mandarino, R.A. DeFronzo, Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach, J. Clin. Endocrinol. Metab. 89 (2004) 463–478.
[23] X. Yang, P.A. Jansson, I. Nagaev, M.M. Jack, E. Carvalho, K.S. Sunnerhagen, M.C. Cam, S.W. Cushman, U. Smith, Evidence of impaired adipogenesis in insulin resistance, Biochem. Biophys. Res. Commun. 317 (2004) 1045–1051.
[24] V. Crespy, O. Aprikian, C. Morand, C. Besson, C. Manach, C. Demigne, C. Remesy, Bioavailability of phloretin and phloridzin in rats, J. Nutr. 131 (2001) 3227–3230.